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Proceedings of an FAO/IAEA Research Coordination Project on Medfly Mating

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MEDFLY AREAWIDE STERILE INSECT TECHNIQUE PROGRAMMES FOR PREVENTION, SUPPRESSION OR ERADICATION: THE IMPORTANCE OF MATING BEHAVIOR STUDIES

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Abstract

The Sterile Insect Technique (SIT) is amongst the most non-disruptive pest control methods. Unlike some other biologically-based methods it is species specific, does not release exotic agents into new environments and does not even introduce new genetic material into existing populations as the released organisms are not self-replicating. However, the SIT is only effective when integrated on an areawide basis, addressing the total population of the pest, irrespective of its distribution. There has been considerable progress in the development and integrated application of the SIT against the Mediterranean fruit fly (medfly), *Ceratitis capitata*, as reflected by operational programs for prevention, suppression and eradication of this pest. There is however, considerable scope for improving the efficiency of medfly SIT, an indispensable requirement for increased involvement of the private sector in any future application. One way to achieve this has been the development of genetic sexing strains, making it possible to release only sterile males. Another is improving sterile male performance through a better understanding of the sexual behavior of this insect. Unlike other insects for which the SIT has been successfully applied, medfly has a complex lek-based mating system in which the females exert the mate choice selecting among aggregated and displaying wild and sterile males. With the objective of developing a better understanding of medfly mating behavior, an FAO/IAEA Coordinated Research Project was carried out from 1994 to 1999. Some of the resulting work conducted during this period with the participation of research teams from ten countries is reported in this issue.

Key Words: Mediterranean fruit fly, medfly, *Ceratitis capitata*, areawide IPM, sterile insect technique, SIT, mating behavior, lek, quality control

Resumen

La técnica del insecto estéril (TIE) está entre los métodos de control de plagas menos perjudiciales. A diferencia de otros métodos con base biológica, la TIE es específica a nivel de especie, no transfiere agentes exóticos hacia nuevos ambientes y ni siquiera introduce nuevo material genético dentro de las poblaciones existentes debido a que los organismos liberados no se pueden auto replicar. Sin embargo, la TIE es solamente efectiva cuando se integra en forma extensiva, considerando el total de la población de la plaga, sin importar su distribución. Ha habido considerable progreso en el desarrollo y la aplicación integral de la TIE contra la mosca del Mediterráneo, *Ceratitis capitata*, tal como lo reflejan los programas operacionales para la prevención, supresión y erradicación de esta plaga. Existe sin embargo, un considerable campo para mejorar la eficiencia de la TIE de la mosca del mediterráneo, un requerimiento indispensable por aumentar la participació del sector privado en cualquier aplicación futura. Una forma de lograr esto ha sido a través del desarrollo de razas genéticamente sexadas, haciendo posible la liberación solamente de machos estériles. Otra es el mejoramiento del desempeño de los machos estériles por medio de un mejor entendimiento del comportamiento sexual de este insecto. A diferencia de otros insectos para los cuales la TIE ha sido aplicada exitosamente, la mosca del Mediterráneo presenta un complejo sistema de apareamiento basado la agregación de machos en un "lek", deutro del cual la hembra ejerce la selección de pareja escogiendo entre el total de los machos salvajes y estériles en cortejo. Con el objetivo de desarrollar un mejor entendimiento del comportamiento de apareamiento de la mosca del Mediterráneo, un proyecto de investigación coordinado por FAO/IAEA se llevó a cabo de 1994 a 1999. En esta edición se reportan algunos de los trabajos conducidos durante este periodo con la participación de equipos de investigación pertenecientes a diez países.

The effectiveness of integrating compatible pest control methods is significantly increased by coordinated implementation over larger contiguous areas to address whole target pest populations (Knipling 1979). This areawide IPM approach to pest management is gaining acceptance for some key insect pests (Tan 2000). Important tephritid fruit fly pests, such as the Mediterranean fruit fly (medfly), *Ceratitis capitata*, a notorious pest of quarantine importance because of its extremely wide host range (Liquido et al. 1991), are such key pests that invariably cause economic damage if left uncontrolled. The case for an areawide IPM approach arises for these key pests as they cannot be effectively controlled at the local orchard level without the systematic use of insecticide that disrupts the biological control of secondary fruit pests and also interferes with the use of other biologically-based control methods (Ehler & Endicott 1984).

Among biologically-based methods, the Sterile Insect Technique (SIT) is the most target-specific and non-disruptive method. Unlike some other biologically based methods it is species specific, does not release exotic agents into new environments and does not even introduce new genetic material into existing populations as the released organisms are not self-replicating. However, to be effective the released mass-reared and sterile males have to successfully transfer their sperm carrying dominant lethal mutations to a large majority of females of the target population. As when mating disruption using pheromones is applied, already-mated females that move into an area under treatment, are largely unaffected by the presence of sterile males, and proceed to lay their eggs into fruit. As a result, SIT is only effective when applied on an areawide basis addressing the pest simultaneously over urban, commercial, non-commercial and wild host areas. The areawide integration of SIT with other control methods, results in significant benefits for growers, providing them with enough incentives to associate and to cooperate.

STATUS OF SIT FOR MEDFLY

Over the last quarter century there has been considerable progress in the development and integrated application of SIT against medfly, as reflected by ongoing operational programs for eradication, prevention, and suppression leading to a rapid increase in sterile fruit fly production capacity (Fig. 1). The expanding use of medfly SIT is followed by similar trends for other fruit flies, particularly economically-important *Anastrepha* and *Bactrocera* species. The benefits accruing to the domestic and export markets for fruit and vegetable of all these programs have been of the order of hundreds of millions of U.S. dollars annually (Hendrichs 2000).
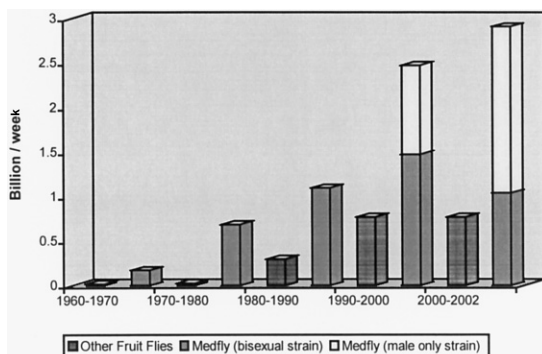
Fig. 1. Current worldwide production capacity of sterile fruit flies.

Medfly SIT Eradication Programs

The application of SIT against medfly focused initially on the concept of eradication, following the successful example of the screwworm, *Cochliomyia hominivorax*, which over the last fifty years has been eradicated from the U.S., Mexico and recently also from all of Central America and most of Panama (Wyss 2000). A number of medfly SIT eradication programs have eliminated populations of this species, succeeding in the establishment of medfly-free regions or whole countries. The first large SIT program against medfly was initiated in southern Mexico in 1977, with the construction of a 500 million sterile fly mass rearing facility in Tapachula. The aim of the Moscamed program was to prevent the spread of medfly, which had become established in Central America, into Mexico and the U.S.A. Establishment of medfly in Mexico would have threatened a multi-million fruit and vegetable export trade with the U.S.A. The program succeeded in 1982 in eradicating medfly from areas it had already infested in southern Mexico (Hendrichs et al. 1983) and since then a sterile fly barrier has been maintained from southern Belize through Guatemala to southern Mexico to assure the fly-free status of Mexico, U.S.A. and a large part of Guatemala (Villaseñor et al. 2000). In Chile, following many unsuccessful attempts to eradicate the pest using insecticides (Olalquiaga & Lobos 1993), eradication from the northern part of the country was achieved in 1995 with the integration of SIT, opening trade opportunities estimated over five years at a benefit to the Chilean fruit industry of ca. U.S.$ 500 million (SAG 1996). In Argentina, also as a result of SIT programs against medfly that started in the early 1990's, fly-free areas have been developed in various Patagonia valleys, and Argentina recently succeeded in negotiations with Chile to transport fruit from Mendoza and Patagonia provinces through medfly-free Chile for export from Chilean ports (De Longo et al. 2000).

Medfly SIT Prevention Programs

There are a number of examples were SIT is being applied as a preventive control to avoid the establishment of exotic or invasive fruit flies. These include Southern Australia, where sterile males are being released near Adelaide to prevent the establishment of medfly coming from Western Australia (Bill Woods, personal communication), and Okinawa, Japan where preventive releases of sterile melon flies are in progress along the southern-most islands of the archipelago to avoid re-establishment of melon fly coming from Taiwan (Kuba et al. 1996).

Probably most visible, are the repeated medfly introductions into high-risk areas in California and lately also Florida, threatening the exports of a multi-billion dollar fruit industry (Siebert & Cooper 1995). These have required recurrent emergency eradication actions, mainly consisting of insecticide applications, costing annually millions of U.S. dollars (Penrose 1995). Allowing the establishment of medfly in California would cost California ca. U.S.$ l.5 billion a year and result in a drastic increase of insecticide use (Siebert 1999). In view of the public opposition to recurrent aerial bait-spraying over urban areas (CDFA 1994), and the failure to eradicate these outbreaks with insecticides, authorities embarked on the area-wide use of SIT over the whole Los Angeles basin (LAB) starting in 1994, involving the aerial release of over 300 million sterile flies per week (Dowell & Penrose 1995). The SIT strategy was so successful technically, politically and environmentally, but also from the economic point of view (costing on average less than half that of recurrent emergency programs), that after eradication in 1996, areawide aerial releases were continued on a permanent basis over 5,500 km² of high risk areas in the LAB (Dowell et al. 1999; Dowell et al. 2000).

This Preventive Release Program (PRP) has been in operation since 1996 without major outbreaks of medfly occurring in the LAB. It has been expanded to ca. 6400 km² to include additional high-risk areas contiguous to the LAB. From 1987 until the inception of the PRP, the State of California faced repeated major medfly infestation in the LAB, with an average of 7.5 medfly infestations detected each year. Since the inception of the PRP this has dropped to 0.2 infestations per year (97% reduction) in the PRP area (CDFA, 2000). The few very confined medfly detections within the PRP boundaries prove the assumptions on which the PRP is based: a) that California, especially southern California is under constant threat of medfly invasion and b) that the PRP can prevent the development of medfly populations from these invasions. There is not a more biologically efficacious, environment-friendly and cheaper method to exclude medfly from southern California (CDFA 2000).

Medfly SIT Suppression Programs

Two factors have been responsible for an increasingly more cost-effective application of medfly SIT. The first factor is the development of strains for male-only release, made possible by continuing research supported and co-ordinated by the IAEA and FAO over the last two decades (Franz et al. 1996). Improved genetic sexing strains with higher production, increased stability and which are molecularly marked are now in use in almost all operational SIT programs (Robinson et al. 1999). The use of male-only strains is now the state of the art for medfly SIT and has resulted in a number of benefits among which are increased applicability of the SIT and also increased effectiveness of the sterile males in the absence of sterile females (Hendrichs et al. 1995, Rendon et al. 2000). The economic implications are significantly reduced costs of applying SIT per square kilometer per week in comparison to the use of bisexual strains (Enkerlin et al. in preparation).

Second, as is the case in any industrial production, biological or non-biological, significant economies of scale can be derived from larger mass rearing factories. Whereas the cost per million for sterile flies in small facilities producing tens of millions per week is relatively high, this cost decreases by more than half when mass rearing facilities reach a capacity of hundreds of millions per week (Enkerlin, unpublished data). The El Pino facility in Guatemala, which has reached a weekly production of over one billion sterile males, actually has a sliding scale of costs for their sales of sterile medfly males, that is inversely related to the production levels (Table 1).

Some of the initial medfly pilot SIT projects in the 1960s and early 1970s confirmed the effectiveness of SIT (De Murtas et al. 1970, Mellado et al. 1970, Ros et al. 1981, Rhode 1970, Kamburov et al. 1975, Cheikh et al. 1975); nevertheless, application of the SIT against medfly did not expand mainly because sterile fly costs were higher at that time when compared to conventional insecticide sprays. However, progress on both of the above factors has opened the possibility of using of SIT for routine medfly suppression, rather than only for eradication programs that are of a limited duration. Using SIT for suppression has the major advantage of not requiring the establishment of quarantines to protect free areas. In addition, in view of the increasing sensitivity to environmental concerns, there is new interest in using the SIT, particularly in the Mediterranean region where tourism and commercial fruit orchards co-exist, with the aim of producing low-insecticide or organic fruit. Sales of organically produced food, though still small, have been growing by 20% a year in the U.S.A. and in some European countries as much as 40% a year (The Economist 2001). This has stimulated the initiation of pilot SIT suppres-

TABLE 1. COSTS PER MILLION STERILE MALES AT VARYING PRODUCTION LEVELS AT THE MOSCAMED MEDFLY MASS
REARING FACILITY, EL PINO, GUATEMALA.[1]

Level of sterile male medfly production	Running costs[2] per million sterile males	Production costs[3] per million sterile males	Total cost per million sterile males
300 million per week	$199.29	$178.67	$377.96
400 million per week	$149.47	$178.67	$328.14
500 million per week	$119.47	$178.67	$298.24
600 million per week	$ 99.64	$178.67	$278.31
700 million per week	$ 85.41	$178.67	$264.08
800 million per week	$ 74.73	$178.67	$253.40
900 million per week	$ 66.43	$178.67	$245.10
1.0 billion per week	$ 59.78	$178.67	$238.45
1.1 billion per week	$ 54.35	$178.67	$233.02
1.2 billion per week	$ 49.82	$178.67	$228.49
1.3 billion per week	$ 45.99	$178.67	$224.98
1.4 billion per week	$ 42.76	$178.67	$221.43
1.5 billion per week	$ 39.87	$178.67	$218.54
1.6 billion per week	$ 37.50	$178.67	$216.17

[1]From Vollmershausen 2001.
[2]Includes direct administrative costs, depreciation, maintenance utilities, security and R&D.
[3]Includes diet materials, supplies, personnel and transportation.

sion programs in Tunisia (Cayol & Zarai 1999), Israel and Jordan (Rössler et al. 2000), Madeira (Pereira et al. 2000), and South Africa (Barnes et al. 2001). These SIT pilot projects have been effective in reducing insecticide applications and fruit losses, as well as rejections of transboundary shipments due to pest presence in fresh fruit exports (Barnes et al. 2001).

The economic feasibility of using SIT for medfly suppression has been confirmed by benefit-cost analyses (Enkerlin & Mumford 1997; Mumford 2000). Even without including the environmental benefits, costs per hectare per year of protecting orchards is now lower for an integrated areawide approach with SIT than for non-areawide conventional cover sprays, and approximately equal to the areawide application of bait-sprays. These savings to the fruit industries and the general environmental benefits already indicate the potential for the establishment of commercial SIT mass rearing facilities. The continuous demand for sterile males in SIT suppression programs should open the way for commercialization of the SIT. Nevertheless, further increases in the cost-effectiveness of medfly SIT are a precondition before serious private sector investment takes place in mass rearing facilities and sterile fly production.

There is a third area, in addition to the use of genetic sexing strains and the implementation of economies of scale, where there is considerable scope for improving the efficiency of medfly SIT. This is the relatively poor performance of the mass produced sterile males, which on average are approximately only one third to one half as competitive as the wild males (FAO/IAEA/USDA 2002). To compensate for this low effectiveness, high sterile to wild over-flooding ratios are routinely applied. A better understanding of medfly sexual behavior and the way it is affected by the processes of colonization, mass rearing and irradiation, could lead to improvements in sterile male performance, thus lowering current over-flooding ratios and overall costs of SIT application.

MATING SYSTEMS AND SIT

The nature of mating systems in any given species is determined primarily by ecological factors such as the distribution of resources (Emlen & Oring 1977). Based on resource distribution, male mating systems of insect pests that are the target of the SIT can be divided into three broad polygynous categories (Thornhill & Alcock 1983). First, there are the resource-defense systems where the potential for mate monopolization by males is high due to a clumped distribution of females and the resources that are attractive to receptive females (Table 2). Here, male mating success is largely determined by *intra-sexual* competition at these resources required by females, both to intercept females and to prevent other males from gaining access to females. Second, there are non-resource-based mating systems where mating takes place away from resources required by females. The potential for males to economically defend resources and females is rather low in this case because the resources and females are more widely dispersed, and intra-male selection involves a prolonged searching polygyny. Males participate in a type of continuous scramble competition, attempting to out-race their competitors to receptive females

TABLE 2. COMPARISON OF MALE MATING SYSTEMS OF INSECT PEST SPECIES THAT ARE THE TARGET OF APPLICATION OF THE STERILE INSECT TECHNIQUE.

Type of male mating system[1]	Male intra-sexual selection	Defense of female-required resources	Potential for monopolization of females	Pheromone release	Non-resource-based mating territories	Visual sound and tactile male courtship behaviors	Inter-sexual selection and female mate Choice	Examples of insect pest targets of SIT application
Resource defense polygyny	Yes	Yes	High	No	No	No	No	New and Old World Screwworm; Tsetse spp.
Prolonged searching polygyny	Yes	No	Low	Females	No	No	No	Codling Moth; Pink Bollworm; etc.
Lek polygyny	Yes	No	Low	Males	Yes	Yes	Yes	Medfly and other Tephritid fruit flies

[1]Classification of mating systems after Thornhill & Alcock 1983.

that are releasing pheromones. Third, there are lek-mating systems, also non-resource-based, where the potential for males to monopolize resources and females is also rather low. However, in these cases, males establish "symbolic" mating territories, and compete by attracting females and attempting to exclude competitors from the mating arenas. In lek mating systems, unlike the two previous mating systems, *inter-sexual* selection components are also involved, with females exerting mate choice by visiting aggregated males and selecting a mate from amongst them.

Tsetse flies, *Glossina* spp. and screwworm flies *Cochliomyia hominivorax* and *Chrysomyia bezziana*, are examples of the first type of mating system that are mainly resource-based (Table 2). Males seek mates on animal hosts where females forage for food (blood in the case of tsetse) or food and oviposition sites (animal wounds in the case of screwworms). In these species, the interaction between the sexes is relatively simple in view of the absence of any courtship and males compete at such encounter sites trying to get hold of females (Jaensson 1979). Once a male manages to grab a female, and confirms through tarsal contact species-specificity of the female based on her cuticular hydrocarbon profile particular to each species, copulation takes place (Pomonis et al. 1993, Carlson et al. 2000). Mating success in these species is therefore largely determined by the sexual aggressiveness of the males, sterile or wild, in intercepting receptive females at these important resources.

On the other hand, the mating system of such pest Lepidoptera as codling moth (*Cydia pomonella*) and pink bollworm (*Pectinophora gossypiella)* is non-resource based, however, also generally involves no male courtship, nor direct female mate choice (Table 2). Sterile and wild males participate in a type of scramble competition following pheromone trails originating from receptive females, with the winner being first in reaching the females and transferring the spermatophore (Snow et al. 1976).

A majority of the tropical and subtropical tephritid fruit flies, including medfly and a majority of *Anastrepha* and *Bactrocera* spp. have a lek polygyny, involving both male *intra-sexual* selection and also *inter-sexual* selection (Prokopy 1980). Males have to find and join leks (male aggregations in mating arenas) within which they have to participate in aggressive encounters with other males to defend sites from which to signal and court females. Receptive females are attracted by male pheromone to the leks for the sole purpose of soliciting courtships from various males, thus comparing their performance and eventually accepting one for mating.

While in tephritids with a lek mating system the population as a whole has a sex ratio of close to 1:1, females are not synchronized in their sexual maturation and thus only a small proportion of females will visit leks at any given time. Therefore the operational sex ratio, the number of courting males for each attracted mature and receptive female in a lek, is largely biased in favor of males. As a result, male intra-sexual competition is intense and the differential mating success can be large, with many males getting no mates, and a few males obtaining many matings (Arita & Kaneshiro 1985, Hendrichs 1986). Bisexual releases of sterile flies, half of which include sterile virgin females that all become receptive within a short time period, decrease the operational sex ratio of males to females at leks. On the other hand, the use of male-only strains for sterile fly releases results in operational sex ratios that increase even further the male bias at mixed leks compared to wild male leks and thus the need for releasing higher quality sterile males. Increasing sterile to wild male over-flooding ratios in species with a lek polygyny is less effective in overcoming reduced sterile male competitiveness than in species with the other mating systems. As wild females actively select and discriminate in favor of males releasing timely pheromone of the adequate profile (Heath et al. 1994) and performing properly visual, sound and tactile courtship behaviors (Eberhard 2000), they may still favor the courtship of a wild male even though he may represent a minority within a mixed lek. Thus the effective application of the SIT for lek species requires a more detailed understanding of the mating systems. They also require a much more sophisticated quality control system to measure and assure the sterile male performance.

STATUS OF MEDFLY SEXUAL BEHAVIOR STUDIES AND QUALITY CONTROL OF STERILE FLIES

Even though various workers had studied various aspects of medfly behavior in the pre-SIT era (Féron 1962), it was the implementation of SIT that stimulated most research into medfly sexual behavior. Concerns about whether mass-reared, or even any laboratory-reared, medfly strains were exhibiting wild-like characters in terms of sexual behavior and competitiveness motivated research as early as the 1970s. Pilot SIT activities against medfly in Central America, Hawaii, Israel and elsewhere resulted in assessments of the mating competitiveness of irradiated and non-irradiated medflies (Causse 1970, Holbrook & Fujimoto 1970, Fried 1971, Rössler 1975), and the publication of a collection of quality control tests for fruit flies in general (Boller & Chambers 1977), which included various tests relevant to medfly mating behavior.

The initiation of the Moscamed program in the late 1970s, resulted in the renewed interest in medfly mating studies. This program, required a quality control system for a weekly production of

500 million sterile flies. As part of this effort, the description of the lek mating behavior of wild medflies, observed under semi-natural conditions in field cages, was provided (Prokopy & Hendrichs 1979), and the first medfly mating compatibility test on a field-caged host tree was carried out to measure female mate choice by allowing wild females to select among competing wild and sterile males under natural conditions (Zapien et al. 1983). In addition, a collection of field tests was developed for confirming and extending a series of laboratory tests (Boller et al. 1981, Chambers et al. 1983) and the first quality control manual for medfly mass rearing and field evaluation was produced (Orozco et al. 1983), which has been used extensively to measure quality of mass produced flies. These publications formed the basis for a USDA quality control manual, compiled to ensure that sterile medflies for SIT programs with USDA involvement met certain quality standards (Brazzel 1986).

Further refinements have come with behavioral studies in the open field to validate the medfly behaviors observed on field-caged host trees (Hendrichs & Hendrichs 1990, Hendrichs et al. 1991, Whittier et al. 1992). More recent studies addressed many other aspects including the various effects of mass-rearing (Calkins et al. 1994, Calkins et al. 1996), male size (Orozco & Lopez 1993), nutritional status (Blay & Yuval 1997), mating-induced changes in female behavior (Jang 1995, Jang et al. 1998), strain differences (Liedo et al. 1996), and behavioral incompatibility between wild and mass reared flies (McInnis et al. 1996).

The conclusion from all these studies has been that although sterile mass reared medflies do join and compete within leks, achieve a portion of matings with wild females, and transfer sperm and induce female refractoriness and sterility in offspring, they are clearly less competitive than their wild counterparts. These behavioural changes appear not to be caused by mating incompatibility among different medfly populations (Cayol 2000a), but rather by mass-rearing conditions, the irradiation process and the years a strain is held in colonization (Cayol 2000b).

FAO/IAEA Sponsored
Coordinated Research Project

The Joint Division of Nuclear Techniques in Food in Agriculture of the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) sponsors Coordinated Research Projects (CRPs) or research networks that focus participating scientists from both developing and developed countries on applying nuclear techniques to specific problems relevant to agriculture. A CRP entitled "Medfly Mating Behavior Studies under Field Cage Conditions" was initiated in 1994 with three objectives: a) to develop a detailed understanding of medfly courtship behavior and female choice through experimentation and slow-motion video analysis, b) to measure mating compatibility worldwide among medfly populations, and c) to develop harmonized mating tests to measure competitiveness and compatibility of sterile flies. This CRP concluded in 1999 and four Research Coordination Meetings (RCMs) were held to review results and plan future research, Vienna, Austria (4-7 October, 1994), Tapachula, Mexico, (19-23 February 1996), Tel Aviv, Israel (15-19 September) and Antigua, Guatemala (29 June-03 July 1999). Twelve research teams from ten countries (Argentina, Austria, Costa Rica, France, Greece, Guatemala, Israel, Kenya, Mexico, U.S.A.) participated and conducted research on different aspects of medfly sexual behavior. The research findings resulting from this CRP, published as refereed publications in scientific journals and as a series of papers in this issue are included in the listing of relevant references in Table 3.

APPLICATION OF RESEARCH FINDINGS:
INTERNATIONAL FRUIT FLY
QUALITY CONTROL MANUAL

On the basis of all the above studies, as well as information from various fruit fly quality control manuals, a concerted effort was made to develop an FAO/IAEA/USDA manual on "Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies" (FAO/IAEA/USDA 2002). The objective was to incorporate the improved understanding of medfly mating behavior into quality control protocols, to harmonize procedures and thus allow comparison of sterile fly quality over time and across rearing facilities and field release programs.

Based on the CRP findings, the FAO/IAEA/USDA international manual emphasizes mating competitiveness, sexual compatibility and post-mating factors and de-emphasizes the widely used laboratory mating propensity test. This test is carried out only with mass reared males and females, under high densities and in small Plexiglas cages, all conditions that favor sterile males and thus it routinely overestimates sterile male performance. Even worse, it measures the wrong parameter, namely speed of pair formation between mass-reared males and females, even though sterile males trying to achieve fast mating, short-cutting steps in the courtship sequence, are only successful under crowded colony rearing conditions but are unsuccessful in mating with wild females (Briceño et al. 1996, Briceño & Eberhard 1998).

The international manual recognizes that the most important indicator of sterile male quality control is their successful interaction with wild females of the target population. Thus a standard field-cage test is required as an ultimate measure of quality, where wild females of the target population

TABLE 3. LISTING OF RELEVANT STUDIES CONDUCTED ON VARIOUS ASPECTS RELATED TO MEDFLY SEXUAL BEHAVIOR AND MATING COMPETITIVENESS.

Field of Study	Relevant References
Courtship behavior in relation to sexual competitiveness	Feron 1962; Sivinski et al. 1989; Whittier et al. 1994; Whittier & Kaneshiro 1995; Briceño et al. 1996; Liimatainen et al. 1997; Briceño & Eberhard 1998; Lance et al. 2000; Briceño & Eberhard 2002; Briceño et al. 2002; Lux et al. 2002b
Lekking behaviour in relation to sexual competitiveness	Prokopy & Hendrichs 1979; Arita & Kaneshiro 1985; Arita & Kaneshiro 1989; Shelly et al. 1993; Shelly & Whittier 1995; Yuval et al. 1998; Kaspi & Yuval 2000; Field et al. 2002
Field cage evaluations in relation to sexual competitiveness	Wong et al. 1983; Zapien et al. 1983; Rendon et al. 1996; Cayol et al. 1999; Katsoyannos et al. 1999; Calcagno et al. 2002; Economopoulos & Mavrikakis 2002
Open field studies and evaluation of field competitiveness	Hendrichs & Hendrichs 1990; Whittier et al. 1992; McInnis et al. 1994; Rendon et al. 2000; Shelly 2000
Feeding behavior and nutritional status in relation to sexual competitiveness	Hendrichs et al. 1991; Warburg & Yuval 1997; Blay & Yuval 1997; Cangussu & Zucoloto 1997; Bravo & Zucoloto 1998; Field & Yuval 1999; Kaspi & Yuval 2000; Papadopoulos et al. 2001; Shelly et al. 2002; Yuval et al. 2002
Predation in relation to sexual behavior	Hendrichs et al. 1993; Hendrichs & Hendrichs 1994; Hendrichs & Hendrichs 1998
Morphometric traits in relation to sexual competitiveness	Churchill-Stanland et al. 1986; Orozco & Lopez 1993; Hunt et al. 1998; Menez et al. 1998; Blay & Yuval 1999; Hunt et al. 2002; Hasson & Rossler 2002; Rodriguero et al. 2002
Irradiation in relation to sexual competitiveness	Causse 1970; Holbrook & Fujimoto 1970; Hooper 1971; Hooper 1972; Calkins et al. 1988; Heath et al. 1994; Lux et al. 2002a
Age in relation to sexual competitiveness	Liedo et al. 1996b; Papadopoulos et al. 1998; Taylor et al. 2001; Liedo et al. 2002
Factors affecting female post-mating and re-mating behavior	Boller et al. 1994; Jang 1995; Hendrichs et al. 1996; Jang et al. 1998; Jang 2002; McInnis et al. 2002; Vera et al. 2002
Effects of mass-rearing in relation to sexual competitiveness	Liedo et al. 1996a; Calkins 1991; Calkins et al. 1994; Calkins et al. 1996; Cayol 2000b
Inter-population compatibility and isolation studies	McInnis et al. 1996; Cayol 2000a; Cayol et al. 2002;
Comparative approaches to sexual behavior in Tephritidae	Myburgh 1962; Prokopy 1980; Sivinski et al. 2000; Yuval & Hendrichs, 2000; Quilici et al. 2002

are the final arbiters of sterile male quality. This test is carried out on a routine basis under semi-natural conditions on field-caged host trees. Further fine-tuning of the manual is a continuous process through which the procedures evolve as new findings emerge. In support of this process, a new 6-year FAO/IAEA Coordinated Research Project on "Quality Assurance of Mass Produced and released Fruit Flies" has been initiated involving participants from all major fruit fly SIT programs.

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DECISIONS DURING COURTSHIP BY MALE AND FEMALE MEDFLIES (DIPTERA, TEPHRITIDAE): CORRELATED CHANGES IN MALE BEHAVIOR AND FEMALE ACCEPTANCE CRITERIA IN MASS-REARED FLIES

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ABSTRACT

Analyses of more than 300 videotaped courtships of wild and mass-reared medflies from Costa Rica showed that the tendency for male and female to align themselves facing directly toward each other increased, and that the distance between them decreased as courtship proceeded. More direct alignments and shorter distances between the flies at the moment the male jumped onto the female were correlated with greater female acceptance of copulation. There were no consistent differences in durations of components of intermittent buzzing songs or male size between successful and unsuccessful courtship in either strain. Several possible cues may release different courtship responses: males of both strains tend to initiate both continuous vibration and intermittent buzzing after reduction of the distance to the female; slow creeping toward the female was associated with longer courtships that had failed to lure the female close; and females tended to turn to face more directly toward the male soon after the male began continuous vibration, and especially after he began intermittent buzzing. Females became progressively more immobile as courtship progressed, especially soon after intermittent buzzing began. There were numerous differences between strains. Mass-reared males were more likely to mount females without previous courtship than were wild males. Wild males initiated continuous wing vibration when farther from the female and when the female was looking less directly toward them, but the two strains did not differ in the distances and angles at which males initiated intermittent buzzing and jumped. Wild males were more likely to creep toward the female during intermittent buzzing. Mass-reared females but not wild females were more likely to copulate when the proportion of time the male had spent in intermittent buzzing was low, and if the courtship began when the flies were nearer each other. Wild but not mass-reared females were less likely to copulate if courtship was shorter. Possible coevolution of female responses with the five different male courtship traits that differ between mass-reared and wild flies are discussed.

Key Words: medfly, sexual selection, courtship behavior, mass-rearing, female choice

RESUMEN

Análisis de más de 300 cortejos video-grabados de moscas del Mediterráneo silvestres y criadas en masas de Costa Rica demostraron que la tendencia de los machos y las hembras de alinearse cara a cara el uno frente al otro aumentó, y que la distancia entre ellos ha disminuó a medida que el cortejo procedía. Alineaciones más directas y distancias mas cortas entre las moscas en el momento en que el macho salta sobre la hembra se correlacionaron con mayor aceptación por parte de las hembras. No hubo diferencias consistentes en la duración de los componentes de los zumbidos intermitentes de las canciones o el tamaño del macho, entre los cortejos efectivos y no efectivos en ninguna de las dos razas. Varios estímulos posibles podrían inducir diferentes respuestas en el cortejo: machos de ambas razas tienden a iniciar vibraciones tanto continuas como intermitentes que después de se reduce la distancia a la hembra; un lento acercamiento hacia la hembra se asoció con cortejos más largos que no lograran inducir el acercamiento de la hembra; y hembras que presentaron la tendencia de girar y encarar más directamente al macho pronto después de que el macho inició las vibraciones continuas, y especialmente después de que iniciaron los zumbidos intermitentes. Las hembras se hicieron progresivamente más inmóviles a medida que el cortejo continuaba, especialmente poco tiempo después que el zumbido intermitente se inició. Existieron numerosas diferencias entre las razas. Los machos criados en masa montaron a las hembras sin ningún tipo de cortejo previo, con mayor frecuencia que los machos silvestres. Los machos silvestres iniciaron vibración continua cuando se encontraban a mayor distancia de la hembra y cuando las hembras se orientaron menos directamente hacia ellos, pero las dos razas no difirieron en las distancias y ángulos a los cuales los machos iniciaron su zumbido intermitente y saltaron. Los machos silvestres se acercaron lentamente hacia las hembras con mayor frecuencia durante el zumbido intermitente. Las hembras criadas en masa, a diferen-

cia de las hembras silvestres presentaron mayor tendencia a copular cuando la proporción de tiempo que el macho utilizó en el zumbido intermitente fue baja, y si el cortejo comenzó cuando las moscas estaban mas cerca las unas a las otras. Hembras silvestres a diferencia de las hembras criadas en masa, tendieron a copular menos si el cortejo era mas corto. La posibilidad de coevolución en las respuestas de la hembra hacia los cinco rasgos del cortejo que difieren entre las moscas criadas en masas y las mosca silvestres se discuten.

The success of the massive efforts to control pest populations of the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann using mass-reared sterile males depends on the abilities of these males to successfully induce wild females to copulate with them. Nevertheless, current understanding of why it is that some courtships result in copulation, while the majority do not, is only fragmentary. The commonly observed mating inferiority of mass-reared males as compared with wild males when they are paired with wild females (e.g., Rössler 1975b, Calkins 1984, Shelly et al. 1994, Hendrichs et al. 1996) is apparently due to their inadequate courtship per se, rather than to inferior abilities to find and attend leks, or to attract females pheromonally and begin to court them once they are at a lek (Shelly et al. 1994, Shelly & Whittier 1996, Hendrichs et al. 1996, Liimatainen et al. 1997, Lance et al. 2000.). Differences in courtship behavior between wild and mass-reared males are, however, only starting to be studied (Briceño et al. 1996, Liimatainen et al. 1997, Briceño & Eberhard 1998, 2000 in press).

Courtship in medflies was first studied in detail by Feron (1962) and current knowledge was reviewed by Eberhard (2000). Usually courtship follows a relatively standard sequence of events, during which the male courts actively but stays more or less in one place. The female performs little if any overt courtship behavior, but moves toward the male and aligns herself facing him. Active male behavioral courtship begins when the male (usually while he is in the pheromone releasing posture—stage I of Feron) responds to the presence of the female (apparently on the basis of visual cues—Feron 1962, Kaneshiro 2000) by turning toward her. He bends his abdomen ventrally and starts to vibrate his wings (stage II of Feron, "continuous wing vibration" of Eberhard 2000). The abdominal pleura and the rectal sac, which are everted during stage I and presumably release pheromone (e.g., Nation 1981, Headrick & Goeden 1994 on other tephritids with similar structures), remain everted, and the wing vibrations, which involve rapidly twisting the wings on their longitudinal axes, presumably causes pheromone to be wafted toward the female (Arita & Kaneshiro 1989, Briceño & Eberhard 2000). The abdominal pleura often pulse during continuous wing vibration (unpublished data). After a variable amount of continuous wing vibration, the male switches abruptly to a second type of wing movement. He moves his wings rhythmically forward and back while continuing to vibrate them rapidly, and he also intermittently rocks his head from side to side and forward and backward (stage III of Feron, "intermittent wing buzzing" and "head rocking" of Eberhard 2000). In some cases the male "creeps" slowly toward the female with small steps that are taken each time he initiates a buzz (Briceño & Eberhard in press). Chemical signaling is probably altered and may be suspended during stage III, since the rectal sac is retracted when intermittent wing buzzing begins (Figure 3-3 of Feron 1962, Briceño et al. 1996). Head rocking often results in contact between the male's aristae and those of the female (Briceño & Eberhard in press).

After a variable amount of intermittent wing buzzing, the male jumps onto the female if she is appropriately positioned in front of him. If she does not dislodge him by flying or falling, as frequently occurs (Eberhard 2000, Lance et al. 2000), he aligns himself on her dorsum, and, if she everts her aculeus from the tubular eversible membrane, he grasps it with his genitalic surstyli and intromits (Eberhard & Pereira 1995). Males also perform apparent courtship movements during copulation itself (Eberhard & Pereira 1995), suggesting that the male also attempts to influence further female decisions (e.g., transport sperm—Yuval et al. 1996) after his genitalia have entered the female's body (Eberhard 1991). This stage of the male-female interaction will not be considered further here, and the term "courtship" will refer only to precopulatory behavior. There is at least one alternative male behavioral sequence. The male does not court, but simply jumps onto the female and immediately attempts to copulate (Prokopy & Hendrichs 1979).

These descriptions show that both the male and the female make a series of behavioral "decisions" or transitions during courtship. The male makes at least five decisions: whether to begin courtship or to jump immediately; when, if he is going to court, to begin continuous wing vibration; when to switch from continuous vibration to intermittent wing buzzing; whether to creep toward the female during intermittent buzzing; and when to terminate intermittent buzzing and jump onto the female. Females also make decisions, although some are less easily characterized. Indirect data (Briceño et al. 1996) indicate that female behavior which results in her being immobile, directly in front of the male and facing directly toward him, increases the chances that he will jump onto her. Such female responses may include turning or not turning, and walking or not walking. Once the male jumps, two further female decisions are

more easily categorized: whether or not to dislodge the male; and whether or not to evert her aculeus (and thus allow him to intromit).

Understanding the factors that influence the decisions of both males and females will probably help clarify why some courtships succeed and others fail. One attractive possibility is that males and females exchange signals during courtship (Lux & Gaggl 1996, Liimatainen et al. 1997). For instance, the male decisions to switch from continuous wing vibration to intermittent buzzing or to jump could be triggered by some particular female behavior indicating that she is receptive. A review of available data showed, however, that there is no quantitative evidence that any particular female behavior has a triggering effect on male behavior (Eberhard 2000). Briceño & Eberhard (1998) found three possible female signals that showed significant associations with eventual mounting attempts (strike the male with her head; lean slowly rearward and sometimes crouch; and tap the male's legs with her front legs); but none had a significant effect on the likelihood that the male would mount after the female performed them. In other words, males appeared not to pay attention to these possible signals from the female.

One possible cue that could be used in male-female dialogues is the female's position with respect to the male. There were differences between positions at the moment the male jumped onto the female as compared with positions when the courting male desisted from courting (Briceño et al. 1996). The present study constitutes an attempt to use similar correlational evidence, in this case from a much larger sample of courtships. Not only the male's decision to jump, but also his decision whether to court rather than jump immediately, when to initiate courtship, and when to switch from continuous wing vibration to intermittent buzzing, as well as the female's decision whether or not to allow a male to mate after he has mounted her are analyzed.

MATERIALS AND METHODS

Wild flies were raised from fallen tangerines and oranges collected at the Estación Experimental Fabio Baudrit of the Universidad de Costa Rica, el. about 900 m near Alajuela, Alajuela Province, Costa Rica. Mass-reared flies were from a strain that had been founded about three years previously with flies collected in the same area, and kept as adults thereafter (about 51 generations) in $2.30 \times 0.35 \times 0.50$ m breeding cages with approximately 60,000 flies/cage. Flies were separated by sex the day after emergence, and kept in $32 \times 32 \times 32$ cm cages with free access to water and a mixture of sugar and hydrolyzed protein. Immediately before taping sessions flies were aspirated into mating chambers (9.5 cm diameter plastic Petri dishes with millimeter ruled paper

on the top of the lid) that had either one pair/dish or that were divided into four equal sectors by cardboard walls with one pair/sector. A subset of the males was preserved by freezing, and later measured using an ocular micrometer at 30× (maximum width of head, maximum length and width of the thorax in dorsal view).

All data came from analyses of videotaped courtships in which the male mounted the female. To avoid pseudoreplication, only the first courtship of each pair of animals was analyzed, and the only courtships included were those in which the male performed a single episode of continuous wing vibration followed by a single episode of intermittent wing buzzing and then leapt onto the female (i.e., courtships in which, for example, the male resumed continuous vibration after intermittent buzzing and then eventually mounted were excluded). Each animal was used only once. Several variables were measured at each of seven moments that were associated with three transitions in male behavior: one s before and one s after the male initiated continuous wing vibration as well as the moment when he initiated this behavior; one s before, one s after, and the moment when the male initiated intermittent buzzing; and the moment when the male jumped onto the female. Data preceding and following transitions were analyzed to elucidate stimuli associated with particular decisions. For instance, a change in the female's position from one s prior to the initiation of buzzing to the moment when buzzing began could be the stimulus used by males to trigger buzzing. In contrast, only data from the moments that transitions occurred were used in analyses involving female acceptance of copulation.

Three variables that were measured to estimate the flies' relative positions are illustrated in Fig. 1: the distance between the centers of the two animals' prothoraces; the orientation of the female with respect to the direction in which the male was oriented (the angle she made with his longitudinal axis—"male angle" in Fig. 1); and the orientation of the male with respect to the direction in which the female was oriented (the angle he made with her longitudinal axis—"female angle" in Fig. 1). These variables were measured using the NIH Image program (public domain software) by grabbing a frame from the video with a Genius videocapture card (Pro II series) and importing it into a computer. Walking behavior was counted as forward movements, and did not include when the fly turned but the center of its thorax was immobile.

Other variables measured included the duration of each stage of courtship, and the length of time the female had been immobile preceding the moment the male leapt onto her. When the male jumped onto the female, the time elapsed until the female began to resist, whether or not she re-
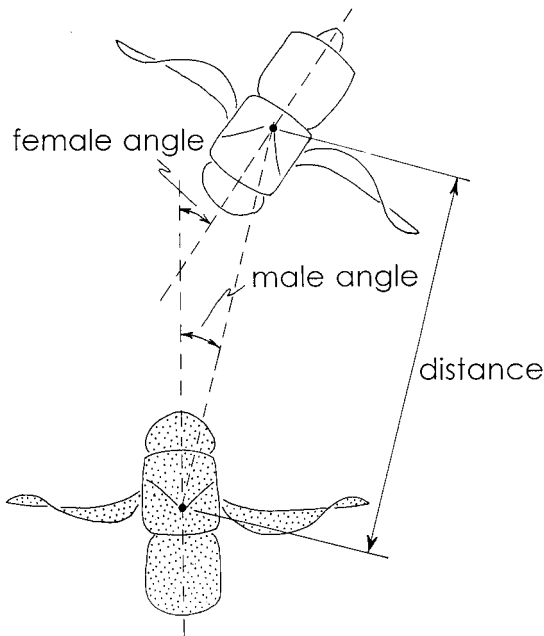
Fig. 1. Angles and distances measured (male stippled).

sisted, and the time the male took to turn and align himself facing in the same direction as the female, were also measured. Successful mounts were those in which the immobile female did not dislodge the male within 60 s of his having landed on her.

The sounds produced during intermittent buzzing were recorded using a small, Sennheiser MZK 80ZU, microphone inserted through a hole in the side of the mating chamber and connected to the camera. Recordings of sounds were imported from video recordings into a PC 486dx2 computer using a 16 bit card. Durations of buzzes and the intervals between buzzes were measured using the real time display in the program Avisoft® using cursors to mark the beginning and the end of the envelope curve displayed in the main window of the program (see Briceño et al. in press for further details). The precision of these measurements was determined by remeasuring the durations of 10 buzzes and 10 intervals in each of 8 different courtships. The average differences were 2.0 ms in buzz duration, and 2.2 ms in interval duration.

Most variables were not normally distributed, and means and standard deviations are presented for illustrative purposes only. Except where noted otherwise, all statistical tests of differences employed two-tailed Mann-Whitney U Tests.

We performed three types of analysis. First we made simple, variable by variable comparisons between strains and between unsuccessful and

successful courtships that led to successful mounts. For instance, we compared the length of intermittent buzzing preceding unsuccessful and successful mounts within both strains, and between strains. These analyses had the possible problem that some independent variables are probably correlated. For instance, an apparently significant effect of variable A on the female decision to copulate rather than reject the mounted male, might actually result from this variable's association with another independent variable B that truly does affect the female's decision. This possible dependence was tested with additional analyses, using the statistics program SYSTAT. These were organized into three questions:

1. Which aspects of the female's behavior during courtship are associated with increased probability that she will allow the male to mate when he mounts?

2. Which aspects of male courtship behavior may have induced this female receptivity?

3. Which cues are used by males to initiate continuous wing vibration to intermittent buzzing?

For those questions with a discontinuous response variable (e.g., unsuccessful, successful), we used stepwise logistic regressions (SYSTAT forward stepwise option). For the others we used ordinary multiple regressions. For each analysis we provided the program with a list of variables with possible effects, on the basis of the stimuli likely to be available to the fly making the response that was being tested. The program first selected from this list the variable that had the largest effect on the response variable, and calculated this effect. It then repeated the process with the remaining variables on the list while correcting for the effect of the first variable, and it continued this process until none of the remaining variables had significant effects on the response variable ($P < 0.05$). In each round the effects of all variables that had already been selected in previous rounds were held constant.

An additional complication is that flies may have multiple threshold criteria for some decisions. Thus, for instance, the female may only allow a mounted male to copulate when the distance between them is below some critical value and in addition the duration of his courtship is above some other critical value. Such interactions could impede detection of decision criteria. We thus performed an additional set of logistic regressions in which we tested for interactions between pairs of independent variables in their effects on the response variable. Regression models were constructed for different questions as before, but in this case we checked for significant interactions between each of the variables that

had been found to have a significant effect in the first model when each was combined with all of the other variables in the list which had not had significant effects in the original model. These interactions analyses were performed separately from other analyses because even with our large sample sizes it was not possible to find significant effects for more than about 4 variables at a time with these regression techniques.

It is important to keep in mind that a given behavioral variable may be influenced by both the male and the female. Consider, for example, the length of time the female was immobile before the male jumped onto her. The male clearly makes the decision to jump, and it would thus seem reasonable to include this time in the model for question 2 (male effects on female receptivity to copulation), but not in the model for question 1 (female indicators of receptivity). But it is also obvious that the female herself determines whether or not she moves, and female movement probably inhibits jumping by the male. This kind of interdependence sometimes made it difficult to decide which variables should be included which models. In some cases the same variable was included in different lists.

RESULTS

Table 1 and Figures 2, 4, and 6-8 present variable-by-variable analyses of behavioral traits with respect to strain and male copulation success. Figures 3 and 5 illustrate changes just before and just after male initiation of continuous vibration and intermittent buzzing behavior. Table 2 presents the results of regression analyses testing for independence of the effects of different variables. Table 3 gives the results of logistic regression analyses of the interactions between those variables with significant effects in the models in Table 2 and the rest of the variables that did not have significant effects in these models. In general, the regression analyses confirmed the results of the variable-by-variable analyses, but did not reveal many additional relationships. We will discuss the results variable by variable.

Distances between the Male and Female

The distance between the male and female tended to decrease as courtship proceeded in both mass-reared and wild flies (Fig. 2) ($P < 0.0001$ with Kruskal-Wallis Test for each strain; a posteriori Duncan tests showed that differences between all three pairs of values at the moment of transition were significant in both strains $P < 0.001$). Wild males initiated continuous vibration at significantly greater distances females than did mass-reared males (Figs. 2 and 3, Table 1). Combined values for successful and unsuccessful courtships differed by more than a factor of 2 between the two strains (0.64 ± 0.40 cm vs. $1.60 \pm$

2.12 cm, $P < 0.001$). Distances when the male initiated subsequent stages in courtship did not differ between the two strains (Figs. 2 and 3, Table 1), even when successful and unsuccessful courtships were combined ($P > 0.05$). The distance from which the male jumped onto the female was relatively less variable within each strain than the other distances (e.g., error bars in Fig. 2; Barlett's homogeneity of variances test showed significant differences between the variances in all three variables in both strains, $P < 0.001$).

In both strains the distance between male and female decreased significantly during the s prior to initiation of continous vibration, and during the s prior to initiation of intermittent buzzing (BC vs. C and BI vs. I in Fig. 3), suggesting that reduction in the distance may be a cue used by males to trigger both behavior patterns. Distances did not decrease significantly during the s after continuous vibration began, nor during the s after intermittent buzzing began, suggesting that these male activities did not immediately induce the female to approach him. There was no significant difference associated with successful vs. unsuccessful mounts in either strain with respect to any of the distances measured (Table 1).

Male Angles

The male angles at the moments of transition changed very little during the course of courtship in both strains (Fig. 4) ($P = 0.021$ with Kruskal-Wallis Test on mass-reared flies, but no pairs were significantly different with a posteriori Duncan Tests; the male's angle at the start of continuous wing vibration was slightly larger than either of the other two in wild flies with similar tests). There was, however, a tendency in both strains for the male to turn to face more directly toward the female in the s preceding initiation of both continuous vibration and intermittent buzzing (Fig. 5). The strains did not differ consistently (Figs. 4 and 5, Table 1).

Mounts by both mass-reared and wild males were more likely to be successful when the male angle was lower at the moment the male jumped (Table 1), although in mass-reared flies this effect was not significant in regression analyses (Table 2, Model 2). The male angle at the moment he jumped was clearly smaller preceding successful as compared with unsuccessful courtships when data from the two strains were combined ($2.8 \pm 6.7°$ vs. $4.6 \pm 5.3°$; $P < 0.001$). Male angles at other stages showed less consistent effects (Fig. 4, Table 1), and were not significant in regression analyses (Table 2, Model 2). Summarizing, the male remained oriented looking more or less directly toward the female during the entire courtship, and he turned to face her even more directly just before beginning both continuous vibration and intermittent buzzing. A male's mount was more

TABLE 1. MEANS AND STANDARD DEVIATIONS OF VARIABLES MEASURED IN COURTSHIPS OF MASS-REARED AND WILD FLIES, AND SIGNIFICANCE OF DIFFERENCES WITH MANN-WHITNEY U TESTS. VARIABLES ARE DESIGNATED AS FOLLOWS: *CONTFEM*—FEMALE ANGLE AT MOMENT CONTINUOUS VIBRATION BEGAN; *CONTMAL*—MALE ANGLE AT MOMENT CONTINUOUS VIBRATION BEGAN; *INTERFEM*—FEMALE ANGLE AT MOMENT INTERMITTENT BUZZING BEGAN; *INTERMAL*—MALE ANGLE AT MOMENT INTERMITTENT BUZZING BEGAN; *JUMPFEM*—FEMALE ANGLE AT MOMENT MALE JUMPED ONTO FEMALE; *JUMPMAL*—MALE ANGLE AT MOMENT MALE JUMPED ONTO FEMALE; *DISTCONT*—DISTANCE BETWEEN FLIES WHEN MALE INITIATED CONTINUOUS VIBRATION; *DISTINTER*—DISTANCE BETWEEN FLIES WHEN MALE SWITCHED FROM CONTINUOUS TO INTERMITTENT BUZZING; *DISTJUMP*—DISTANCE BETWEEN FLIES WHEN MALE JUMPED ONTO FEMALE; *FEMQUIET*—TIME FEMALE WAS MOTIONLESS PRIOR TO THE MALE'S JUMP; *VIBRATE*—DURATION OF CONTINUOUS WING VIBRATION; *BUZZ*—DURATION OF INTERMITTENT WING BUZZING; *COURT*—DURATION OF ENTIRE COURTSHIP

| | Mass-reared (m) | | Wild (w) | | Significance of differences | | | |
	ms Successful (N = 36)	mu Unsuccessful (N = 204)	ws Successful (N = 34)	wu Unsuccessful (N = 68)	ms-mu	ws-wu	ms-ws	mu-wu
Angles (°)								
contmal	2.4 ± 2.7^a	6.3 ± 8.8^b	10.2 ± 9.2^e	5.4 ± 8.5^f	**	ns	**	ns
contfem	39.4 ± 65.7^a	29.3 ± 33.3^b	52.6 ± 33.2^e	38.9 ± 30.4^g	*	ns	***	*
intermal	4.2 ± 3.3^a	4.7 ± 4.6^b	2.8 ± 3.6	3.7 ± 4.3	ns	ns	ns	*
interfem	9.3 ± 6.2^a	$11.5 \pm 12.4b$	7.3 ± 9.9	14.9 ± 12.4	ns	*	*	ns
jumpmal	4.0 ± 8.5^a	4.5 ± 5.3^c	1.1 ± 2.1	4.6 ± 5.5	**	*	ns	ns
jumpfem	4.0 ± 6.6^a	11.0 ± 11.6^c	3.9 ± 4.2	10.3 ± 15.6	***	*	ns	ns
Distances (cm)								
distcont	0.61 ± 0.35^a	0.64 ± 0.41^c	1.36 ± 0.81^e	1.69 ± 2.44^g	ns	ns	**	***
distinter	0.29 ± 0.12^a	0.32 ± 0.15^c	0.30 ± 0.12	0.31 ± 0.14	ns	ns	ns	ns
distjump	0.12 ± 0.02^a	0.15 ± 0.08^c	0.14 ± 0.02	0.13 ± 0.03	ns	ns	*	ns
Durations (sec)								
femquiet	4.07 ± 4.61^a	7.26 ± 8.00^d	5.78 ± 3.63	5.8 ± 3.77	**	ns	*	ns
buzz	7.53 ± 8.09	11.36 ± 9.21	10.7 ± 6.6	10.92 ± 8.92	**	ns	***	ns
vibrate	9.05 ± 15.70	6.23 ± 7.70	18.7 ± 23.8	13.2 ± 17.97	ns	*	ns	*
court	16.08 ± 16.30	15.76 ± 11.85	23.2 ± 22.2	18.0 ± 19.45	ns	*	*	ns

[a] N = 32.
[b] N = 134.
[c] N = 178.
[d] N = 172.
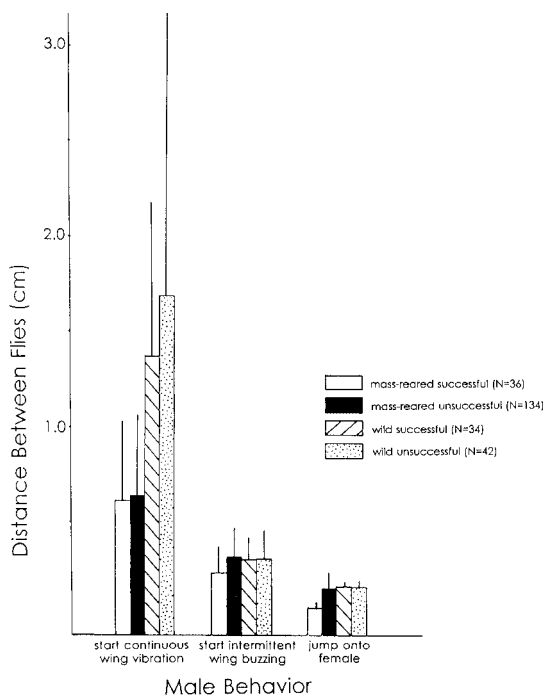[e] N = 16.
[f] N = 44.
[g] N = 42.

Fig. 2. Distances between male and female at different stages during male courtship in mass-reared and wild flies during successful and unsuccessful courtships. The flies gradually came closer together as courtship proceeded. Distances when courtship began were significantly greater in wild flies.

likely to be successful if he launched his jump while looking more directly toward the female.

Female Angles

Female angles clearly decreased during courtship (Figs. 5 and 6) ($P < 0.0001$ with Kruskal-Wallis Test in each strain comparing angles at the initiations of continuous vibration, intermittent buzzing, and the male's jump in combined data from successful and unsuccessful courtships). A posteriori Duncan tests showed that the female angle when the male began continuous wing vibration was significantly larger than each of the other angles in both strains (all $P < 0.001$). Females of both strains turned to face more directly toward males during the s following initiation of intermittent and continuous buzzing, but this trend was only weak in wild flies (Fig. 5).

Courtship was more likely to be successful in both strains when the female was looking more directly toward the male at the moment he jumped (Fig. 6, Table 1), though in wild flies this effect was not significant in regression analyses (Table 2, Model 1). The mean female angles for both strains combined at the moment the male jumped were 4.0 ± 5.6 vs. $10.8 \pm 12.8°$ comparing

successful and unsuccessful mounts ($P < 0.001$). The female angles at earlier stages of courtship did not show significant differences between successful and unsuccessful courtships in mass-reared flies, and only inconsistent differences in wild flies (Tables 1 and 2). Combining successful and unsuccessful courtships, mass-reared males initiated courtships when females were facing more directly toward them (female angle 31.2 ± 41.5 vs. 42.7 ± 31.6 in wild flies, $P < 0.001$). Summarizing, the female looked more directly toward the male later in courtship, and mounts that occurred when she was looking more directly toward him were more likely to result in copulation. Initiation of continuous and intermittent buzzing apparently induced the female to turn toward the male, the trend was weak in wild flies. Mass-reared males initiated courtship when females were looking more directly toward them.

There was a positive correlation in both strains between male and female angles, so when the male was looking more directly toward the female, she tended to be looking more directly toward him (Fig. 7). This correlation seemed stronger later in courtship, but the changes were not significant.

Female Immobility

Females were nearly always immobile when the male jumped. The amount of time the female had been quiet before the male jumped was significantly shorter in successful courtships of mass-reared flies than in those preceding unsuccessful mounts, but there was essentially no difference in wild flies (Fig. 8, Table 1). This difference was independent of the effects of other variables in mass-reared flies (Table 2, Model 1).

Female immobility was significantly shorter when the distance between the flies at the moment of the jump was larger, and it was larger when the distance at the beginning of intermittent buzzing was larger in mass-reared but not in wild flies (Table 3, Model 1). Mass-reared females that were successfully mounted had been motionless for a marginally shorter time than wild females that were successfully mounted ($P = 0.03$), but there was no difference between strains for the females that were unsuccessfully mounted ($P > 0.05$). Combining successful and unsuccessful courtships in each strain, the mean durations of female immobility did not differ significantly between strains (6.76 ± 7.65 s vs. 5.84 ± 3.71 s for mass-reared and wild flies ($P > 0.05$).

Absolute and Relative Durations

As was found previously using different flies and a different, older mass-reared strain from Cost Rica (Briceño & Eberhard 1998), several aspects of courtship by mass-reared males were shorter than those by wild males (Table 1). The

Fig. 3. Distances between male and female 1 s before initiation of continuous vibration (BC), at the moment continuous vibration began (C) and 1 s after it began (AC), and 1 s before (BI), 1 s after (AI), and at the moment of initiation (I) of intermittent buzzing in two strains. Dots accompanying lines between bars indicate significant differences between the two bars (one dot $P < 0.05$; two dots $P < 0.01$; three dots $P < 0.001$).

Fig. 4. Male angles at different stages during male courtship in mass-reared and wild flies during successful and unsuccessful courtships. Males tended to be oriented toward the female throughout courtship. Differences between strains, and between successful and unsuccessful courtships were not significant.
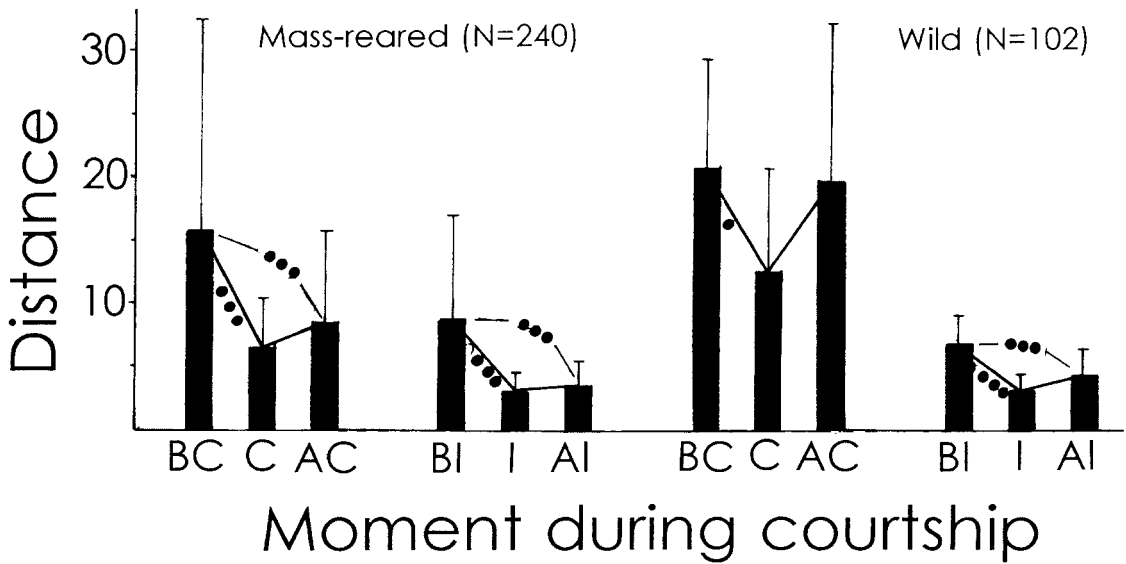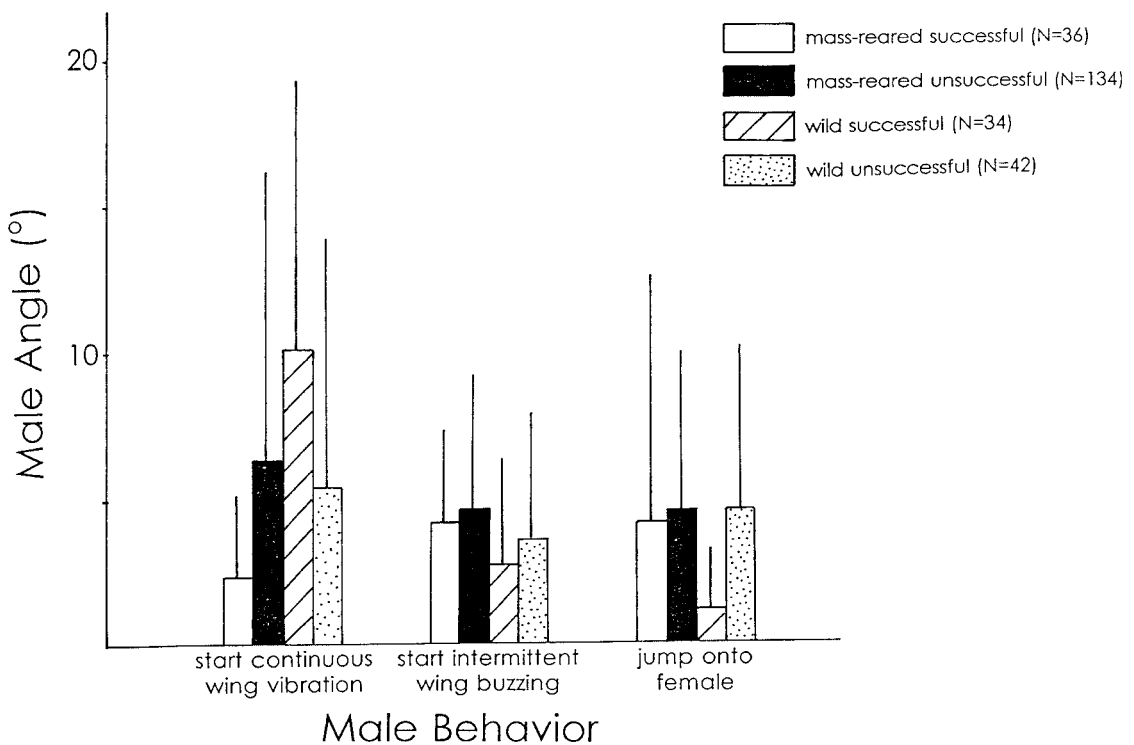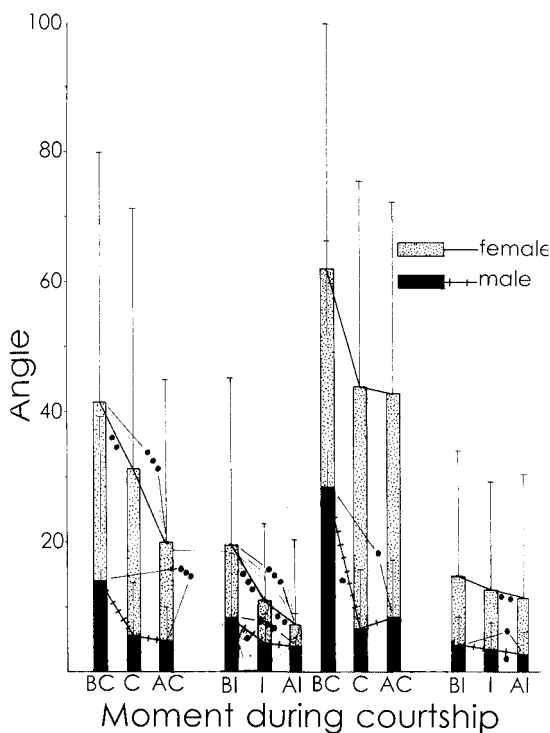
Fig. 5. Male and female angles 1 s before initiation of continuous vibration (BC), at the moment continuous vibration began (C) and 1 s after it began (AC), and 1 s before (BI), 1 s after (AI), and at the moment of initiation (I) of intermittent buzzing in two strains (mass-reared on left, wild on right). The dots accompanying lines between bars indicate significant differences between the two bars (one dot $P < 0.05$; two dots $P < 0.01$; three dots $P < 0.001$).

difference in total courtship duration between mass-reared and wild flies when successful and unsuccessful courtships were combined was also significant (15.81 ± 12.50 s vs. 20.18 ± 20.70 s, $P < 0.05$). This difference was due to different durations of continuous vibration (6.68 ± 9.77 s vs. 15.86 ± 20.20 s, $P < 0.001$) rather than differences in intermittent buzzing ($P > 0.05$). The time spent in intermittent buzzing was shorter in successful courtships than in unsuccessful courtships of mass-reared flies, but not in wild flies (Table 1), while longer continuous vibration and total courtship led to greater success in wild flies but not in mass-reared flies (Table 1). Combining data from mass-reared and wild flies, the mean time spent in intermittent buzzing in successful courtships was less than that in unsuccessful courtships (8.80 ± 7.63 and 11.08 ± 9.14 s, ($P < 0.001$). The corresponding difference in durations of continuous vibration was not significant.

Mass-reared females showed a strong tendency to copulate when the proportion of time

during the courtship that was spent in intermittent buzzing was especially low. The mean proportion of time spent buzzing prior to successful mounts was 57.0%, while the corresponding value prior to unsuccessful mounts was 77.6% (Student's t comparing arcsine transformations of these proportions was 4.38, $P < 0.00002$). This effect was both independent of and stronger than the effects of the other durations (Table 2, Model 2). Although wild flies showed the same trend to copulate when the proportion of the time spent in intermittent buzzing was lower (66.4% vs. 74.7%), the difference was not significant (Tables 1, 2). The decrease in this proportion in mass-reared flies was due in large part to the decrease in the duration of intermittent buzzing preceding successful mounts rather than to longer durations of continuous vibration (Table 1). In wild flies, however, there was not even a hint of a similar difference (Table 1).

There was a weak negative correlation in both strains between the total duration of courtship and the percentage of courtship dedicated to intermittent buzzing ($r = -0.29$, -0.60 in mass-reared and wild flies respectively; in both cases $0.01 < P < 0.05$). There was no correlation in either strain between the absolute duration of intermittent buzzing and continuous vibration.

Durations of Individual Buzzes and the Rate of Buzzing

A comparison of the mean durations of the last 10 individual buzzes during intermittent buzzing before the male jumped onto the female in a subsample of the courtships of mass-reared and wild flies that led to copulation (N = 19 and 25 respectively) with those in courtships that did not lead to copulation (N = 121 and 109 respectively) showed that buzz duration did not differ between successful and unsuccessful courtships in either strain (respective means were 156 ± 71 ms vs. 152 ± 70 ms for mass-reared flies, and 114 ± 16 ms vs. 113 ± 16 ms for wild flies; $P = 0.80$ and 0.76 respectively) (the durations of buzzes did not change significantly during intermittent buzzing – unpublished data). Similarly, comparisons between the average intervals between buzzes during successful and unsuccessful courtships in these same pairs also failed to show consistent significant differences (respective means were 164 ± 50 ms vs. 175 ± 52 ms for mass-reared flies, and 155 ± 60 ms vs. 184 ± 117 ms for wild flies; $P = 0.10$ and 0.03 respectively). Similar comparisons showed no differences in the overall rates of intermittent buzzes (number/sec) in successful and unsuccessful courtships (respective means 2.51 ± 0.7 buzzes/s vs. $3.0 \pm +1.0$ buzzes/s for mass-reared flies, and 3.73 ± 0.40 buzzes/s vs. 3.52 ± 0.71 buzzes/s for wild flies; $P = 0.25$ and 0.42 respectively).
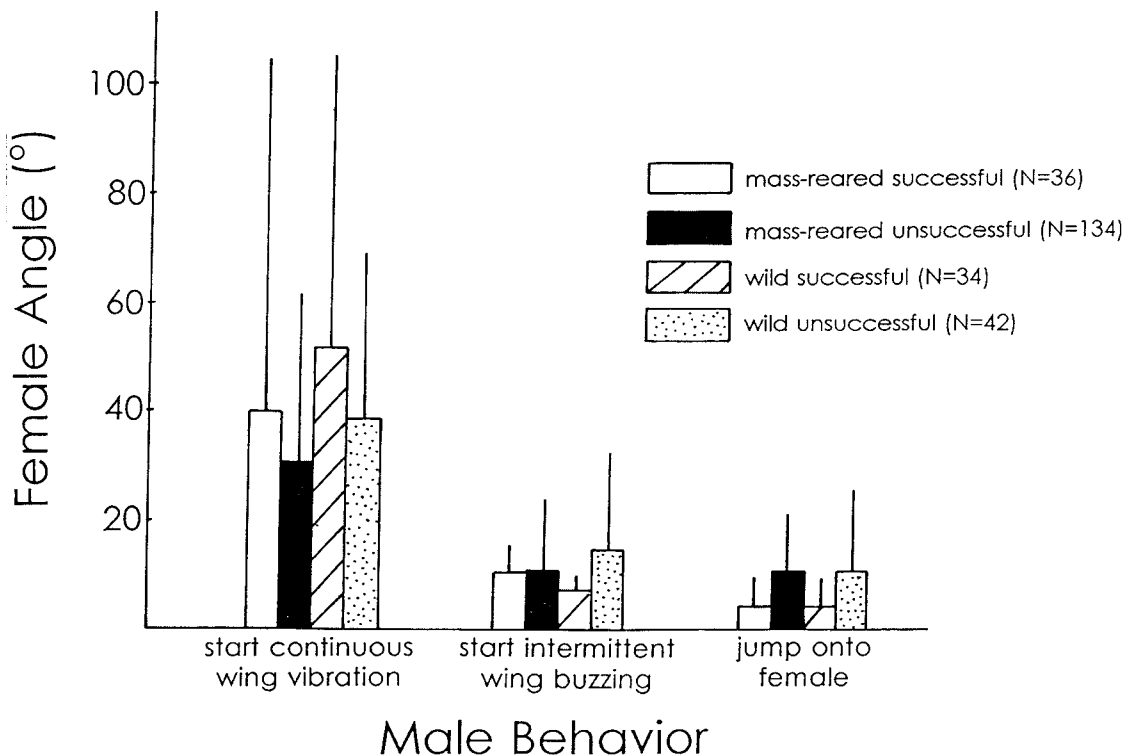
Fig. 6. Female angles at different stages during male courtship in mass-reared and wild flies during successful and unsuccessful courtships. The female tended to turn to face more toward the male after continuous wing vibration began and before intermittent wing buzzing. Differences between strains and between successful and unsuccessful courtships were not significant.

Walking Behavior

There were no consistent differences between successful and unsuccessful courtships with respect to whether the female or the male was immobile (= not walking) when continuous vibration or intermittent buzzing began, or one s before or afterward. In both strains female immobility increased as courtship progressed. The female was more likely to be immobile when intermittent buzzing began than she had been when continuous vibration began ($P < 0.0001$ with Chi^2 for both), and to be immobile one s after buzzing began than she had been 1 s before it began ($P < 0.0001$ with Chi^2 for both). There was a similar, but inconsistent trend for females to be immobile more often one s after continuous vibration began than they had been one s before ($P = 0.002$ for mass-reared flies; $P = 0.45$ for wild flies).

There were several between-strain differences. Mass-reared females were less likely to be immobile after continuous vibration began ($P = 0.0003$ with Chi^2), and one s after intermittent buzzing began ($P = 0.006$). Mass-reared males were less likely to be immobile during the s after intermittent buzzing began ($P = 0.001$ with Chi^2), but were

less likely to creep slowly toward the female during intermittent buzzing ($P = 0.0001$ with Chi^2).

In general, courtships were shorter in both strains when the female was immobile one s before or one s after continuous vibration began (means were smaller in all eight within-strain comparisons; differences were significant in five). There was also a significant association between longer courtships and male creeping behavior during intermittent buzzing (P = 0.008 and 0.01 in mass-reared and wild flies respectively). Thus males apparently decided to creep toward the female when relatively long courtships failed to lure her close enough.

Male Size

Mass-reared males were smaller than wild males in head width 0.85 ± 0.06 vs. 0.89 ± 0.06 mm), thorax length (2.63 ± 0.15 vs. 2.81 ± 0.13 mm), and thorax width (1.73 ± 0.10 vs. 1.83 ± 0.09 mm) (all $P < 0.001$ with t tests). Although mass-reared males performed more mounting attempts than wild males (respective means were 8.3 and 3.7; $P = 0.019$), there was no consistent relation within either strain between male body size and
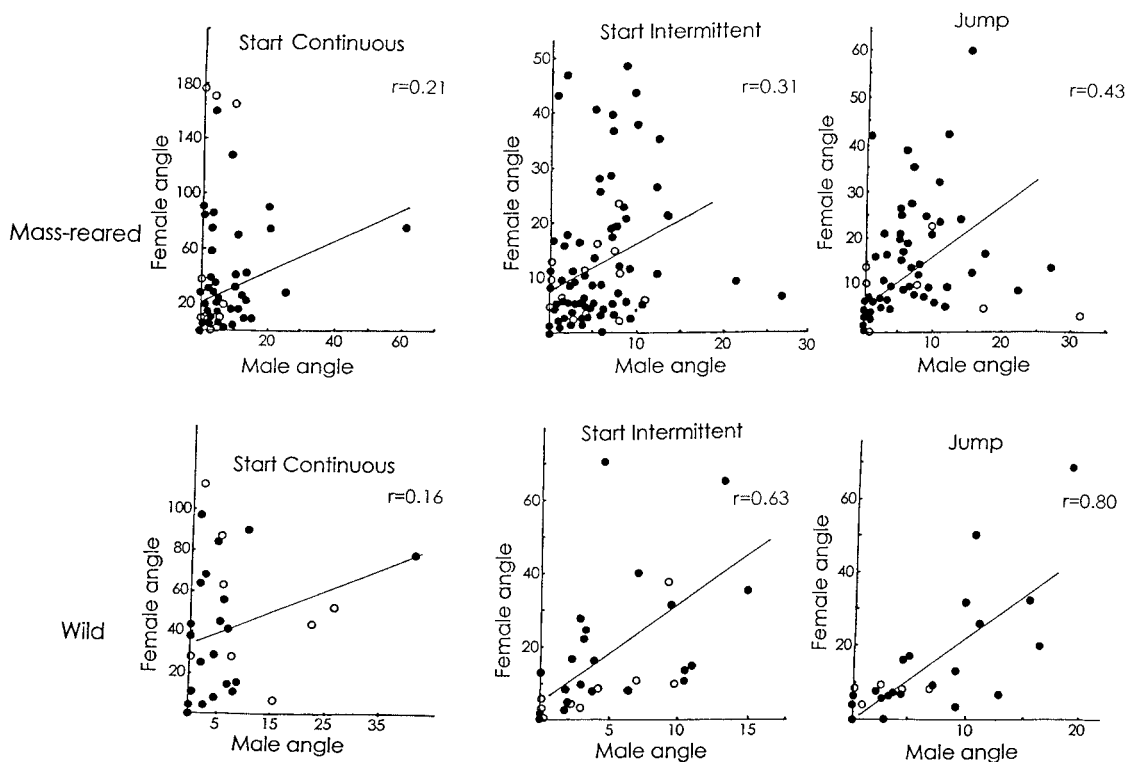
Fig. 7. Relationships between male and female angles in mass-reared and wild flies at different stages of courtship.

the rate of mounting failure (number of mounts that led to copulation/ total number of mounts). The p values for linear regression slopes of the rate of failure on the three size measurements were 0.047, 0.149 and 0.479 for wild flies (N = 21), and 0.472, 0.606 and 0.939 for mass-reared flies (N = 48). Males that copulated were not larger than those that did not copulate among either mass-reared or wild flies.

Mounts without Prior Courtship

Males and females frequently encountered each other as they walked about in the confines of the Petri dish. Mass-reared males were more likely to jump onto the female during such an encounter without previous courtship (40.8% of 142 mounts were not preceded by courtship) than were wild males (22.0% of 109 mounts without prior courtship) (Chi^2 = 9.9, df = 1, p = 0.0016). Mounts without a previous courtship were less likely to result in copulation in mass-reared flies (6.9% of 58 mounts without previous courtship vs. 19.0% of 84 with previous courtship; Chi^2 = 4.24, df = 1, $P < 0.05$), There was no difference in acceptance rates in wild flies (corresponding values were 20.8% of 24 vs. 24.7% of 85; $P > 0.05$).

DISCUSSION

Cues associated with Courtship Decisions

The data presented here are probably related at two different levels of causation to the decisions made by males and females during courtship. The difference between levels involves cause-and-effect relations as opposed to simple correlations. Some measurements, such as the distance between the flies (which is largely a function of whether or not the female approached the male), are probably "indicator variables" that represent the probability that a particular decision has been or will be made. They may constitute, for instance, indicators from the female's overt behavior of the likelihood that she will eventually accept the male's copulation attempt when he jumps onto her. These variables may have little or nothing to do with *why* the female made the decision to accept or reject the male, but rather be consequences of her having made a decision. A second set of possible "cue" variables represent possible stimuli that trigger particular decisions by males or females. For example, the male's size and his song characteristics, represent possible cues that might be used by females in making the
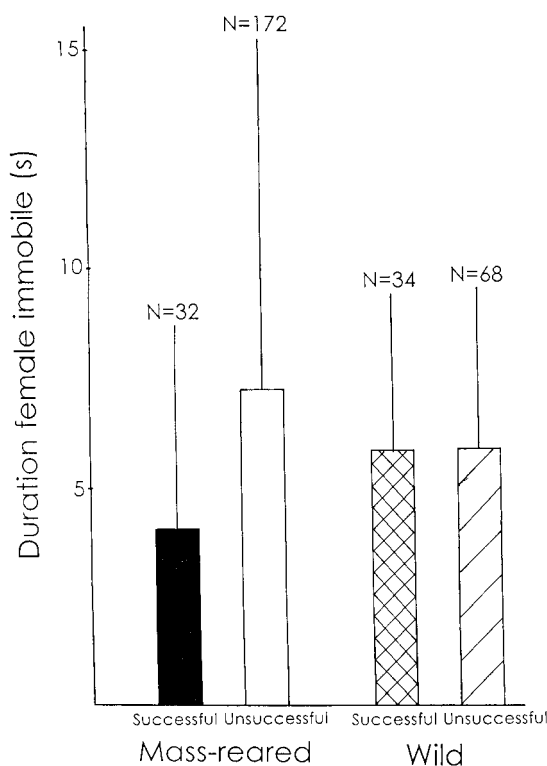
Fig. 8. Length of time female was immobile before the male mounted her in successful and unsuccessful courtships of mass-reared and wild flies. Mass-reared females were immobile for significantly shorter periods preceding successful mounts as compared with unsuccessful mounts; and successful mounts of mass-reared flies were preceded by significantly shorter periods of female immobility than successful mounts of wild flies.

decision whether or not to accept copulation. These two questions are discussed separately, although it is possible that some variables may play more than a single role. For instance, female behavior that is associated with likely acceptance may be used as a cue by the male to trigger particular courtship behavior of his own. These differences have not always been clear in previous discussions of medfly courtship.

Possible Indicator Variables. Our results confirm and quantify several conclusions regarding possible female "acceptance" variables from previous studies. The gradual reduction in the distance between male and female, the increase in the female's tendency to look more directly toward the male, and her increased immobility in the later stages of courtship are in accord with the idea that one result of successful male courtship behavior is to induce the female to approach him (or allow him to approach her), to look directly toward him, and to remain still. Feron (1962) derived these ideas from qualitative observations of

the relative mobility of females compared to courting males, but gave no quantitative support. Briceño et al. (1996) came to similar conclusions from comparing A) the positions of flies at the moment a male leapt onto the female (both successful and unsuccessful leaps were included), and B) positions when males decided to abandon courtship (presumably relatively extremely unfavorable conditions).

The present data are much more extensive and quantitative. They are also more convincing regarding the biological importance of male and female angles and the distance between the two flies at the moment that the male jumps, because they establish correlations with the likelihood that the female will allow copulation to occur, rather than just whether or not the male will jump. It must be kept in mind, however, that the data are only correlations, and thus do not allow confident deductions regarding cause and effect. It is thus not yet certain whether male-female alignment and close proximity is a cause of female acceptance of copulation, or whether it is correlated with female receptivity that is due to other causes.

It is entirely possible that we have documented here only manifestations of the female's likelihood of accepting copulation, and not the reasons why sometimes they were receptive and sometimes not. On the other hand, our results call into question the usefulness of studies of male-female interactions and possible interchanges of signals that do not take the angles and distances between the flies into account (e.g., Lux & Gaggl 1996, Liimatainen et al. 1997). It is now clearer than before that these factors are indeed associated with the success and failure of male courtships.

The tendency for the female to have spent less time moving prior to courtships that terminated with successful mounting is also in accord with the idea that male courtship functions to arrest female movement. The correlation between male and female angles may be due to the difficulty of continuing to look directly toward the female when the female is looking (and perhaps moving) in a direction other than toward the male.

Possible Cue Variables. The changes in distances and angles that occurred just before and after males began continuous vibration and intermittent buzzing suggest the following interpretation. Males are stimulated to begin continuous vibration when the female approaches, and to begin intermittent buzzing when she approaches still closer. The male may also be induced to initiate these behavior patterns when the female is oriented facing more directly toward him. Mass-reared males began both types of behavior when the female was facing them more directly, while similar trends in wild flies were not significant. Female decisions may be affected by stimuli from continuous vibration and intermittent buzzing by the male.

TABLE 2. SUMMARY OF RESULTS FROM LOGISTIC REGRESSIONS. VARIABLES ARE DESIGNATED AS IN TABLE 1.

Model 1. Female behavioral variables that were associated with altered probability that the mount would be successful (copulation) instead of failure (female rejection). Variables included: 1. angles: contfem, interfem, jumpfem; 2. distances: distcont, distinter, distjump; 3. durations: femquiet.

	Mass-reared flies				Wild flies			
	Estimate	S.E.	t ratio	P value	Estimate	S.E.	t ratio	P value
Constant	0.148	0.460	0.322	0.747	-1.10	0.74	-1.49	0.135
jumpfem	-0.105	0.035	-3.03	0.002	No significant effect			
femquiet	-0.177	0.066	-2.69	0.007	No significant effect			
interfem	No significant effect				-0.41	0.14	-2.84	0.005
contfem	No significant effect				0.074	0.025	2.93	0.003

Model 2. Male behavioral variables that were associated with altered probability that the mount would result in copulation rather than in female rejection of the male. Variables included: 1. angles: contmal, intermal, jumpmal; 2. distances: distcont, distinter, distjump; 3. Durations: vibrate, buzz, vbuz, femquiet.

	Mass-reared flies				Wild flies			
	Estimate	S.E.	t ratio	P value	Estimate	S.E.	t ratio	P value
Constant	2.15	0.92	2.32	0.02	1.85	1.29	1.43	0.15
vbuz	-3.02	0.92	-3.27	0.001	No significant effect			
distcont	-2.19	0.94	-2.34	0.02	No significant effect			
jumpmal	No significant effect				-2.63	1.10	-2.40	0.017
intermal	No significant effect				-0.69	0.32	-2.14	0.032
contmal	-0.14	0.07	-1.95	0.05	0.22	0.10	2.11	0.035

Model 3 (Multiple regression). Female behavioral variables that were associated with altered distance at which the male switched from continuous vibration to intermittent buzzing. Variables included: 1. angles: contfem, interfem; 2. distances: discont; 3. Durations: vibrate.

	Mass-reared flies				Wild flies			
	Estimate	S.E.	t ratio	P value	Estimate	S.E.	t ratio	P value
Constant contfem	-0.001	0.00	-0.23	0.005	No significant effect			

Model 4. Variables associated with differences in distance at which continuous wing vibration was initiated. Variables included: 1. angles: contmal, contfem.

	Mass-reared flies				Wild flies			
	Estimate	S.E.	t ratio	P value	Estimate	S.E.	t ratio	P value
Constant	No significant effect				No significant effect			

Although neither type of male behavior was associated with reductions in the distance between the two flies, females tended to turn to orient themselves more directly toward the male in the s after intermittent buzzing began, and, less consistently, in the s after continuous vibration began.

These interpretations must be evaluated carefully. They assume that reductions in the distance between the flies are due to female rather than male movements (nearly always true prior to and during continuous vibration and the early stages of intermittent buzzing, but not later in buzzing). They also attribute cause and effect relations to what are at present only correlations (above).

Our results shed little light on the cue or cues that trigger female acceptance of a male, other than ruling out several possibilities. The durations of individual buzzes and the intervals between them during intermittent buzzing, the rate of buzzing, the duration of the intermittent buzzing stage, the duration of the continuous wing vibration stage, and the male's size all failed to show significant differences between successful and unsuccessful courtships. There was a weak tendency in wild flies for successful courtships to have been longer than unsuccessful courtships; the lack of such a female criterion in mass-reared flies may be due to selection on females in mass-rearing cages (Briceño & Eberhard 2000), where males with shorter courtships are favored (Briceño & Eberhard 1998). The significance of the very strong tendency for increased female acceptance of copulation in mass-reared flies when the proportion of time during the courtship spent in intermittent wing buzzing was low with respect to the time spent in continuous wing vibration is not clear, especially in view of the lack of a significant trend in wild flies

Our finding that male size does not affect female acceptance is similar to the results of several studies in Hawaii (Arita & Kaneshiro 1988, Whittier et al. 1992, 1994, Whittier & Kaneshiro 1995), but differs from the equally clear tendency for larger males to be more readily accepted in Israel (Blay & Yuval 1997). It appears that there may be geographic variation in this trait. Female choice criteria are known to vary geographically in other species (e.g., Andersson 1994).

Differences between Mass-reared and Wild Flies

Our results constitute the second set of observations showing that courtship duration is reduced in a mass-reared strain compared with the wild strain from which it was derived (Briceño & Eberhard 1998). Males of two other mass-reared strains also perform relatively short courtships, but in one case the behavior was recorded under different conditions, and in the other nothing is known of behavior of the wild flies from which it was derived (Briceño & Eberhard 1998). The degree of reduction in the present study of a 3 year-old strain (78% of wild flies) is somewhat less than that seen previously with older, 4.5 year-old mass-reared strain (69%), as would be expected if inadvertent selection due to interruptions under mass-rearing conditions produced a gradual shift toward shorter courtships.

The tendency for mass-reared males to initiate continuous wing vibration when the female was closer and when she was facing more directly toward him may represent additional adaptations by mass-reared males to the crowded conditions of mass-rearing cages. High thresholds for initiating courtship may well be advantageous in a cage containing 60,000 flies. These changes may be accompanied on the female side by a greater tendency in mass-reared but not wild females to accept courtships that were initiated at a shorter distance. This difference was not significant in the variable-by-variable analyses in Table 1, but was significant in regression analyses of mass-reared flies when the effect of relative duration of intermittent buzzing was held constant (Table 2, Model 2), and also showed a significant interaction with the female angle when the male jumped (Table 3, Model 1). Thus the acceptance criteria of mass-reared females may have coevolved in accord with a reduction in the distance at which the male begins courtship.

The greater selectivity in mass-reared males with respect to when to initiate courtship was not accompanied by a possibly coevolved preference by females favoring courtships that began with smaller female angles. However the selectivity shown by wild females with respect to the effects of the female's angle at the moment when continuous and when intermittent wing vibration began was absent in mass-reared females (Table 2, Model 1). The loss of such selectivity in mass-reared females would thus favor males that initiate courtship at smaller female angles, which is the trend found when mass-reared and wild males are compared. Thus this female acceptance criterion may also have changed in mass-reared flies (via abandonment of a former bias) in step with changes in male behavior that probably resulted from selection in crowded mass-rearing cages, just as greater acceptance of shorter courtships has also coevolved with abbreviated courtship in mass-reared flies (Briceño & Eberhard 2000). Males in cages may thus be under selection to respond only to females at shorter distances to avoid interruptions; and female criteria may have evolved to favor males whose sons were more likely to perform uninterrupted courtships. Further data are needed to test these ideas.

One further point regarding differences and similarities in distances concerns the uniformity, both between and within strains, in the distance between male and female when the male initiated his jump onto the female. It might have been

TABLE 3. SUMMARY OF RESULTS OF INTERACTIONS BETWEEN VARIABLES IN LOGISTIC REGRESSIONS. VARIABLES ARE DESIGNATED AS IN TABLE 1. INTERACTIONS WITH THE OTHER VARIABLES IN THE LIST WERE TESTED SEPARATELY FOR EACH OF THE VARIABLES THAT HAD A SIGNIFICANT EFFECT IN THE REGRESSIONS IN TABLE 2.

Model 1. Female behavioral variables that were associated with altered probability that the mount would result in copulation rather than in female rejection of the male. The variables which showed significant effects in previous regression analysis, and with which other variables (Table 2) were combined to test for interactions, were jumpfem, femquiet, and distcont.

	Mass-reared flies				Wild flies			
	Estimate	S.E.	t ratio	P value	Estimate	S.E.	t ratio	P value
Interactions with jumpfem								
constant	0.38	0.46	0.82	0.412	-1.25	0.64	-1.98	0.048
distcont* jumpfem	-0.25	0.075	-3.33	0.001	-0.10	0.05	-1.85	0.064
femquiet	-0.19	0.068	-2.83	0.005	0.18	0.12	1.50	0.13
Interactions with femquiet								
constant	0.76	0.51	1.48	0.14	-1.05	0.48	-2.21	0.027
jumpfem	-0.11	0.034	-3.13	0.002	-0.10	0.05	2.10	0.036
distjump* femquiet	-4.64	1.53	-3.03	0.002	0.59	0.65	0.90	0.37
distinte* femquiet	0.86	0.41	2.10	0.035	0.065	0.31	0.21	0.84
Interactions with contfem	No significant effect				No significant effect			
Interactions with interfem	No significant effect				No significant effect			

TABLE 3 (CONTINUED). SUMMARY OF RESULTS OF INTERACTIONS BETWEEN VARIABLES IN LOGISTIC REGRESSIONS. VARIABLES ARE DESIGNATED AS IN TABLE 1. INTERACTIONS WITH THE OTHER VARIABLES IN THE LIST WERE TESTED SEPARATELY FOR EACH OF THE VARIABLES THAT HAD A SIGNIFICANT EFFECT IN THE REGRESSIONS IN TABLE 2.

Model 2. Male behavioral variables that were associated with altered probability that the mount would result in copulation rather than in female rejection of the male. The variables which showed significant effects in previous regression analysis, and with which other variables (Table Variables which had not showed significant effects previously and were combined to check for interactions included the angles contfem, interfem and the distances: distinter, distjump. 2) were combined to test for interactions, were vbuz and distcont.

	Mass-reared flies				Wild flies			
	Estimate	S.E.	t ratio	P value	Estimate	S.E.	t ratio	P value
Interactions with vbuz								
Constant	0.083	0.43	0.19	0.85	-1.87	0.58	-3.23	0.001
vbuz* court	-0.18	0.054	-3.20	0.001	No significant effect			
vbuz* contmal	No significant effect				0.27	0.11	2.43	0.015
Interactions with distcont								
Constant	-0.17	0.38	-0.44	0.66	-0.61	0.83	-0.74	0.46
distcont* buzz	-0.26	0.083	-3.14	0.002	No significant effect			
distcont* jumpmal	No significant effect				-3.54	18.40	-0.19	0.85#
distcont* femquiet	No significant effect				0.86	0.32	2.68	0.007
distcont* intermal	No significant effect				-0.73	0.29	-2.56	0.01
Interactions with jumpmal								
Constant	-1.31	0.27	-4.93	0.000	No significant effect			
jumpmal* vibrate	0.006	0.004	1.73	0.083				
jumpmal* buzz	-0.034	0.016	-2.12	0.034				
jumpmal* intermal	0.048	0.017	2.84	0.005				
jumpmal* contmal	0.035	0.016	-2.13	0.033				

#Was significant at P = 0.024 when first entered the model.

thought that the shorter courtships of mass-reared males (e.g., Briceño & Eberhard 1998; above) occurred because the males fail to wait until the distance to the female has decreased, and jump onto the female from farther away. This seems not to be the case. The probable reason is that male stimulation by the female using his aristae (Briceño & Eberhard in press) cannot occur until the flies are quite close together.

Summarizing the results of this and previous studies, there are now five differences known between the sexual behavior of mass-reared males and wild males. Mass-reared male courtship prior to mounting is shorter, is initiated at a shorter distance from the female and when the female is looking more directly toward the male, the male is less likely to creep toward the female during intermittent buzzing, and the male is more likely to attempt to mount a female they have encountered without prior courtship. Acceptance criteria in mass-reared females appear to have changed to favor the first two and possibly also the third of these male changes, but to act against the fifth. The first two changes fit a Fisherian sequence of evolution by sexual selection (see Briceño & Eberhard 2000). Changes in female criteria have also been documented in other mass-reared insects (Liu & Haynes 1994, Zhu et al. 1997).

The data documenting differences between strains must be interpreted cautiously, because only pairs of flies of the same strain were observed. Given the probable effects of the behavior of one sex on that of the other, it will not be possible to attribute differences to one sex or the other with certainty until cross-strain pairs are studied.

Limitations of These Analyses

Many male courtships do not end in a mount; the male terminates courtship when the female moves away or otherwise fails to respond appropriately (Feron 1962, Briceño et al. 1996). Courtships that do not lead to a mounting attempt could obviously affect a male's success, but were omitted in the present analyses. Also omitted were those courtships in which the male returned to continuous wing vibration after having begun intermittent wing buzzing. Perhaps additional answers to why some courtships succeed and others fail will be revealed by analyses of these types of interaction.

This study revealed several strong trends with respect to probable male and female cues and responses during the course of courtship, and also demonstrated several clear behavioral differences between mass-reared and wild flies. But it was much less fruitful in uncovering clear differences between successful and unsuccessful courtships. The critical reader cannot help but be struck by the large standard deviations and substantial overlaps in nearly all of our data. Indeed, large variations are ubiquitous in nearly all quantitative data on medfly courtship behavior (Briceño et al. 1996, Liimatainen et al. 1997, Briceño & Eberhard 1998, Quilici in press; an exception is behavior involving contact between male and female aristae—Briceño & Eberhard in press). It is clear, for instance, that when the distance to the female is shorter and when she is facing more directly toward the male, there is a greater likelihood that the male will jump (Briceño et al. 1996), and that the female will accept copulation with him when he does (Figs. 2 and 6). But there were numerous rejections when both the distance and the female angle were low, and acceptances when they were both high. Similar variation also occurs at earlier stages of courtship (see large error bars in Figs. 2 and 6).

These large variations and substantial overlaps have several consequences. On a practical level, they mean that relatively large samples of courtships are needed to document significant differences between successful and unsuccessful courtships or differences between strains. They also signal our lack of detailed understanding why some courtships are successful and others are not. There are several possible explanations of this failure. Perhaps we simply have not yet focused on the male trait or traits that have the most powerful effects on female acceptance. Such "mystery traits" could involve factors that cannot be measured in videotapes (e.g., sound intensities in male songs, male pheromones).

A second possibility is that the basic approach of searching for triggering stimuli is not biologically appropriate. Perhaps female acceptance is sometimes "spontaneous", and does not depend on the presence of particular stimuli. The consistent superiority of wild males over mass-reared males (above) and of some males over others (e.g., Whittier & Kaneshiro 1995) argues, however, that this cannot be the complete explanation. Another possibility is that each particular stimulus only slightly increases the probability of acceptance, rather than guaranteeing that it will occur. Discriminating between the possibilities of mystery traits and small incremental effects may be especially difficult in medfly courtship, where a large variety of possible stimuli are involved. Experimental manipulation of traits may be the best tactic for future studies.

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VARIATION IN THE INTERMITTENT BUZZING SONGS OF MALE MEDFLIES (DIPTERA: TEPHRITIDAE) ASSOCIATED WITH GEOGRAPHY, MASS-REARING, AND COURTSHIP SUCCESS

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ABSTRACT

Many aspects of the temporal pattern of sounds produced during the intermittent buzzing displays of pre-copulatory courtship by male medflies varied between wild flies from Costa Rica, Argentina, and Hawaii, and between mass-reared flies from Costa Rica, Argentina, Mexico, and Hawaii. There were no consistent differences when mass-reared strains were compared with the wild strains from the area where they originated in Costa Rica, Argentina and Hawaii. Buzzing sounds produced prior to successful mounting attempts did not differ consistently from those preceding unsuccessful mounts in flies from Costa Rica and Argentina. In strains from all sites, however, courtships in which buzzes were interrupted were more likely not to result in mounting of the female. There was a weak tendency for interruptions to be more common in mass-reared strains.

Key Words: Courtship sounds, medfly, geographic differences

RESUMEN

Varios aspectos de los patrones temporales de los sonidos del zumbido intermitente producido durante el cortejo pre-copulatório de los machos de la mosca del Mediterráneo variaron entre moscas silvestres de Costa Rica, Argentina y Hawaii, y entre moscas de sepas de cría masiva de Costa Rica, Argentina, Mexico y Hawaii. No se presentaron diferencias consistentes cuando se compararon las sepas de cría masiva en Costa Rica, Argentina y Hawaii con moscas silvestres de los sitios de origen. Los sonidos producidos durante cortejos que terminaron en cópulas no difieron de los sonidos producidos durante cortejos que llevaron a montas que fracasaron en moscas de Costa Rica y Argentina. Pero en sepas de todos los sitios los zumbidos intermitentes que incluyeron pequeñas pausas tuvieron una mayor probabilidad de no terminar en un intento de monta. Los zumbidos intermitentes de las moscas de las crias masivas tuvieron una tendencia leve a interumpirse mas frecuentemente.

Male medflies (*Ceratitis capitata* Wied.) produce two types of wing vibration during pre-mount courtship (Féron 1962, Rolli 1976, Webb et al. 1983, see review of behavior in Briceño et al. 1996, Briceño & Eberhard 2002). During an early stage of courtship, the male produces the "calling song" with an average fundamental frequency of about 350 Hz (Webb et al. 1983), by vibrating his wings continuously while he looks toward the female and holds his abdomen bent ventrally so that the pheromone-producing everted rectal epithelium is ventral to the rest of his body. Probably a plume of pheromone is thus wafted toward the female. After an average of about 6 sec of continuous wing vibration, the rectal epithelium is retracted, and the male begins a series of intermittent wing buzzes (Fig. 1) which continue until he leaps onto the female or abandons courtship. Each buzz is associated with a more intense sound that has a lower average fundamental frequency of about 165 Hz (Webb et al. 1983). A single buzz lasts on the order of 0.1 sec and is produced during the time wings are vibrated rapidly with a large amplitude, from anterior of the male's head to back over his body (Briceño & Eberhard 2000). The softer calling song continues during the intervals between buzzes. During intermittent buzzing behavior the male often also rocks his head rapidly from side to side, often tapping the female with his aristae (Briceño & Eber-
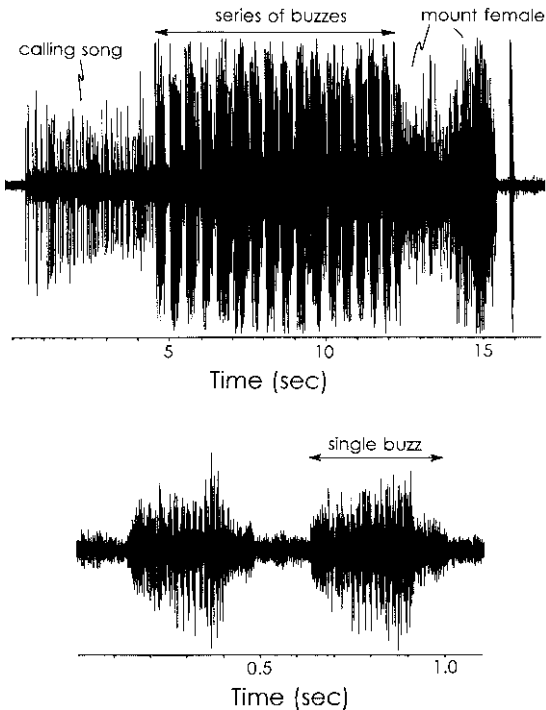
Fig. 1. Sounds produced by a male *Ceratitis capitata* during courtship and mounting.

hard in press). Finally the male leaps onto the female, shakes his body briefly rapidly forward and backward while producing another strong buzzing sound (Fig. 1). Sound production ends as the male then attempts to turn and establish genitalic contact; males do not resume sound production during copulation.

The significance of sounds produced during courtship in medflies is as yet unclear. Possible effects of sounds produced during courtship on copulation success, and of changes in the female's ability to perceive them have been investigated experimentally. Keiser et al. (1973) found that the percentage of females that were inseminated when male wings were removed dropped by about half, and Nakagawa et al. (1973) and Levinson et al. (1987) found that removal of the female antennae nearly completely eliminated copulation. However, the first experiment also modified possible visual stimuli, the second also modified possible tactile stimuli from the male's aristae, and both may have also modified chemical stimulation of females. There are thus as yet no conclusive demonstrations that any sounds are functionally important in courtship; it is possible that they are all only incidental consequences of other activities (i.e., creation of air currents) (see review in Eberhard 2000).

There are several reasons to expect that present day populations of medflies may not have uniform courtship songs. The geographic range of the species, which is native to Africa, has increased dramatically, and several population bottlenecks have probably occurred in recent times (Huettel et al. 1980, Fuerst 1988). Thus both drift and divergence under sexual selection in geographically isolated populations may have occurred. In addition, mass rearing of sterile males has often been used in attempts to control pest populations of medflies, and mass-reared strains have often been conserved for many years. Reproduction in these strains occurs under conditions that differ sharply from those in nature in several respects. Old mass-reared strains thus represent the results of inadvertent experiments in which several environmental conditions have been changed. It is not obvious, however, which song traits would be more advantageous under mass-rearing conditions. Rolli (1976) reported a lack of differences between the songs of wild medflies from Tunis and Morocco and mass-reared males in Germany (the age of the mass-reared strain was not specified). The sample sizes were small, however, and it is not clear which song characters were compared.

This paper tests the possibility of geographic divergence and of changes under mass-rearing in the temporal pattern of the intermittent buzz and the mounting songs of males of seven strains of flies: wild flies from Costa Rica, Argentina and Hawaii; mass-reared strains derived from these strains 6.5, 10, and more than 40 years previously; and a three-year old mass-reared strain from Mexico. Songs of successful and unsuccessful courtships are also compared.

MATERIALS AND METHODS

Mass-reared flies from Costa Rica were from a strain which had been initiated in 1990 using wild flies collected near Alajeula, Costa Rica, and maintained subsequently at the Laboratorio de Manejo Integrado de la Mosca de la Fruta mass-rearing facility. Wild flies were raised from larvae that emerged from infested tangerines collected in Jan-April, 1997, at the Estación Experimental Faubio Baudrit near Alajuela, Costa Rica.

Argentinian mass-reared flies came from the Mendoza strain, which had been derived from flies collected about 10 years before our observations in Mendoza province, Argentina. Wild flies were a laboratory G2 derived from flies raised from fruit collected in the field in the Alto Valle region of Patagonia. Mass-reared flies in Mexico were from a three year-old strain derived from flies collected as larvae from coffee in Costa Cuca, Quetzaltenengo, Guatemala. Hawaiian mass-reared flies were from the Hilab strain derived from wild flies more than 40 years previously, while wild flies were reared from coffee fruit collected on Kauai.

Adult flies of all strains were separated by sexes when they were less than two days old, and fed mixtures of sugar and protein hydrolysate. Male-female pairs of mass-reared flies were placed together for video taping when they were five days old; male-female pairs of wild flies, whose sexual maturation is more delayed, were placed together only after they were 10 days old.

Pairs of flies in Costa Rica and Hawaii were videotaped in 13.7 cm diameter and 1.8 cm deep mating chambers (clear Petri dishes) on a glass table using a Sony CCD Video Hi 8 camera equipped with + 6 closeup lenses. The camera was below the table, allowing taping from below (most courtships occurred on the ceiling of the mating chamber). A small microphone (Sennheiser System MZK 80ZU) was inserted through a hole in the side of the chamber and connected to the camera. Pairs in Argentina and Mexico were videotaped in a clear plastic cylinder 7.3 cm high and 9.0 cm in diameter. Each morning a fresh leaf from a citrus tree was attached to the ceiling of the cage, and a male was released in the cage. Five minutes after the male began emitting pheromone, a female was released into the cage, and the flies' behavior was recorded for 30 min or until they copulated.

All recording environments were noisier than that used by Webb et al. (1983), and both types of mating chambers produced strong echoes. Other than verifying that the apparent fundamental frequencies of the songs of the Costa Rican flies were similar to those observed by Webb et al. (1983), we did not attempt to analyze the frequencies or power spectra of songs. Instead we concentrated on the temporal patterns of the songs. There are indications in other flies that temporal patterning may be an especially important aspect of songs (Bennet-Clark & Ewing 1969, Kyriacou & Hall 1982, Tomaru & Oguma 1994, Aspi & Hoikkala 1995, Neems et al. 1997, and Hoikkala & Kaneshiro 1997 on *Drosophila*).

Recordings of sounds were imported from video recordings into a PC 486dx2 computer using a 16 bit card. The mean durations of buzzes and intervals between buzzes were measured using the real time display in the program Avisoft® when cursors marked the beginning and the end of the envelope curve displayed in the main window of the program (Fig. 1). The precision of these measurements was determined by re-measuring the duration of 10 buzzes and 10 intervals in each of 8 different courtships. The average differences were 2.0 ms in buzz duration, and 2.2 ms in interval duration. In addition, the number and duration of interruptions during the buzzes was determined. An interruption was defined as any interval between buzzes that was more than twice the mean of the intervals immediately preceding and following it (Fig. 2).

We examined two different sets of buzzes in Costa Rican flies: the first 10 buzzes in the court-
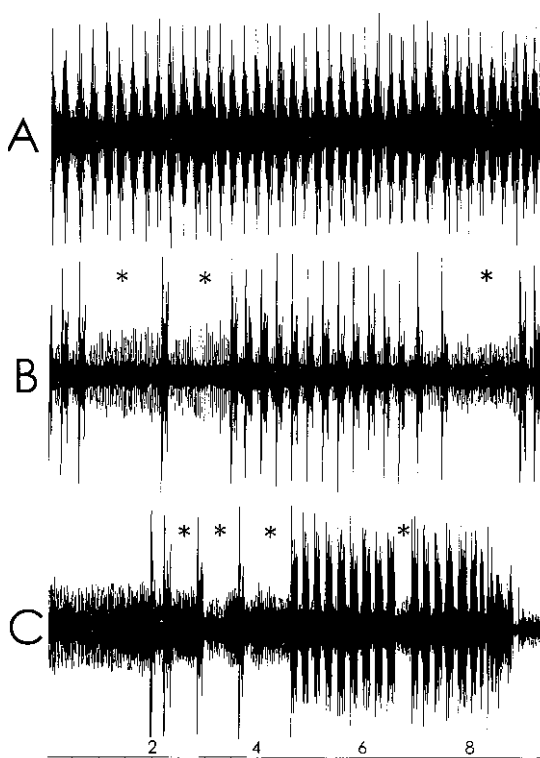

Fig. 2. Three intermittent buzzing courtships in which interruptions (*) were absent (A) and present with slower (B) and more rapid buzzing (C) (all with same time scale).

ship of a male; and the last 10 buzzes before the male either leapt onto the female or ceased courting, to determine whether the characteristics of buzzes varied systematically during courtship.

Courtship outcome was classified in three classes: *no mount*—the male ceased courting without attempting to mount the female (failure to mount is often associated with failure of the female to align herself properly with the courting male and to remain still—Briceño et al. 1996, Briceño & Eberhard 2002); *failed mount*—the male mounted but was dislodged within 10 s when the female struggled; and *successful mount* - the male mounted the female and achieved genitalic contact.

All statistical tests were non-parametric Mann Whitney U Tests due to the highly skewed distributions of many variables. Means are presented followed by one standard deviation for illustrative purposes only.

RESULTS

There was no clear, consistent tendency for buzz duration or interbuzz duration to differ between the first and last 10 buzzes of a courtship in Costa Rican or Argentinian flies (Fig. 3). Wild flies

from Costa Rica, Argentina, and Hawaii differed significantly in all five pre-mount song traits (Table 1). Comparing wild flies, those from Argentina produced longer buzzes, those from Hawaii produced the largest number of buzzes/courtship, and those from Costa Rica had longer intervals between buzzes and a lower overall rate of buzzing. Wild Hawaiian flies had the longest mount buzzes. Mass-reared flies from the three sites also differed in many traits. When mass-reared and wild flies from the same site were compared, no traits showed the same trends at all three sites.

In an attempt to understand the possible selective factors which might influence song characteristics, we compared several aspects of intermittent buzzes in courtships that led to copulation as compared with those that ended in female rejection of a mount or a failure to mount in different strains (Tables 2 and 3). There were no consistent directional differences for traits related to durations and frequencies of intermittent buzzing (Table 2). The duration of the mount buzz was marginally longer in successful mounts in the mass-reared strain from Costa Rica, but there were no similar trends in wild Costa Rican flies or in strains from Argentina and Hawaii.

In contrast, there was a consistent trend in each of the six strains with good sample sizes for interruptions in intermittent buzzing to be more frequent in courtships that did not lead to a mounting attempt (first two columns in Table 3); combining the data from all strains, the frequency with which buzzing was interrupted was 72.3% of 166 courtships which did not lead to mounting, 39.2% of 263 in which the male mounted but was then rejected, and 28.4% of 81 in which the male mounted and succeeded in copulating. Hawaiian flies were more likely to interrupt buzzing. Among the other sites, mass-reared males in Costa Rica were less likely to interrupt their courtships than mass-reared males from Argentina or those from Mexico, but there was no difference in this respect between wild flies from Costa Rica and Argentina. There were no differences when mass-reared flies were compared with their respective wild counterparts in Costa Rica, Argentina, and Hawaii with respect to the proportion of courtships in which buzzing was interrupted, the number of interruptions, or their durations. But there were similar trends for more interruptions to occur in mass-reared strains, and when data from different sites were combined, mass-reared flies were slightly more likely to interrupt buzzing (52.7% of 262) than were wild flies (41.7% of 156) ($P = 0.0297$ with Chi Squared Test).

The apparent female bias against interrupted buzzing did not extend to events that occurred after mounting. There was no significant association in any strain between the occurrence of an interruption, the number of interruptions, or the mean duration of interruptions and the likelihood that the female would reject the male once he had mounted. Similarly, when all courtships in which mounts occurred were combined for all strains, there was no significant relation between male copulation success and whether or not buzzing had been interrupted.

When song parameters were correlated with male body size (estimated by the maximum width of the head in dorsal view), there was only one significant relationship, with the total number of buzzes/courtship ($r = 0.26$ with log-transformed numbers, $P < 0.05$). Other correlation coefficients were -0.01 ($P = 0.99$) for the mean duration of each buzz, -0.12 ($P = 0.29$) for the mean duration of interval between buzzes, -0.06 ($P = 0.62$) for the number of buzzes/s, 0.22 ($P = 0.20$) for the log of total duration of buzzing, and 0.17 ($P = 0.41$) for the duration of the mount buzz.

Discussion

Although there were several differences between mass-reared and wild Costa Rican flies in duration and number of buzzes, similar differences did not occur between mass-reared and wild flies from Argentina and Hawaii. This suggests that the differences in the Costa Rican flies may not be due to mass-rearing per se. This result is in accord with a less-detailed study of flies from Tunis and Morocco (Rolli 1976), and with the lack of obvious differences in selective pressures on the details of intermittent buzzing behavior under mass-rearing conditions.

Similarly, there were no consistent differences between successful and unsuccessful courtships in Costa Rican, Argentinian and Hawaiian flies in many of the song variables that we measured. However the possibility of stabilizing sexual selection on these traits cannot be excluded until further analyses are performed. We also did not measure additional aspects of the song, such as its intensity and basic frequency, and there are other ways that vibrations may be transferred to the female, such as through substrate and near field medium motion (Markl 1983). Song intensity is an important determinant of female acceptance in the tephritid *Anastrepha suspensa* (Loew) (Sivinski et al. 1984). Thus while presently available data do not support the idea that song traits influence courtship success, the possibility cannot be ruled out.

The strongest association we found with the eventual outcome of courtship was that between interruptions of intermittent buzzing behavior and failure to mount (Table 3). The significance of this association is not clear. On the one hand, our criterion for distinguishing interruptions was obviously arbitrary. The strength and consistency of the association we found with failure to mount leaves little doubt that there is an association of some sort with the pattern of buzzes, but it is uncertain whether or not the biologically relevant criterion is the one which we used.

TABLE 1. SONG CHARACTERISTICS FOR COURTSHIPS THAT LED TO BOTH SUCCESSFUL AND UNSUCCESSFUL MOUNTS IN WILD AND MASS-REARED MEDFLIES FROM COSTA RICA, ARGENTINA, MEXICO, AND HAWAII (MEAN ONE STANDARD DEVIATION). VALUES IN THE SAME ROW FOLLOWED BY THE SAME LETTER AND NUMBER I DIFFER SIGNIFICANTLY WITH MANN-WHITNEY U TESTS ($a = P < 0.05$; $b = P < 0.01$; $c = P < 0.001$).

	Costa Rica		Argentina		Mexico	Hawaii	
	Wild	Mass-reared	Wild	Mass-reared	Mass-reared	Wild	Mass-reared
Intermittent buzzes:							
Duration of each buzz (ms)	113 ± 16 $c_1 c_2 c_3$	152 ± 70 $b_1 c_3 c_4 c_5$	127 ± 26 $c_3 c_4 c_7$	135 ± 16 $a_1 c_5 c_6 c_9$	113 ± 16 $a_1 b_2 c_2 c_{10}$	114 ± 12 $c_6 c_7$	95 ± 12 $c_4 c_8 c_9 c_{10}$
Duration of each interval between buzzes	178 ± 108 b_1	173 ± 52	145 ± 44 b_1	149 ± 47	182 ± 167	152 ± 46	151 ± 102
Total number of buzzes/courtship	34.7 ± 19.0 $c_1 b_1$	29.1 ± 14.6 $b_2 c_3$	24.6 ± 14.0 $b_3 c_1$	25.5 ± 15.5 $b_1 c_2 c_4$	45.8 ± 31.1 $b_2 b_3 c_2$	55.0 ± 43.4	47.4 ± 21.4 $c_3 c_4$
Rate (number/sec)	3.57 ± 0.67 $a_1 c_1$	3.03 ± 0.95 $b_1 c_2 c_3 c_4 c_5$	3.75 ± 0.85 $a_1 b_1 b_2 c_1$	3.65 ± 0.84 $b_2 c_4$	4.11 ± 0.65 $c_2 c_4$	4.04 ± 0.53	4.30 ± 1.30 c_5
Total duration of buzzing (s)	9.64 ± 5.98 $a_1 b_1$	11.07 ± 8.49 $a_2 b_1 b_2$	7.58 ± 5.16 $a_1 b_2 b_3$	7.16 ± 4.31 $a_2 c_1 c_2$	15.03 ± 12.65 $c_1 b_3$	14.2 ± 11.2	11.7 ± 10.3 c_2
N (courtships)	90	142	66	46	55	11	44
Mount buzz:							
Duration (s)	1.77 ± 0.41 $a_1 a_2 c_1$	2.76 ± 0.41 $a_1 b_1 c_2$	1.23 ± 0.44 c_1	1.58 ± 0.86 b_1	1.10 ± 0.52 b_2	3.22 ± 2.70 a_2	4.86 ± 4.52 c_2
N (courtships)	37	36	40	30	36	9	28

TABLE 2. SONG CHARACTERISTICS PRECEDING SUCCESSFUL (S) AND UNSUCCESSFUL (U) MOUNTS BY MASS-REARED AND WILD COSTA RICAN, ARGENTINIAN, AND HAWAIIAN FLIES (MEAN ± STANDARD DEVIATION). VALUES IN THE SAME ROW FOLLOWED BY THE SAME LETTER AND NUMBER DO NOT DIFFER SIGNIFICANTLY WITH MANN-WHITNEY U TESTS ($\alpha = P < 0.05$).

| | Costa Rica | | | | Argentina | | | | Hawaii | |
| | Mass-reared | | Wild | | Mass-reared | | Wild | | Mass-reared | |
	S	U	S	U	S	U	S	U	S	U
Intermittent buzzes:										
Duration of each buzz (ms)	156 ± 71	152 ± 69	114 ± 16	113 ± 16	137 ± 18	135 ± 15	134 ± 22	125 ± 25	105 ± 14 [a1]	92.4 ± 10.5 [a1]
Duration of each interval between buzzes (ms)	164 ± 51	175 ± 52	155 ± 59	184 ± 118	138 ± 40	155 ± 50	132 ± 41	148 ± 45	115 ± 32	159 ± 110
Total number of buzzes/courtship	24 ± 10	30 ± 15	35 ± 16	37 ± 26	28 ± 16	24 ± 16	26 ± 17	26 ± 18	39 ± 19	49 ± 22
Rate (number/s)	2.5 ± 0.7	3.0 ± 0.9	3.7 ± 0.4	3.5 ± 0.7	3.9 ± 0.9	3.5 ± 0.8	3.6 ± 1.0	3.6 ± 0.9	4.4 ± 1.7	4.3 ± 1.1
Total duration of buzzing (s)	9.7 ± 3.6	11.2 ± 8.9	9.0 ± 4.0	9.7 ± 6.4	7.6 ± 4.6	6.9 ± 4.2	8.6 ± 6.7	7.3 ± 4.8	7.5 ± 4.6	12.5 ± 6.5
N	32	110	30	60	15	31	13	53	18	30
Mount buzz:										
Duration (s)	6.9 ± 4.3 [a2]	2.6 ± 1.8 [a2]	1.5 ± 1.9	1.8 ± 0.5	1.2 ± 0.4	1.9 ± 1.0	1.4 ± 0.5	1.2 ± 0.4	1.2 ± 0.1	1.3 ± 0.4
N	14	22	17	20	15	31	13	53	8	25

TABLE 3. ASSOCIATION BETWEEN THE OCCURRENCE OF AN INTERRUPTION IN INTERMITTENT BUZZING, AND THE NUMBER AND DURATION OF INTERRUPTIONS WITH THE OUTCOMES OF COURTSHIPS IN DIFFERENT STRAINS. MEANS WERE CALCULATED ONLY FOR THOSE COURTSHIPS IN WHICH INTERRUPTIONS OCCURRED, AND ARE FOLLOWED BY ± ONE STANDARD DEVIATION. THE SAMPLE SIZES (IN PARENTHESES) FOR MEAN NUMBER OF INTERRUPTIONS REFER TO THE NUMBER OF COURTSHIPS; FOR MEAN DURATION THEY REFER TO THE NUMBER OF INTERRUPTIONS. MC = MOUNT AND COPULATE; MF = MOUNT BUT FAIL TO COPULATE DUE TO FEMALE RESISTANCE; NM = NO MOUNT. ASTERISKS DENOTE NM INTERRUPTION FREQUENCIES THAT WERE SIGNIFICANTLY LOWER THAN THE FREQUENCIES FOR MC AND MF IN THE SAME STRAIN USING A CHI^2 OR A FISHER EXACT TEST (F). IN NO STRAIN DID MC FREQUENCY DIFFER SIGNIFICANTLY FROM MF FREQUENCY, NOR WAS THE FREQUENCY FOR ALL MC (DATA FROM ALL STRAINS COMBINED) DIFFERENT FROM THAT FOR ALL MF (CHI^2 TEST). VALUES FOLLOWED BY THE SAME LETTER ARE SIGNIFICANTLY DIFFERENT (*a* AND * = $P < 0.05$, *b* AND ** = $P < 0.01$, *** = $P < 0.001$).

Strain and outcome		Interrupt?		Mean number of interruptions	Mean duration of each interruption
		Yes	No		
Costa Rica					
Mass-reared	MC	0	15	—	—
	MF	15	45	2.8 ± 3.29 (15)	1.46 ± 2.19 (43)
	NM***	15	9	4.07 ± 3.24 (14)	1.40 ± 1.35 (39)
	Total $\underline{a}_1\underline{b}_1\underline{c}_4\underline{c}_5$	30	69	3.36 ± 3.20 (20)	1.44 ± 1.75 (82)
Wild	MC	1	9	2.0 (1)	1.80 ± 1.64 (2)
	MF	9	34	1.66 ± 1.65 (9)	1.98 ± 4.33 (15)
	NM***(F)	10	2	2.36 ± 1.93 (10)	0.81 ± 0.43 (26)
	Total $\underline{c}_3\underline{c}_6$	20	45	2.15 ± 1.87 (20)	1.26 ± 2.60 (43)
Argentina					
Mass-reared	MC	8	12	2.00 ± 1.15 (7)	1.81 ± 1.75 (15)
	MF	15	18	2.23 ± 1.42 (13)	1.93 ± 3.30 (30)
	NM*(F)	11	2	1.92 ± 2.60 (11)	1.47 ± 1.24 (31)
	Total $\underline{a}_4\underline{b}_1\underline{b}_2$	34	32	2.34 ± 1.84 (31)	1.72 ± 2.33 $\underline{b}_2$ (76)
Wild	MC	4	9	1.75 ± 0.95 (4)	1.47 ± 0.67 (7)
	MF	14	30	1.37 ± 0.71 (14)	1.47 ± 1.94 (27)
	NM**(F)	8	2	2.15 ± 1.35 (8)	1.10 ± 1.09 (17)
	Total $\underline{c}_2\underline{c}_7$	26	41	1.64 ± 0.98 (26)	1.34 ± 1.56 (51)
Mexico					
Mass-reared	MC	0	3	—	—
	MF	23	21	3.30 ± 4.07 (23)	1.08 ± 1.28 $\underline{a}_3$ (80)
	NM***	20	25	5.31 ± 5.44 (20)	1.27 ± 1.30 $\underline{a}_3$ (116)
	Total $\underline{a}_1\underline{b}_3\underline{c}_1$	43	49	4.28 ± 4.82 (43)	1.19 ± 1.29 $\underline{b}_2$ (196)
Hawaii					
Mass-reared	MC	9	9	3.75 ± 2.75 (4)	0.375 ± 0.22 (4)
	MF	19	11	5.10 ± 4.86 (19)	1.02 ± 1.21 (19)
	NM	46	3	3.80 ± 2.62 (46)	1.17 ± 1.50 (46)
	Total $\underline{b}_2\underline{c}_{1\text{-}4}$	74	23	4.15 ± 3.40 (69)	1.08 ± 1.40 (69)
Hawaii					
Wild	MC	1	1	3 (1)	1.13 (1)
	MF	8	1	3.25 ± 2.86 (8)	1.20 ± 1.23 (8)
	NM	10	3	4.00 ± 2.58 (10)	0.77 ± 0.45 (10)
	Total $\underline{a}_4\underline{b}_3\underline{c}_{5\text{-}7}$	19	5	3.63 ± 2.60 (19)	1.01 ± 0.94 (19)

In addition, cause and effect relations in this association are uncertain. Further analyses will be required to distinguish between two possibilities. Interruption of buzzing may be a cue used by females, and they may exercise selection against those males whose buzzes are interrupted, failing to allow the male to mount by positioning themselves properly. Alternatively, females may not use interruption of the buzz as a cue, but rather the male may interrupt his buzzing when he per-
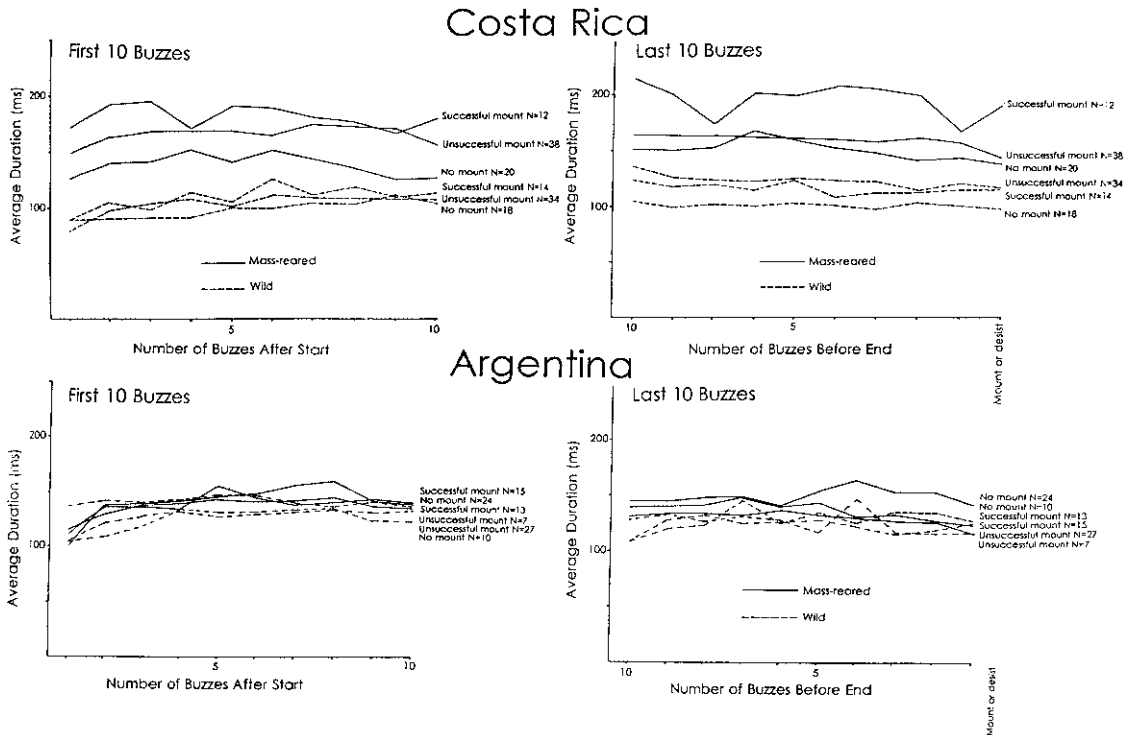
Fig. 3. Durations of the first and last 10 buzzes in courtships of wild and mass-reared Costa Rican and Argentinian flies that led to successful mounts (copulation), unsuccessful mounts (female rejection), and failure to mount (n = number of courtships).

ceives from the female's behavior that she is about to reject him. However this question is resolved, it does not appear that our results will help explain the common inferiority of mass-reared males as compared with wild counterparts, as we found only a weak difference in interruption frequencies between mass-reared and wild males from the same site.

There were several geographic differences between both wild and mass-reared strains in the details of intermittent buzzing. Possible causes of divergence include founder effects and divergent sexual selection in different populations. The lack of consistent association between these details of male songs and copulatory success argues against the possible significance of sexual selection.

Sizes of mass-reared males from Hawaii showed little sign of strongly influencing the different song parameters we measured in this study.

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COMPARISON OF MATING PERFORMANCE OF MEDFLY (DIPTERA: TEPHRITIDAE) GENETIC SEXING AND WILD TYPE STRAINS: FIELD CAGE AND VIDEO RECORDING EXPERIMENTS

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ABSTRACT

To improve the efficiency of the sterile insect technique (SIT) efforts are being devoted to obtain genetic sexing strains (GSS). The present work was carried out in order to compare the mating efficiency of flies from the GSS [(Ty34228 y$^+$/X)sw^x] and from a wild type strain (Mendoza). Females of the GSS (T228) exhibit longer embryonic development, while males develop in a normal time period. In a field-cage experiment, mating competitiveness was compared between the T228 and the Mendoza, Argentina mass reared strain. The number and duration of matings and the location of copula in the tree were recorded. The analysis was repeated using irradiated males of T228. The results showed that mating efficiency of the GSS is good in comparison with that of the Mendoza strain. Although copulatory success in T228 is reduced by the radiation treatment, the high numbers of sterilized males released would compensate this effect in the control programs. In a second experiment, under laboratory conditions, videorecording techniques were applied. In this case two virgin males, one of the GSS and one emerged from wild collected fruits, competed during 30 min for a virgin wild female. The proportion of successful males did not differ between strains, but some differences were observed between strains in the time spent in different stages of the courtship. Males of the T228 were more aggressive, and they attempted to copulate with the other male more frequently than did wild males. These differences may be due to selection for more aggressive individuals under the overcrowded laboratory breeding conditions for this strain.

Key Words: mating behavior, sexual selection, sperm transfer, copulatory success

RESUMEN

Para aumentar la efectividad de la técnica del insecto estéril (TIE) se están dedicando grandes esfuerzos a la obtención de líneas de sexado genético (LSG). El presente trabajo se realizó con el fin de evaluar la eficiencia en el apareamiento de una LSG [(Ty34228 y$^+$/X)sw^x], en comparación con moscas de una línea de tipo salvaje (Mendoza). Las hembras de la LSG (T228) exhiben un desarrollo embrionario más lento, mientras que los machos tienen un tiempo de desarrollo normal. En un experimento realizado en jaulas de campo se comparó el éxito en el apareamiento entre las líneas T228 y Mendoza. Se registró el número y duración de cópulas y la ubicación de las parejas en el árbol. El análisis se repitió utilizando machos irradiados de la línea T228. Los resultados mostraron que la eficiencia de la LSG es buena en comparación con la de la línea Mendoza. Aunque el éxito copulatorio de la línea T228 disminuye por efecto de la radiación, este efecto se podría compensar en los programas de control por el alto número de machos esterilizados liberados. En un segundo experimento se realizaron, en condiciones de laboratorio, videograbaciones del cortejo. En este caso dos machos vírgenes, uno de la LSG y otro salvaje emergido de frutas colectadas en el campo, compitieron durante 30 minutos por una hembra virgen salvaje. La proporción de machos exitosos no difirió entre las líneas, pero se observaron algunas diferencias entre ellas en los tiempos empleados en las distintas etapas del cortejo. Los machos de la línea T228 fueron más agresivos e intentaron copular más frecuentemente con el otro macho que los salvajes. Estas diferencias podrían deberse a selección a favor de individuos más agresivos en la LSG como consecuencia de la alta concentración de individuos característica de la cría en laboratorio.

Although the most widespread method of insect pest control is the use of chemical insecticides, multiple disadvantages have favored the current tendency toward replacing them by bio-insecticides or methods of biological or genetic control.

The sterile insect technique (SIT) for the control or eradication of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is being applied successfully in different countries (Wong et al. 1986, McInnis et al. 1996, Arauni et al 1996, Cayol et al. 1999). Genetic sexing strains (GSS)

have been isolated in Argentina in which males and females can be differentiated by the color of the larval posterior spiracles, and the color or size of pupae (McInnis et al. 1994, Manso & Lifschitz 1991). Although these strains have a genetic sexing system compatible with mechanical sex selection, they are not accompanied by a reduction of rearing costs. In the IG/CICA/INTA Castelar, Argentina, a mutant carrying a different eye color, and a slower embryonic development, was isolated (Manso & Lifschitz 1991, Pizarro et al. 1997). While 90% of the wild type eggs hatch after a 36 h incubation period at 23°C, the mutants can not complete egg development before 76 h. The corresponding gene pair was linked to the sex chromosome through a chromosomal translocation that yielded linkage of the wild allele with the Y chromosome. In this strain, named (Ty34228 y$^+$/X)sw^x (or simply T228), males have a normal embryonic development time, while females have the mutant phenotype, allowing an early separation of sexes (Manso & Lifschitz 1991, Pizarro et al. 1997). This strain seems to be promising for use for mass-rearing in SIT programs.

Although SIT is being implemented thoroughly, it can only benefit from a better knowledge of medfly biology in relation with courtship behavior, sexual selection, and the mating system. Variability in male copulatory success can result from differences in the activity of males prior to female arrival, differences in male activity displayed in its presence, and/or female choice (Whittier et al. 1994).

An interesting example is the case of a pilot SIT test carried out in Hawaii to eradicate the medfly from Kauai. The program failed after several years of continuous releasing, partially due to the fact that the wild females of the treated area in Kauai altered their mating preference and began to reject more of the laboratory males during courtship (McInnis et al. 1996). Females that discriminate mass-reared males could perpetuate this ability through their descendants. The presence of a genetic basis for the discrimination would select for females that are able to reject mass reared and sterilized males (McInnis et al. 1996).

Furthermore, preliminary analyses (Favret et al. 1995) indicate that, depending on the dose, irradiation not only causes a reduction in the ability to mate, but also to transfer sperm (Favret et al. 1995). This is important because it is necessary that released sterile males are not only able to copulate with wild females; they should also be able to transfer sperm. If they fail to transfer sperm, females will continue mating until finding a male, fertile or not, that can fill its spermathecae, weakening the method.

A basic technique to conduct research on medfly mating behavior is an analysis in field cages (Prokopy et al. 1987, McInnis et al. 1996, Cayol et al. 1999). Another method is video-recording that allows detailed analysis of the courtship stages, the factors affecting mating success, and the occurrence of inherited differences in courtship behavior between different laboratory and wild flies (Liimatainen et al. 1997, Calcagno et al. 1999).

In the present work, mating success and duration of copula were compared between irradiated and non-irradiated T228 males, and non-irradiated Mendoza mass-reared males under field cage conditions. Sperm transfer was also checked in mated females. Besides, a competition experiment was conducted under laboratory conditions through the video recording of successful and non-successful courtships. In this case, T228 and wild males were compared.

MATERIALS AND METHODS

Field Cage Experiment

Insects. In this experiment two medfly strains were used: (i) the Mendoza (Argentina) mass reared strain of wild-type phenotype; (ii) the genetic sexing strain (Ty34228 y$^+$/X)sw^x (thereafter T228), isolated from the Mendoza strain at the IGEF, CICA, INTA Castelar, Argentina (Favret et al. 1995). The methods of egg collection and pupae and adult rearing were described by Teran (1977). Adults and pupae were kept in breeding chambers at 23-25°C, under a photoperiod of 12:12 (L:D). Half of the T228 pupae were irradiated 48 h before emergence with an X-ray dose of 10 Krad (100 Grays) in normal air atmosphere with a Phillips irradiator.

The day following emergence adults of each strain were sexed. The T228 females were discarded and the rest of the individuals were transferred into 2750 ml flasks, and separated according to sex, strain, and irradiation treatment. Adults were fed with sucrose:yeast (3:1), and water was provided in the form of 1% agar. Flies were tested at 9 ± 1 d old to make sure they were sexually mature and to avoid differences in copulatory success due to biological development. The males of each strain and treatment were identified by labels painted on their pronotum with water-based paint.

Experiment. Mating capability was compared among the three classes of males (irradiated and non-irradiated T228, and non-irradiated Mendoza) using in all cases non-irradiated Mendoza strain females as the target. The experiment was conducted in two field cages (2.9 m diameter × 2.0 m height) in the experimental field of the Ciudad Universitaria campus of the Universidad de Buenos Aires. Each field cage contained a young potted citrus tree inside (1.5-m height, 0.80-m diameter). The experiment lasted from February 28 to March 20, 1998. During this period, temperature ranged from 15 to 32°C.

A total of 9 replicates were made in each cage. In each replicate, 60 males (30 of each strain) were released into each cage at 7.00 AM. In one cage, T228 males were irradiated while in the other they were non-irradiated. Males were allowed to establish territories and join leks for one hour. At 8.00 AM, 30 virgin Mendoza females were released into each cage. From 9.00 AM until 4.00 PM, the number of mating pairs and their position within the tree were recorded once an hour. Mating pairs were removed and carefully transferred into 300 ml vials. The vials were kept in a shady place in order to avoid mating disruption. Copula duration was also recorded for each pair. At the end of the day, mated females were kept frozen (-20°C) until they were checked for sperm transfer.

Sperm transfer. The spermathecae of mated females from the above experiment were dissected and placed onto a slide. They were stained with 2% acetic orcein, then softly squashed with a coverslip. The presence of spermatozoa could then be observed under a light microscope (20×). A total sample of 60 females were analyzed, involving 20 females mated with each of the three groups of males tested (irradiated and non-irradiated T228, and non-irradiated Mendoza).

Video Recording Experiment

Insects. T228 flies were compared with wild flies emerged from infected guava, *Psidium guajava,* collected from Concordia, Entre Ríos Province, Argentina. Pupae from both strains were kept under controlled conditions (23-25°C; L:D 12:12) until adult emergence. Flies were maintained under conditions described for the previous experiment, until they were 11 ± 1 day old.

Experiment. The experiment was conducted from April 30 to June 29, 1998. Males of both origins were placed in mating cages with wild, virgin females. The cages (70-mm height × 85-mm diameter) were made of a clear acrylic tube closed on the top by a Petri dish. The bottom of the cage was open and placed onto a transparent 2 mm thick glass plate. Recordings were made through this glass from below. The experiment was conducted in a room maintained at ca. 23°C, and was acoustically isolated. The following recording equipment was used: a Sony Hi 8 (Model CCD-TR805, Japan) video camera with a Novoflex Video Macro Lens (Germany), a Phillips (Model 14GX1510/77B, Argentina) color TV, a JVC (Model H-J401EN, Japan) videocassette recorder, and a Sennheiser (Model K6P/MKE102, Germany) microphone.

A fresh lemon, *Citrus limon*, leaf was placed inside the cage at the top in order to simulate natural conditions (males tend to establish their territories on the underside of leaves in the field [Prokopy & Hendrichs 1979]). The recording technique was the same as in Calcagno et al. (1999). Courtship behavior was recorded from 10 AM to 2 PM, the typical period of highest mating (Calcagno et al. 1999). Five mating cages were prepared each morning at 9.00-9.30 (ca. 30-60 min prior to the expected time of the first mating) with one male of each origin inside. The first cage where both males began calling (i.e., releasing pheromone from the abdomen) was chosen for the first recording. This cage was recorded for 10 min, after which time a female was gently released into the mating cage. Courtship behaviors were recorded during 30 min following female release. A male was considered successful if he copulated within that period. After concluding a recording, the camera was placed under the next cage with calling males. Two recordings were completed each day.

Recordings were analyzed to classify courtship behaviors, and to determine the time spent in each activity. The frame by frame function of the video recorder, which provided 1/30 second resolution, was used when necessary.

Notation for Courtship Activities. The main courtship activities performed by males are the following (Calcagno et al. 1999): stationary (S), mobile (M), calling stationary (CS), calling mobile (CM), fanning (Fa), buzzing (B), violent attempt (VA), peaceful attempt (PA), copulation (C), fight (Fi), and missed jump (MJ). The presence of two males inside the cage and the analysis of female activities, requires the description of additional activities listed in Table 1.

Statistical Analysis

In the field cage experiment, the proportion of mated and unmated males of each group, and the corresponding distribution of couples in the tree, were compared using a homogeneity Chi square test (contingency tables). Copula duration was compared among groups through a one way analysis of variance (ANOVA), and non-planned contrasts were made by Scheffe's method using the program Statistica (Statsoft 1996). In the video recording experiment, time spent in each activity for each strain was compared through a non-parametric Mann-Whitney test, using the program Statistica (Statsoft 1996).

RESULTS

Field Cage Experiment

Males of both strains formed leks together. Leks were usually found in the central third of the tree and involved 3 to 6 males. Upon female arrival, male displays both acoustic and visual signals. According to previous results (Calcagno et al. 1996) and under local conditions, the highest mating rate occurred between 10:00 AM and 2:00 PM, the period with the highest light intensity.

The copulatory success of T228 irradiated (I) and non-irradiated (NI) males were compared

TABLE 1. MALE AND FEMALE ACTIVITIES OBSERVED IN THE BISEXUAL VIDEO RECORDING TEST THAT WERE NOT DESCRIBED IN PREVIOUS UNISEXUAL EXPERIMENTS.

	Name	Symbol	Description
Male-female	wing signaling	WS	Soft front and backward wing movement. Wings in vertical and lateral position
	Stationary	S_L	the female remains still, close (<3 cm) to the laboratory male
		S_w	the same as previous but refers to the wild male
	male-female fight	$Fi\rightarrow m_L$	the female attacks the laboratory male or there is mutual aggression
		$Fi\rightarrow m_w$	the female attacks the wild male or there is mutual aggression
		$Fi\leftarrow m_L$	the laboratory male attacks (fights with) the female
		$Fi\leftarrow m_w$	the wild male attacks (fights with) the female
Male-male	male-male fanning	$Fa\rightarrow m$	the male under observation is displaying fanning as a response to the proximity of another male
	male-male buzzing	$B\rightarrow m$	the same as the former but referred to buzzing
	male-male attempt	$A\rightarrow m$	the male under observation attempts copulation with the other male
		$A\leftarrow m$	the other male attempts to copulate with the male under observation
	male-male fight	$Fi\rightarrow m$	the male under observation attacks (fights) the other male or there is mutual aggression
		$Fi\leftarrow m$	the male under observation is attacked by the other male, or there is mutual aggression
	head-to-head	$H\leftrightarrow H$	the males confront each other, head-to-head, and remain in this attitude immobile for several seconds
Female	Mobile	M	the female walks or flies
	Ovipositing	Ov	the female remains still but explores with the ovipositor as if trying to lay eggs

with that of Mendoza (M) males (Table 2). The proportion of successful males did not differ among strains, but irradiated T228 males did mate significantly less than other males.

Average copula duration (min) of NI, T228 I and M males (217, 179, and 208 respectively) (Fig. 1) differed statistically (F = 3.7; df = 2,350; P = 0.026). The comparisons of means by Scheffe's method indicated that the difference between I and NI males was significant (P = 0.029), but the remaining contrasts were not significant (P =

TABLE 2. NUMBER AND PERCENTAGE (IN PARENTHESES) OF MATED MALES OF EACH STRAIN IN EACH EXPERIMENT, NI= NON-IRRADIATED T228 MALES; I= IRRADIATED T228 MALES.

Strain	NI	I
T228	145 (54)	51 (26)
Mendoza	123 (46)	145 (73)
Total number of mating	268 (82)	196 (89)
Chi Square (DF= 1)	3.26	76.2
P	0.07	$<10^{-6}$

0.106 and 0.439 for the comparisons I-M and NI-M respectively).

The distribution of copulas in the cage did not differ among groups (Tables 3 and 4). For the three groups, most couples were recorded on the underside of leaves and in the central third of the tree.

Sperm Transfer Analysis

A total of 60 mated females were checked for sperm transfer. Out of the 60 pairs of spermathecae, 59 contained sperm. The only empty spermatheca belonged to a female that had mated for 60 min with an I male.

Video Recording Experiment

According to Calcagno et al. (1999), a successful courtship usually exhibits the following sequence of activities: calling, fanning, buzzing, peaceful attempt, and copulation. In the present work, the courtship pattern was analyzed for 40 trios involving one virgin wild female, one virgin T228 male, and one virgin wild male. The number
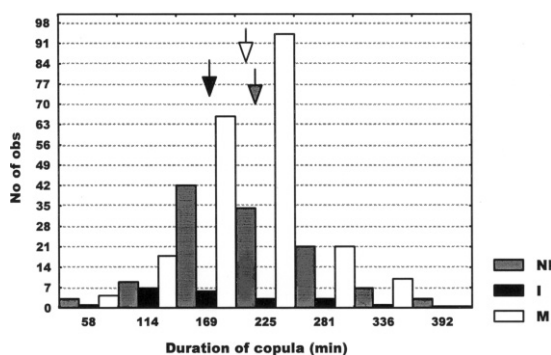
Fig. 1. Duration of copula (min) for males of the Mendoza (M) strain and non-irradiated (NI) and irradiated (I) males of the T228 strain. The arrows indicate the mean value for each group.

of successful males of each origin was the same (7 out of 40).

Significant differences were observed between successful and unsuccessful males and between strains in the time spent in some activities.

The analysis of the activities displayed by males during the 10 min prior to female arrival (Table 5) showed differences between successful and unsuccessful males of the T228 strain. The eventually successful males spent more time doing mobile calling (MC) while the others tended to be still (S).

The comparison between strains of the activities displayed in the absence of the female indicated that males of the T228 strain do more attempts to copulate (A→m) and more buzzing (B) than wild males. By contrast, wild males tend to spend more time in CS (Table 5).

The comparison of male activities in the presence of a female indicated that T228 males spent more time buzzing (B→m) and in mating attempts (A→m). On the contrary, wild males are courted (A←m) and attacked (Fi←m) by T228 males (Table 6). These results suggest that GSS males are more aggressive, and display activities in front of the other male that should be displayed during a typical courtship to females.

Some differences were also observed between successful and unsuccessful males. Unsuccessful males tend to spend more time in activities like S, M, Fi→m, and Fi←m, which are not usually connected with mating, and less time in B and PA, usually required to achieve a successful courtship (Table 6). The comparison between successful and unsuccessful females indicated that unsuccessful ones spent more time in S, M, and WS, and attacked wild male (Fi→m[+]), while females that eventually get mated did not (Table 7).

Discussion

The study of biological aspects related to sexual selection, mating systems, and courtship behavior are very important to solve methodological problems of the sterile insect technique (Burk 1991, Calkins 1987, Harris et al. 1988, Whittier & Kaneshiro 1991, 1995). Preliminary analyses (Hooper & Katyas 1971, Favret et al. 1995, Lux et al. 1996, Calcagno et al. 1997) indicate that the sterilization treatment results in a reduction of the male ability to mate and transfer sperm. It is important to clearly determine which courtship activities are of major importance for a successful mating (Calkins 1989, Whittier & Kaneshiro 1995, Calcagno et al. 1996), and which of them are altered during mass-rearing.

The process of sexual selection might be divided into two components: intrasexual and intersexual selection. Intrasexual selection refers to those aspects involved in fights and competition among individuals of the same sex (usually males). Those mechanisms involved in the ability to attract, and be accepted by, individuals of the opposite sex, constitute intersexual selection (Partridge & Halliday 1984). However, determining which of the mechanisms of sexual selection are acting is usually a very difficult task (Whittier et al. 1994).

In this work, the experimental design applied allowed male-male competition, and mating success was determined by both intrasexual and intersexual selection.

Results of the field cage experiment indicate that the T228 genetic sexing strain exhibits a bet-

TABLE 3. DISTRIBUTION (IN PERCENTAGE) OF MATING PAIRS WITHIN THE TREE IN THE FIELD CAGE EXPERIMENT.

		T228	
	Mendoza	Non irradiated	Irradiated
Adaxial leaf side	9	9	2
Abaxial leaf side	71	67	72
Stem	1	2	4
Net	19	22	22
Number of matings	266	145	50

$\chi^2 = 5.41$, df = 6, $P = 0.49$.

TABLE 4. PERCENTAGES OF MATINGS AT DIFFERENT HEIGHTS IN THE TREE IN THE FIELD CAGE EXPERIMENT.

| | Mendoza | T228 | |
		Non irradiated	Irradiated
Top	25	21	24
Middle	35	35	38
Bottom	21	21	16
Net	19	22	22
Number of matings	266	145	50

$\chi^2 = 1.69$, df = 6, p = 0.94.

ter mating performance than the Mendoza mass-reared strain. The radiation procedure and dose used in this experiment reduces copulatory success in T228. This result is not completely extrapolable to SIT programs because we used X instead of gamma rays, however, it shows the relevance of an adequate dosimetry control to improve the method. Harmful effects of irradiation might be also compensated by the huge numbers of sterile males released in control programs. The lower mating success of irradiated males may be a consequence of multiple effects of radiation on different physiological levels (Favret et al. 1995, Haish 1969, Zumreoglu et al. 1979, Burk 1991).

One important result in this experiment is that males of both strains were able to form leks and establish territories on the abaxial side of leaves. The number of males per lek (3-6) is comparable with that observed by Prokopy & Hendrichs (1979) in field cage experiments conducted in Guatemala. The localization of leks in the central third of the tree, which was observed even for irradiated individuals, also agreed with the behavior of wild populations. Although the reasons for this preference are not well understood, some factors such as light intensity, foliage density, and wind protection might be involved (Arita & Kaneshiro 1989, Hendrichs & Hendrichs 1990, Whittier et al. 1992). The circadian rhythm of laboratory and wild flies was similar. The conclusion is that the main aspects of the mating behavioral patterns of wild medflies are preserved in these strains.

The copula duration was significantly shortened in irradiated versus non-irradiated GSS males. Since Seo et al. (1990) observed that very short copulas (less than 15 min) do not result in sperm transfer, one might expect that the difference between irradiated and non-irradiated males might be reflected in sperm transfer differences. However, several studies have indicated that failure in sperm transfer may occur in cases of copulas of normal duration (more than 120 min) (Camacho 1989, Seo et al. 1990). In the current work, the difference in mating duration between irradiated and non-irradiated males was not reflected in sperm transfer differences, since all but one analyzed spermathecae pairs of mated

females contained sperm. Although the number of spermatozoids transferred could not be estimated, this preliminary evidence indicates that T228 males are able to transfer sperm, a property of major importance for a mass-reared strain.

In the video recording experiment, the mating rate (17.5%) was much lower than in previous ones (37.8 to 48.7%) (Calcagno et al. 1999, Norry et al. 1999). One important difference between the current and former experiments is that, in Calcagno et al. (1999) and Norry et al. (1999), intrasexual selection (competition between males) had been avoided by releasing only one male and one female into the cage. The relatively low mating rate observed in the present work might reflect interactions between males in the limited space inside the cages. Intrasexual selection may involve aggressive interactions which reduce the time available to interact with the female. Another cause for the reduced mating rates might be related to the female's origin. Calcagno et al. (1999) and Norry et al. (1999) tested originally wild females that had been reared for two generations under laboratory conditions. In the current work, females emerged from wild collected fruits and, perhaps, were not adapted to the experimental conditions for video recording, which are clearly more similar to laboratory than to wild conditions.

The results of this experiment indicate that the copulatory success of T228 and wild males was similar. However, important behavioral differences were observed between strains that might influence the copulatory process under conditions different from those of the current experiment. Mainly, GSS males display courtship activities such as A→m and B→m toward the other (wild) male, which in normal conditions should be displayed only in presence of females. Moreover, the GSS males were more aggressive (Fi→m) than wild males.

Laboratory rearing conditions are characterized by a dramatic reduction of space, high population densities, and absence of natural constraints (lek formation, fruits, etc.). Mass rearing conditions probably favor fast mating and shortened courtship (Calcagno et al. 1999), and most probably an increase of male aggressiveness.

TABLE 5. COMPARISON OF THE TIME (MEAN) SPENT IN EACH ACTIVITY BY LAB (L) AND WILD (W) MALES DURING THE 10 MIN PREVIOUS TO FEMALE ARRIVAL IN VIDEO RECORDED COURTSHIPS.

| | Time (seconds) | | | | | | Z' statistic for Mann-Whitney test | | | |
| | L | | | W | | | | | L | W |
	S	U	Total	S	U	Total	L vs W	S vs U	S vs U	S vs U
S	2,1	107,4	54.75	7,7	36	21.85	1.288	-1.887	2.150*	0.297
M	32,1	70,8	51.45	39,7	72,5	56.1	0.304	-0.103	0.253	-0.073
CS	446,6	328,7	387.65	431,7	426,0	428.85	-1.993*	0.399	-1.230	0.623
CM	75,1	37,7	56.4	41,6	32,3	36.95	1.315	2.890**	-2.481*	-1.852
Fa	36,7	47,0	41.85	66,7	26,2	46.45	0.533	0.863	-0.037	-1.322
B	0,3	0,6	0.45	0,0	0,3	0.15	2.154*	-0.426	0.296	0.461
A→m	1,1	0,7	0.9	0,0	0,0	0	1.993*	-0.181	-0.029	0.461
A←m	0,0	0,0	0	0,9	0,5	0.7	-1.562	-0.328	0.660	-0.029
Fi→m	0,6	1,9	1.25	1,2	1,2	1.2	1.284	0.239	0.705	-1.108
Fi←m	0,4	0,4	0.4	0,7	0,6	0.65	-1.089	0.908	-0.561	-0.681
H↔H	5,0	3,9	4.45	6,9	3,5	5.2	-0.298	0.415	-0.376	-0.217
WS	0,0	1,0	0.5	2,9	0,9	1.9	-0.386	-0.439	0.957	-0.279

Individual P values are *, $P < 0.05$; **, $P < 0.01$. S, male mated successfully; U, male did not mate successfully.

TABLE 6. COMPARISON OF THE TIME (MEAN) SPENT IN EACH ACTIVITY DURING COURTSHIP BY LAB (L) AND WILD (W) MALES IN THE 30 MIN AFTER FEMALE ARRIVAL IN VIDEO-RECORDED COURTSHIPS.

| | Time (seconds) | | | | | | Z' statistic for Mann-Whitney test | | | |
| | L | | | W | | | | | L | W |
	S	U	Total	S	U	Total	L vs W	S vs U	S vs U	S vs U
S	38.5	348.7	193.6	19.9	271.9	145.9	-0.203	-2.868***	1.381	2.667**
M	34.0	266.7	150.35	66.3	312.8	189.55	-1.392	-3.758***	2.581*	2.802
CS	623.0	837.8	730.4	481.9	973.7	727.8	-0.789	-1.818	0.588	1.940
CM	107.4	85.4	96.4	42.7	95.2	68.95	0.593	-0.672	-0.750	1.621
Fa	1.1	104.2	52.65	5.0	21.9	13.45	0.999	-1.876	2.136*	0.366
Fa→f	77.1	75.2	76.15	15.3	60.2	37.75	0.000	1.825	-1.714	-0.677
Fa→m	8.1	49.5	28.8	0.6	27.6	14.1	1.521	-1.914	1.074	2.112*
B→f	16.4	5.9	11.15	9.4	9.2	9.3	0.337	4.147**	-3.041**	-2.769**
B→m	2.7	3.5	3.1	0.0	0.3	0.15	3.531**	-0.717	0.594	0.660
PA	34.9	0.0	17.45	12.4	0.1	6.25	-0.141	8.591**	-6.207**	-5.884**
VA	0.6	0.8	0.7	0.0	1.2	0.6	0.000	-0.928	0.115	1.203
A→m	0.4	8.2	4.3	0.0	0.1	0.05	3.311**	-0.325	0.308	0.461
A←m	0.0	0.1	0.05	0.9	8.0	4.45	-2.556*	-0.846	0.660	0.632
Fi→m	1.1	3.3	2.2	0.4	2.3	1.35	1.542	-2.256*	1.885	1.132
Fi→f	0.8	0.8	0.8	0.6	1.2	0.9	-0.185	-0.625	-0.251	1.208
Fi←m	0.0	0.3	0.15	0.0	2.3	1.15	-3.420	-2.916**	1.317	2.772**
Fi←f	0.0	0.2	0.1	0.6	0.7	0.65	-1.640	-0.698	0.818	0.280
H↔H	0.9	3.6	2.25	0.0	6.5	3.25	-0.412	-0.297	-0.662	0.957
C	851.4	2.8	427.1	1143.7	0.0	571.85	0.155			
WS	1.4	2.8	2.1	0.4	4.7	2.55	-1.163	-0.555	-0.062	0.755
MJ	0.0	0.0	0	0.0	0.2	0.1	0.981	-0.939	0.818	0.461

Individual *P* values are *, *P* < 0.05; **, *P* < 0.01. S, male mated successfully; U, male did not mate successfully.

TABLE 7. TIME (IN SECONDS) SPENT BY FEMALES IN EACH ACTIVITY. *: SIGNIFICANT; **: HIGHLY SIGNIFICANT.

Activity	Mated	Unmated	Z'
S	642.6	1270.8	3.346**
M	104.2	423.8	3.176**
S_L	11.0	14.7	-0.740
S_w	5.4	20.6	0.807
$Fi{\rightarrow}m_L$	0.2	1.0	1.048
$Fi{\rightarrow}m_w$	0.0	2.7	3.104**
$Fi{\leftarrow}m_L$	0.4	0.3	-0.543
$Fi{\leftarrow}m_w$	0.8	0.5	0.168
WS	2.6	13.9	2.547*
Ov.	11.3	49.2	0.517

These might be the causes for the observed mating attempts with other males. If the behavioral differences between strains have a genetic basis they arose as a selective response to the laboratory rearing conditions.

Despite the behavioral differences observed, the results of the video recording experiment indicate that the T228 strain is compatible with the Concordia wild population. However, the conclusions about sexual selection are not so conclusive.

The general conclusions from both field cage and video recording approaches are consistent in showing that the strain T228 performs acceptably and is a promising strain for medfly genetic control programs.

ACKNOWLEDGMENTS

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SEXUAL COMPATIBILITY IN MEDFLY (DIPTERA: TEPHRITIDAE) FROM DIFFERENT ORIGINS

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ABSTRACT

The use of the Sterile Insect Technique to control and/or eradicate insect pest populations has been extensively applied to medfly. However, patented differences in sexual compatibility between populations or strains from different origins has been a serious concern to a wider use of sterile flies, and in particular sterile males of genetic sexing strains (GSS). In the present experiments, the sexual compatibility and mating performance of flies from 9 countries representing 5 continents and 4 GSS were measured. It is demonstrated that, from a qualitative standpoint, wild medfly populations world-wide have not yet evolved specific sexual behaviors indicative of incipient pre-mating isolation mechanisms under local natural selection. Wild medfly populations are as sexually compatible with GSS as they are with other wild populations. On that basis, the same mass reared strain can now be used world-wide, as long as it fulfills the standard quality control requirements.

Key Words: medfly, *Ceratitis capitata*, sexual compatibility, comparison, wild population, genetic sexing strain

RESUMEN

El uso de la técnica del insecto estéril para controlar y o erradicar poblaciones de plagas insectiles ha sido aplicado extensamente a la mosca del Mediterráneo. Sin embargo, diferencias en compatibilidad sexual entre poblaciones o razas de diferentes orígenes ha sido una seria preocupación para un uso mas amplio de las moscas estériles y en particular machos estériles de Cepas genéticamente sexadas (CGS). En los siguientes experimentos, la compatibilidad sexual y la capacidad de apareamiento de las moscas de 9 países, representando 5 continentes y 4 RGS fueron evaluadas. Se ha demostrado que, desde un punto de vista cualitativo, poblaciones salvajes a nivel mundial de la mosca mediterránea aun no han evolucionado bajo selección natural local comportamientos sexuales específicos indicativos de incipientes mecanismos de aislamiento anteriores al apareamiento. Poblaciones salvajes de moscas mediterráneas son tan sexualmente compatibles con CGS que con otras poblaciones salvajes. Sobre esas bases, la misma cepa criada en masa se puede utilizar ahora a nivel mundial con la condición de que cumpla con los requerimientos estándares de control de calidad.

The Mediterranean fruit fly (medfly), *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), is often referred to as the most important agricultural pest in the world (Liquido et al. 1990) and this "title" is widely justified. From its origin in Eastern Africa (Silvestri 1913, Bezzi 1918), the pest efficiently conquered new countries and new hosts. If medfly was present in North African and almost all European Mediterranean countries by the mid-19th century, its introduction in North, Central and Latin America occurred nearly 100 years later (Dridi 1990). Following the development of fruit and vegetable trade worldwide, and the increasing number of international, including intercontinental, airway connections, medfly successfully spread over five continents in less than 150 years, and is found developing, to date, in more than 350 wild and cultivated host plants of various families (Liquido et al. 1990). Such a threat for agriculture represented by a single species turned medfly into one of the main targets of pest control programs, including the Sterile Insect Technique (SIT) described by Knipling (1953).

The use of SIT requires that rearing facilities be developed to produce large numbers of insects for sterile fly releases. In the early stages of medfly control using SIT, mass reared strains were established by colonizing wild insects collected from, or in the vicinity of, the target area. Such strains have been reared in Mexico, Chile, Hawaii and Guatemala rearing facilities. More recently, with the increasing demand for sterile medflies

and the limited number of mass rearing facilities available worldwide, some of these facilities began to export sterilized medflies to other countries. Eight facilities have now reached production levels, which allow them to export sterile insects (Fisher & Caceres 2000) on a regional or inter-regional basis. When this procedure is used, the flies released have to compete with wild flies of a different geographic origin.

The increasing use of medfly genetic sexing strains (GSS) has also resulted in the same strain being used in different countries. To date, five rearing facilities in the world produce GSS (Fisher & Caceres 2000). Since GSS are assembled from specific components, it is impossible to "colonize" them from each country where sterile GSS flies are needed. The GSS are sometimes outcrossed with insects from the target population to increase the genetic variability (Franz et al. 1996), although in some cases this presents problems (G. Franz, IPCS, FAO/IAEA, Vienna, unpublished data). In practice, a single wild population is used as a basis for the synthesis of the GSS. Consequently, the same GSS based on the same wild genetic material may be used in various countries/continents and the question was raised concerning the sexual compatibility of these strains with wild medfly populations in different countries.

In the present work, the sexual compatibility of wild populations originating from nine countries, representing five continents, was measured in pairwise comparisons under semi-natural field cage conditions. In a second series of experiments, flies from four GSS were evaluated.

MATERIALS AND METHODS

Wild Material

Wild insects were collected as pupae from infested fruits in their country of origin. Pupae were shipped by express air mail to Seibersdorf, Austria (or hand-carried), except for field cage tests run in Argentina where wild flies were tested on site (Cayol et al. 1999). Wild insects originating from Argentina (Patagonia region), Australia (Perth), France (Reunion Island), Greece (Crete Island), Guatemala (Antigua), Israel (near Tel Aviv and from the Arava Valley), Kenya (near Nairobi), Portugal (Madeira Island) and South Africa (Western Cape Province) were tested. Their host of origin was guava (Israel, both locations; Portugal; South Africa), coffee (Guatemala, Kenya), orange (Australia, Greece), fig and peach (Argentina) and milkwood (France). Upon reception of a shipment, pupae were weighed and counted. On emergence, flies were sexed and kept in separate ventilated Plexiglas cages ($11 \times 15.5 \times 11$ cm) until tested and provided with adult food (sugar and yeast in 3:1 ratio) and water.

Genetic Sexing Strains

Flies of several genetic sexing strains (GSS) were obtained as pupae from the FAO/IAEA facility at Seibersdorf for green house tests. In the field cage tests in Argentina, GSS flies were provided by the KM8 facility in Mendoza (Cayol et al. 1999). The following four GSS were tested. SEIB 6-96 is a GSS carrying a white pupa (*wp*) mutation (Rössler 1979) in combination with the translocation T(Y;5) 2-22 (Franz et al. 1994). VIENNA 4/TOL-94 is a GSS carrying *wp* and temperature sensitive lethal (*tsl*) mutations in combination with the translocation T(Y;5) 1-61 (Franz et al. 1994). VIENNA 7-97 is a GSS carrying *wp* and *tsl* mutations in combination with the translocation T(Y;5) 3-129 (Kerremans & Franz 1995). AUSTRIA 6-97 is a triple mutant strain carrying *wp*, *tsl* and yellow body (*y*) (Rössler & Rosenthal 1992) selectable markers in combination with the translocation T(Y;5) 2-22 (G. Franz, IPCS, FAO/ IAEA, Vienna, unpublished data). The genetic background of SEIB 6-96, VIENNA 7-97 and AUSTRIA 6-96 GSS originates from Egypt. The genetic background of VIENNA 4/TOL-94 originates from Guatemala highlands (Lake Atitlan), following an outcrossing of the original strain (Franz et al. 1996). After sexing on emergence, GSS flies were maintained under the same conditions as wild flies.

Testing Cage

Flies were tested in a greenhouse located at the FAO/IAEA Agriculture and Biotechnology Laboratory (Seibersdorf, Austria). The green house was temperature monitored (temperature ranging between 24 and 32 degrees Celsius). A cage made of netting material was placed inside the greenhouse. The cage contained 6 potted citrus trees (up to 1.8 meter height) in a total volume of 15 m³. In Argentina, flies were tested in outdoor field cages (Chambers et al. 1983) containing a single planted citrus tree (Cayol et al. 1999). In both greenhouse and field cage tests, the cages were covered with a shading cloth filtering 85% of sunlight to avoid any "greenhouse effect".

The strains were tested in pair-wise comparisons. Depending on the availability of biological material, two types of tests could be run: (i) wild-wild comparisons, where wild flies from two different geographic origins were tested and (ii) wild-GSS comparisons, where wild flies originating from one country were tested against GSS flies. In both types of test, the protocol described by Cayol et al. (1999) for "bisexual" type test was applied. Two days before being tested, active and flying flies were selected. Males and females, from alternatively one of the two populations were marked with a dot of water-based paint on the notum for identification during the course of the

tests. On the day of the test, 30 flies of each sex and each strain were released into the cages at dawn. Males were released 30 minutes before females to give them time to establish a territory and start forming leks (Prokopy & Hendrichs 1979). The number of calling males and the environmental conditions (temperature, relative humidity, light intensity and air pressure) were checked every half-hour. The number and type of mating pairs were checked on a continuous basis, and 5 minutes after initiation of mating, the pairs were collected and placed in vials (50 ml volume) to monitor mating duration. The mated flies were not replaced or released back into the cage after separation (Chambers et al. 1983). Tests lasted for 7-8 consecutive hours. Tests were performed from March 1997 until September 1998, whenever flies were available. A total of 19 combinations were tested as shown in Table 1. Due to the availability of flies from different origins, the number of replications for each combination was variable.

Statistical Analysis

Raw data were transformed following an ARC-SIN $[ASIN(\sqrt{(X/100)}*180/PI)]$ transformation to stabilize variance.

For each of the parameters measured and whenever it was relevant, data were first pooled according to the type of combination tested "wild versus wild" or "wild versus GSS" (later called "wild/wild and wild/GSS comparisons"). As a second step

of the analysis, data were pooled according to the origin of the strain (Madeira, Argentina, Vienna 7-97, etc.) (later called "strain comparison").

In both cases, data were analyzed using Systat 9.0 (Systat, 1999) for analysis of variance (ANOVA), followed by Tukey's HSD test.

RESULTS

Participation of Flies in Mating

This measures the suitability of the flies and the environmental conditions of the tests for mating. It represents the overall mating activity of the flies (Table 2). If PM < 0.20 (proportion of mating) then the results of the test must be rejected (IAEA 1997).

Wild/Wild and Wild/GSS Comparison. The mean PM values obtained in comparing wild/wild and wild/GSS combinations confirmed that the test conditions were suitable for mating, as about 40% of the possible matings were achieved. However, there was a highly significant difference between the two mean PM values, 0.407 (wild) and 0.484 (GSS), ($F = 7.530$; df=1,71; $P = 0.008$) showing that somewhat more matings took place when GSS flies were involved in the test (Table 3).

Strain Comparison. When comparing the PM values obtained for each strain tested, even though the overall mating activity was satisfactory in each case (PM > 0.20), some significant differences can be found among the strains ($F = 2.789$; df = 12,134; $P = 0.002$). Significantly more matings were achieved in tests involving wild flies from Australia (PM = 0.554) than in tests involving wild flies from Kenya, Madeira or Austria 6-96 GSS flies (PM values 0.349, 0.386 and 0.345 respectively). Those differences might reflect various adaptations to the test conditions or a generally higher mating activity of Australian flies.

Sexual Compatibility

In all of the 19 comparisons involving any of the wild populations or GSS tested, each of the four possible types of mating was encountered confirming that there was no absolute behavioral incompatibility among these populations. The sexual compatibility among the flies from different origins was assessed using the Isolation Index (ISI) (Cayol et al. 1999) as described in Table 2. The ISI ranges from -1 ("negative assortative mating", i.e. flies only mate with a "foreign" partner) to +1 ("positive assortative mating" or total sexual isolation, i.e. flies only mate with partner of the same origin), through an equilibrium at 0 (uniform sexual compatibility, i.e. no mating preferences).

Wild/Wild and Wild/GSS Comparison. There was no significant difference between the overall mean value of ISI obtained when comparing wild versus wild populations and wild versus GSS ($F =$

TABLE 1. TYPE OF MATING COMBINATIONS TESTED.

| Wild population | Tested against | |
	Wild	GSS
Argentina[a]		Seib 6-96
Australia	Crete Israel	Vienna 4/Tol-94
Crete	Australia	Seib 6-96
Guatemala	Israel Kenya Madeira	Vienna 4/Tol-94
Israel	Australia Guatemala Madeira Reunion	Vienna 4/Tol-94 Austria 6-96 Vienna 7-97
Kenya	Guatemala Madeira	Vienna 4/Tol-94
Madeira	Guatemala Israel Kenya	Vienna 4/Tol-94 Vienna 7-97
Reunion	Israel	
South Africa		Vienna 7-97

[a]Tested in field cages in San Miguel de Tucuman (Argentina) (Cayol et al. 1999).

TABLE 2. INDICES USED TO MEASURE SEXUAL COMPATIBILITY OF MEDFLY STRAINS FROM DIFFERENT ORIGINS.[a]

Trait measured	Index formula[b]
Participation in mating	$PM = \dfrac{\text{No. of pairs collected}}{\text{No. of females released}}$
Sexual isolation	$ISI = \dfrac{(aa + bb) - (ab + ba)}{\text{Total no. of matings}}$
Male relative performance	$MRPI = \dfrac{(aa + ab) - (bb + ba)}{\text{Total no. of matings}}$
Female relative performance	$FRPI = \dfrac{(aa + ba) - (bb + ab)}{\text{Total no. of matings}}$
Male mating competitivenessa[c]	$RSI = \dfrac{LW}{LW + WW}$

[a]After Cayol et al. (1999) and McInnis et al. 1996.
[b]"ab": number of matings of "a" males with "b" females.
[c]In RSI: "L" for mass reared males and "W" for wild flies (males or females).

0.030; df 1,71; P = 0.864). Even though the two mean ISI values showed a tendency for homologous (male and female of the same origin) mating (Table 3), there was certainly no evidence of sexual isolation. Of utmost importance, these results show that wild flies did not discriminate against GSS flies more than wild flies originating from a different area or continent. In other words, wild populations are as behaviorally compatible with GSS as they are with other wild populations from various geographic origins.

Strain Comparison. The mean ISI values obtained for the 9 wild populations and the 4 GSS did not differ significantly (F = 1.499; df 12,134; P = 0.132) (Table 4). This confirms that, even if there are some minor differences among the various wild populations and GSS tested, none of them developed, to date, a significant behavioral isolation (ISI > 0.50).

Male and female Relative Mating Performance

Two other indices which look at the relative mating performance of males (MRPI) and females (FRPI) of the two strains, regardless of their mating partners, were measured (Cayol et al. 1999). These indices range between -1 (all matings achieved by one type of male (MRPI) or female (FRPI)) and +1 (all matings achieved by the other type of male (MRPI) or female (FRPI)) through an equilibrium at 0 (equal mating performance of males or females of the two strains) (Table 2). These indices complement the ISI value by better describing the role played by males and females of the two strains compared.

Wild/Wild and Wild/GSS Comparison. The male relative mating performance is significantly higher when comparing wild versus wild populations than it is when comparing wild populations versus GSS (F = 4.693; df 1,71; P = 0.034) (Table 3). This demonstrates that, when two types of wild males of different geographic origin are present in the same cage, one of the two types of males mates more than the other. However, when wild and GSS males are present, the relative performance is more "balanced", i.e. both types of males mate in a similar proportion (regardless of

TABLE 3. SEXUAL COMPATIBILITY AND PERFORMANCE MEASURED WHEN TESTING MEDFLY WILD POPULATIONS AGAINST WILD OR GSS.

Parameter measured	Combination tested		
	Wild/wild	Wild/GSS	
PM	0.407 b ± 0.020	0.484 a ± 0.018	F = 7.530; df 1,71; P = 0.008
ISI	0.233 a ± 0.057	0.221 a ± 0.034	F = 0.030; df 1,71; P = 0.864
MRPI[a]	0.375 a ± 0.042	0.265 b ± 0.030	F = 4.693; df 1,71; P = 0.034
FRPI[a]	0.288 a ± 0.033	0.345 a ± 0.032	F = 1.330; df 1,71; P = 0.253

[a]Based on absolute values.
[b]Data are presented as mean ± SEM. Data followed by the same letter on the same row do not differ significantly according to Tukey's HSD test (P > 0.05).

TABLE 4. SEXUAL COMPATIBILITY AND PERFORMANCE OF WILD POPULATIONS AND GSS.[b]

Origin of the flies	Parameter measured			
	PM	ISI	MRPI[a]	FRPI[a]
Argentina	0.488 ab ± 0.032	0.309 a ± 0.062	0.360 b ± 0.023	0.332 ab ± 0.051
Australia	0.554 a ± 0.029	0.069 a ± 0.104	0.480 abc ± 0.069	0.496 a ± 0.075
Crete	0.508 ab ± 0.025	0.108 a ± 0.123	0.586 ab ± 0.066	0.300 ab ± 0.114
Guatemala	0.462 ab ± 0.043	0.188 a ± 0.069	0.462 abc ± 0.072	0.442 a ± 0.085
Israel	0.419 ab ± 0.021	0.200 a ± 0.056	0.316 bc ± 0.043	0.491 a ± 0.047
Kenya	0.349 b ± 0.039	0.319 a ± 0.161	0.171 c ± 0.056	0.420 ab ± 0.102
Madeira	0.386 b ± 0.022	0.196 a ± 0.079	0.600 a ± 0.053	0.156 b ± 0.028
Reunion	0.389 ab ± 0.053	0.377 a ± 0.085	0.278 c ± 0.056	0.361 ab ± 0.081
South Africa	0.477 ab ± 0.043	0.259 a ± 0.064	0.421 abc ± 0.054	0.299 ab ± 0.083
Vienna 4/tol-94	0.522 ab ± 0.038	0.235 a ± 0.070	0.456 abc ± 0.061	0.335 ab ± 0.042
Vienna 7-97	0.457 ab ± 0.029	0.092 a ± 0.084	0.236 c ± 0.058	0.563 a ± 0.061
Seib 6-96	0.494 ab ± 0.028	0.300 a ± 0.038	0.313 bc ± 0.049	0.396 ab ± 0.030
Austria 6-96	0.345 b ± 0.029	0.104 a ± 0.021	0.200 c ± 0.200	0.470 ab ± 0.004
	$F = 2.789$ df = 12,134 $P = 0.002$	$F = 1.499$ df = 12,134 $P = 0.132$	$F = 4.563$ df = 12,134 $P = 0.000$	$F = 3.985$ df = 12,134 $P = 0.000$

[a]Based on absolute values.
[b]Data are presented as mean ± SEM. Data followed by the same letter in the same column do not differ significantly according to Tukey's HSD test ($P > 0.05$).

the type of female). No significant difference was found in the female relative mating performance ($F = 1.330$; df = 1,71; $P = 0.253$) (Table 3). In both cases (wild/wild and wild/GSS), there is a slight tendency for one of the two types of females to outcompete the other. In wild/GSS comparisons, the GSS females often mate more than their wild counterparts (regardless of the type of males).

Strain Comparison. The MRPI value of the Madeira wild population is significantly higher than that of the Kenya and the Reunion wild populations, and that of the Vienna 7-97 and the Austria 6-96 GSS ($F = 4.563$; df = 12,134; $P < 0.000$) (Table 4). Whatever strain they were compared to, Madeira males very often, and by far, outcompeted the other type of males for mates. To the contrary, and under similar conditions, Kenya, Reunion, Vienna 7-97 and Austria 6-96 males were outcompeted by any other type of males they were compared to, even with their own female counterparts. There was a significant difference between the higher FRPI value of the Australia, Guatemala and Israel wild populations and the Vienna 7-97 GSS and that of the Madeira wild population ($F = 3.985$; df = 12,134; $P < 0.000$) (Table 4). This shows that these 4 types of females were more prone to mate than were wild Madeira females. This would indicate that Madeira females were more "selective" in choosing a mate than were the other strains of females.

Mating Competitiveness of GSS Males

The mating competitiveness of GSS males with wild males for wild female mates was mea-

sured by the Relative Sterility Index (RSI) (McInnis et al. 1996) described in Table 2. When RSI = 0.5, wild and GSS males are equally competitive. The mean RSI value has been compared for the 4 GSS tested and results are shown in Table 5.

The analysis showed that, even though all the GSS males did compete with wild males for wild female mates, Vienna 4/Tol-94 males were about twice as competitive as Vienna 7-97 males ($F = 2.967$; df = 3,48; $P = 0.041$) (Table 5). This result confirms a poor relative mating performance for Vienna 7-97 males, which has been previously demonstrated by the relatively low MRPI value.

Duration of Mating

Time spent in copula (duration of mating) was measured and compared for the homologous type of mating (male and female of the same origin) for each GSS and wild population tested and these results are shown in Table 6.

TABLE 5. MATING COMPETITIVENESS OF MALES FROM THE DIFFERENT GSS.[a]

Strain	Relative Sterility Index[b]
Vienna 4/Tol-94	0.448 a ± 0.059
Vienna 7-97	0.217 b ± 0.045
Seib 6-96	0.302 ab ± 0.049
Austria 6-96	0.250 ab ± 0.087

[a]Data are presented as mean ± SEM.
[b]Data followed by the same letter do not differ significantly according to Tukey's HSD test ($F = 2.967$; df = 3,48; $P = 0.041$).

TABLE 6. DURATION OF HOMOLOGOUS MATING (MALE AND FEMALE OF THE SAME ORIGIN) FOR THE DIFFERENT WILD POPULATIONS AND GSS.

Origin of the flies	Duration of homologous pairing (min)[a,b]
Argentina	164.466 cd ± 4.777
Australia	204.920 ab ± 5.962
Crete	195.500 ad ± 6.859
Guatemala	156.970 d ± 4.180
Israel	157.418 d ± 4.728
Kenya	198.464 ac ± 8.412
Madeira	178.095 cd ± 4.559
Reunion	178.538 bcd ± 9.047
South Africa	227.310 a ± 6.241
Vienna 4/Tol-94	164.983 cd ± 3.593
Vienna 7-97	158.072 d ± 4.225
Seib 6-96	124.035 e ± 3.700
Austria 6-96	164.278 cde ± 8.664

[a]Data are presented as mean ± SEM.
[b]Data followed by the same letter do not differ significantly according to Tukey's HSD test (F = 28.710; df = 12,1157; P = 0.000).

A large and significant variation in mating duration has been found (F = 28.710; df = 12,1157; P < 0.000) (Table 6). Average time spent in copula can vary from 2 hours for the Seib 6-96 GSS up to nearly 4 hours for the South Africa wild population. However, this duration seems to be relatively stable for each strain, as demonstrated by the low standard error values. In addition, the GSS flies tend to mate for a relatively shorter period (2 to 3 hours) than do wild flies (3 to 4 hours).

DISCUSSION

These data demonstrate that, from a qualitative standpoint, wild medfly populations worldwide have not yet evolved specific sexual behaviors indicative of incipient pre-mating isolation mechanisms under local natural selection. In addition, it was shown that wild populations are as sexually compatible with GSS as they are with other wild populations.

However, some quantitative differences have been measured among wild populations or GSS, such as a lower or higher male or female relative performance. In the case of Madeira for example, the high male performance, and the relative selectiveness of the females as shown in the present experiments could, in a long run, result in a lower mating acceptance of relatively poorly competitive mass-reared males, such as Vienna 7-97, which could affect the effectiveness of SIT. Additional tests have been run to look at this specific case which tends to show that Madeira females do discriminate against Vienna 7-97 males more than other wild or GSS males (J. P. C., unpublished data).

The high importance of mating behavior studies to the SIT has encouraged the Insect Pest Control Section of the International Atomic Energy Agency to investigate this subject. The coordinated research program started in 1994 by the IAEA examined details of male courtship behavior in wild populations from nine countries (FAO/IAEA 1994) from both qualitative and quantitative standpoints, using slow motion video recording. Some minor differences have been found among the wild populations, as demonstrated by Briceño et al. (2002). When comparing wild flies from Costa Rica and Argentina, the authors showed that some significant differences of the courtship songs could be identified and measured. In addition, it was shown that long term rearing could affect significantly the duration of the mass-reared male courtship (Eberhard & Briceño 1996, Briceño & Eberhard 2002) and love songs (Briceño & Eberhard 1997). The present findings tend to show that copula duration is also shortened in mass rearing. Those differences in mating duration warrant further study in relation to post-mating isolation. Post-mating isolation could affect the efficacy of SIT due to remating of wild females, shortly after a first mating with a sterile GSS male.

Concerns about the sexual compatibility among medflies from different origins represented somewhat of a threat to the shipment of sterile flies from one country to another to support SIT programs. These concerns become more pronounced when a GSS was proposed to be used in many different SIT programs. The findings of the present experiments support the potential use of the same GSS anywhere in the world. Out of the 4 GSS tested in the present experiments, the only one, which was outcrossed with a wild population (Vienna 4/Tol-94), did show the highest mating competitiveness. This strongly supported the idea of building-up a new GSS based on mixing wild populations from various origins. This new and very promising GSS has now been developed and is currently under testing (G. Franz, IPCS, FAO/IAEA, Vienna, unpublished data).

Gasparich et al. (1997) showed that the mitochondrial DNA of medfly populations from 100 different origins was indeed variable and that it probably reflected the colonization pattern of medfly from its origin in Eastern Africa about 200 years ago. However there was no evidence that substantial genetic differentiation had occurred. When a medfly outbreak occurs, program managers sometimes worry that the sterile flies released might not be from the same geographic origin and hence would not mate. A second concern is that the "foreign" flies might introduce new genetic material into the country. The fear is that "foreign" fertile flies would establish a new population with its own genetic and behavioral characteristics. However, the present work based on populations representative of five continents,

clearly demonstrates that there are no significant population specific mating behavior traits. These observations together with the genetic data suggest that the risk of introducing a more virulent form of medfly into a specific country is remote.

In conclusion, strains to be used in SIT programs in any country must be selected to maximize the quality of the flies produced, rather than based on the geographic origin of the strain.

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MATING PERFORMANCE AND SPATIAL DISTRIBUTION OF MEDFLY (DIPTERA: TEPHRITIDAE) WHITE PUPA GENETIC SEXING MALES UNDER FIELD CAGE CONDITIONS

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Abstract

In mixed populations of wild and males from T (Y;5) 1-61 white female pupa genetic sexing strain of *Ceratitis capitata* (Wiedemann), sterilized males of the genetic sexing strain expressed calling, lekking and mating compatibility with their wild counterparts. Nevertheless, their mating performance was most of time poor to very poor. For example, in a series of studies from June-October 1996, only 0- about ⅓ of expected matings (based on insect ratios) by genetic sexing sterilized males was recorded. Similar results were observed in the other years of this study. No substantial differences between gen. sex. male × wild female and wild male × wild female type copulations were detected in spatial distribution of couples in copula on the orange tree. Over 83% of both mating types were detected on the underside leaf surface.

Key Words: *Ceratitis capitata*, medfly, sexing strain, quality control, competitiveness

Resumen

En poblaciones mezcladas de machos salvajes y machos provenientes de pupas blancas de hembras T (Y;5) 1-61 de razas genéticamente sexadas de *Ceratitis capitata* (Wiedemann), los machos esterilizados de razas genéticamente sexadas expresaron la capacidad de llamamiento, la acción de seleccionar un lugar de apareamiento y apareamiento con su contraparte salvaje. No obstante, su capacidad de apareamiento fue en la mayoría de las veces de pobre a muy pobre, por ejemplo, en una serie de estudios llevados a cabo entre junio y octubre de 1996, solamente entre 0 y ⅓ de los apareamientos esperados (basados en proporciones de insectos) por machos estériles genéticamente sexados fueron registrados. Resultados similares se observaron en los otros años de este estudio. No se detectaron diferencias substanciales entre el tipo de copulación entre machos genéticamente sexados × hembras salvajes y machos salvajes × hembras salvajes en las distribuciones espaciales de parejas en cópulas sobre árboles de naranja. Mas del 83% de ambos tipos de apareamiento se detectaron en el lado inferior de la superficie de las hojas.

Artificial mass-rearing and sterilization may affect field effectiveness of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), drastically. Mass rearing conditions, selection genetic changes, irradiation and sterile insect technique (SIT) handling procedures may affect the vigor and behavior of sterile males and reduce strikingly their mating performance with the wild population in the field (Economopoulos 1996). Furthermore, recent evidence suggests development of behavioral resistance in the wild flies against sterile flies (McInnis et al. 1996). The limited success SIT had so far on several key insect pests was connected to a large degree with the quality of released insects. This became evident from the initial steps of the methodology and resulted in the development of tests which monitor the quality of sterile insects and the field effectiveness of the methodology (Calkins et al. 1996, Cayol et al. 1999). This effort culminated in the recent publication of a comprehensive manual of quality control for fruit flies (IAEA 1998).

The basic and closest to field conditions mating performance test available so far is that of field cage (Calkins & Webb 1983). Nevertheless, although the test is applied under natural conditions and involves a host tree, the fact that flies cannot freely fly away or "escape" from the host tree, or newcomers cannot mix with the caged tree flies reduces the value of the test. Recently, the interest on sterile insect competitiveness as deduced from egg hatch, first described in 1971 (Fried), has been renewed. Measurements of egg hatch from field oviposition in mock fruits are used for a more accurate evaluation of mating performance under completely natural conditions (Katsoyannos et al. 1999). Unfortunately, no practical method has been standardized so far on egg hatch measurement of field oviposited eggs in mock fruits.

In this study, the mating performance of a white female puparium strain has been evaluated under field cage conditions in citrus plantation.

MATERIALS AND METHODS

Cylindrical field cages 2m h × 2.9m d were used (Synthetic Industries, Dalton, GA 30720, USA). Each cage was installed over a Navel orange tree in a mixed plantation with Navel and Valencia orange trees. The cage ceiling was covered by thick white fabric to provide shade. For the study of 1996 (Table 1), most wild flies were raised from larvae in sour oranges and loquats (early summer) and figs (late summer). The genetic sexing males were of the white female pupa strain T (Y;5) 1-61(95) (Franz et al. 1994) at generations 5-10. They were gamma-sterilized 1-2 days before adult emergence. Eight experiments were performed from June till October (see also caption of Table 1). Flies for the experiments in 1998 (Tables 2 and 3) were similar except that the genetic sexing males were of generations 33 and 36, respectively, and were not gamma-sterilized. In the June experiments, high mortality was observed on the second and third experiment days because of air-born toxicity due to near-by bait spraying. In both 1996 and 1998 matings were recorded from 09:00-18:00, every half hour.

In all experiments trees were pruned to fit the cages and make easy the census of fly activities and copulations. Males and females were separated soon after emergence and kept on standard adult diet (unless indicated differently in the table) prior to introduction into the field cage. Water was sprayed on the caged trees on the hot hours of the day to provide the flies with drinking water.

RESULTS AND DISCUSSION

In 1996 mating performance experiments are presented in Table 1. All genetic sexing males included were gamma sterilized. In June-August (highest daily temperatures under shade between 30-36°C), the genetic sexing males produced only 0-33% of observed matings while expected values according to insect type ratios were 50-90%, i.e. 13 observed instead of 87 expected matings in total. In 3 out of the 5 experiments organized in this period, the genetic sexing males contributed zero to near zero of mating activity observed, while in the other 2 experiments their mating share was 1/2.7 and 1/2.5 of expected values, respectively. It is noted that the reduced performance of genetic sexing males in the June 18 experiment could had been intensified by the young age of laboratory flies mixed in this test (refer to Table 1 caption). On October 10 and 17 (when daily temperatures were between 16-29°C), genetic sexing males succeeded in getting 1/3.4 to 1/3.1 of their expected mating activity, i.e. 8 observed instead of 28 expected matings in total. That is, although the results in October were not as bad as in July, the genetic sexing males competed again poorly with wild males for matings with wild females. In all experiments the difference was highly significant ($P < 0.001$). Both type matings were recorded almost exclusively on the underside

leaf surface, with sterile matings located relatively more at lower canopy than the wild type matings. The majority of both type matings was recorded at the same tree canopy sectors.

The results of the 1998 experiments are shown in Tables 2 and 3. All genetic sexing males involved were not sterilized. The genetic sexing strain was already at generations 33 or 36 with extensive break down. In the June experiment (warm weather) the genetic sexing males, at 1:1:1 ratio (wild males: wild females: gen. sex. males) and fed complete diet, obtained more than expected matings, while at 1:1:3 ratio they obtained significantly fewer than expected matings. In September (cooler weather) at both insect ratios they obtained mating percentages lower than expected. It was observed that their performance was higher in the second and third experiment days as compared with the first one. At the ratio of 1:1:3, sugar fed laboratory flies obtained fewer than expected matings in both June and September. The difference was not significant in June, while in September it was highly significant. When insect ratios of 1:1:3 are compared with 1:1:1 ratios in June, the genetic sexing males of the high overall male: female ratio (4:1) obtained mating values lower than expected, while in the low overall male: female ratio (2:1) they obtained mating values higher than expected ones. In September the genetic sexing males of the low male: female ratio did not perform as well. It could be that the reduced male: female ratio improves the sterile male performance under warm weather. Also, if we compare the mating performance of genetic sexing males fed complete diet with sugar fed ones at 1:1:3 ratio, we observe that in the June experiments the sugar fed males had superior mating performance as compared with the complete diet fed ones. The opposite was true in September. In conclusion, the mating performance of non-irradiated genetic sexing males was considerably improved as compared with the performance of irradiated males in the 1996 experiments. This could be the result of no irradiation-damage and/or even of strain performance improvement because of sexing break down between 1996-98. The mating performance of genetic sexing males fed complete adult diet as compared with sugar only feeding, did not clearly prove superiority of any of the treatments.

The study of copulation site (1998, Table 3) concluded that the preferred site by both type matings is the underside of the leaf. There were some differences on mating site preference between June (higher temperatures) and September (lower temperatures). In June the preferred mating site by both type matings was the lower canopy while in September, matings moved higher in the canopy, the phenomenon been more striking with the genetic sexing type matings. As to tree sector and although differences were not significant, in June both type matings appeared to concentrate in the north and west of the tree canopy, the phenomenon

TABLE 1. MATING PERFORMANCE OF GAMMA-STERILIZED, MASS-REARED T (Y;5) 1-61/95 GENETIC SEXING MALES (Ls) WHEN MIXED WITH WILD FLIES FROM SOUR ORANGES, LOQUATS OR FIGS (W), IN A SERIES OF ORANGE-TREE FIELD CAGE STUDIES FROM JUNE-OCTOBER 1996.

Experiment dates[a]	Temp. range (°C)	Insect combinations tested W:W:Ls	Total no. of medflies per field cage	Total no. of matings observed	Type of matings % Ls × W O[b]	Type of matings % Ls × W E	Chi-square test χ²	Chi-square test df	Chi-square test P
June 18 (4)	17-30	1:1:9	220	23	4	90	192.56	3	<0.001
July 5 (2)	17-36	1:1:9	220	9	33	90	32.67	3	<0.001
July 25 (2)	18-34	1:1:1	60	21	0	50	21.00	3	<0.001
August 1 (3)	21-34	1:1:3	100	37	3	75	103.25	3	<0.001
August 8 (3)	23-35	1:1:3	100	27	30	75	35.29	3	<0.001
October 10 (3)	16-25	1:1:3	100	18	22	75	26.80	3	<0.001
October 17 (3)	17-29	1:1:3	100	17	24	75	24.56	3	<0.001
October 24 (3)[c]	10-18	1:1:3	100	1	0	75	—	—	—

[a]In parenthesis is the duration of the experiment in days.
[b]O = observed, E = expected % matings based on insect ratios. Except Oct.24 when no statistical comparison was made because only 1 mating was recorded, in all other experiments observed matings were found significantly fewer from expected at P = 0.001 (χ² test).
[c]Due to cold, rainy weather only one mating (between wild flies) was observed.
[d]Upon mixing, wild flies were 6-13 days old in the different experiments, while genetic sexing males were 3-5 days old or older, except the first 2 days of the June 18 experiment when they were 1-3 days old. In each experiment 2 field cages were used, each with the indicated total flies and insect ratios. The total number of matings recorded are for both cages of each experiment. All flies were fed complete diet except Experiments Oct.10 and Oct.17 in which half of sterilized males were fed only sugar, with no substantial effect observed on their mating performance.

TABLE 2. MATING PERFORMANCE OF NON-STERILIZED, MASS-REARED T (Y;5) 1-61/95 GENETIC SEXING MALES (LN) WHEN MIXED WITH WILD FLIES FROM SOUR ORANGES OR FIGS (W), IN A SERIES OF ORANGE-TREE FIELD CAGE STUDIES ORGANIZED IN JUNE AND SEPTEMBER 1998.

Experiment dates[a]		Temp. range (°C)	Insect combinations tested W:W:Ln[b]	Total no. of medflies per field cage	Total no. of matings observed	Type of matings% Ln × W		Chi-square test		
						O[c]	E	χ^2	df	P
June	18/21 (3)	22-35	1:1:1 (CD)	100	22	72.7	50	4.60	3	$P < 0.25$
June	18/21 (3)	22-35	1:1:3 (CD)	100	10	40.0	75	6.53	3	$P < 0.10$
June	18/21 (3)	22-35	1:1:3 (S)	100	19	57.9	75	2.97	3	$P < 0.25$
September	22/25 (3)	16-29	1:1:1 (CD)	100	42	30.9	50	11.06	3	$P < 0.025$
September	22/25 (3)	16-29	1:1:3 (CD)	100	42	59.5	75	9.08	3	$P < 0.05$
September	22/25 (3)	16-29	1:1:3 (S)	100	47	48.9	75	23.69	3	$P < 0.001$

[a]Two successive experiments were organized in June and another two in September at the indicated starting dates. In parenthesis is the duration of each experiment in days.

[b]CD: laboratory males fed on complete diet, S: laboratory males fed on sugar only.

[c]O = observed, E = expected % matings based on insect ratios. Except June 1:1:1 (CD) and 1:1:3 (S) when no significant differences were detected in all other experiments observed matings were significantly different from expected (chi-square test). Within the O column when 1:1:1(CD) was compared with 1:1:3 (CD) the difference was found significant at $P = 0.10$ in both June and September; when 1:1:3 (CD) was compared with 1:1:3(S) the difference in June was found significant at $P = 0.05$ while in September the difference was not significant (t-test).

[d]Upon mixing, wild flies were 8-12 days old in the different experiments, while genetic sexing males were 4-6 days old. In each experiment 3 field cages were used, each with the indicated total flies and insect ratios. The total number of matings recorded is for the two successive experiments of each month and for all 3-days of the specific cage experiment. In the experiments of June, high mortality of both wild and mass-reared flies occurred due to pesticide application near the experimental area.

TABLE 3. PERCENT OF TOTAL MEDFLY COPULATIONS OBSERVED ON THE DIFFERENT ORANGE TREE CANOPY SITES IN 1998. CAGED-TREE EXPERIMENTS WITH WILD FLIES (W) FROM SOUR ORANGES OR FIGS MIXED WITH NON-IR-RADIATED GENETIC SEXING MALES OF STRAIN T (Y;5) 1-61/95 (LN) AT GENERATIONS 33-36.

Tree-canopy site	June 1998		September 1998	
	W × W	Ln × W	W × W	Ln × W
Leaf surface				
Top	0.0 a	3.3 a	5.6 a	1.6 a
Bottom	85.7 b	83.3 b	88.7 c	90.2 c
Other sites (fruit, tree trunk, cage screen and floor)	14.3 a	13.4 a	5.6 a	8.1 a
Height				
High	5.3 a	3.4 a	14.9 ab	23.7 bc
Middle	15.8 a	10.3 a	40.3 c	47.5 c
Low	78.9 bc	86.2 c	44.8 c	28.8 bc
Tree sector				
West	30.0	35.7	20.9	11.5
South	10.0	3.6	35.8	36.1
East	10.0	3.6	13.4	24.6
North	40.0	50.0	7.5	8.2
Center	10.0	7.1	22.4	19.7

[a]The above data are based on 181 matings recorded in total. Data of different sex ratio and adult feeding treatments were pooled together because no substantial differences were observed. In the columns and the rows means followed by different letter are significantly different: $P < 0.001$, F = 9.033, df 11, 24 (Leaf surface) or $P < 0.05$, F = 2.182, df 11,24 (Height). No significant difference was found in Tree sector: $P < 0.166$, F=1.433,df 19,40 (Tukey's test).
[b]Upon mixing, virgin wild flies or genetic sexing males were 8-12 or 4-6 days old, respectively. Experiments were organized in June and September with 2 three-day replicates of 3 field cages each time. One hundred total flies per field cage were always used at W:W:Ln 1:1:3 or 1:1:1 sex ratios, the first sex ratio tested with Ln flies fed either complete or sugar only diet and W flies fed always complete diet. In June environmental conditions were 25-35°C, 32-48% RH and 2000-15000 Lux, while in September the conditions were 16-29°C, 36-78% RH and 600-12500 Lux, respectively.

been again more intense with the genetic sexing male matings. In September, wild type matings concentrated primarily in south, west and center while genetic sexing type matings concentrated in south, east and center of tree canopy. It is interesting to note that in June about half of total both-type matings concentrated in the cooler northern sector of the tree, while in September only 8% of matings preferred this part of the tree canopy.

In conclusion, genetic sexing sterilized males were found much inferior than their wild counterparts in mating performance under field cage conditions. Nevertheless they performed sexual activity mostly on the same canopy sites as the wild flies. Further research is needed, especially to elucidate the effect of protein feeding before releasing on the survival and mating effectiveness of sterile males.

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WHY DO CALLING MEDFLIES (DIPTERA: TEPHRITIDAE) CLUSTER? ASSESSING THE EMPIRICAL EVIDENCE FOR MODELS OF MEDFLY LEK EVOLUTION

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ABSTRACT

Recent years have seen a proliferation both in theoretical approaches to understanding lek evolution and in empirical work on the lek mating system in the Mediterranean fruit fly. This paper aims to integrate these two lines of research and to identify practical insights of relevance to those engaged in medfly research. We begin by considering the definition of a medfly lek and recognize the existence of male aggregations at two distinct spatial scales: entire trees, or groups of trees within a given habitat; and small areas (ca 30 cm diameter) within the canopy of a given tree. After summarizing the assumptions and predictions of the main candidate models for lek evolution (predation avoidance, hotspot, hotshot and female preferences) we review empirical evidence from previous and ongoing research that is relevant to medfly lek evolution at both spatial scales. Far from being attributable to a single cause, we conclude that the evolution and selective maintenance of lekking behavior in the medfly can be attributed to a complex mosaic of interacting ecological pressures. We recognize that much more empirical work is needed to resolve outstanding questions on medfly lek evolution, and highlight potential benefits from the interaction between pure and applied lines of research on medfly mating behavior.

Key Words: medfly, *Ceratitis capitata*, lek, model, evolution

RESUMEN

En años recientes se ha visto una proliferación tanto en aproximaciones teóricas para entender la evolución del comportamiento en la selección del lugar de apareamiento y en trabajos empíricos sobre los sistemas de selección de los lugares de apareamiento en la mosca del Mediterráneo. Este trabajo está dirigido hacia la integración de estas dos líneas investigativas y hacia la determinación de información practica y de relevancia para las personas envueltas en la investigación de la mosca del Mediterráneo. Comenzamos por considerar la definición de el método de selección del lugar de apareamiento de la mosca del mediterráneo y en reconocer la existencia de grupos de machos en dos escalas espaciales diferentes: árboles completos, o grupos de árboles dentro de un hábitat dado; y pequeñas áreas (30 cm de diámetro) dentro del área foliar de un árbol determinado. Después de resumir las suposiciones y predicciones del candidato como modelo principal para la evolución del comportamiento en la selección del lugar de apareamiento (evitar depredación, punto clave, personaje y preferencias de la hembra) se analizó la evidencia empírica proveniente de investigaciones anteriores y aún en proceso, que son relevantes para la evolución del comportamiento en la selección del lugar de apareamiento de la mosca del Mediterráneo en ambas escalas espaciales. Lejos de poderse atribuir a una sola causa, concluimos que la evolución y mantenimiento selectivo del comportamiento en la selección del lugar de apareamiento de la mosca del Mediterráneo puede ser atribuido a un complejo mosaico de presiones interactivamente ecológicas. Reconocemos que es necesario mucho más trabajo empírico para contestar las sobresalientes preguntas en cuanto a la evolución del comportamiento en la selección del lugar de apareamiento de la mosca del Mediterráneo, y para destacar los beneficios potenciales de la interacción entre líneas puras y aplicadas de investigación en el comportamiento de apareamiento de la mosca del Mediterráneo.

WHAT IS A MEDFLY LEK?

Animals from a wide range of taxa, with a diverse array of life-histories, are classified as having a lek mating system (Hoglund & Alatalo 1995). Although the term 'lek' has been used by ornithologists for over a century, only recently have attempts been made to give it a rigorous ecological definition (see Hoglund & Alatalo 1995). Bradbury (1977, 1981, 1985) suggested four crite-

ria: 1) the absence of male parental contribution; 2) most mating occurs at a specific site(s) where males aggregate and display; 3) the display sites contain no significant resources required by females; 4) females have the ability to choose a mate when visiting the display site. However, in light of numerous exceptions and ambiguities, Hoglund & Alatalo (1995) advocated a more relaxed definition based simply on the presence of aggregations of males and the absence of pair bonding and paternal care. This definition, "aggregated male display that females attend primarily for the purpose of fertilization", nearly reverts to that previously proposed by Emlen & Oring: "communal display area where males congregate for the sole purpose of attracting and courting females and to which females come for mating". Under this classification, more than 240 species scattered across the animal kingdom have so far been reported to exhibit a lek mating system (Hoglund & Alatalo 1995).

One of these species is the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). Although the details of its mating behavior are today of great economic significance, this only came about with the development of the Sterile Insect Technique (SIT) in the 1960s. Numerous studies on medfly mating ensued, but it was not until the first systematic study of sexual behavior under semi-natural conditions was undertaken by Prokopy & Hendrichs (1979) that the existence of lekking as the major component of the mating system was recognized. Since that time, there have been two distinct operational definitions of medfly leks used in the literature. Prokopy & Hendrichs (1979) originally defined medfly leks as "3-6 males grouped within ca. 30 cm of one another, each on a separate leaf". Hendrichs & Hendrichs (1990) more or less followed this classification, as did Field & Yuval (unpublished data), having independently arrived at the same definition based on field observations. However, Whittier et al. (1992), followed by Shelly et al. (1994) and Shelly & Whittier (1995), instead defined a lek as an entire tree, because they usually found only one aggregation formed per tree and males moved readily between leaves, making it difficult to delimit the boundaries of an aggregation.

Before proceeding with a discussion of lek evolution in medflies, it is important to clarify exactly what is meant by the term 'lek' as applied to medflies. We note that both existing definitions have merits and flaws and, in the interests of gaining ecological insight, we advocate the retention of both pending further investigations. We thus propose the recognition of medfly leks at two distinct spatial scales: "small-scale leks" as defined by Prokopy & Hendrichs and "large-scale leks" as defined by Whittier et al. (1992).

With respect to small-scale leks, our own experience has independently supported the original conclusion of Prokopy & Hendrichs (1979) that aggregations of males in the tree canopy within a diameter of ca 30 cm are indeed functional components of the mating system. However, we should note that confirming the objectivity of this working definition will require studies that quantify the spatial distribution of calling males within and among trees, and demonstrate that males are more clustered at this spatial level than would be expected by chance. Nevertheless, a crucial aspect of lek mating systems, and one that makes them so interesting in terms of sexual selection, is that behavioral interactions such as dominance contests, competitive courtship and mate comparisons take place within them, and can heavily influence male reproductive success. Although in medflies communication over longer distances, particularly by chemical means, are likely to be of importance in male mating interactions, much of the behavioral detail that determines the course of sexual selection in lekking species can occur *only* when males are in close proximity. The intensive study of small-scale medfly aggregations is therefore vital to our understanding of sexual selection in medflies and the continued use of this tentative definition is therefore amply justified.

One advantage of the large-scale lek definition is that it is easier to verify quantitatively and indeed is already supported by empirical evidence. Whittier et al. (1992), for example, showed that a small subset of available trees accounted for a large majority of recorded incidences of male calling. Unpublished observations in Israel have suggested the existence of a similar phenomenon (R. K. & B. Y., unpublished data). Such large-scale aggregations differ considerably from, for example, classic avian leks, in which females enter a discrete male aggregation within which both male interactions and relative display efforts are readily observable. Male medflies within a tree, or group of trees, are relatively much more dispersed, separated and obscured from one another and from females by foliage and thus cannot interact or be observed to anywhere near the same extent as males in classic leks. However, it is conceivable that behavioral dynamics could at least resemble a classic lek. Male distribution throughout the tree could be influenced by agonistic interactions, and females could enter a tree and move about it comparing males in an analogous fashion to a female visiting a small group of males on adjacent leaves. This is especially so if, as Whittier et al. (1992) report, males can be somewhat clustered within a single tree canopy at certain times of day. Although the existence and generality of large-scale leks, like their small-scale counterparts, require further quantitative confirmation, present evidence suggests the existence of a distinct pattern of aggregation that must be investigated if we are to achieve a fuller understanding of medfly sexual behavior.

At present, therefore, we can recognize two distinct spatial scales of medfly aggregation. Our aim in this paper is to investigate the evolutionary causes of both, using existing theoretical models and emerging empirical evidence. We first enumerate the candidate models, then present the relevant evidence from previous and ongoing medfly research. In light of this, we identify critical gaps in our understanding of medfly lek evolution and suggest some practical steps that can be undertaken to fill them. We conclude with the prediction that pursuing the path toward such an understanding may bring many insights of benefit to programs aimed at managing medfly populations.

CANDIDATE MODELS FOR LEK EVOLUTION

The main hypotheses that have been put forward to explain lek evolution (see Hoglund & Alatalo 1995) are: i) predation risk; ii) hotspots; iii) hotshots; iv) female preferences. Others, such as the "passive attraction", "information-sharing" and "black-hole" models, are either not applicable to the biology of medflies, or are otherwise of dubious importance, and will not be considered here.

Hotspots. If resources used by females are patchily distributed, or female home-ranges overlap, then by displaying at certain sites (hotspots) in the habitat through which females are constrained to pass when traveling, males may obtain an increased probability of female visitation.

Hotshots. If males differ in quality and certain males are highly attractive to females (hotshots), lower quality males may increase their mating probability by clustering around the hotshots.

Female Preferences. Females may exhibit preferences for mating in male aggregations due to benefits from: a) predator deflection; b) opportunity to compare males; c) increased average quality of males. Alternatively, such a preference could evolve as an arbitrary Fisherian trait (Andersson 1994).

Predation Risk. If males incur a risk of predation due to sexual display, and the *per capita* risk decreases with increasing group size, then selection will favor aggregations.

It should be emphasized that although each of these models generates distinct empirical predictions, they are not mutually exclusive possibilities for the evolution of lekking, and any combination of mechanisms could be operating in a given system. Indeed, some recent attempts at modeling lek evolution have recognized the value of combining elements of several of these models. Below we summarize the main theoretical features of such models and discuss how they can be used to understand lek evolution. As there are no formal mathematical models of the influence of predation on lek evolution, we will concentrate on the hotspot, hotshot and female preference hypotheses.

Before doing so, it is necessary to introduce a critical concept in the theory surrounding lek evolution, *viz.*, the mating skew. This parameter can be calculated in many different ways (Kokko & Lindstrom 1997, Kokko et al. 1998), but essentially measures the degree to which matings are non-randomly distributed among males in a lek. The interest in mating skew stems from the common empirical observation that matings in leks are monopolized by one or a few high-ranking males. Given that such differences exist, theoreticians have attempted to explain how leks could evolve, in spite of the fact that low-ranking males seemingly have so little to gain by joining.

Hotspots and Hotshots

Widemo & Owens (1995) proposed that the answer to this question lies in increased levels of aggression and disturbance on the lek as group size increases, and a lesser ability of the high-ranking males to monopolize matings. This lowers the mating skew, and leads to the prediction that low-ranking males will have larger optimal lek sizes than high-ranking males. Thus leks represent the interests of low-ranking rather than high-ranking males, the latter being trapped into staying in the lek due to constraints imposed by habitat limitation or the hotspot effect. Data from the ruff, *Philomachus pugnax*, in which low-ranking males actively increase the lek size by soliciting other males to join, support this conclusion.

However, Hernandez et al. (1999) recently demonstrated that the situation is more complicated than this model suggests. Further to the interaction between lek size and mating skew, it is essential to know how relative competitive differences (RCDs) change as lek size increases. It is not automatic that competitive differences among males will decrease as more males join the lek. It is conceivable that even if the mating skew decreases with increasing lek size as expected, RCDs could actually *increase*, so that the increasing lek size favors the interests of *high*-ranking males rather than low-ranking ones. The analysis of Widemo & Owens therefore represents a special case, which may well be applicable to the ruff, but is unlikely to be general.

Both of these models simultaneously incorporate aspects of the hotspot and hotshot hypotheses. First, they assume that males are constrained in the locations they can choose for display (the essence of the hotspot hypothesis), and hence they do not abandon the lek even if it grows in size beyond what is optimal for them as individuals. Second, they assume that large differences in male quality exist, and, at least for some combinations of RCD, mating skew and lek size, that the lower-ranking males are benefiting from the attractiveness of the high-ranking males (the central idea of the hotshot hypothesis).

Female Preferences

The evolution of leks by female preferences has been modeled separately, using a game-theoretic approach (Kokko 1997). Here females assess males by comparison both within and between leks, subject to two constraints: within leks, they are not able to assess the best male perfectly; and among leks, travel costs mean they are unable to visit and assess all available aggregations. From the female side, the optimal strategy can be deduced with a straightforward mathematical calculation: when choosing among leks, they should prefer the larger ones, because the average male quality is higher; and within leks, they should choose the male they assess as being top-ranking, no matter what their probability of making an error. However, from the male side, the same problem remains, *viz*. why should low-ranking males join? One obvious answer is the imperfection of female choice, which gives low-ranking males at least a small chance of being chosen by mistake. But female travel costs are also important; if costs are low enough so that females can sample a large number of leks (recall they will prefer the larger ones), then it pays males to join large leks, which will receive the most female visits. Conversely, if travel costs are high, optimal aggregation sizes will remain low and lek evolution is constrained.

Empirical Evidence Concerning Lek Evolution in Medflies

Any narrative for lek evolution in medflies must begin by accounting for why mating does not occur on the host fruit, as it does in many temperate fruit flies. The accepted explanation, propounded by Prokopy (1980) and Burk (1981), invokes several ecological factors (multivoltinism, polyphagy and high predation risk) that in combination make it impossible or at least unprofitable for males to monopolize resources important to females. The enormous host range and multivoltine life cycle of *C. capitata* certainly qualify it to fit this model. Furthermore, the reduction of oviposition to a short time-window in the afternoon, added to the high levels of predation on females observed on fruit (Papaj et al. 1989, Hendrichs et al. 1991, Hendrichs et al. 1994, Hendrichs & Hendrichs 1998), offer strong support for the role of predation. The secondary mating tactic seen in *C. capitata*, of males guarding fruit and attempting to copulate females without courtship, can be seen as an evolutionary relic in a mating system initially resource-based but driven away from resources over evolutionary time by the combined effects of the above-mentioned ecological pressures. Alternatively, it could be an alternative mating strategy used by low-ranking males.

Large-Scale Leks

Having accounted for the evolutionary shift in medflies away from mating on host fruit, it remains to address the question of why sexually active males might aggregate at the first spatial level, that of the tree, or group of trees, within the habitat. The first thing to recognize is that although mating away from the vulnerability of host fruit would have reduced predation risk, it by no means would have eliminated it. Predators such as yellowjacket wasps, *Vespula germanica* (F.) (Hymenoptera: Vespidae), which use a combination of visual and olfactory cues to hunt amongst tree foliage for calling males and resting or copulating flies of both sexes (Hendrichs et al. 1994, Hendrichs & Hendrichs 1998), would continue to pose a substantial risk.

Predation. There are two evolutionary routes by which predation could lead to male aggregation in certain trees. The first is by providing a selective pressure for males to display from the most protected sites available in the habitat, *viz*. trees with a dense canopy structure that would impede the movement of larger predators and provide a variety of refuges and escape routes. This view has been supported by observations made by Hendrichs & Hendrichs (1990) in Egypt, and an analysis by Shelly & Whittier (1995) of lek distribution in Hawaii, showing that leks were clustered in the trees of largest volume, which also seemed (qualitatively) to possess the greatest horizontal foliage density. Nevertheless, it remains for this hypothesis to be explicitly tested. The second route is by males gaining a decreased per-capita risk of mortality by displaying with other males. This can occur either because the per-capita attractiveness to a predator of an aggregation decreases with aggregation size or due to the benefits of group vigilance. As yet there are no data addressing per-capita predator attraction at the large-scale lek level. However, we can deduce that the benefits of vigilance are unlikely to apply on a scale as large as an entire tree. A single wasp attack in one part of the canopy would only alert males in the immediate vicinity and not elsewhere in the tree, unless attacked males produce a specifically designed signal, e.g. an alarm pheromone.

Hotspot. The hotspot hypothesis is also an appealing explanation for the formation of large-scale leks. By choosing to display in fruiting host trees, males could maximize their chances of encountering females, which must visit these sites for feeding and oviposition. Some evidence from the field supports the hotspot interpretation, by showing that male sexual activity is indeed concentrated on fruiting host trees. Hendrichs & Hendrichs (1990, unpublished data cited therein) found most male sexual activity occurred on fruiting citrus. Hendrichs et al. (1991) found that most leks and matings occurred in foliage of the pri-

mary host (orange), where females went to oviposit after visiting leks. Most recently, Kaspi & Yuval (1999b) have produced several lines of experimental support from a field-cage study. They showed that wild males preferred to display on trees containing real fruit and preferred trees containing a combination of visual and olfactory fruit stimuli over trees with either stimulus alone or no host stimulus. However, although these studies are consistent with the hotspot hypothesis, they are also consistent with the hypothesis that males choose such trees because of their accessibility to food sources. As lekking is energetically costly (Warburg & Yuval 1997, Yuval et al. 1998) this may be an important consideration in male lek site selection.

Evidence inconsistent with the hotspot hypothesis has also been obtained. In a field study in Hawaii, Shelly & Whittier (1995) found lek sites did not correspond with female oviposition or feeding sites, as males settled preferentially on certain persimmon trees that did not have ripe fruit at the time. Similar observations have been made in Israel, where in a mixed orchard, 2 particular pitanga trees have been found to harbor a disproportionate number of calling males. Although the pitanga trees were fruiting at the time, there were also an abundance of suitable citrus, guava (and other pitanga) trees immediately adjacent that harbored few or no males (R. K. & B. Y., unpublished data). These observations strongly suggest that if a hotspot effect is involved, it is by no means the only factor driving male aggregation into large-scale leks.

Many authors have noted the possibility that in addition to offering protection from predation, trees with a dense canopy structure could offer a more suitable microclimate for male calling activity (Arita & Kaneshiro 1985, 1989, Hendrichs & Hendrichs 1990, Whittier et al. 1992, Shelly & Whittier 1995). Although there are as yet no quantitative data on microclimate variations *among* trees of different size and canopy structure, Kaspi & Yuval (1999b) have recently shown that *within* trees, male positioning throughout the day tracks changes in temperature, relative humidity, light intensity and the azimuth of the sun. In particular, males exhibited a marked preference for leaves with microclimate characteristics closely matching those in full shade. Should males actively choose among trees according to such preferences (or be beholden to the decisions of females concerning where to mate), this would provide a complementary explanation to predation avoidance for male clustering in certain trees that have unusually dense canopies.

Hotshot and Female Preferences. With respect to the hotshot and female preference models, we argue that these mechanisms are not viable explanations for clustering at the whole-tree level or at best constitute only weak evolutionary forces. If low-quality males are to gain any benefit from settling near high-quality males, they must be in sufficiently close proximity that visiting females will detect them and possibly mistake them for the high-quality male to which they were initially attracted. Obviously the best position to achieve this is as close to the high-quality male as possible, so the evolutionary effect of the hotshot mechanism would be the formation of groups of males that are small and tightly clustered at a scale well below the level of an entire tree. Similarly, if female preferences for large leks are driving male aggregation, males that settle adjacent to other male(s) will be favored over males that take up position in the same tree, but in a relatively distant part of the canopy. For these reasons we feel the hotshot and female preference hypotheses are more applicable to small-scale male aggregations and delay discussion of them until the following section.

Summary. In overview, a combination of ecological factors appear to have acted to drive medfly mating activity firstly away from host fruit and secondly to become concentrated in certain trees within the habitat. Evidence suggests the action of a composite predation and hotspot effect, modulated by microclimate preferences in habitat selection. There are compelling reasons why hotshot and female preference effects would not be potent evolutionary forces at this spatial level. However, this may not be the case when considering multiple spatially distinct leks within the canopies of particular trees, as we discuss in the next section.

Small-Scale Leks

Predation. As noted above, the need to avoid predation may have driven the mating system away from host fruit, but it is an unlikely explanation for the clustering of males into large-scale leks. However, at the level of small-scale leks, it becomes plausible by the following possible mechanisms. If the number of predator attacks does not increase in proportion to group size, animals in the center of an aggregation are better protected than those on the periphery (Hamilton 1971), and/or efficiency in predator detection is increased by sharing the task of vigilance with conspecifics (Pulliam 1973). If any of these operate, then predation pressure can act as a driving force in lek evolution.

Hendrichs & Hendrichs (1994, 1998) have shown that medfly males lekking at a site in Chios, *Greece* are at substantial risk from yellowjacket wasp attacks, especially when calling, an activity that appears to significantly reduce their vigilance. Most recently they have found strong support for the evolutionary impact of predation by showing that the per-capita number of wasp attacks decreases with lek size, making larger ag-

gregations safer for calling males (M. & J. Hendrichs, Insect Pest Control Section, IAEA, Vienna, unpublished data). As the experiment was conducted with artificial leks of caged males, there are no data on the number of *successful* attacks as a function of group size. However, accounting for the benefits of group vigilance, we can infer males in large leks would be even better protected than these data imply. It is also relevant to consider the effect of disruption of mate attraction activities by predator attacks. Each time a predator attacks, all males in the lek are forced to disperse and resume searching for a suitable site, temporarily excluding them from mate-attraction activities. The lower per-capita level of predation in larger leks would reduce interruptions and increase the amount of time available for mate attraction. Provided the per-capita rate of female visitation remained comparable with that seen in smaller aggregations (see below), calling in larger groups would result in fitness benefits.

Hotspot. The prospects for hotspot effects to be operating at this spatial level seem remote. A common criticism of the hotspot model is that areas of high female density (driven by resource patchiness) might not be localized enough to account for the tightness of male clustering (Westcott 1994). This would certainly seem to be the case with small-scale medfly leks.

Hotshot. The hotshot hypothesis also deserves attention. It assumes that variations in male quality occur, such that high quality males are able to monopolize matings and less attractive males then benefit by clustering around them. Evidence certainly points to the necessary variation in male quality existing, as laboratory studies have repeatedly found a non-random distribution of matings among males. Although this has not yet been expressed in terms of mating skew (sensu Kokko & Lindstrom 1997), or been verified in a natural setting, it is nevertheless suggestive of variations in male quality that females are able to detect and use as a basis for discrimination during mate choice. However, for the hotshot mechanism to work in medflies, the variations in quality must be apparent to both females and males during long-range mate attraction, i.e. pheromone calling. This is because the majority of small-scale medfly leks disperse without ever receiving a female visit (S.A.F., unpublished data), making it impossible for males to identify hotshots on the basis of the number of matings they achieve, as can occur in other species (see Beehler & Foster 1988). Hotshots could conceivably be identified by the quality of a given male's pheromone, as an indicator of the likelihood that he will attract a female. This could potentially be achieved by first using the presence of pheromone to locate leks and then the relative proportions of certain components in the pheromone blend to discriminate among males of different quality.

However, current evidence on the question of whether males are attracted to the pheromone emissions of other males at all, let alone whether they discriminate among males of different quality, is inconclusive. It had previously been widely assumed that males are attracted to leks by the male pheromone and that the powerful attraction of males to chemicals such as trimedlure was due to its mimicking key components of the male pheromone (Burk & Calkins 1983, Sivinski & Calkins 1986). However, chemical analyses have since refuted this hypothesis (Millar 1995, cited in Eberhard, 1999), leaving the mechanism by which male clustering occurs an open question. Evidence against male attraction to pheromones has come recently from Shelly (UH, Hawaii, unpublished data), who found very low attraction of released males to artificial leks formed in the field using caged calling males. However, positive evidence has also been obtained by Kaspi & Yuval (1999a), who showed that when selecting a calling site in a field cage, males were more likely to settle on a tree from which caged males were emitting pheromone, than on a control tree containing only caged dead males. However, we must take into account differences in methodology. The artificial leks formed by Kaspi & Yuval (1999a) contained 30 males and they released 100 males into a confined space, whereas Shelly (UH, Hawaii, unpublished data) used only 12 males in the leks and released 300 males into the wild. The former experiment thus tipped the balance in favor of detecting an effect, however weak. Nevertheless, the result raises the possibility that males do indeed use the male pheromone as a cue in lek site selection, an issue worthy of further study.

It remains to be directly studied whether males and females are able to distinguish among calling males of different quality using variation in pheromone blends. However, while two studies have indicated that male mating success depends on the quantity of pheromone produced (Whittier et al. 1994, Shelly, UH, Hawaii, unpublished data), there is as yet no evidence favoring an effect of pheromone quality. Shelly found that males with high previous mating success attracted more females than males with low success, but apparently only because they spent more time calling. It also seems clear that the difference in mating success frequently found between sterile and wild flies is not due to females discriminating against them on the basis of pheromone composition; females arrive at leks of both types of males with equal frequency and appear to discriminate only during close-range courtship (Calkins et al. 1994, Shelly et al. 1994, Shelly & Whittier 1996, Shelly 1999. Nevertheless, this could mean simply that the sterilization and/or mass-rearing process affects courtship rather than pheromone quality. The hypothesis that there exist variations in pheromone quality among males remains worthy of investigation.

A prediction from the hotshot hypothesis would be the presence of "satellite" males, who join leks but engage in little or no calling, but nevertheless attempt to court females that are attracted to the lek by the pheromone emissions of others. Observations by several authors suggest that this may be occurring. Shelly et al. (1994) observed non-calling males in leks in the field. In our observations in the field in Israel, a substantial proportion of males in scan samples of leks were not calling. Further, in a field-cage study (S. A. F. & B. Y., unpublished data), non-calling males in a lek sometimes switched immediately to directed wing-fanning when a female flew past. This may have been simple opportunism but could also represent an evolved strategy. The occurrence of variation in calling activity among males with a uniform rearing history and environment (Shelly, UH, Hawaii, unpublished data) also hints that investigating the possibility of a genetic influence on individual calling strategies may be worthwhile.

Female Preferences. To demonstrate that female preferences are a significant factor in lek evolution, it must be shown that more females arrive per male as lek size increases, causing an increase in per capita male mating success. Three studies have examined this question. Shelly (UH, Hawaii, unpublished data) found the increase in female visitation rate to artificial leks in the field remained in constant proportion to the number of calling males, whether the males had a successful or unsuccessful mating record in the laboratory. Similarly, Kaspi & Yuval (unpublished data) found that the per-capita rate of female visits to artificial leks in a field cage remained constant over the range of lek sizes likely to be encountered in nature (2-8). As such a response can be accounted for by passive attraction of females to a larger olfactory signal, we may be tempted to conclude that there is no evidence for a large lek preference in female medflies. However, the third study, by M. & J. Hendrichs (IPCS, IAEA, Vienna, unpublished data), adds a new twist by simultaneously considering the effect of predation. They found that when wasp predators were allowed to attack leks, the females preferred the smallest of four lek sizes offered. However, when predation was removed, not only did females prefer larger leks, they preferred them out of proportion to the number of males, so that the per-capita rate of female arrival did indeed increase with lek size. This suggests that females in fact do actively prefer large leks, but are constrained in their preference due to the risk of predation. Future studies of female preferences must take into account the possible interaction of this factor with predation.

SUMMARY

To summarize, the evolutionary causes of small-scale leks within trees appear to be distinct from those favoring aggregation into large-scale leks. Hotspot effects, apparently one of the key factors driving large-scale lek formation, is of little relevance for small-scale lek evolution, whereas protection from predation, hotshot effects and female preferences could all be important. Although plausible, no direct empirical evidence is yet available to support the hotspot hypothesis. The evidence is perhaps strongest for the effect of predation acting to increase aggregation size. Evidence also exists for female preference for large aggregations, although this appears to be modulated by predation risk, pointing to an intriguing conflict of interest between the sexes with respect to lek size.

FUTURE EXPERIMENTAL PRIORITIES AND PRACTICAL IMPLICATIONS

Despite the passage of two decades since the medfly was identified as a lek-mating organism, our understanding of the evolutionary forces driving lek formation in this species remains very rudimentary. It seems this is not due to a lack of research effort into medfly mating behavior but rather because such research has rarely been framed with evolutionary issues specifically in mind or been used to explicitly test evolutionary hypotheses. Understandably, the emphasis has been on experiments designed to bring immediate improvements in the quality of mass-reared males for SIT or to understand the proximate mechanisms determining successful courtships. However, basic and applied research questions are never mutually exclusive, and we believe that attempting to place medfly mating behavior in an explicit evolutionary setting can yield practical benefits, just as practically-oriented research has already begun to benefit our evolutionary understanding, by providing critical empirical data for testing theoretical models. Most importantly, an evolutionary framework can facilitate ongoing critical evaluation of empirical studies, aiding the resolution of experimental ambiguities and contradictions, and speeding the conversion of an otherwise haphazard accumulation of results into an orderly, coherent body of knowledge.

Although the studies cited above have provided a useful start towards understanding medfly lek evolution, many questions and uncertainties remain. Below we identify lines of research that appear to hold promise for teasing apart the influences of various ecological factors and suggest some experiments critical to resolving outstanding issues.

Firstly, most of the tentative conclusions concerning lek evolution drawn above rely on evidence from only one or a few studies. Inevitable variations among studies in the origin, rearing and handling of insects, experimental methodology and analysis make it likely that even the most

carefully designed and executed studies can produce ambiguous or inconclusive results. In medflies, the potential for discrepancies between studies is perhaps compounded by the fact that this insect has relatively recently colonized a variety of new habitats worldwide, and different populations have possibly undergone (or are undergoing) adaptation to local conditions. It therefore may be necessary to accumulate numerous tests of the same hypothesis under differing ecological conditions before robust conclusions emerge. Ideally, consensus on the evolutionary influence of an ecological factor should be quantitatively assessed after taking multiple similar studies into account (Arnqvist & Wooster 1995). Far from being a redundant exercise, repeating experiments performed by other researchers on different medfly populations may highlight critical ecological factors that influence mating behavior and thus prove essential to the task of understanding its evolution.

At the large-scale lek level, it would be useful to repeat studies like that of Shelly & Whittier (1995), which applied a multivariate analysis to confirm which factors determine the favored sites for male display within the habitat. Ideally, such studies would be longitudinal in nature and would track the location of calling males in relation to seasonal patterns of host availability within seasons and fluctuations in these patterns among seasons. Combined data from different medfly populations, climates, and habitats would provide a rich database with which to identify universal factors determining large-scale lek locations. Should the pattern of males being clumped into large-scale leks be borne out by such studies the next task would be to confirm that this is due to a hotspot effect rather than males simply choosing to lek near nutritional resources. This would require tracking of female distributions (feeding sites, oviposition sites and movements among them) and the demonstration of a correlation between female distribution and the male calling sites.

Concerning specific hypotheses for lek evolution, the effect of predation is one area that has received intense empirical attention recently (Hendrichs et al. 1994, Hendrichs & Hendrichs 1998) and should be pursued further. To clarify whether the formation of large-scale leks in trees with large volumes and dense canopies is in part a response to predation, it would be desirable to measure predation rates in trees of different size and canopy structure. At the level of small-scale leks, the information already obtained on attack rates at different lek sizes could be supplemented by data on the rate of *successful* attacks at different lek sizes, which would indicate whether individuals displaying in larger groups benefit from increased vigilance. As this would entail measurement of predation rates on naturally displaying males, the data would be difficult to obtain

but would be well worthwhile as they would clinch the argument for the role of predation in driving male aggregation.

Further investigations of the hotshot hypothesis should focus on testing whether the proximate mechanism by which males aggregate is indeed by cueing on the pheromone emissions of other males, and if so, which are the active components in the blend. A positive result would add credibility to the hypothesis that low-quality males are attracted to leks occupied by hotshot males that can be distinguished by the quality of their pheromone. This hypothesis could then be investigated in an experiment similar to that performed by Shelly (UH, Hawaii, unpublished data), who tested attraction of males and females to calling males of low and high mating ability, with the difference that the calling males should be classified with respect to their ability to attract conspecifics on the basis of their pheromone alone. Classifying them by their mating success leaves open the possibility that the high-mating males were successful not due to quality of their pheromone, but due to the efficacy of their courtship, which is of no evolutionary consequence for lek formation. If both females and males concurred in their choice of males, the pheromone blends of attractive and unattractive males could then be compared and the physiological basis for the hotshot effect identified.

One of the most interesting research directions to pursue is the putative interaction between predation and female preferences for large leks in determining optimal lek size in small-scale leks. While large leks appear to increase male survival by decreasing per-capita attack rate, this may not be true for females, at least judging by the behavior observed by J. & M. Hendrichs (IPCS, IAEA, Vienna, unpublished data). This may be explained by the fact that female vigilance toward predators is at its lowest when receiving courtship, so females may be particularly sensitive to the risk of predation when visiting a lek. This not only brings female interests into conflict with that of males with respect to predation, but also sets up a counterbalance to any preference females might have for mating in larger leks due to the opportunity to compare males. Thus it could be an important selective force acting to set an upper boundary to lek sizes. Further experiments measuring female arrival rates while manipulating lek sizes and predator attack rates would be extremely valuable.

Although the research directions outlined above are primarily directed towards answering a theoretical question in behavioral ecology, there also exist potential avenues whereby such research could make a positive contribution to improving the efficacy of medfly control programs. Tests of the hotspot hypothesis will provide us with detailed knowledge about preferred lek locations in various habitats and climates, enabling

more judicious selection of sites for monitoring traps and thus improving the ability to detect and respond to infestations. Successful pursuit of the hotshot hypothesis could provide the key to understanding variations in male attractiveness, and be a step forward in improving the quality and mating competitiveness of mass-produced males *vis a vis* their wild counterparts. If specific components of the male pheromone could be identified as responsible for a hotshot effect, they could also be used to manufacture more effective chemical baits and lures. It is our hope that the future will see more interaction between applied empirical research on medfly mating behavior and theoretical modeling of lek evolution, to mutual profit.

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CHARACTER-SPECIFIC HOMEOSTASIS DOMINATES FLUCTUATING ASYMMETRIES IN THE MEDFLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Fluctuating asymmetry (FA) indicate random variation in size of bilaterally-produced traits, which occurs during development, and hence measures the degree of developmental instability. Whole-individual homeostasis has been assumed responsible for the negative correlation that has often been found between FA of many sexually selected traits and their size. We show that, theoretically, character-specific homeostasis can provide an equally convincing explanation for this correlation. Furthermore, we tested these two hypotheses for (1) a sexually dimorphic character and (2) sexually monomorphic characters of the male Mediterranean fruit fly, *Ceratitis capitata*, by manipulating density during larval development. Our results clearly support character-specific homeostasis.

Key Words: Fluctuating asymmetry, developmental homeostasis, sexual selection medfly, *Ceratitis capitata*

RESUMEN

La fluctuación asimétrica (FA) indica variación aleatoria en el tamaño de los rasgos producidos bilateralmente, los cuales ocurren durante el desarrollo, y por lo tanto mide el grado de inestabilidad del desarrollo. La homeóstasis de individuos completos ha sido asumida como la responsable de la correlación negativa que frecuentemente ha sido encontrada entre FA de muchos rasgos sexualmente seleccionados y de su tamaño. Se demuestra teóricamente, que la homeóstasis carácter-especifica puede proporcionar una explicación igualmente convincente para esta correlación. Además, se probaron estas dos hipótesis con respecto a 1) un carácter sexualmente dimórfico y 2) caracteres sexualmente mono-mórficos de los machos de la mosca del Mediterráneo, *Ceratitis capitata*, por medio de la manipulación de la densidad durante el desarrollo larval. Nuestros resultados claramente apoyan la homeóstasis carácter-específica.

Minute variations in growth rate that occur randomly during development accumulate and result in small variations in the final size of organs. These variations (or developmental 'noise') can be quantified by comparing the final sizes of a character that is produced more than once under the influence of the same genome, at the same developmental stage, while being exposed to the same environmental conditions. Because development repeats itself twice during growth of bilaterally produced organs, bilateral asymmetry can be used to give an estimate of developmental instability (Soule 1982, Soule & Cuzin-Roudy 1982, Leary & Allendorf 1989, Parsons 1990). This type of asymmetry is known as 'fluctuating asymmetry' (FA), and is usually measured as the absolute difference between the size values of left and right, divided by their mean (to control for the effect of size). A number of studies have shown that FA increases following environmental and genetic stress. The most common environmental factors that are known to increase FA are extreme temperatures, starvation and chemical stresses (Soule 1982, Parsons 1990). New mutations, in-

creased homozygosity, recombination and directional selection are documented genetic factors that increase FA (Parsons 1990, Moller & Pomiankowski 1993b).

Recently, the study of FA has gained much interest because of its role in sexual selection. Females of different species, including the scorpionfly, *Panorpa japonica* (Thornhill 1992), barn swallow, *Hirundo rustica* (Moller 1992) and human (Thornhill & Gangestad 1992, Thornhill et al. 1995) have been shown to mate more readily with symmetric males. This, and other potential uses and implications of FA (e.g., Leary & Allendorf 1989, Moller & Pomiankowski 1993b), requires a good understanding of the mechanisms that affect FA.

There is a general agreement that symmetry is enhanced by genetic modifiers that increase the harmony of development (Leary & Allendorf 1989). Other parallel terms that have been used are 'developmental homeostasis', 'developmental stability' (Soule & Cuzin-Roudy 1982), and 'intrinsic genetic coadaptation' (Leary & Allendorf 1989), all of which imply a state of evolutionary

equilibrium. More explicitly, these terms reflect the general view that evolutionary transitions away from symmetry are followed by an evolution of modifiers that restore symmetry (McKenzie & Klarke 1988, Leary & Allendorf 1989). The assumed nature of this stability, however, is debatable. Some studies argue that developmental homeostasis evolves separately for each of the individuals' characters, and hence that FA measures the degree of character-specific developmental stability. Others contend that homeostasis is holistic, affecting the development of whole organisms. Attempts to discriminate between these two hypotheses by examining within-individual correlations between FA of several characteristics have provided conflicting evidence (Soule & Cuzin-Roudy 1982, Watson & Thornhill 1994).

An important reason for the conflicting evidence shown by these correlations is well explained by Whitlock (1996). He points out that for each character in a bilaterally symmetric individual there exist only two samples of the variance of developmental instability. Because each of the measurements of FA is an unreliable statistical measure of variations within the individual (assuming common cause for different characters), then even if developmental instabilities of different characters are correlated, the within-individuals correlations of FA are often too coarse to show them. Therefore, to decrease the role of statistical errors due to random sampling, other types of correlations are required, ones that can use repetitions across individuals, or that involve only one bilaterally symmetric character. A decision about these other measures requires a better understanding of the possible mechanisms that induce fluctuating asymmetry.

The known list of causes of asymmetry suggests that there might be more than one mechanism involved. The distinction between character-specific and whole-individual homeostasis is often suggestive about these mechanisms. Whole-organism homeostasis is known to be a result of certain external factors, such as extreme temperatures, which results in instability in the whole organism. Whole-organism homeostasis is often assumed to be, however, also a function of overall individual 'quality' that may vary between individuals due to heritable or non-heritable differences in the ability and/or opportunity to capitalize and use resources. Heritable differences that may exist between individuals are expected to be diminished by natural selection, until a state of equilibrium between natural selection and the source of the heritable variations is reached (Falconer 1981). This point of equilibrium, characterized by an improved overall adaptedness, should be reflected by reduced FA.

In contrast, character-specific homeostasis is frequently assumed to be a result of heritable or non-heritable variations in size of each character.

Extreme character sizes reduce harmony of development (and, as a result, increase FA) because they are usually not accompanied by corresponding changes in developmental organization, or in supportive tissues such as muscle, blood vessels or nerve cells, required for the enhancement of developmental stability. For an evolving character, under a regime of directional selection, this might be just a temporary stage followed by the evolution of these other modifications that will, eventually, increase developmental homeostasis of the character in its final state.

Investigations of FA, in relation to studies of sexual selection, have consistently interpreted results assuming that developmental homeostasis is a whole-organism characteristic, rather than character-specific. This assumption nicely links sexual selection theory with the empirical data that frequently (but not always) show a negative correlation between FA of sexually dimorphic traits and their size, a pattern that is not found in other traits (Moller & Höglund 1991, Moller & Pomiankowski 1993a). This pattern is readily explained by assuming that (i) FA is determined by a whole-organism developmental homeostasis, (ii) this homeostasis is correlated with the individual's overall quality or some of its components, and (iii) the sexual trait is a handicap. The term 'handicap' refers to a signal, such as a sexual ornament, which is costly to produce or maintain such that a male's optimal trait size (i.e., his investment in advertising) is correlated with his physical condition. As a result, better males produce more expensive sexual ornaments (Zahavi 1975, 1987, Nur & Hasson 1984, Pomiankowski 1988, Grafen 1990). Theoretical studies show that at evolutionary equilibrium, the male residual quality (after developing its sexual ornament) is expected to remain higher for high quality males, despite the fact that absolute investment in sexual ornaments by these males is greater (Nur & Hasson 1984, Grafen 1990, Iwasa et al. 1991). Hence, if developmental stability is caused by whole-organism mechanisms that depend on the male quality during development, larger sexual ornaments should be also more symmetric.

Despite the consistency of the negative correlation between the size of a sexual trait and FA with the whole-organism stability hypothesis, it can only provide support for this hypothesis if character-specific mechanisms cannot explain this pattern. Here we show that this is not the case. In the next section we show that size-dependent character-specific homeostasis provides an equally reasonable explanation for the negative correlation between FA of many sexual traits and their size. In the rest of the paper we present a simple experiment that tests these two hypotheses. Its results strongly support the character-specific mechanism.

SIZE-DEPENDENT HOMEOSTASIS

The single character size-dependent homeo-
stasis hypothesis suggests that individuals in a
population evolve modifiers that improve homeo-
stasis for a certain character size. Characters
that develop to match this size will be the least
asymmetric. Hence, we can call this size the least
asymmetrical size (LAS). Sizes that deviate from
LAS (either bigger or smaller) develop to be asym-
metric. They are expected to be more asymmetric
the more they deviate from LAS (Soule 1982,
Soule & Cuzin-Roudy 1982). Therefore, traits that
undergo stabilizing selection should normally
produce a more or less symmetric V- or U-shaped
distribution of FA around the mean size of the
trait (the form of the distribution depending on
whether the effect of deviation from LAS on FA is
linear or exponential, respectively).

If there are no other size-dependent effects,
such as directional natural or sexual selection,
modifiers are most strongly favored if they im-
prove developmental stability (hence, symmetry)
of the size that they most frequently encounter

(the term 'modifiers' has been used vaguely in the
literature of FA; here, we use this term to simply
refer to a set of heritable traits that affect a char-
acter's developmental stability, as a function of its
size, although other size-dependent responses are
also possible). Consequently, the most common
size is also expected to be the LAS. However, the
actual shape of the relationship between FA and
size, its strength (i.e., its variance around the least
squared regression line), and the value of LAS it-
self should heavily depend on the distribution of
trait sizes in the population. For a normal distri-
bution of sizes of a trait undergoing stabilizing se-
lection, LAS is expected to correspond with the
population's mean, mode and median, all of which
fall onto a single point. A high standard deviation
creates a weak selection on modifiers that improve
homeostasis of any particular size, including the
mean, because they are less likely to encounter
that size. In contrast, selection pressure on modi-
fiers that improve developmental stability of the
LAS when standard deviation of sizes is small,
must be stronger, hence also more canalizing
(Fig. 1). Furthermore, a small standard deviation
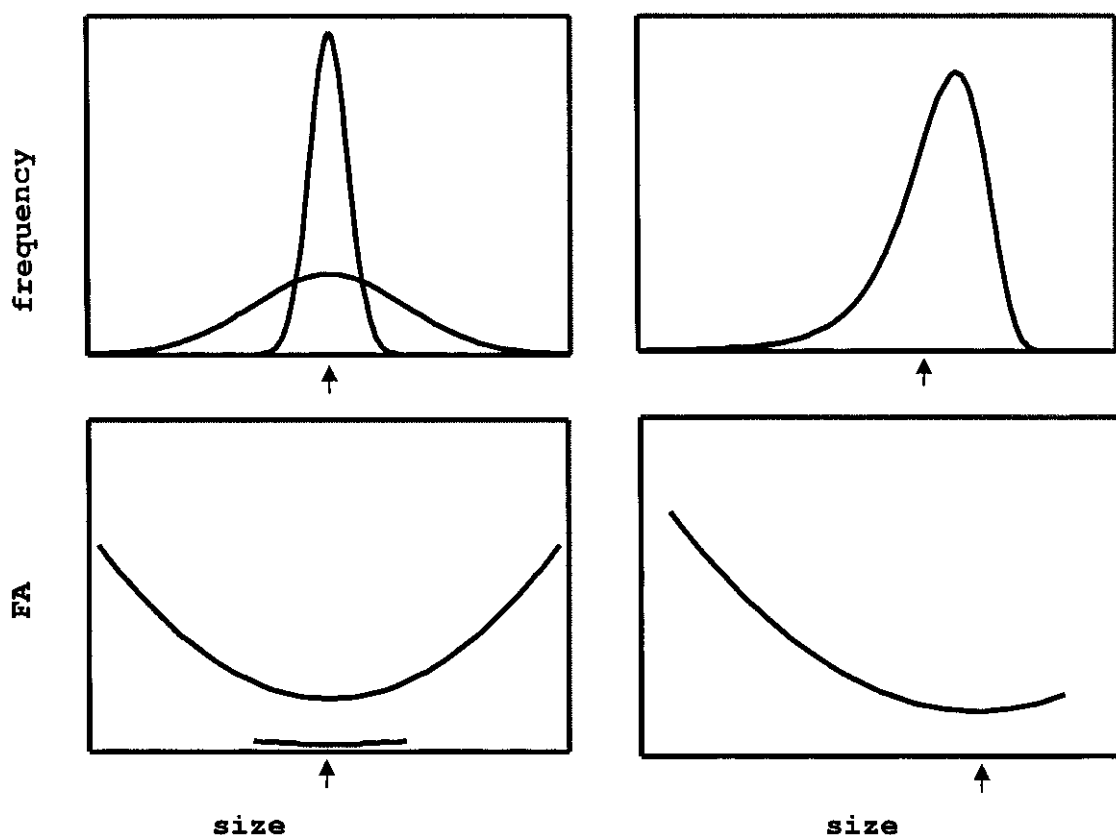
Fig. 1. The expected relationships between FA (bottom) and size frequency distribution (top), assuming charac-
ter-specific developmental homeostasis. The pointers show average size (top), and the least symmetric size (bottom)
for two normal curves that differ in their standard deviation (left) and a skewed curve to the left (right).

of sizes should also lead to a small average FA, because the population is distributed tighter around the LAS. Empirical data show that non-sexual traits have either a U-shape distribution of FA with relation to size, or no pattern at all (Moller & Pomiankowski 1993a), which may reflect different standard deviations of sizes around the mean.

What if distribution of sizes is not normal, but skewed to one side? This question is of particular interest here, because many sexual ornaments may be at equilibrium between a strong directional sexual selection toward larger sizes, and a weak opposing force induced by biased mutations (Iwasa et al. 1991, Pomiankowski et al. 1991, Pomiankowski & Moller 1995). These opposing tendencies are likely to result in a skewed distribution of sizes to the left. In such cases, LAS is expected to be on the right side of the range of the sizes frequency distribution rather than in its center (Fig. 1). As a result, the relationship between FA and size should not be U shaped with symmetrical arms, but a short arm on the right, and a long arm on the left. As before, strong deviations from LAS in either direction should produce, on average, poor symmetry. Because this distribution results in a relatively weak selection on modifiers for developmental stability at any particular size, the tightness of the correlation between FA and size described by Figure 1 (bottom right) should be relatively weak. Consequently, a distribution of sizes that is strongly skewed to the left is likely to produce an apparent negative linear regression line between FA and size.

Furthermore, if reproduction is higher for individuals whose trait size is larger, which is the case for many sexually selected traits, then LAS is expected to shift even further to the right, because modifiers for large sizes are frequently associated with a higher than average reproduction rate. This will reduce further the right arm of the already asymmetric U shaped correlation between the trait's FA and size, and improve their apparent negative linear correlation.

PREDICTIONS

The findings of negative correlations between FA and size in sexual traits may, therefore, be consistent with both the whole-organism and the character-specific homeostasis hypotheses. Both hypotheses suggest that greater deviations from LAS should produce, on average, greater degrees of asymmetry at the individual level, and greater average values of FA, at the population level. However, there are still some different predictions that can be made to distinguish between the effects of these two hypotheses:

A. The primary factor that affects FA under the regime of a character-specific homeostasis, is the shape of the frequency distribution of trait sizes (weighted by the expected fitness benefits to the genetic modifiers of each particular size). Also, LAS is always expected to be intermediate between minimum and maximum sizes. If the distribution of sizes is highly skewed to the left, LAS is expected to be near the maximum, hence may be difficult to be empirically distinguished from it.

In contrast, the primary factor that affects developmental stability and FA at equilibrium within a whole-organism homeostasis regime, is the individual's adaptedness and the capacity to gain, store and use resources. For a sexually selected handicap at equilibrium, LAS is expected to correspond with the maximum trait size.

B. For character-specific homeostasis, stabilizing selection is expected to maintain LAS in the neighborhood of the mean. In response to directional selection, where mean size is driven away from equilibrium, the evolution of modifiers should lag behind, leaving LAS on one side of the mean, opposite to the direction of selection (e.g., for a trait that increases its size, LAS is expected to be smaller than the mean). The correlation between FA and size is therefore expected to be positive if the character is in a process of increasing in size (Moller & Pomiankowski 1993b, making, here, an implicit assumption of character-specific developmental stability).

Whole-organism homeostasis is expected to respond similarly in the case of stabilizing selection, but to result in an opposite correlation in the case of directional selection (negative for increasing size, positive in the case of decreasing size), because individuals who carry the novel extreme size have higher overall adaptedness.

METHODS

Following the predictions in section (A) above, this study is based on the specific prediction that if the ability to gain and use resources (reflecting developmental stress) can be better estimated by a parameter other than the particular trait's size, asymmetry of the trait should nevertheless be best correlated with its size if homeostasis is character-specific, but better correlated with that other parameter of quality if homeostasis is a whole-organism trait.

We used for our experiment a laboratory stock of the Mediterranean fruit fly (medfly) *Ceratitis capitata*, which has been raised by one of us (YR) under constant conditions since 1964. The adult population has been kept constant at about 3000 individuals, which is normally large enough to avoid frequent incidences of genetic drift (Roughgarden 1979). Temperature has been kept approximately constant at 25°C. Hence, relative to wild flies, this population has been probably kept under relatively narrow temporal fluctuations in selective pressure with regard to developmental conditions.

To manipulate developmental stress we raised larvae by collecting eggs of the same age, and put-

ting them, at the same day, on 3 gm food at densities of 10 (8 repetitions), 20 (4), 40 (3) and 80 (3). We estimate typical densities of larvae in the culture stock to vary between 50 to a 100 per 3 gm food. The average egg hatch was 0.87 (SD = 0.08), and the proportion of larvae that pupated (from the hatched eggs) was 0.92 (SD = 0.05). Although the output numbers were a little smaller than the input numbers, for clarity we continue to refer to the original densities. Within this range of densities, the effect of density on survivorship was insignificant (we avoided a higher density, of 160 eggs per 3 gm, which, according to a preliminary test, reduced survivorship considerably). We collected the flies on the day of emergence, kept them alive and unfed overnight to let them fully expand their organs, and then put them individually in plastic tubes and stored them in a freezer, until we measured them. To avoid temporal biases in measurements, we sampled male flies taken from different densities at random until we got about 20 males of densities 10 and 20 (for which there were fewer males). We then continued to sample males of these two densities alone until we measured 22 males of each. Our final sample sizes were 22 (for density 10), 22 (20), 40 (40) and 29 (80). Some measurements were not recorded in all individuals because of physical damage to the adult flies. To minimize unconscious bias, measurements were made by a research assistant who had no knowledge of the motivation of this research or the significance of our marks on the plastic tubes in which the frozen flies were stored.

To determine the relationship between phenotypic quality and fluctuating asymmetry of traits that are not sexually dimorphic we measured head width, thorax width and length, and both wings width and length. For a sexually dimorphic trait we measured maximum width and length of the bilateral supra fronto orbital (SFO) bristle in males, which is the most modified sexually dimorphic organ in the medfly. On females this bristle is similar in shape and size to other bristles found on the head. On males, this bristle has an elongated stem and a modified wide spatula at the distal end, oriented forward (Fig. 2). Two recent studies suggest that the medfly SFO bristles are sexually selected character: Mendez et al. (unpublished) find indications that female medflies prefer intact males as opposed to males whose SFO bristles were removed, and Hunt et al. (1998) show that females prefer more symmetric SFO bristles (although not longer ones).

To measure the flies, we transferred their magnified video images from a dissecting microscope to a Power Macintosh, and used NIH Image for scaling and measurement. We measured both left and right of bilateral organs (bristles and wings), where the individual's means were computed as the average value of left and right. We removed the wings and bristles from the flies and put them
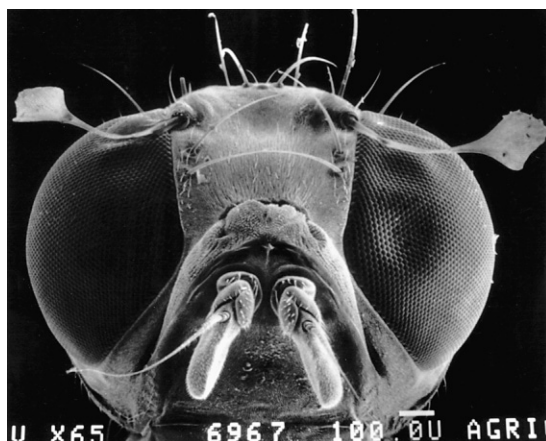

Fig. 2. The modified supra fronto orbital bristles on the head of a male Mediterranean fruit fly.

on a glass microscope slide, using a cover glass to flatten them before taking measurements. Because the bristles' stems usually broke during removal, we used measurements of the bristle's lengths that were made on the head. Although the bristle is slightly curved, our technique of keeping in focus its two distal ends proved, by comparing these measurements with those made for stems of bristles that remained intact (21), to be highly consistent. Size differences between wings and bristles had no effect on the relative degree of the measurement error because both were enlarged to about the same size before their image was digitized.

We calculated standardized values of FA of bilateral organs as the absolute value of left minus right, divided by their mean size. We standardized sizes by dividing each of them by the trait's mean size. This shifted the population mean of each trait to unity, enabling comparisons of distributions and standard deviations of traits of different sizes. For statistical analyses we used JMP, Version 3.1.5 for the PC (by SAS Institute, Inc.).

RESULTS

Larval density had a significant effect on all measured sizes (Fig. 3) but one (bristle width). The strongest effect was on thorax length, which we therefore use as our best indicator of overall ability to gain and use resources. Head and thorax widths gave lower Chi-square values (Kruskal-Wallis tests) than that of thorax length, and we omit them from the analyses. For most purposes we were interested in looking, in individuals taken from the same gene pool, at the effect of variations in trait size on FA, regardless of the source for these variations. This roughly represents a state where individuals of different, usually unknown developmental history, are collected
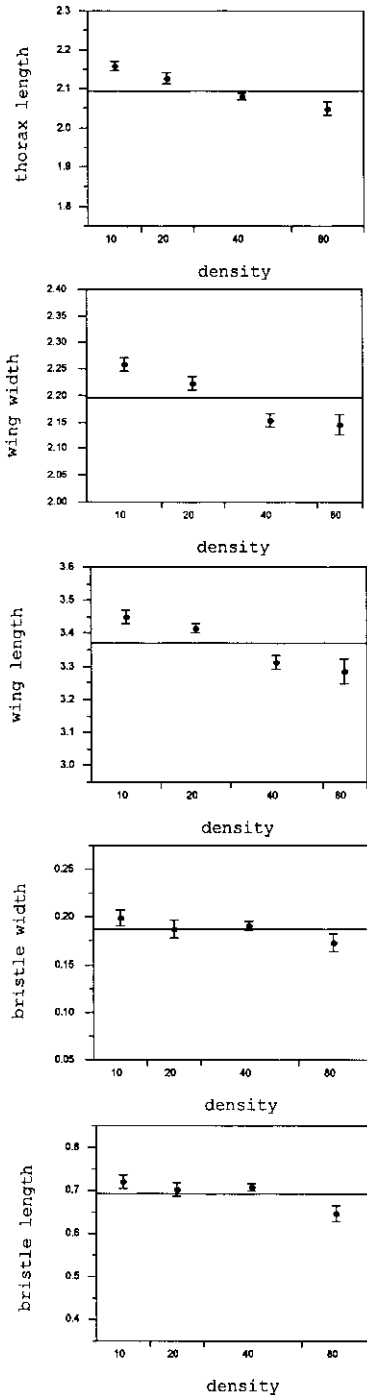
Fig. 3. The effect of larval density on adult size. Each figure shows all densities' mean (vertical line across), and the mean and standard error of each density. Distances between the horizontal ticks represent the relative sample size of each density. Statistical results (Kruskal-Wallis tests) from top down—thorax length: $O^2_{[3]} = 26.88$, $P < 0.001$; wing width: $O^2_{[3]} = 26.74$, $P < 0.001$; wing length: $O^2_{[3]} = 25.85$, $P < 0.001$; bristle width: $O^2_{[3]} = 3.30$, $P = 0.35$; bristle length: $O^2_{[3]} = 13.63$, $P = 0.0035$.

at random in nature. Unless stated otherwise, we therefore used pooled data.

We found tight relationships between traits' FA and size distributions (Fig. 4). FA of bristle width and length both showed significant negative correlations with their corresponding size (Table 1). They also showed a highly skewed distribution to the left, and high standard deviation. In contrast, FA of wing length showed no correlation with size, and FA of wing width showed a parabolic, U-shape correlation with size ($y = 2.021 - 3.966x + 1.955x^2$; $F_{[2,72]} = 7.83$, $P < 0.001$). Wing length was also the only measure with a distribution that was not statistically different from normality. In accordance with their much larger standard deviations, the average FA of the bristle's width and length was about an order of magnitude larger than that of the wing's width and length.

In contrast with the tight association between FA and size of characters, the relationships between FA and the best quality indicator, thorax length, were weak, and fluctuating asymmetries of the bristle's parameters, both width and length, were much less affected by thorax length than by the corresponding trait size (Table 1). Similarly, FA of the wing width, which produced a significant quadratic (U-shape) relation to wing width (previous paragraph), showed no such pattern when correlated against thorax length ($y = 0.653 - 1.303x + 0.662x^2$; $F_{[2,72]} = 0.702$, $P = 0.5$). We also estimated least asymmetric size (LAS) of bristle's width and length by using moving averages: we sorted the data by size of the corresponding trait (Fig. 5, top) and grouped data points in tenths according to their ranked size. This resulted in groups of ten individuals, from small to large, ranked as 1-10, 2-11, 3-12 and so on till the end of the list. For each group we computed average FA and estimated LAS as the average size of the least asymmetric group. The average FA of the estimated LAS was significantly lower than the average FA of the ten points of maximum bristle width, but not in bristle length. The moving averages technique also exposed, once again, the tight relationships between FA and trait size. When we repeated the same procedure by grouping sizes by thorax length rather than by bristles' width and length (Fig. 5, bottom), the continuous trend found in the first two figures (Fig. 5, top), was lost.

Furthermore, if symmetry develops in response to whole-organism homeostasis, and the latter is affected by the individual's (residual) quality, then symmetry should be a good indicator of quality. Because larval density affected phenotypic quality (as indicated by its effect on adult fly size), this enabled another test of the relationships between FA and quality. We found no effect of larval density on FA (bristle length: $O^2_{[3]} = 2.769$, $P = 0.42$; bristle width: $O^2_{[3]} = 1.607$, $P = 0.66$; wing width: $O^2_{[3]} = 3.1247$, $P = 0.373$; wing length: $O^2_{[3]} = 1.359$, $P = 0.72$; Kruskal-Wallis tests).
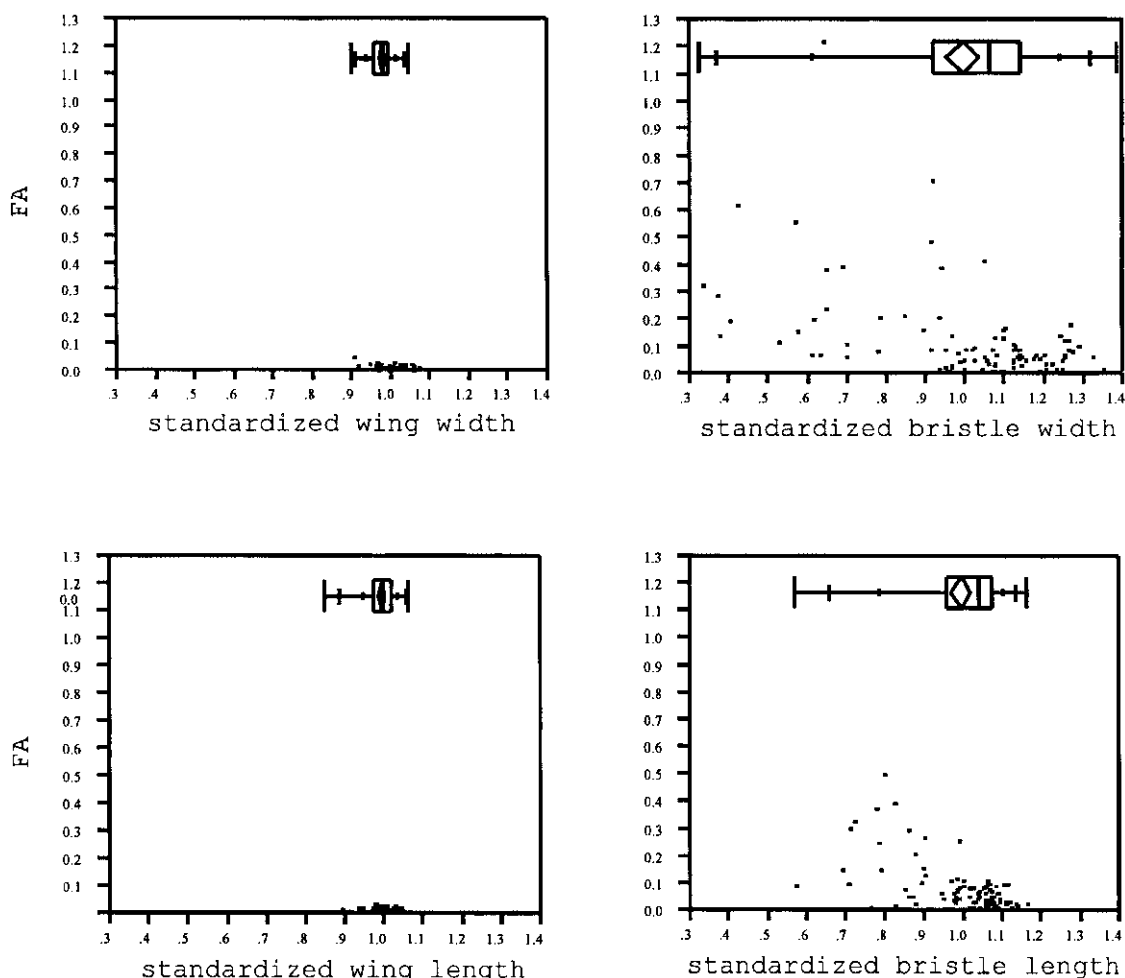
Fig. 4. The relationships between size frequency distributions and FA. Distributions are represented by quantile plots: each box shows the median as a vertical line across the middle, and the quartiles (25th and 75th percentiles) as its ends. The means diamond identifies the mean of the sample and the 95% confidence interval about the mean. Ticks represent 90%, 97.5%, 99.5% and maximum size from the box to the right, and 10%, 2.5%, 0.5% and minimum size, to the left.

Finally, only bristle's width and length measurements showed a positive correlation between their FA's ($N = 104$, $r_s = 0.2918$, $P = 0.0027$; Spearman rank correlation). All other pairs of measurements showed no correlation between their FA values (giving $-0.124 < r_s > 0.089$, and $0.31 < P > 0.77$).

DISCUSSION

Our study indicates that character-specific homeostasis plays a major role in determining the degree of FA in the medfly. This, however, should not undermine the role of whole-organism homeostasis, supported by studies that show within individual correlations in FA of different characters (Soule & Cuzin-Roudy 1992, Watson & Thornhill 1994). The current study, and the fact that some of

these previous studies support the whole-organism homeostasis, may suggests that both may be important, perhaps under different conditions, or for different organisms. Some sources of instability, for example, such as the effect of certain chemicals, or of extreme temperatures (Soule 1982, Parsons 1989) may directly affect instability of development of all traits, irrespective of their sizes, resulting in a true whole-organism effect on developmental homeostasis. However, then the source of instability affects some characteristics more than others, a greater tendency toward character-specific homeostasis should be detected. Alatalo et al. (1988) showed that sexually dimorphic characters have larger size variations than other characters. They did not look at the shapes of the size frequency distributions, but the

TABLE 1. ASSOCIATION OF BRISTLE WIDTH AND LENGTH'S FA WITH BRISTLE CORRESPONDING SIZE AND WITH THORAX LENGTH (USING SPEARMAN'S RANK CORRELATIONS).

	N	r_s	Probability
Bristle width's FA × bristle width	104	-0.4060	<0.0001
Bristle length's FA × bristle length	104	-0.5023	<0.0001
Bristle width's FA × thorax length	103	-0.2447	0.0127
Bristle length's FA × thorax length	103	-0.1120	0.2599

large size variations of sexually dimorphic characters may make their FA more vulnerable to character-specific effects (size variations) than to whole-organism homeostasis.

In the study that we present here, larval densities affected bristles' length, but did not produce corresponding changes in their FA. This result corresponds with other studies that show low heritability for FA, relative to that of quantitative morphological traits' sizes (Parsons 1990). This suggests that in our study other sources of variation in FA were stronger than the stress created by
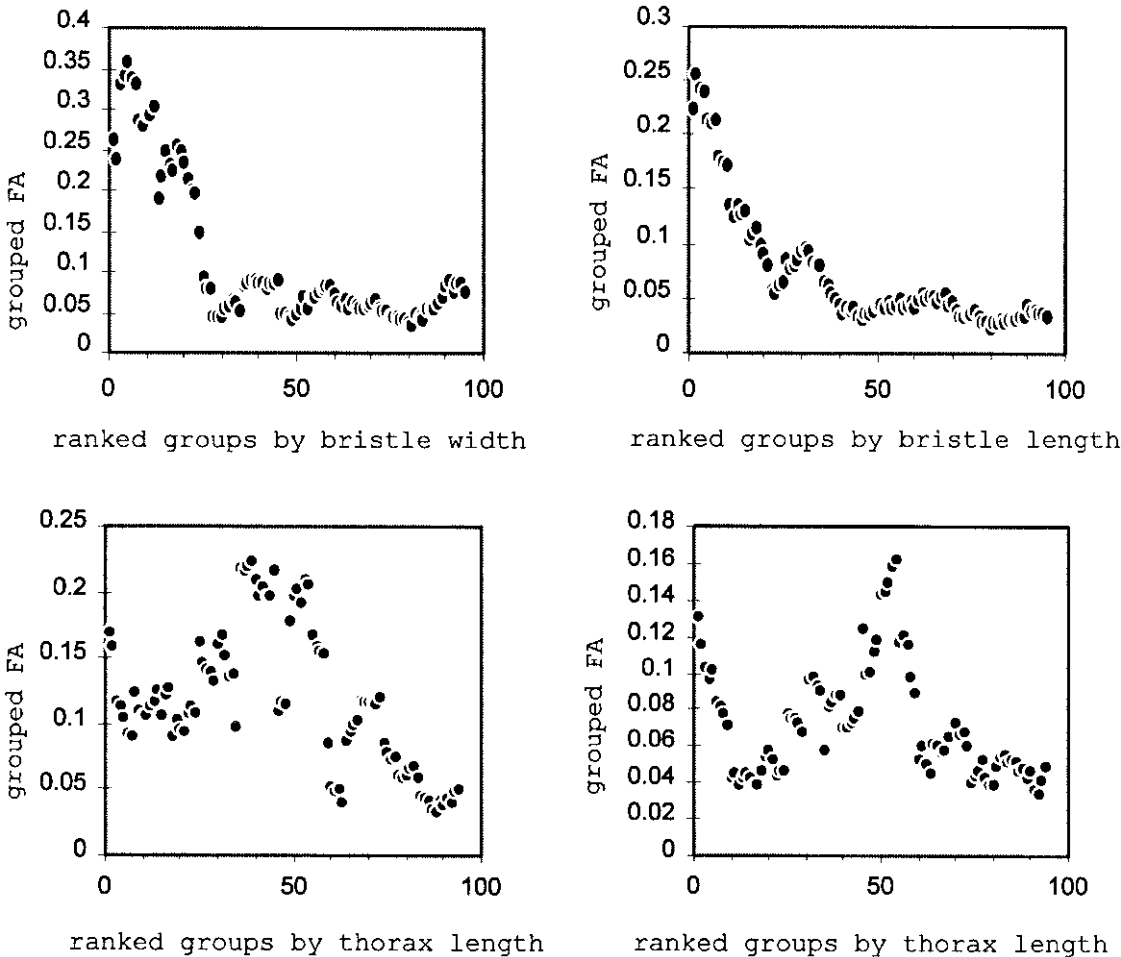
Fig. 5. The relationships between moving averages of FA and groups ranked by sizes. Each closed circle represents its minimal rank point (horizontal axis) and the group's average FA. For bristle width, minimum average FA is 0.0355, representing the rank order 81-90. Its corresponding average standard size is 0.224 mm. The minimum average FA is significantly lower than that of the top ten sizes (ranked 95-104), 0.0760 (one-tail t-test for unequal variances, $t_{[18]}$ = -2.35; P = 0.018). For bristle length, minimum average FA is 0.0242, rank order, 80-89 (N = 104), and corresponding average standardized size, 0.755 mm. This minimum average is not statistically different from the average FA of the top ten sizes (ranked 95-104), 0.0349 (one-tail t-test for equal variances, $t_{[18]}$ = -0.80; P = 0.216).

density alone. Here, variations in FA appear to be the consequence of the sensitivity of character-specific developmental homeostasis to size frequency distributions. The high variance of sizes of a trait (e.g., the highly skewed bristle length) should weaken selection on modifiers that form stability of each particular size. This increases the frequency of modifiers for sizes that are at some distance from LAS. Consequently, phenotypic changes in average size in response to density should have a relatively weak effect on average FA. Size-dependent character-specific homeostasis should, therefore, weaken the response of FA to environmental variables, especially when these variables induce changes in size that are well within the natural range of variations of the trait size (also considering fluctuations in time). Hence, FA of a trait should generally be less sensitive to stress than the trait size itself. This only strengthens Whitlock's (1996) argument for the low heritability of FA.

FA has been assumed to be a measure of overall quality in a variety of studies of sexual selection (Watson & Thornhill 1994). However, if it is instead strongly influenced by character-specific modifications and size frequency distributions, then FA should not be regarded as a direct measure of overall quality but, at best, as its approximation. When size frequency distribution of a sexual handicap is skewed then, despite the general correlation between symmetry and quality, the very best individuals (whose sexual characters are largest) should develop, on average, a greater asymmetry than males with somewhat smaller handicap (Figs. 1 and 5). In other words, LAS should not correspond with trait size of best individuals. Thus, although symmetry is generally informative and correlated with quality, within a certain range of sizes it becomes non-informative, maybe even misleading criterion for quality (depending on the degree of female selectivity, and on the nature of the trait's size frequency distribution).

Our results show that we need to re-evaluate the current use of FA. For example, the assumption that FA is determined by whole-organism homeostasis, underlies the suggestion that a negative correlation between FA and male ornament size indicate handicaps, and a lack of it points at Fisherian ornaments, which are not informative about any of the male "qualities" except for attractiveness to females (Moller & Pomiankowski 1993c). If, however, symmetry of sexually dimorphic characters is dominated by character-specific homeostasis, differences in correlations between traits' FA and size may only reflect different size frequency distributions. The correlation between characters' FA and size would nevertheless differentiate between handicaps and Fisherian sexual traits only if handicaps consistently show highly skewed distributions and Fisherian sexual traits do not. Hence, in or-

der to make this argument, it is essential to study also the frequency distribution of trait sizes.

The possibility that FA is less sensitive to environmental stress than traits' size poses another important question: why do female swallows, for example, use both tail symmetry and tail length as criteria of the male quality (Moller 1992)? This should only make sense in an adaptive manner if each provides a certain different additive component of information regarding the male quality (Hasson 2000). The character-specific homeostasis can provide an intriguing answer: while a well developed handicap advertises a male's superior phenotypic quality, the bilateral symmetry of a handicap may indicate, by presenting modifiers for its particular size, that the male is a descendant of a long line of similarly good phenotypes. Hence, while a handicap improves information about the male phenotypic quality, the handicap's symmetry indicates the probability that this phenotype is a product of "good genes".

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THE EFFECT OF SUPRA-FRONTO-ORBITAL (SFO) BRISTLE REMOVAL ON MALE MATING SUCCESS IN THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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Abstract

Here we present the findings of a laboratory study in which male Mediterranean fruit flies (medflies), *Ceratitis capitata*, (Wiedemann) had one or both supra-fronto-orbital (SFO) bristles artificially removed, for comparison with unoperated controls. All the flies were weighed and had their wings measured. The time at which a male began pheromone-calling was correlated with its weight, lighter males beginning calling earlier, but there was no effect of weight on mating success. Mated males had significantly longer wings than unmated males although there was no correlation with wing width. Although males missing both bristles were rejected more by females than those with one or two bristles, the loss of a single bristle had no effect on female response. The presence of bristles was not essential for successful mating. This study does not support the idea of females visually assessing males on the basis of their bristle symmetry.

Key Words: *Ceratitis capitata*, female choice, fluctuating asymmetry, medfly, sexual selection

Resumen

A continuación se presentan los descubrimientos de un estudio de laboratorio en el cual machos de la mosca del Mediterráneo, *Ceratitis capitata* (Wiedemann) presentaban una o ambas setas supra-fronto-orbitales (SFO) artificialmente removidas, para comparación con los controles no modificados. Todas las moscas fueron pesadas y sus alas fueron medidas. El tiempo en el cual un macho inició su llamado a través de una feromona se correlacionó con su peso, los machos más livianos iniciaron su llamado más temprano, pero no hubo ningún efecto por parte del peso en el suceso de apareamiento. Los machos apareados presentaban alas significativamente mas largas que los machos no apareados, sin embargo, no se presentó ninguna correlación con el ancho de las alas. Aunque los machos que carecían de ambas setas fueron mas rechazados por las hembras en comparación con aquellos que poseían una o las dos setas, la perdida de una sola de las setas no produjo ningún efecto en la respuesta de las hembras. La presencia de las setas no fue esencial para un apareamiento exitoso. Este estudio no apoya la idea de que las hembras analizan visualmente a los machos en base a la simetría de sus setas.

Mediterranean fruit flies (medflies), *Ceratitis capitata*, are sexually dimorphic, with males possessing features that are either less exaggerated in the female or absent. These include more brightly colored eyes, front legs with more numerous and longer hairs, a brighter white 'chin' or labrum (which in the female is a duller cream color), and a pair of supra-fronto-orbital (SFO) bristles which are elongated and modified to form a spatula shape at the tip (Féron 1962).

The medfly has a lek mating system (Prokopy & Hendrichs 1979, Arita & Kaneshiro 1985) in which males gather beneath neighboring leaves on a fruit tree and release a pheromone to attract females. They then perform a courtship ritual which involves more pheromone calling, wing fanning and wing buzzing, and head rocking (Féron 1962) before the male jumps onto the female and attempts to mate.

The female may reject the male at any stage up to and including the jump, simply by dropping away from the leaf. Male mating success varies greatly, with a small proportion of the males gaining most of the matings (Arita & Kaneshiro 1985, Whittier et al. 1992, 1994). It has been estimated that in the wild over 93% of all courtships end in rejection by the female (Whittier et al. 1994). Given these facts, there is considerable scope for both male competition and female choice to be taking place.

Hunt et al. (1998) carried out laboratory studies to investigate a possible role for the SFO bristles in the mating success of the male. Groups of 50 males and 50 females were allowed two hours to form pairs. Symmetrical males had higher mating success than asymmetrical males. The experiments were repeated in field cages in Guatemala and Crete using wild flies, and again

symmetrical males were found to be more successful (Hunt et al. in prep.).

When wild males emerging from oranges in Crete were housed in wooden (rather than plexiglass) cages they were sometimes observed to have lost one or both of their bristles. It was found that males missing both bristles were less likely to be accepted by females than males with one or two bristles (Hunt et al. in prep.).

Interestingly there was no significant reduction observed in the female acceptance rate of males when only one bristle was missing. It seemed therefore that this extreme form of asymmetry had no effect on a male's mating success, a conclusion which rested on the assumption that males that had lost a single bristle formed a random sample of all fitness types. It remained possible, however, that less fit males may have been more prone to accidental bristle loss. Here we report a repeat of the experiment under laboratory conditions, during which bristles were removed surgically from a random sample of flies.

Materials and Methods

A sample of flies collected from coffee beans in Guatemala and sent directly to Manchester as pupae, formed the basis of the laboratory colony used in the present investigation. This colony had been under laboratory conditions for approximately 50 generations and was reared according to the techniques described by Hunt et al. (1998).

Virgin adults were collected in the laboratory within 24 hours of eclosing. For both rearing and mating tests, the flies were maintained at $25 \pm 2°C$ and $68 \pm 4\%$ rh. The sexes were kept separately until the adults were seven days old. The day before an experiment, a group of nine flies was transferred by aspirator and held immobile in a mosquito net bag ($15 cm \times 30 cm$) and marked individually with a dot of paint on the thorax. While still in the bag the flies were placed under a binocular dissecting microscope and both SFO bristles were removed from three of the flies with a fine pair of curved forceps. Three others had one bristle removed and the remaining three were manipulated in a similar manner, although both bristles were left intact. The flies were then returned to their rearing cage.

For the mate trials we simulated the natural conditions of field cage trials by developing a smaller indoor version (50 cm height × 40 cm diameter) containing a small potted orange tree measuring approximately 45 cm in height. The cage was made of fine black mesh so the flies and their marks were easily visible though it. The experiments were begun 30 minutes after the lights were switched on. No food and water was provided during the test due to its short duration.

On the morning of the seventh day the nine males were released into the cage and allowed fifteen minutes to acclimatize before nine marked females were released. All interactions between the flies were noted including the time at which the males began pheromone calling, the time at which each courtship attempt took place and the outcome. Each experiment lasted for two hours and was replicated 20 times. The small number of flies being observed allowed accurate recordings to be made using paper and pencil. For this purpose event tables were used.

At the end of each experiment, the flies were removed from the cage and immobilized in the freezer for one minute before they were weighed, after which they were preserved in 70% ethanol. Both wings were dissected and fixed onto microscope slides under a cover-slip using glycerol gelatin (Sigma Diagnostics, St. Louis, USA). The length and width of the wings were measured under a binocular microscope using a graticule eyepiece.

Statistical Analysis

Mating success was defined as whether a male mated or not within the two hours of the experiment. The mean acceptance rate of a male by females was calculated by dividing the number of its successful copulations by the number of its courtship attempts (matings plus rejections). This index was designed to take into account the amount of 'effort' a male had to expend before being accepted or not by a female. It therefore excluded those males that made no courtship attempt. T-tests were used to determine if there was a difference in the lengths and widths of wings in the mated and unmated males, and between males that attempted pheromone calling or courtship and those that did not. Tests of association between wing dimensions and acceptance rates were calculated using Spearman's rank correlation. The association between the number of bristles and male mating success, pheromone calling behavior and courtship attempts was calculated using Chi-squared tests. Associations between the number of bristles possessed by a male and its mating success, the time at which it began pheromone calling and the time to acceptance were determined using Kruskal-Wallis tests. The associations between time to pheromone calling and time to acceptance with weight were determined using Spearman's rank correlations. Logistic regression was used to determine if there was a difference in the weight of mated and unmated males. ANOVA was used to test for any association between the number of bristles, male weight and male wing dimensions. All statistical tests were carried out using the statistical package SPSS.

Results

Out of a total of 180 male flies used in the experiments, 31 died during the trial or could not be used, leaving a final total of 149 males, out of which 86 (57.7%) mated. In the 20 replicates, the percentage of males mating ranged from 25 to 100%.

There was no difference in weight between males with 0, 1 or 2 bristles ($F_{2,146}$ = 1.294, P = 0.277). Nor was there any difference in mean wing length ($F_{2,146}$ = 0.048, P = 0.953) or mean wing width ($F_{2,146}$ = 0.234, P = 0.788). The mean wing dimensions and weight in the different bristle categories are listed in Table 1.

Male SFO Bristles

The numbers of males that mated in each bristle category are shown in Table 2. There was no association overall between mating success and the number of bristles (χ^2 = 1.601, df = 2, P = 0.449). Comparisons between 0, 1 and 2 bristles also showed no significant differences (*0,1* χ^2 = 0.344, df = 1, P = 0.558; *0,2* χ^2 = 1.593, df = 1, P = 0.207; *1,2* χ^2 = 0.334, df = 1, P = 0.563).

There was no overall association between acceptance rate and number of bristles (Kruskal-Wallis χ^2 = 5.64, df = 2, P = 0.06), although this value was close to significance. However significant differences were observed between the acceptance rate of males with no bristles and males with either one bristle (Mann-Whitney U = 345.5, P = 0.039) or two bristles (Mann-Whitney U = 446.0, P = 0.045) (see Fig. 1 and Table 1). No significant difference was found in the acceptance rate of flies with one versus two bristles (Mann-Whitney U = 629.0, P = 0.883).

The number of bristles possessed by a male was not associated with whether it pheromone called or not (χ^2 = 1.029, df = 2, P = 0.598) or whether or not it initiated a courtship (χ^2 = 0.757, df = 2, P = 0.685).

There was no significant difference in the time taken to begin pheromone calling between flies with 0, 1 or 2 bristles (Kruskal-Wallis χ^2 = 0.328, df = 2, P = 0.849), nor was there a significant difference in time taken to begin copulation (Kruskal-Wallis χ^2 = 0.077, df = 2, P = 0.962). See Table 1 for mean values in each bristle category.

Male Body Weight and Wing Dimensions

There was a significant positive correlation between wing length and width (r_s = 0.859, N = 149, P < 0.001), wing length and body weight (r_s = 0.651, N = 149, P < 0.001), and wing width and body weight (r_s = 0.657, N = 149, P < 0.001).

Mated males had significantly longer wings than unmated males (χ^2 = 7.251, df = 1, P = 0.007) (Fig. 2), although there was no difference in wing width (χ^2 = 2.415, df = 1, P = 0.120).

No association was found between male mating success and male body weight (χ^2 = 1.185, df = 1, P = 0.276).

Neither mean wing length (R_s = 0.099, N = 149, P = 0.321) nor mean wing width (R_s = 0.001, N = 149, P = 0.995) were associated with acceptance rate.

The weight of a male was not associated with its acceptance rate (R_s = -0.004, N = 102, P = 0.967).

Neither wing length nor wing width were associated with whether or not a male engaged in pheromone calling (length: t = 1.045, df = 147, P = 0.294, width: t = 0.306, df = 147, P = 0.760). There was also no difference in wing width between those males which initiated a courtship and those that did not (t = 1.665, df = 147, P = 0.098), but males which initiated a courtship had significantly longer wings than those which did not (t = 2.663, df = 147, P = 0.009).

The weight of a male was not associated with whether it pheromone called or not (t = 1.472, df = 147, P = 0.143) or whether or not it initiated a courtship (t = 0.262, df = 147, P = 0.793).

There was no significant association between time to begin pheromone calling and either wing length (R_s = 0.02, N = P = 0.835) or wing width (R_s = 0.045, N = P = 0.637). However there was a correlation with weight, the lighter males beginning pheromone calling earlier (R_s = 0.261, N = 111, P = 0.006), although the weight of a male was not correlated with the time it took to begin copulation (R_s = -0.079, N = 149, 86, P = 0.489).

DISCUSSION

Males with no supra-fronto-orbital bristles were less readily accepted by females than males with either one or two intact. This suggests that the SFO bristles play some role in either the ability of the male to perform an adequate courtship, or in the decision of a female to accept a male as

TABLE 1. THE NUMBERS (AND PERCENTAGES) OF MALES IN EACH BRISTLE CATEGORY THAT MATED AND THOSE THAT DID NOT.

	0 bristles	1 bristle	2 bristles
Pheromone called	35 (77.8%)	34 (69.4%)	42 (76.4%)
No pheromone calling	10 (22.2%)	15 (30.6%)	13 (23.6%)
Attempted courtship	30 (66.7%)	32 (65.3%)	40 (72.7%)
No attempt	15 (33.3%)	17 (34.7%)	15 (27.3%)
Mated	23 (51.1%)	28 (57.1%)	35 (63.6%)
Unmated	22 (48.9%)	21 (42.9%)	20 (36.4%)

TABLE 2. MEAN WEIGHT, WING LENGTH AND WIDTH, TIME TO PHEROMONE CALL (PC) AND ACCEPTANCE RATE OF MALES IN EACH BRISTLE CATEGORY.

	0 bristles (±se)	1 bristle (±se)	2 bristles (±se)
Mean weight (mg)	6.956 (0.172)	6.584 (0.154)	6.764 (0.152)
Mean wing length (mm)	4.322 (0.025)	4.327 (0.023)	4.332 (0.020)
Mean wing width (mm)	2.302 (0.011)	2.291 (0.013)	2.296 (0.010)
Mean time to pc (secs)	1105.086 (262.167)	1420.353 (370.162)	1088.214 (276.478)
Mean acceptance rate	0.558 (0.075)	0.763 (0.064)	0.748 (0.059)

a mate. However under the experimental conditions reported here, the influence of the bristles was not strong enough to affect a male's final mating success.

Several theories have been proposed to explain the role of the SFO bristles in the medfly. Arita & Kaneshiro (1985) suggest that the most important determinant of male mating success is its ability to successfully direct a pheromone towards the female during courtship, and the SFO bristles may be playing a role in this (Kaneshiro cited in Hunt et al. 1998). However Briceño et al. (1996) and Mendez et al. (1998) note that during the wing buzzing and head-rocking phase, when bristles are in movement, the male is no longer emitting pheromones.

Briceño et al. (1996) and Mendez et al. (1998) believe it is more likely that bristles function as display devices due to the fact that the stalk and spatulate regions are different colors, the stalk being clear and the spatulate end black. However there seems to be geographical variation in this feature since many male bristles in Hawaii are all black (D.O. McInnis, USDA/ARS, Honolulu, unpublished data).

It has been suggested that fluctuating asymmetry (FA) is an important component of sexually selected characters (Moller 1990), reflecting the quality of a male, and that males with low levels of FA are more successful than their more asymmetrical rivals. Hunt et al. (1998) found that laboratory mated male medflies with bristles that were symmetrical in their length had a higher mating success than males with asymmetrical bristles, a result later confirmed in the wild in tow separate locations, in Guatemala and Crete (Hunt et al. in prep.). However it was impossible to tell from these studies whether symmetrical males were being actively selected by females or whether such males were simply better or more assiduous in their courtship.

Our field studies on the effect of accidental loss of one or both bristles (to be published later) suggested that female choice on the basis of visual symmetry could not be supported. Acceptance rates of males with one or two bristles present were significantly higher than those of males with no bristles. The present laboratory study also indicates that the loss of one bristle has no adverse effect on acceptance rate. Males missing one bristle are absolutely asymmetrical and yet they have the same acceptance rate as males with two bristles intact. The fact that these results could be replicated in the laboratory discounts the possibility that in the wild flies, only the less fit males were losing bristles. The present experiments also show that the actual removal of the bristles in the laboratory does not adversely affect the males
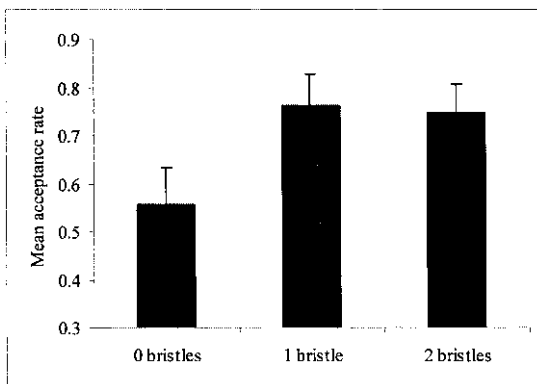

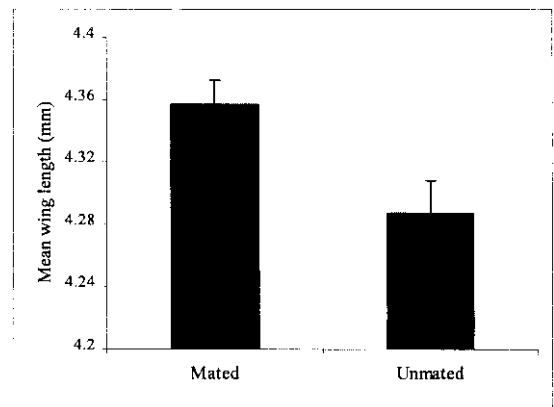Fig. 1. The effect of male bristle number on acceptance rate.

Fig. 2. The mean wing lengths of mated versus unmated males.

mating behavior, and that the relatively poor acceptance rate of males with no bristles was due to their absence rather than the process by which they were removed or any resulting trauma.

The question still remains about how the bristles are actually functioning. Accepting that their effect is visual, a recent suggestion is that they create a 'halo' effect above the male's head as they move from side to side during head-rocking (O. Hasson & P. Taylor, pers. comm.). If this is so, it might explain why males with one bristle are as readily accepted by females as males with two bristles, since one bristle should still be able to create the 'halo' effect above the males head.

At a different level it is possible that the bristles could be functioning as a species recognition signal, as discussed by Hunt et al. (1998). To investigate this theory it would be necessary to study the other species of *Ceratitis* in which the males possess SFO bristles.

In summary our work on SFO bristles presents a complex picture. Although we have demonstrated that symmetrical males have higher mating success, whether in the laboratory (Hunt et al. 1998) or in the field (Hunt et al. in prep.), the present study does not support the conclusion that this occurs as a result of female mate choice on the basis of visual symmetry. This agrees with the suggestion of Mendez et al. (1998) that symmetry in bristle length is unlikely to be acting as an indicator of male quality to the female, because the position of the bristles on the male's head makes it difficult for the female to assess either their size or symmetry with any degree of accuracy. Despite this, we found that the presence of one bristle, although making the male absolutely asymmetrical, is better than having no bristles at all. We can therefore conclude from these experiments that the SFO bristles are indeed important in encouraging acceptance by females, although the reason remains unclear.

We have previously demonstrated in the Guatemalan field cage studies that males with wider wings had a higher mating success (Hunt et al. in prep.). In the current study, we did not find this effect, but instead found that males with longer wings were more successful, and were also more likely to initiate a courtship. These results have since been shown to be repeatable in a second laboratory study (M. K. H., unpublished data). Furthermore, in the Guatemalan field study, male mating success was close to being significantly positively associated with wing length. It is clear that the wings are important in male mating success. The importance of wings in determining mating success is not surprising as they are used in both olfactory and auditory stimulation in the courtship sequence. A particular shape or size of wing may be better than others at performing these functions although wing morphology will obviously be constrained by natural selection on

flying ability as well as its function in courtship. The present studies indicate that the optimum wing design for courtship may differ between samples or between laboratory and field cages. The reason for this variation is unclear.

Investigations of male body size made by Arita & Kaneshiro (1988) and Whittier et al. (1992, 1994 & 1995) led to the conclusion that size has no influence on male mating success. Arita & Kaneshiro (1988) found that smaller males from coffee were preferred over larger males from cherry in some mate trials, but they concluded that 'characters other than body size are essential determinants of mating success of the males'. In contrast, both Churchill-Stanland (1986) and Orozco & Lopez (1990) found that large laboratory males had a greater mating success than small males, although Orozco & Lopez (1990) found that size was less important in wild strains. In our work in field cages (Hunt et al. in prep) we found that male body size may be important to some degree, since smaller (lighter) males begin pheromone calling earlier. In the current study we have demonstrated that this also occurs in laboratory experiments, suggesting that it may be evidence of an alternative mating strategy by the males. Perhaps smaller males need more time to achieve the same degree of mating success. Dunn et al. (1999) found that smaller males of several seaweed fly species were more willing to mount a female than larger males and suggested that this may be because smaller males are more active, or because they develop faster than large males and thus have first access to females. They also suggested that larger males may be longer lived and therefore have a longer time in which to gain access to females. Several studies have shown a positive association between male size and longevity, for example Butlin & Day (1985) studying seaweed flies, and Banks & Thompson (1985) studying damselflies. An association between size and longevity in medflies has not been reported, although Sivinski (1993) found it in domestic *Anastrepha ludens*, a species of Tephritid related to the medfly.

Other investigations into male body size have focused on the nutritional status of the male. Blay & Yuval (1997) found that protein-deprived males are smaller and have lower mating success then the protein-fed males which mate earlier and have a higher probability of mating. Yuval et al. (1998) found that males of all sizes (measured by wing length) participate in leks but that lekking males tend to be heavier and they explain this as a result of these males having greater nutritional reserves. These findings cannot explain the results from our current study in which smaller males pheromone call earlier. Their small size cannot be explained by any nutritional deficiencies. If this were the case we would expect them to begin calling later rather than earlier. However, Blay & Yuval (1997) conclude that male medfly

size has no effect on the overall reproductive success of the female, which suggests that size may not be an important criterion for female choice.

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PHYSIOLOGY OF MATING BEHAVIOR IN MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE): CHEMORECEPTION AND MALE ACCESSORY GLAND FLUIDS IN FEMALE POST-MATING BEHAVIOR

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ABSTRACT

Studies on behavior of tephritid fruit flies have historically focused on the interaction of external stimuli such as temperature, semiochemicals, seasonality, etc., or the interactions of flies between and among species for an number of observed behaviors such as mating, pheromone calling and oviposition. While descriptive behaviors represent much of what we know about these pest species, less is known about the underlying physiological mechanisms which function in priming or modulation of the observed behaviors. In the Mediterranean fruit fly, virgin females are preferentially attracted to the volatile male pheromone over host fruit odors. This behavior switches as a result of mating. Factors from the male accessory gland have been shown to facilitate the switch suggesting a male role in modulation of female olfactory-driven behaviors. Other physiological factors are likely to further influence the degree to which female behaviors are influenced.

Key Words: medfly, *Ceratitis capitata*, mating behavior, pheromone, host odors, accessory glands

RESUMEN

Los estudios en cuanto al comportamiento de las moscas tefritidas de la fruta se han enfocado históricamente en la interacción del estimulo externo tal como temperatura, semio-químicos, estacionalidad, etc., o en las interacciones de las moscas, entre y dentro de las especies, para un numero de comportamientos observados tales como apareamiento, llamado por feromonas y oviposicíon. Mientras comportamientos descriptivos representan la mayor parte de lo que sabemos de estas especies de plagas, menos se conoce de los mecanismos fisiológicos subyacentes cuya función es de preparar o modular los comportamientos observados. En la mosca del Mediterráneo, las hembras vírgenes son preferentemente atraídas a la volátil feromona masculina por encima de los olores de fruta de su hospedero. Este comportamiento cambia como resultado del apareamiento. Factores provenientes de la glándula accesoria masculina han demostrado facilitar este cambio sugiriendo el papel del macho en la modulación de los comportamientos olfato conductores femeninos. Otros factores fisiológicos son probables que influyan mas adelante en el grado en que se influye sobre el comportamiento femenino.

Much of our current knowledge on mating behavior of the Mediterranean fruit fly, *Ceratitis capitata* (medfly) has historically focused on the role of external stimuli such as temperature, pheromones, time of day, seasonality, etc., or the interactions of flies with their physical surroundings (host trees, canopy size, lek sites, etc). Descriptive behaviors represent a sizable portion of what we know about this pest. Less is known about the underlying physiological mechanisms which function in priming or modulation of pre and post mating behaviors. For us to fully understand mating behavior in medfly we must have knowledge of the multiple and often complex internal factors which are involved, and the path / mechanisms by which external stimuli results in observed behaviors. The physiological basis of behavior is a vastly understudied research area which could provide important information on how peripheral receptors receive environmental cues, the transduction and coding of information centrally (from these receptors to the brain) and how behavior is regulated biochemically. The integration of physiology to help explain behavior is central to the goal of understanding the mechanisms of mating behavior and improving the sterile insect technique (SIT) in this species. In this report I would like to review some of the work in our laboratory looking at the link between mating in female medfly and chemoreception.

Chemoreception and Physiological State in Mediterranean Fruit Fly

We have been studying the mechanisms of chemoreception and its link to behavior in the medfly in such areas as olfaction, feeding, mating and oviposition. Our approach is based on the hypothesis that tephritid behavior is influenced by olfactory, gustatory, visual and tactile informa-

tion inputs as primers of behavior and physiology which internally modulates behavior. Behaviorally, tephritids are often classified within a continuum from monophagus to polyphagus, and from "highly chemoreceptive" to "mostly visual" (Jang & Light 1996). However what is known about the underlying receptor systems which accompany these difference in behavior? Do highly chemoreceptive species have different systems than the non chemoreceptive species for detecting semiochemicals? Do differences exist peripherally (the receptor) or more centrally (the brain)?

In earlier studies on female medfly, we focused our research on the influence of plant volatiles on chemoreception and male pheromone on attraction of females. Both sexes exhibited sensitvity to a range of C6-C8 aldehydes and alcohols but did not show sex specific selectivity (Light et al. 1988). Cosse et al. (1995) identified difference in antennal sensitivity to mango volatiles. We also identified over 50 compounds produced by calling male medflies and compared electroantennogram responses of males and females to the identified chemicals (Jang et al. 1989). Although we found some differences in sensitivity and selectivity between male and female antennae, female sensitive compounds were not attractive when assayed individually in the wind tunnel. A blend of the five most prevalent identified chemicals were found to be attractive to females (Jang et al. 1994). While conducting assays of female attraction to male pheromone and/or host fruit volatiles we noted that virgin females behaved differently than mated females which triggered our interest in the effects of physiological state on behavior.

Physiological state is an important concept which, when applied to behavior, helps to explain some of the differences observed in response of medfly to standard stimuli. Physiological state can be the result of multiple "effectors" which result in generalized states which we commonly identify as age, mating status, nutritional history, or more specific molecules such as accessory gland fluids, sex peptides, hormones, etc. which may be involved either directly or indirectly in a specific behavior. These specific molecules represent an understudied area of research due to their secondary recognition as real modulators of behavior. Over the last several years we have seen increasing recognition of the influence of physiological state on behavior which has heightened our awareness of its importance to behavior.

Male Accessory Gland Fluid and Female Post Mating Behavior

In many adult tephritids, semiochemicals serve important roles in life history (Jang & Light 1996; Light & Jang 1996). Specific behaviors such as feeding, attraction to pheromone, and oviposition are all probably influenced by semiochemicals such as food odors, pheromones, and host fruit odors. Our current research in Hawaii has focused on the physiological factors which are responsible for olfactory-driven female behavior. Virgin female medflies are attracted to and prefer male pheromone over host fruit odors. However, mating triggers profound physiological (and behavioral) changes resulting in a switch in preference by females to host-fruit odors (Jang 1995). Other physiological and behavioral effects such as inhibition of remating (Nakagawa et al. 1971, Delrio & Cavalloro 1979) have also been reported. When females were injected (abdominally) with extracts of accessory gland fluids from males, they switched their behaviors like their naturally mated counterparts (Table 1). Namely, a switch from preference to male produced pheromone to host fruit (guava) odors. Females injected with saline only did not switch their behavior and continued to be attracted to the pheromone over the host fruit odors. In addition to changes in the

TABLE 1. RESPONSE OF FEMALE MEDITERRANEAN FRUIT FLIES TO MALE-PRODUCED PHEROMONE AND GUAVA HOST ODOR.

	AGF injected	Mated	Unmated	Saline injected
a. Time on Sphere				
Pheromone	4203 ± 786**	2061 ± 312**	8143 ± 1287**	9039 ± 1208**
Guava	14022 ± 2220	18174 ± 1520	3832 ± 1520	3766 ± 645
b. Landings				
Pheromone	6.8 ± 1.9**	4.6 ± 0.7**	12.8 ± 2.4**	12.6 ± 2.1**
Guava	16.2 ± 1.7	17.2 ± 1.2	5.8 ± 1	5.6 ± 1.2
c. Eggs				
Pheromone	0 ± 0*	0 ± 0**	0 ± 0	0 ± 0
Guava	43 ± 13	163 ± 18	6.2 ± 4.6	8.1 ± 5.6

**Significance at $P < 0.01$ level.
*Significance at $P < 0.05$ level.
AGF = Accessory gland fluid.

TABLE 2. RESPONSE OF VIRGIN FEMALE MEDITERRANEAN FRUIT FLIES TO MALE-PRODUCED PHEROMONE AND GUAVA HOST ODOR.

	LRN	LRS	Wild
a. Time on Sphere			
Pheromone	11026 ± 3275**	2779 ± 574	1706 ± 555*
Guava	3859 ± 2116	2148 ± 704	259 ± 152
b. Landings			
Pheromone	11.94 ± 0.93**	6.83 ± 1.47	3.2 ± 0.66**
Guava	5.56 ± 0.78	4.17 ± 1.01	0.6 ± 24
c. Eggs			
Pheromone	0 ± 0**	0 ± 0	0 ± 0
Guava	20.88 ± 3.5	0 ± 0	0 ± 0

**Significance at $P < 0.01$ level.
*Significance at $P < 0.05$ level.
LRN = laboratory reared normal.
LRS = laboratory reared sterile.

olfactory-stimulated switch in attraction from pheromone to host fruit odors, females which were naturally mated or injected with accessory gland fluid, laid significantly more eggs than non-mated or saline injected controls. Females did not exhibit the switch in behavior immediately after mating suggesting that secondary mechanisms may be involved in the regulation of the behavioral switch.

Sterile Males and Female Postmating Behavior

If factors from male accessory glands are important in control of female driven olfactory behavior, what, if any, is the effect of irradiation on the ability of sterile males to switch the behavior of wild-type females? To test this question we set up a series of laboratory flight tunnel assays in which laboratory normal, irradiated and wild-type females were mated to conspecific males and observed for their response to pheromone or host-

fruit (guava) odor. Both laboratory-reared normal and wild virgin females preferred male odor over guava odor (Table 2) (Jang et al. 1998). Laboratory reared normal and wild females mated to normal males switched their behavior and preferred the guava odor over male pheromone. Both laboratory-reared and wild females also switched their behavior when mated to sterile males (Table 3). In both of these tests, sterile females did not exhibit a significant switch in behavior. These studies concluded that irradiated males were equally adept at altering female behavior as non-irradiated males (Jang et al. 1998).

Field Cage Studies on Female Post Mating Behavior

Laboratory-reared and wild female medflies were assayed in outdoor field cages to assess the impact of the mating-induced behavioral switch on mating behavior and oviposition activity. Laboratory and wild type virgin females mated more

Table 3. Response of laboratory normal, laboratory sterile and wild females mated to sterile males.

	LRN	LRS	Wild
a. Time on Sphere			
Pheromone	4556 ± 531**	260 ± 162**	270 ± 140**
Guava	11368 ± 825	1856 ± 445	2821 ± 612
b. Landings			
Pheromone	6.07 ± 0.67**	0.5 ± 0.27**	0.67 ± 0.14**
Guava	14.2 ± 0.88	3.25 ± 0.59	3.83 ± 0.46
c. Eggs			
Pheromone	89 ± 14**	0 ± 0	0.17 ± 0.17
Guava	189 ± 23	0 ± 0	8.33 ± 4

**Significance at $P < 0.01$ level.
LRN = laboratory reared normal.
LRS = laboratory reared sterile.

with males on leaves and branches than females which had previously mated with either laboratory normal or irradiated males (Fig. 1) (Jang et al. in press). More of the mated females could be found alighting and ovipositing in artificial spheres emitting guava odor (or authentic apples) hung in host guava trees (Figs. 2 and 3). Females mated with either normal or sterile males exhibited the behavioral switch which we had seen in earlier laboratory studies (increased landings, time on sphere and oviposition/eggs laid). Some quantitative differences where observed between responses of laboratory reared and wild females. Both wild and laboratory reared mated females laid significantly more eggs in artificial spheres emitting guava odor than virgin females (Fig. 4).

Effects of Anti-JH Compounds and Chemosterilants on Behavior

In medfly, anti-juvenile hormone compounds such as precocene and the chemosterilant benzyl 1,3- benzodioxole have been shown to effect synthesis and release of JH from the corpora allata (Chang et al. 1994). This interference in JH production has been shown to affect sex attractancy of male medflies (Chang & Hsu 1982) as well as ovarian development in females (Hsu et al. 1989). Preliminary studies with females treated with these compounds suggest that JH may also be involved in modulation of olfactory behavior.

CONCLUSION

Chemoreception, transduction, age, mating status and nutritional state all play important roles in regulating behavior. Semiochemicals
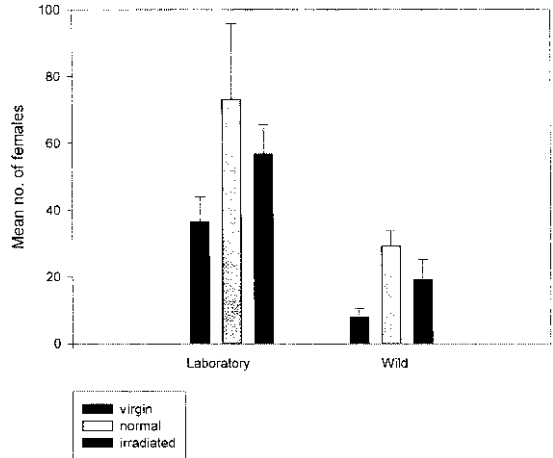

Fig. 2. Cumulative fly counts on spheres of laboratory and wild virgin and mated female Mediterranean fruit flies.

serve as behavioral primers while hormonal activities and cellular homeostasis are further "downstream" in the regulatory process but no less important. In our example we have shown that mating and transfer of accessory gland fluid had a direct impact on female medfly olfactory behavior which probably works through biochemical intermediates and possibly hormones. The specific mechanisms which drive female behavior await further research and promise to be complex but exciting to uncover. Irradiation used in sterilizing mass-reared males for sterile insect release control programs does not appear to affect the ac-

Fig. 1. Mating behavior of virgin and mated laboratory and wild Mediterranean fruit flies in a field cage.
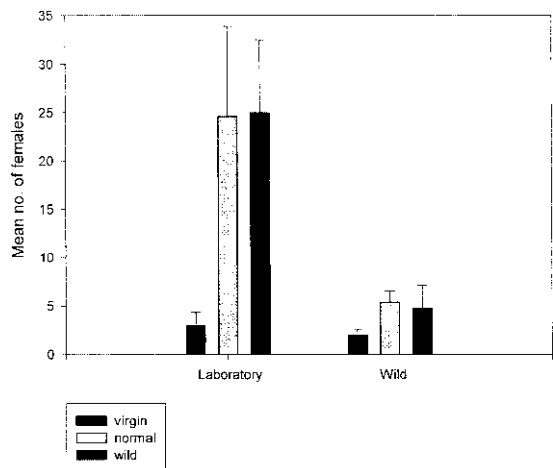

Fig. 3. Cumulative ovipositions on spheres by virgin and mated laboratory and wild female Mediterranean fruit flies.
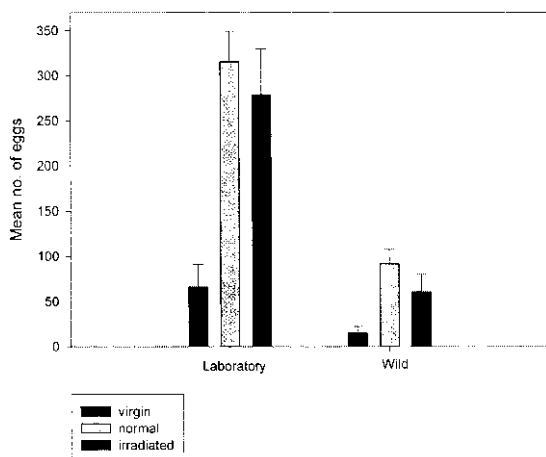
Fig. 4. Number of eggs deposited/h by virgin and mated laboratory and wild female Mediterranean fruit flies.

cessory gland fluid which switches behavior. Improvements in our knowledge of these processes and their control will be the key to development of strategies which target behavioral processes against these pests.

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EFFECT OF AGE ON THE MATING PROPENSITY OF THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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Abstract

The effect of age on the mating propensity of both wild and laboratory-reared Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann) was investigated under laboratory and field cage conditions. The optimal age for wild flies ranged from 7 to 13 days, whereas in laboratory-reared flies it was between 3 and 5 days old. Virgin flies were selective and more prone to mate than flies that were held with both sexes combined and therefore, had a chance to mate before the test. The difference among ages in laboratory-reared flies was significant only in virgin flies. Virgin females showed a tendency to increase their mating propensity as they got older, whereas virgin males showed a bimodal pattern, with peaks at 4 and 11 days old. When flies of both strains and different ages were combined, laboratory-reared females accounted for 72% of the all the matings and most matings were by 4-day-old females. Wild males accounted for 67% of all the matings and the maximum number of matings were by 10-day-old males. For quality control purpose, flies should be virgin and at their optimal age, this will produce more robust data for statistical analysis. For control purpose, it is recommended to release sterile flies at 1-2 days old, because flies in the field will be at their maximum mating propensity. Our results support the concept that releasing males only will make the Sterile Insect Technique more effective, since sterile males will be virgin and therefore, more prone to mate.

Key Words: medfly, *Ceratitis capitata*, Sterile Insect Technique, quality control, mating behavior

Resumen

Se investigó el efecto de la edad en la propensión al apareamiento de moscas del Mediterráneo *Ceratitis capitata* (Wiedemann), silvestres y de cría masiva, en condiciones de laboratorio y jaula de campo. La edad óptima para las moscas silvestres estuvo entre los 7 y los 13 días de edad, para las moscas de laboratorio estuvo entre los 3 y los 5 días. Las moscas vírgenes fueron más selectivas y más propensas a aparearse que aquellas que se mantuvieron mezcladas antes de la prueba. Las diferencias entre edades solamente fueron significativas en moscas vírgenes de laboratorio. Las hembras vírgenes mostraron una tendencia a aumentar su propensión al apareamiento conforme aumentaba su edad, mientras que los machos mostraron un patrón bimodal, con picos a los 4 y 11 días de edad. Cuando se combinaron ambas cepas y diferentes edades, las hembras de cría masiva realizaron el 72% de todos los apareamientos y la mayoría fue por hembras de 4 días. Los machos silvestres realizaron el 67% de los apareamientos y el máximo fue por machos de 10 días de edad. Para fines de control de calidad, las moscas deben ser vírgenes y en su edad óptima, esto producirá datos más robustos para los análisis estadísticos. Para fines de control, se recomienda liberar las moscas estériles cuando tienen 1-2 días de edad, así las moscas estarán en el campo cuando sean más propensas a aparearse. Nuestros resultados apoyan el concepto de que liberar machos solamente hará mas efectiva la Técnica del Insecto Estéril, ya que los machos serán vírgenes y por lo tanto más propensos a aparearse.

The successful application of the Sterile Insect Technique (SIT) to suppress the Mediterranean fruit fly *Ceratitis capitata* (Wied.), as well as new developments to improve this technique, have motivated a wider use of SIT worldwide (Hendrichs et al. 1995). As the demand for more environmental friendly control methods increases, it is likely that the use of the SIT will expand. For wider and more efficient applications, new developments that improve it are required.

As with any other pest control method, it is generally accepted that a better knowledge and understanding of the biology, behavior and ecology of the target pest will result in improvements and more effective applications. One factor that is likely to improve the effectiveness and efficiency of the SIT is the development of new and better methods to estimate or evaluate male mating competitiveness and to determine the factors that are important for successful mating.

After pioneering work by Prokopy & Hendrichs (1979), the "Field Cage Test" (Calkins & Webb 1983) has been used as a research and quality control tool to characterize and understand the mating behavior of fruit flies and to evaluate the mating competitiveness of laboratory-reared ster-

ile flies (i.e. Chambers et al. 1983, Hendrichs 1986, Guerra et al. 1986, Robinson et al. 1986, Orozco & Lopez 1993, McInnis et al. 1996, Lance et al. 1996, Cayol et al. 1999, Calcagno et al. 1999, IAEA 1999). However, there is not a detailed protocol on how the test should be run for quality control purposes, and factors such as density, sex ratio, and age of the flies vary widely. Fine tuning of these factors is important to compare results from different locations and strains, to standardize quality control procedures and make them more efficient, and to determine the conditions that make the test more sensitive, so it can be used as an early warning for mass rearing decision making.

The general goal of this research project was to investigate the effect of age on the mating propensity of both wild and laboratory-reared Mediterranean fruit flies. Our specific goals were to determine: 1) if mating propensity changes with age, and 2) if there is an effect of the mating status (virgin vs. non-virgin) on the mating propensity of males and females.

MATERIALS AND METHODS

Five different studies were carried out. In all cases, laboratory-reared flies (L) were obtained as irradiated pupae from the Moscamed facility in Metapa, Mexico. Wild flies (W) were obtained as larvae from infested coffee berries collected in Southwestern Guatemala. After leaving the fruit, mature larvae were placed in screened plastic containers for pupation. For both strains, at eclosion, adults were sorted by sex and placed in plastic cages with food (sugar + yeast hydrolyzate 3:1 ratio) and water. Adult flies were kept virgin before the tests, except in those cases in which the effect of the mating status was investigated. In these cases, in one group males and females were held together, so they had a chance to mate before the test (mixed). In the other group, males were held in one cage and females in another cage, so they could not mate before the test (virgin).

When different age groups or strains (laboratory-reared or wild) were tested in the same cage, flies were marked with a small spot of water paint on the thorax the day before the test. A different color was used for each age group and strain. So far we have not detected that these color markings have any effect on the mating performance of the flies.

Field cage studies were carried out in the standard 3 m in diameter by 2 m high cages, with a coffee bush inside (Calkins & Webb 1983). In those cases in which wild flies were used, the tests were done in a coffee plantation in Southwestern Guatemala. When only laboratory-reared flies were used, the cages were located in the "coffee garden" of ECOSUR, in Tapachula, Chiapas, Mexico.

During the test, the behavior of the flies was observed and mating pairs were detected. When a

mating was observed, the pair was collected in a vial and the following information was recorded: 1) Time in copula. The time at which the mating was formed was recorded and the vials with the mating pairs were observed frequently to record the time at which the copulation was finished; 2) Site of mating, whether it was on the cage screen or over the coffee plant. In the case of matings on the plant, whether they were on the top or bottom part of the leaf, on a branch or on a fruit; and 3) Kind of mating, recording the age, and strain (where applicable) of the male and the female in each mating, according to the colors used.

Field and laboratory observations were made from 07:00 to 13:00 h. Temperature conditions ranged from 22 to 32°C, relative humidity from 65 to 90%, and a photoperiod of 12:12 (L:D).

Wild Flies, Mixed Ages (W1)

This was a field cage study with only wild flies. The ages of the flies were 7, 9, 11 and 13 days old. This age range was selected based on our previous unpublished observations and due to the limiting number of flies available. Adult flies were released in the cages between 07:00 and 08:00 h. In each cage, 10 males and 10 females of each age group were released. Flies that could not fly or died during the observation period were replaced. The experiment was repeated 10 times (5 cages per day, two days).

Laboratory-Reared Flies, Mixed Ages (L2)

This was a field cage study similar to the previous one but with laboratory-reared flies. The effect of the mating status (virgin vs. mixed) was evaluated by running two sets of tests, one with virgin flies (L2v) and the other with mixed flies (L2m).

The density in the cage was greater than the one with wild flies as was the age range tested. This was done to increase the number of potential matings during the test and given the greater availability of flies. Four different age groups of flies were released in each cage. Twenty five males and 25 females of each age group, so the total number of flies per cage was 200 (100 males and 100 females). The ages of the flies for the first day were: 2, 5, 8, and 11 days old; the second day the ages were: 3, 6, 9, and 12; and the third were: 4, 7, 10, and 13. Both mated and dead flies were replaced with individuals of the same sex, age and mating status, so the density, as well as the sex and age ratios in the cages were constant. Four replicates were done for both virgin and mixed flies.

Laboratory-Reared Flies, Same Age (L3)

This was a field cage study with laboratory-reared flies in which all the flies in the cage were the same age (cohort). One hundred pairs (100 males and 100 females) of 2-day-old flies were released initially in the field cage. Flies were ob-

served from 07:00 to 13:00 h every day. Mated pairs were vial collected and records on time and location of mating were taken. At 13:00 h all the flies from the cage were collected and transferred to the laboratory where they were maintained in glass cages provided with food and water. The sexes were sorted out to prevent matings during the time when flies were not observed (13:00 to 07:00 h). Two different approaches were followed. In one case, both mated and dead flies were replaced with virgin individuals of the same age and sex, observations were made during 15 consecutive days (L3a). In the other case, both mated and dead flies were not replaced (L3b), so the test was finished when no more females were available. Four replicates of each test were done.

Laboratory-Reared Flies, Mixed Ages for Males and Fixed Age for Females (L4)

This was a laboratory cage study with laboratory-reared flies. In a $1.0 \times 0.6 \times 0.9$ m screened cage with a potted coffee plant inside, 20 males and 5 females were released. Males were of 4 different age groups (5 males per age group) and females were of a fixed age, older than the young males and younger than the old males. The first day of the test, males were: 2, 5, 8, and 11 days old and female age was 7 days old. The experiment was conducted over 3 consecutive days, so the age range for the males was from 2 to 13 days, and for the females was from 7 to 9 days. Both mated and dead flies were replaced with individuals of the same age and sex. This experiment was carried out with virgin females and with females that were exposed to males before the test. Males were always virgin. Four replicates of each approach were done.

Wild and Laboratory-Reared Flies, Mixed Ages (WL5)

This was a field cage study. In each cage, 60 laboratory-reared sterile flies (30 males and 30 females) and 60 wild flies (30 males and 30 females) were released. Flies were sorted in 3 age groups

and the ages of the flies were the same for both strains. The ages of the flies for the first day were 2, 5, and 8 days old; for the second day were 3, 6, and 9 days old; the third day were 4, 7, and 10 days old, the fourth day were 5, 8, and 11 days old, and the fifth and last day were 6, 9, and 12 days old (range tested was 2 to 12 days old). All flies were virgin before the test. Three replicates were done.

RESULTS

Wild Flies, Mixed Ages (W1)

A total of 260 matings were recorded in the 10 replicates. Although differences among ages were not significant, 7-day-old flies showed the lowest mean number of matings for both sexes. Males showed a gradual increase in the number of matings as they aged. The maximum number of matings was achieved by 13-day-old males. Females showed the greatest number of matings at 11 days old (Table 1). The most common combination was between 13-day-old females with of 7-day-old males (9.3% of all combinations), but was very similar to other combinations, such as 11-day-old females with 11-day-old males (8.8%) and 13-day-old females with 13-day-old males (8.4%). The least common combinations were between 7-day-old males and females (2.6%) and 9-day-old females with 7-day-old males (3.5%).

Laboratory-reared Flies, Mixed Ages (L2)

There was a significant effect of the mating status on the mating propensity of both, males and females ($P = 0.0001$). Greater number of matings were recorded from virgin flies than from mixed flies at all ages, except on 2-day-old females, where the mean number of matings was the same (Fig. 1A).

Differences among ages in both, virgin and mixed females, were not significant ($P = 0.057$ for virgin females, and $P = 0.497$ for mixed females). However, in both types of females there was a tendency to increase mating propensity from 2 to 4

TABLE 1. MEAN (SE) NUMBER AND PERCENTAGE OF MATINGS, PER SEX AND AGE OF WILD MEDITERRANEAN FRUIT FLIES UNDER FIELD CAGE CONDITIONS. AGE GROUPS WERE COMBINED AND FLIES WERE VIRGIN (N = 260).

Sex	Age	Mean Number of matings (SE)	Percent
Females[a]	7	4.8 (0.70)	18.46
	9	6.3 (0.77)	24.23
	11	8.3 (1.22)	31.54
	13	6.7 (0.42)	25.77
Males[b]	7	5.5 (0.40)	21.15
	9	6.7 (0.67)	25.77
	11	6.5 (0.56)	25.00
	13	7.3 (0.42)	28.08

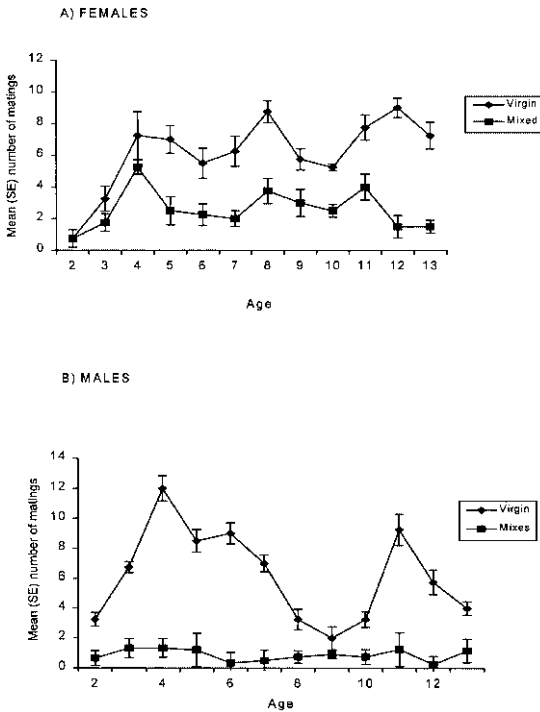[a]F = 2.38, D.F. = 3, $P = 0.070$.
[b]F = 1.88, D.F. = 3, $P = 0.134$.

Fig. 1. Age-specific mean number (SE) of matings by laboratory-reared virgin flies and flies in which the two sexes were mixed before the test. A) Females, B) Males.

days. Later, in the case of mixed females, their mating propensity decreased. In the case of virgin females, there was a second peak when females were 8 days old and the third and greatest peak was recorded when females were 12 days old.

There was a significant difference ($F = 2.92$, $P = 0.007$) among ages in virgin, but not in mixed males ($F = 0.81$, $P = 0.63$). The maximum mean number of matings was attained by 4-day-old virgin males (Fig. 1B). Virgin males showed a bimodal pattern in their mating propensity, increasing from 2 to 4 days old, then decreased to a minimum at age 9 and peaking again when they were 11 days old. The mean number of matings by mixed males was never greater than 2 and without any clear pattern associated with age.

Laboratory-reared Flies, Same Age (L3)

There were significant differences among ages for both replacement ($F = 4.39$, $P = 0.0001$) and non-replacement ($F = 10.28$, $P = 0.0001$) tests. The maximum number of matings was recorded when flies were 3 days old (Fig. 2). Mating activity gradually decreased with age. In the non-replacement test, by age 9 all the flies had mated or died. In this test, over 70% of all matings were by 3 and 4-day-old flies. When mated and dead flies were replaced, the fraction of matings at these same ages was only 39.9% of all matings.

Laboratory Cage, Mixed Ages for Males, Fixed Age for Females (L4)

There was a significant differences among male ages when females were virgin, but not when females were mixed. The greatest number of matings were achieved by 5-day-old males (Table 2). The total number of matings with mixed females was 34, whereas in the test with virgin females was 66. Although this greater mating propensity, virgin females apparently were more selective than mixed females, regarding the age of the males and based on the statistical analysis.

Field Cage—Wild and Laboratory-Reared Flies (WL5)

There was a significant difference between laboratory-reared and wild flies, both in the number of matings achieved and the age for maximum mating activity. Laboratory-reared females showed greater mating propensity and the age of maximum mating activity was earlier in life, compared to wild females (Fig. 3). These females accounted for 72.0% of all the matings and the greatest number of matings were recorded when they were 4 days old.

In the case of males, wild males achieved more matings (67.1%) than laboratory-reared males and the greatest number was recorded when they were 10 days old (Fig. 4). The maximum mean number of matings was by 4-day-old lab females with 10-day-old wild males.

Time in Copula

The time in copula was recorded in all field cage tests. A summary of these data is presented in Figure 5. There was not a clear pattern or con-
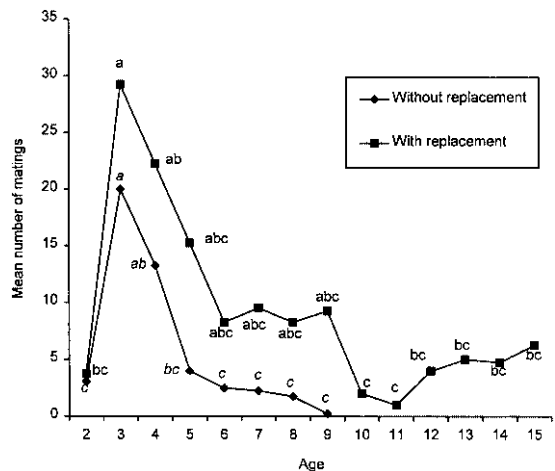

Fig. 2. Age-specific mean number of matings by laboratory-reared flies in tests with and without replacement of mated and dead flies. Same letters at each point (mean) indicate non significant differences within each test by the Tukey Multiple Range Test.

TABLE 2. MEAN (SE) NUMBER AND PERCENTAGE OF MATINGS BY MALES OF DIFFERENT AGES UNDER LABORATORY CONDITIONS. FEMALES WERE OF A GIVEN MEDIAN AGE, AND THEY WERE KEPT MIXED WITH MALES OR KEPT VIRGIN BEFORE THE TEST (N = 34 AND 66 MATINGS FOR MIXED AND VIRGIN, RESPECTIVELY).

	Mean (SE) number of matings		Percentage of matings	
Age	Mixed[a]	Virgin[b]	Mixed	Virgin
2	0	0.25 (0.29) b	0	1.51
3	0.25 (0.29)	0.75 (0.87) ab	2.94	4.54
4	0	2.00 (2.31) ab	0	13.63
5	1.25 (1.44)	3.50 (4.04) a	14.70	21.21
6	0.75 (0.87)	1.75 (2.02) ab	8.82	10.61
7	0.75 (1.73)	1.25 (1.44) ab	8.82	7.58
8	1.00 (1.15)	2.00 (2.31) ab	11.76	12.12
9	1.00 (1.15)	1.25 (1.44) ab	11.76	7.58
10	0.50 (0.57)	1.00 (1.15) ab	5.88	4.54
11	1.00 (1.15)	0.75 (0.87) b	11.76	7.57
12	1.00 (1.15)	0.75 (0.87) b	11.76	1.51
13	1.00 (1.15)	1.25 (1.44) b	11.76	7.57

[a]Differences were not significant by the Chi square test, $P = 0.218$, d.f. = 11). Data were transformed by $\sqrt{X}$ for analysis.
[b]Means followed by the same letter are not significantly different (F = 2.85, $P = 0.0086$) by the Tukey Multiple Range test. Data were transformed by $\sqrt{X}$ for analysis.

sistent effect of age on the duration of copula. However, this parameter was consistently affected by the strain. Generally, wild flies showed greater mean time in copula than laboratory-reared flies.

Considering all the tests together, the mean time in copula for wild and laboratory-reared females was 125.8 and 103.5 minutes, respectively. For males, the means were 140.6 and 104.8 minutes
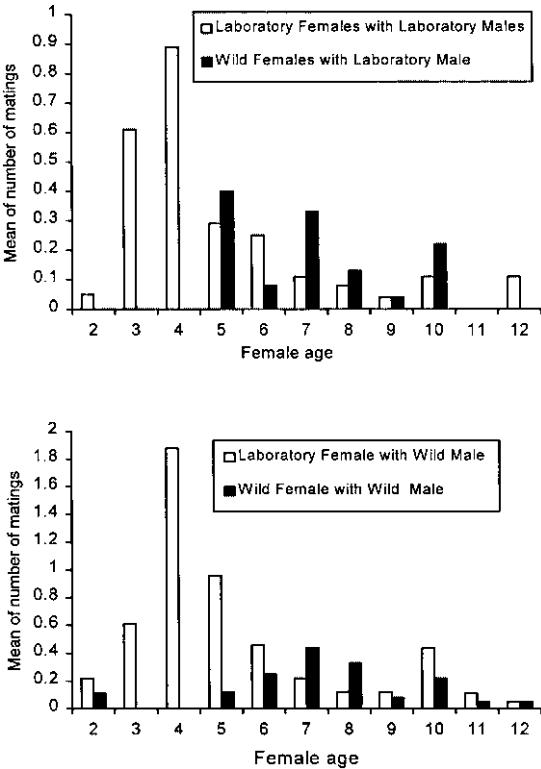

Fig. 3. Age-specific mean number of matings by wild and laboratory-reared females under field cage conditions.
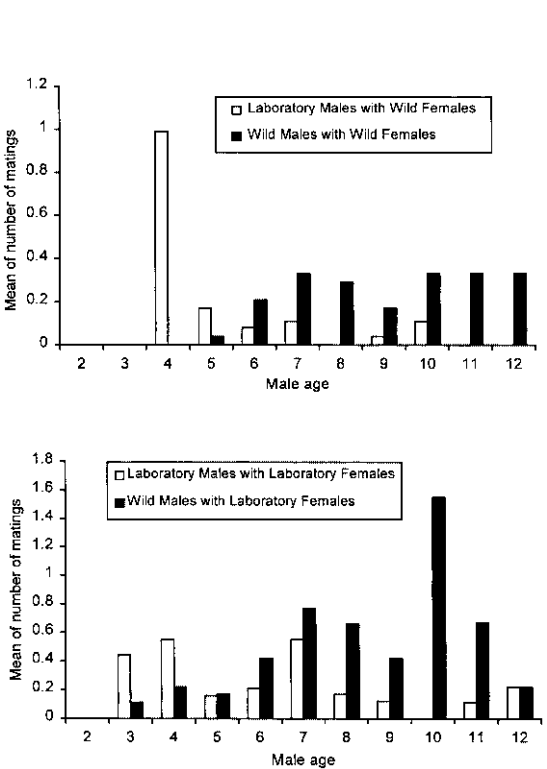

Fig. 4. Age-specific mean number of matings by wild and laboratory-reared males under field cage conditions.
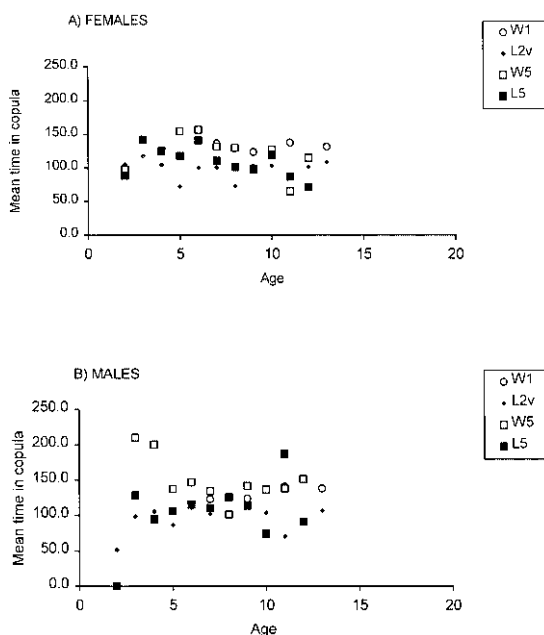
Fig. 5. Mean time in copula of wild and laboratory-reared flies as a function of age. W1 represents data from experiment 1 in which only wild flies were tested. L2v represents data from virgin laboratory-reared flies tested in experiment 2. W5 represents data from wild flies in experiment 5 in which wild and laboratory-reared flies were tested together. L5 represents data from laboratory-reared flies in experiment 5.

for wild and laboratory reared, respectively. Copulas that last less than one minute were discarded for the analysis. The maximum time in copula was 308 minutes (13-day-old wild male with 11-day-old wild female). The mating status of the flies (virgin or mixed) showed no significant effect on the duration of copula ($F = 1.25, P = 0.25$).

Location of Matings

When only wild flies were observed, 52.5% of the matings took place on the bottom part of the leaves, 3.5% occurred on the top part of the leaves, and the rest were observed on the cage screen (44.0%). When both, wild and laboratory reared flies were tested, the same pattern was observed, with 78.3 and 6.3% of the matings located at the bottom and top part of the leaves, respectively. The rest (15.4%) occurred on the screen cage.

Regarding orientation, most matings occurred in the East and Southeast part of the cages (45.9%), which was the sunny part when most mating activities took place (08:00-11:00 h).

Mating activity was strongly associated with temperature conditions. A multiple regression analysis was done with the number of matings and the temperature, for the four field cage tests

with laboratory-reared flies (Fig. 6). Mating activity occurred within a range from 23 to 31°C. The greatest number of matings occurred when the temperature was 26°C.

DISCUSSION

Considering the two tests with wild flies (experiments 1 and 5), we conclude that the age range for greater mating activity (optimal range) in wild flies is between 7 and 13 days. The non significant difference among ages in wild flies (Table 1), could be attributed to the range tested (7-13) that was too small for resolution. However, in the experiment with wild and laboratory-reared flies (WL5), we find no matings by 3 and 4-day-old wild females and 2-day-old wild males (Figs. 3 and 4). This is consistent to what was reported by other authors following similar methods, regardless of the geographic origin of the flies. In Reunión Island, Quilici & Franck (1996) recorded the maximum number of matings by 9-day-old flies (optimal range: 7-9 days old), the range they tested was from 3 to 9-day-old flies. In Argentina, Calcagno et al. (1996), tested a range from 3 to 17 days old and they recorded the maximum number of matings from 13-day-old flies (optimal range: 11-17 days old). In Greece, Economopoulos & Mavrikakis (1996) recorded the maximum number of matings by 14-day-old flies (optimal range: 8-14 days old), the range they tested was 2 to 14-day-old flies. There were two common features in all these tests, 1) males mature one or two days earlier than females, and 2) in most cases, the age with the maximum number of matings, was the oldest age tested. The only exception was in Argentina, where also was the only
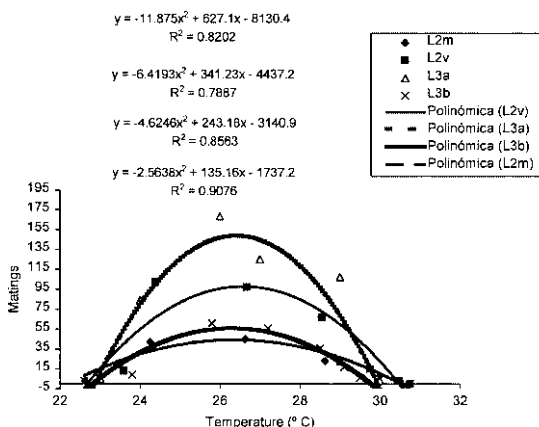

Fig. 6. Multiple regression analysis between number of matings and temperature. L2m and L2v represent data from experiment 2 with mixed and virgin laboratory-reared flies, respectively. L3a and L3b represent data from laboratory-reared flies used in experiment 3 with and without replacement, respectively.

case where flies older than 13 days old were tested. We believe that this characteristic might be due, at least in part, to the fact that flies were kept virgin before the test (see below).

The optimal age range for laboratory-reared flies was between 3 and 5 days old. This is consistent with demographic data that shows that mass-rearing conditions select for early maturation, and flies adapted to this conditions start laying eggs much earlier than wild flies normally do (see Liedo & Carey 1996 and references therein).

Another characteristic of laboratory reared flies, was that females were more prone to mate than wild females. This was particularly clear in the test in which wild and laboratory-reared flies were compared (WL5). Despite that the particular strain we tested was only two years under mass-rearing conditions, 72.0% of all matings were by laboratory-reared females. Again, this is a common characteristic of mass-reared flies (Calkins 1984, Harris et al. 1986, Hendrichs 1986).

The mating status of the flies showed a strong effect on their mating propensity. Virgin flies were more prone to mate, as we were expecting. This might explain the increase in mating propensity with age (i.e., this increase could be an artifact of the experimental design, using virgin flies) and can be attributed to physiological changes that happen in the females after mating. If the females have not mated, they will still be responding to male signals. However, once mated, females will be more interested in finding a host to laid their eggs. The reduced number of matings by mixed males (L2), could be attributed to the low mating propensity of the mixed females.

The mating status could also affect female choice. Virgin females were more selective with respect to male age than mixed females (Table 2).

We believe there are three important applied implications of these results. For quality control purposes, we recommend use of virgin flies at their optimal age for mating propensity (7 to 13 for wild and 3 to 5 for laboratory-reared flies) in field cage tests. This will increase selectivity by females and will increase the mating probability, producing more robust data for statistical analysis and will make quality control efforts more efficient. Also, we believe that our laboratory test (L4) represents an alternative for quality control programs, particularly when the availability of wild flies is a limiting factor and/or when field cage tests represent a risk for fruit fly free zones or areas under eradication or suppression.

Regarding the release of sterile flies, our results indicate that releases when sterile flies are 1 or 2 days old, is the optimal condition. This will reduce the chance of mating before release, and therefore, these flies will be virgin and with a high propensity to mate. Keeping the flies for longer time before release would reduce their mating propensity, could result in high mortality due to crowding conditions, and will increase unnecessarily program costs because of the space required.

Finally, our results support the concept that releasing males only will result in more effective sterile fly release programs, since this will avoid matings between sterile flies, and this will result in males more motivated to court females and to mate.

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EFFECTS OF IRRADIATION ON THE COURTSHIP BEHAVIOR OF MEDFLY (DIPTERA, TEPHRITIDAE) MASS REARED FOR THE STERILE INSECT TECHNIQUE

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Abstract

The effects of routine irradiation of the mass-reared males of the medfly, *Ceratitis capitata* on their mating performance were re-evaluated. Male courtship behavior was observed and quantified both in laboratory (video recording cages) and field cage conditions. For the experiments, samples of the strains routinely mass-reared for SIT operations at Seibersdorf, Austria; Mendoza, Argentina and Metapa, Mexico, were used. No major qualitative differences were found in the courtship pattern between irradiated and non-irradiated males. However, the results revealed that the process of routine irradiation as commonly used in the mass rearing facilities at the time of the experiments, reduces the mating performance of the sterilized males nearly two-fold. A whole range of quantitative differences between the irradiated and non-irradiated males were detected and described, and their implications for the efficiency of SIT operations are discussed. In contrast, partial sterilization with low doses of radiation did not affect the mating competitiveness of the treated males to a noticeable degree. In view of the results obtained, and due to the current wider use of "male-only" strains in SIT operations, a re-evaluation of the sterilization strategy and irradiation doses for males used in SIT is recommended.

Key Words: medfly, *Ceratitis capitata*, mating behavior, courtship, sterilization, irradiation, SIT

Resumen

Los efectos de irradiaciones rutinarias de machos criados en masa de la mosca del Mediterráneo, *Ceratitis capitata* sobre su capacidad de apareamiento fueron re-evaluados. El comportamiento del cortejos de los machos fue observado y cuantificado tanto en el laboratorio (grabaciones de videos en jaulas) y en condiciones de jaulas en campo. Para los experimentos, muestras de las razas que rutinariamente son criadas en masa para operaciones SIT en Seibersdorf, Austria; Mendoza, Argentina y Metapa, México fueron utilizados. No se encontraron diferencias cualitativas mayores en los patrones de cortejo entre machos irradiados y no irradiados. Sin embargo, los resultados revelaron que el proceso de irradiación rutinaria comúnmente utilizado en las instalaciones de cría en masa para el momento de los experimentos, reduce la capacidad de apareamiento de los machos estériles aproximadamente dos veces. Una gran variedad de diferencias cuantitativas entre los machos irradiados y los no irradiados fue detectado y descrito, y sus implicaciones en la eficiencia de las operaciones SIT son discutidas. En contraste, esterilización parcial con bajas dosis de radiación no afectó la competitividad de apareamiento de los machos tratados en un grado notable. En vista de los resultados obtenidos, y debido al uso más amplio y corriente de razas "solo-machos" en las operaciones SIT, una re-evaluación de la estrategia de esterilización y las dosis de irradiación utilizadas para los machos en SIT es recomendada.

The effectiveness of Sterile Insect Technique (SIT) programs is determined by the ability of released sterile males to successfully mate with and inseminate wild females. However, during the process of establishing a strain (or its creation), along the rearing process, and during treatment before release, the insects are subject to highly artificial conditions, including extreme population

densities, a sterilization process, and sometimes, genetic manipulation. These factors adversely affect the biological fitness of the treated insects and their performance during SIT operations. In fact, a certain degree of reduction in insect quality seems to be an unavoidable cost in maintaining the economic efficiency in the mass rearing process.

In the practice of SIT operations, a moderate reduction in the quality of the mass-reared insects may be tolerated, because it can be compensated by higher release rates. This is especially true when the target insect pest has a simple mating system, closely resembling a stochastic process. In such cases, well represented by the tsetse, *Glosina morsitans morsitans* (Saini 1985), the courtship is usually reduced and females have a limited chance of rejecting males. Mating decisions are made by males and direct male-male competition is limited. Hence, the ability of the mass-reared tsetse males to copulate seems to be sufficient for SIT to be effective. However, the quality requirements for mass-reared insects may greatly increase when the SIT operation targets a pest having a more sophisticated mating system.

Recent studies on the medfly, *Ceratitis capitata* (Wiedemann), mating system have shown that wild medfly males form leks to collectively attract females. This provides the females with an opportunity to compare, nearly at the same time, the qualities of several males present in a lek. To mate, the males have to engage in an elaborate courtship and wild females are known to be choosy in selecting a male for mating (Arita & Kaneshiro 1985, Calcagno et al. 1996, Calkins 1987, Harris et al. 1988, Hendrichs et al. 1991, Lux et al. 1996, Prokopy & Hendrichs 1979, Whittier & Kaneshiro 1991, Whittier & Kaneshiro 1995, Whittier et al. 1994). Consequently, the success of SIT for medfly control largely depends on the mating competitiveness of released irradiated males being directly challenged by their wild counterparts in competition for position in leks and the attention of wild females. Hence, simply the ability to copulate may not be sufficient to fully guarantee their efficiency. This implies that even minor deficiencies in the mass-reared sterilized males may have profound consequences for their mating success in the field, and consequently, on the costs and efficiency of medfly control operations.

Currently, irradiation is the only method available to effectively sterilize insects. The impact of an irradiation on the quality of the mass reared medfly males, in particular on their ability to mate, has been tested in the past. It has been concluded that the irradiation process causes no major mating deficiencies, if conducted according to the recommended standards (Hayashi & Koyama 1981, Holbrook & Fujimoto 1970, Hooper 1970, Hooper 1971a, Hooper 1971b, McInnis et al. 1985,

Wong et al. 1983, Wong et al. 1982). Hence, possible detrimental effects of irradiation, though reported by several authors (Favret et al. 1995, Haish 1969, Haish 1970, Hooper & Katiyar 1971, Lux et al. 1996, Lux et al. 1997), are generally considered to be of negligible importance for the effectiveness of SIT operations.

Indeed, the SIT has been used successfully in the USA and Latin America in numerous operations for large-scale medfly control or eradication (Hendrichs 1996, Penrose 1996). Though the technique is effective, efforts to improve its efficiency even further, continue. With increasing emphasis being placed on the quality of the produced and released insects, the present study was initiated to re-investigate the impact of irradiation on the performance of medfly males. To obtain more detailed insights into the subtleties of medfly courtship behavior, video recordings of courtship sequences and quantitative ethological analyses were made.

Materials and Methods

The experiments were replicated in three locations: Seibersdorf (Austria), Mendoza (Argentina) and Metapa (Mexico), using three different medfly strains routinely mass-reared for SIT operations. To ensure unbiased data collection as well as comparability of the results, the video recording experiments were conducted by independent researchers from the three locations following a standard protocol, while most of the ethological analysis of the recorded material was conducted by the same team at ICIPE using a consistent methodological approach.

Biological Material

The following medfly strains were used:

– In 1994-1995, the G-47 temperature sensitive lethal (*tsl*) strain, created and produced in the mass-rearing facility of FAO/IAEA Joint Division, Seibersdorf, Austria, was used. At the time of the experiments, the strain was mass-produced for a pilot SIT demonstration programme in Tunisia and, for this study, samples of routinely produced and irradiated flies were used. Later on, maintenance of the G-47 strain was discontinued. The pupae of the G-47 strain were gamma-irradiated (minimum absorbed dose: 14 Krad) in oxygen containing atmosphere, following the procedures used at IAEA at the time of experiments. Courtship behavior of both irradiated and non-irradiated medfly males was video-recorded for 90 min.

– In 1996, the wild type Mendoza strain adapted to mass-rearing conditions was used. This strain, produced in the mass-rearing facility located in Mendoza, Argentina, was used for

the large-scale medfly control/eradication program in Cuyo and Patagonia regions. For logistic reasons, a sample of flies from Mendoza facility was transferred to the INTA laboratory in Buenos Aires and reared for one generation following Teran's (1977) method as described by Calcagno et al. (1996). Pupae were irradiated 48 h before emergence with a Philips X ray emission device, under normal atmosphere. The applied doses were as follows: 14, 7 and 3.5 Krad, and an additional non-irradiated group was used as a control. Pupae were kept under controlled conditions (23-25ºC, L:D 12:12). Every day, emerged adults were sexed, to insure virginity of both males and females. The effects of irradiation were tested under field cage conditions and video-recorded for 30 min in the laboratory in small chambers. For the field tests, pupae were X-irradiated with doses of 14, 7, 3.5 Krad and non-irradiated control. For the video recording experiment only 14 Krad and control (non-irradiated) males were used. The dose of 14 Krad is equivalent to the irradiation dose used in Seibersdorf in the above-described experiments.

– In 1998, a bisexual strain was used, which was mass-produced in the MOSCAMED facility in Metapa, Mexico for medfly suppression operations along the Mexican—Guatemalan border. The insects were taken directly from the mass rearing facility. The pupae were gamma-irradiated (minimum absorbed dose: 14 Krad) in hypoxia (closed full container, at least 30 min before exposure), following the procedures used at MOSCAMED at the time of experiments. Courtship behavior of both irradiated and non-irradiated medfly males was video-recorded for 30 min.

In all the experiments, both the irradiated and non-irradiated males were paired with non-irradiated mass-reared females originating from the same strain. All insects were virgin and mature, 7-12 days old.

Field-cage Experiments

The field cage was built around a young citrus tree. Every day 30 virgin mature males from Mendoza strain representing each class of irradiation dose (14, 7, 3.5 Krad and non-irradiated control) were released into the cage at 08.00 AM (local time). After one hour, in which they had the opportunity to establish the territory and integrate into leks, 30 virgin non-irradiated females were released into the cage. Two days before the tests, the males were etherized and marked on the scutellum with a water-based paint, using color codes to identify the corresponding radiation dose.

Every hour from 10:00 AM to 14:00 PM, the cage was monitored and number of mating pairs was recorded. The mating pairs were removed and the number of pairs along with the male category (radiation dose) and the position of the mating pair inside the cage were recorded. Every day after the test, all non-mated individuals were removed from the cage. The experiment was repeated 8 times between April 30 to May 9 1996.

Video Recording

The recordings were conducted using the methodology and set-up proposed by Lux (1994). A sound-proof room was maintained at approximately 23-26°C. The equipment consisted of a Sony Hi 8 video camera (Model CCD-TR805, Japan) with a Novoflex (Germany) macro lens, a color TV, a Hi 8 videocassette recorder, and a microphone (Sennheiser, Model K6P/MKE102, Germany).

Cylindrical mating cages (70 mm × 85 mm diam.) were used, made from acrylic pipe. The top of the cage was covered with a Petri dish and the open basis was placed on a 2 mm thick transparent glass plate. The video recording was carried out through the glass from below. To simulate natural field conditions, a fresh lemon or coffee leaf was placed inside the cage and fixed to its top cover and, as it usually happens in the field, the males tended to establish their territories on the underside of the leaves. A microphone for recording sound signals was inserted through a lateral hole in the cage. Another lateral hole permitted the release of flies into the cage.

In the experiments with the Seibersdorf strain, the males used for video recording were selected at random from the mass reared population. In the case of Mendoza and Moscamed strains the males were randomly pre-selected. Each morning, several mating cages were prepared. Approximately 30-60 min prior to the start of the recording, a male was gently placed into each mating cage and allowed to calm down and establish a territory. The first male that began to call (i.e., release pheromone from the abdomen) continuously for 5 min was chosen for the first recording.

Five min after releasing the male into the cage (Seibersdorf) or five min after the males started calling (Moscamed and Mendoza), a female was gently released into the same cage. Immediately following release of the female, behavior of the male and its interactions with the female were recorded for 30 min, except the Seibersdorf strain, where male behaviour was recorded for 90 min. The video recordings were carried out between 10:00 AM and 02:00 PM, which was found to be the period of the highest diurnal mating activity rate. About 30-40 recordings were conducted for each strain and treatment. A male was considered successful if copulation occurred within the observation period.

Data Analysis

Male behavior was categorized into the following activities/stages: 1. Stationary (the male remains still, cleaning), 2. Stationary Calling (the male remains still and calling, i.e. emitting pheromone), 3. Mobile Calling (calls as in the previous case but being mobile), 4. Fanning (continuous fanning-wing vibration upon detecting the presence of a female so that pheromone is directed towards her), 5. Wing Buzzing (intermittent buzzing that is produced by means of an intense wing vibration, performed alternately with short periods of head rocking, which starts when a female approaches and remains in front of the male), 6. Head Rocking (rapid movements of the head rotating toward both sides, which accompany the Buzzing), 7. Missed Jump (male jumps, trying to mount the female, but he fails to achieve it), 8. Violent Mounting Attempt (male jumps onto a female but she rejects him violently, only exceptionally this activity is followed by copulation), 9. Peaceful Mounting Attempt (male jumps onto a female and reorients parallel to her, the female is cooperative and does not object, usually this activity is followed by copulation), 10. Copulation (after jumping and reorienting on the female the male is accepted, which is followed by intromission of the aedeagus), 11. Fight (aggressive interaction initiated by the male, the female or both).

Quantitative ethological analysis was performed using QuantEtho software (Lux 1989). The program selected a specified part of each observation and calculated:

- mean duration of each activity (its variability and standard error),

- number of occurrences and total time spent for each activity,

- probability of passing from other activities to the given one (input chances),

- probability of passing from the given activity to others (output chances), and

- ratio of time spent for each activity.

For each analyzed part of an observation, a chart of time-budget was prepared showing the percentage of time spent on each activity during the observation.

Each time when the two observed individuals (a male and a female) were closer to each other than 3 cm, responses of both, the male and female, as well as the interactions between them, were quantified. When necessary, the frame-by-frame function of the time-lapse video recorder was used, which allowed a 1/30 second reviewing resolution and analysis of very short-lasting responses, such as touching a male by the front legs of a female.

The analysis of all recordings produced a large body of numerical data and charts and only a small part of it is presented in this paper. The complete data set was recorded on CD-ROM (Lux et al. 1999) and deposited at FAO/IAEA at Seibersdorf, Austria and at ICIPE, Nairobi, Kenya.

RESULTS AND DISCUSSION

A typical sequence of a successful male courtship was described by Feron (1962) and is comprised of the following main steps: calling, fanning (wing vibration), wing buzzing, mounting attempt and copulation. Several other behavioral elements were described by Lux & Gaggl (1995), which indicate that medfly courtship is a kind of a "dialogue" with intensive exchange of signals between sexes. Females display a rich repertoire of responses to courting males, such as touching a male with her head or front legs, brief jumping towards the male, short wing vibrations and stretching wings just after mounting. Most of these activities are very short-lasting, within a range of 1/30-1/10 second, and thus were not noticed in the earlier studies.

For all the strains tested, no major qualitative differences were found in the courtship pattern between irradiated and non-irradiated males. In both cases, the courtship sequences were composed of the same major behavioral steps and each activity, if it occurred, was performed in a similar manner. The irradiated males were generally able to display courtship and mate. However, several consistent quantitative differences between irradiated and non-irradiated males were noticed.

The most important difference was that the average mating performance of the irradiated males was reduced nearly two-fold as compared to the non-irradiated males. However, during 30 min of the video-recording experiment, the mating success of males from the Seibersdorf strain was about 8 times lower compared to that of the Moscamed and Mendoza strains observed over the same period. With such an extremely low numbers of mating males, the difference between the irradiated and non-irradiated Seibersdorf males was difficult to interpret and was not significant (Table 1). The low performance of this strain is most likely to have been caused by the fact that the males used for recording were selected at random from the entire mass-reared population. In contrast, the males from the other two strains were pre-selected before recording, and only sexually motivated and calling males were used. Indeed, in the case of the Moscamed and Mendoza strains, the number of matings was much higher, and in both strains, the reduction in mating performance caused by the irradiation process reached 40%. However, only 30 pairs were observed from the Moscamed strain, and the difference between the irradiated and non-irradiated males, though close to the borderline of

TABLE 1. EFFECT OF IRRADIATION ON MATING SUCCESS (DURING 30 MIN OBSERVATION).

Strain origin	Irradiated males			Non-irradiated males			Reduction in performance	
	Mating success	S	F	Mating success	S	F	percent	$P*$
Seibersdorf G-47	3.6%	1	27	7.1%	2	26	50.0%	0.553
Moscamed	30.0%	9	21	50.0%	15	15	40.0%	0.114
Mendoza	37.5%	15	25	62.5%	25	15	40.0%	0.025

*Significance level in one-sided chi² contingency test.
S—successful males which started copulation within 30 min.
F—failures, number of unsuccessful males which failed to copulate within 30 min.

statistical significance ($P = 0.114$), was not significant. In the case of the Mendoza stain, more pairs (40) were observed, and the same level of reduction was highly significant ($P = 0.025$) (Table 1). It was also noticed that the irradiated males engaged in mating later than the non-irradiated ones, a trend well represented in the time budgets of the Moscamed strain (Fig. 1). Remarkably, the differences between irradiated and non-irradiated males were noticed even though the video-recordings were conducted in extremely limited space and each female was forced to repeatedly interact with a given single male, having only a "NO-choice and NO-retreat" option.

When given more time, the males from the Seibersdorf strain finally reached a similar mating frequency as the two other strains. However, when the females were forced to interact with the males for so long in the restricted space, over 40 meetings might occur during the 90 min of observation. Under such conditions, the consistent (albeit not significant) differences in performance between the irradiated and non-irradiated males gradually became relatively less apparent (50%, 15% and 12% in reduction in mating success during 30, 60 and 90 min of observation, respectively). The results appear to indicate that the method for selection of males from the mass-reared population, and the duration of the quality control test, are important factors determining the sensitivity of the test and its ability to detect deficiencies in male quality. More research, however, will be required to confirm and quantify such effects and to optimize the quality control tests.

Analysis of the recordings further revealed that the irradiated males were more passive and less vigorous. They spent relatively more time passive ("resting"), and less time walking or flying (Table 2). Activity indices, ratio of the time spent active (walking or flying) to the time spent passive, as measured for the whole sample of males from each strain, not only represented the overall vigor of the strain, but were also found to be indicative of the strain's mating success (Fig. 2).

It appears that the irradiated males were less motivated sexually; more frequently they did not

call (did not emit pheromone) just before meeting with a female, even though they sometimes mated during the same meeting (Table 3). These non-calling males did not attract females, but rather met them by chance. As a consequence, the frequency of meetings with females was lower for the irradiated males as compared to the non-irradiated males (Table 3). When very close to the approaching female, the irradiated males tended to move towards her, which often provoked a fight and resulted in more frequent aggressive interactions during meetings (Table 3).

During courtship, females interacted with courting males by touching a male with her head or front legs, brief jumping towards the male, short wing vibrations, lowering wings and stretching wings just after mounting. Several differences were detected in the frequency of female responses to courtship between irradiated and non-irradiated males, both during wing vibration and buzzing (Table 4). Though it appears that the frequency and structure of female responses might be indicative of the quality of male courtship, the exact role of these behaviors and their relation to the probability of mate acceptance and successful mating remain unclear.

In general, non-irradiated males were more "patient" and allowed females to approach very closely and interact. During buzzing, they more frequently remained still and were less likely to approach or "push" the courted female (Table 5). Such a steady courtship has been reported to be a general trait of successful males, as opposed to the "impatient" courtship of those males which were usually later rejected by females and failed to mate (Lux & Gaggl 1995, Lux et al. 1996). Interestingly, in those cases where the courtship successfully progressed until a mounting attempt, no differences were noticed between irradiated and non-irradiated males.

Under the described experimental conditions with such drastically restricted space, frequent and random interactions may sometimes result in a successful mating, even in the case of males which are less sexually motivated or less competitive. In the field, however, a non-calling male
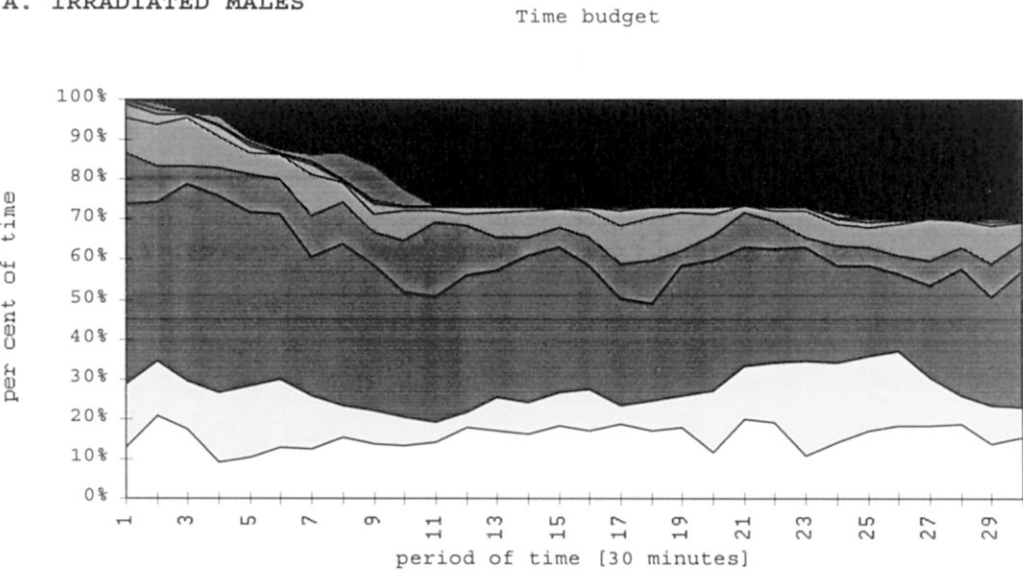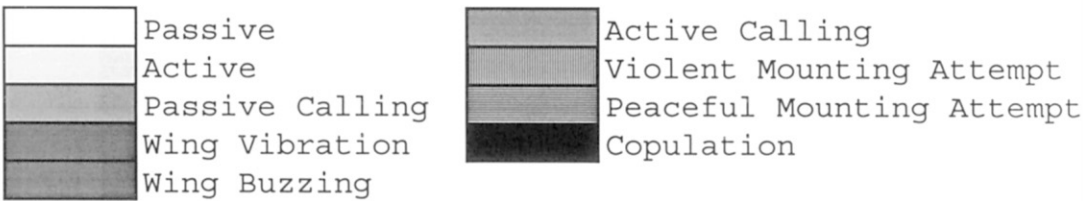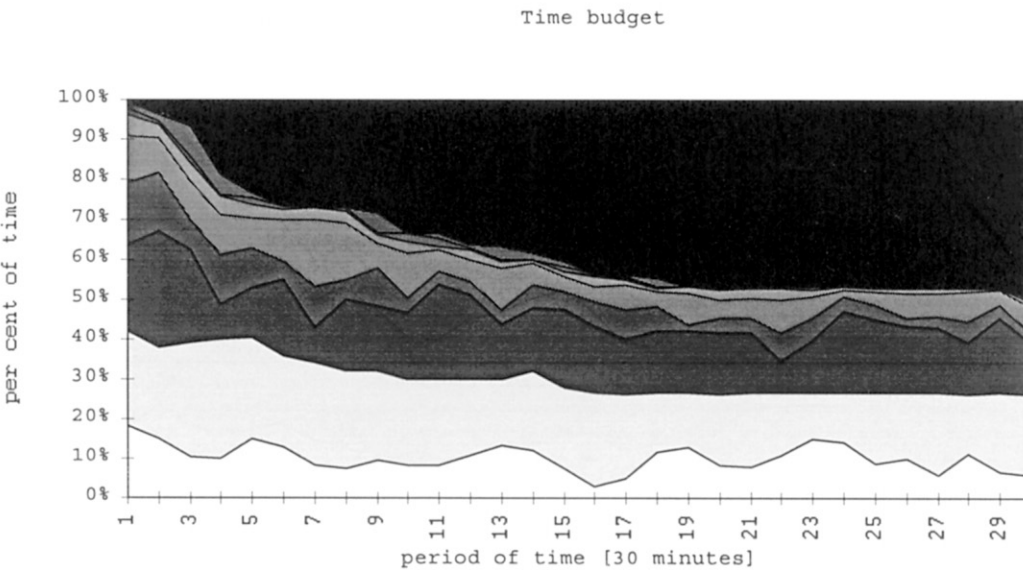
Fig. 1. Effect of irradiation on performance of medfly males (Moscamed strain).

TABLE 2. AVERAGE TIME IN SECONDS SPENT ON VARIOUS ACTIVITIES DURING 30 MIN OBSERVATION.

Activity	Seibersdorf G-47		Mendoza		Moscamed	
	IR	Non-IR	IR	Non-IR	IR	Non-IR
Passive	1306.4	1281.6	90.0	12.6	294.5	187.2
Active	432.1	501.6	158.6	43.7	215.9	372.6
Average activity index: (time Active/time Passive)	0.33	0.39	1.76	3.46	0.73	1.99
Significance level	n.s.		*		*	

*Significant difference (*P* < 0.05) in the average activity index between irradiated and non-irradiated males from the same strain, according to t-test conducted using activity indexes calculated for individual males from each group.
IR = irradiated, Non-IR = non-irradiated.

would have a negligible chance of meeting with a female and mating. Such males would most likely not appear or establish themselves in lek arenas or participate in calling activities, or attract females. Hence, females would be unlikely to find them and mate. Furthermore, those poorly performing males which, although suffering rejection several times, finally managed to mate, would be unlikely to have so many opportunities when released into the field. Therefore, the described differences between irradiated and non-irradiated males should be expected to be far more apparent under field conditions. Indeed, the detrimental effects of irradiation on mating performance were found to be more explicit in the field cage test (Table 6), where much more space was available and the females were given a chance to choose among males irradiated at various doses.

The results also revealed that with increasing dose of radiation, a male's ability to participate in lek formation was reduced. The males which received a high radiation dose (7 or 14 Krad) were recorded on the tree less frequently than the males which received the lowest dose (3.5 Krad) or no radiation at all (Table 7). Although the integration of males into leks was not quantified in relation to the corresponding radiation dose, it was noticed that the males irradiated with 14 Krad tended to stay on the floor and only rarely joined leks on the tree. In addition, these males tended to keep calling on the tree in the afternoon when most of the other males had already stopped calling.

The success of an SIT program relies principally on reduction of the reproductive potential of wild females by induced egg sterility caused by the release of sterile males. This in turn depends on a balance among several factors, such as the degree of sperm sterility induced by irradiation; male mating competitiveness; and a male's ability to transfer sperm to female spermathecae in order to switch off her receptiveness, thus preventing re-mating and inducing egg laying behavior. Interestingly, males irradiated with 3.5 Krad mated in the same proportion as non-irradiated ones, indicating a similar mating competitiveness (Table 6). Therefore, the use of low doses of radiation allows good mating performance, although not ensuring total male sterility. In fact, it has been
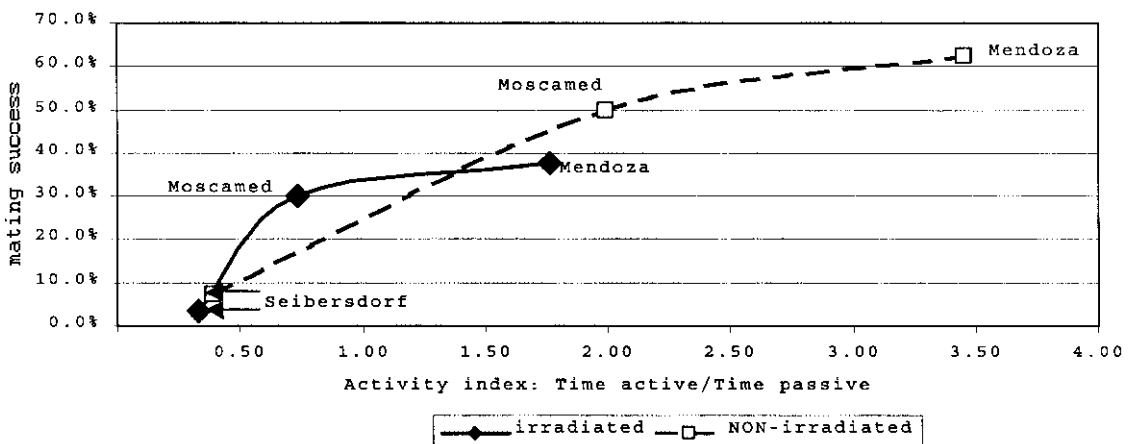

Fig. 2. Relation between activity index and mating success for irradiated and NON-irradiated males.

TABLE 3. THE EFFECT OF IRRADIATION ON MALE-FEMALE INTERACTIONS DURING MEETING.

Meeting (male & female are closer than 3 cm)	Seibersdorf G-47 (90 min observation)			Moscamed (30 min observation)		
	IR	Non-IR	P[a,b]	IR	Non-IR	P[a,b]
Average number of meetings/pair	5.87	7.00	n.s.	4.68	8.92	*
Male calling just before the meeting	57%	73%	0.003	85%	90%	0.201
Male approaching female during meeting	22%	16%	0.154	6%	1%	0.013
Fight during meeting	18%	4%	0.000	10%	6%	0.211

[a]Significance level (presented in as the exact probability) in one-sided chi² contingency test, conducted using the actual frequencies of events.
[b]Significance level (presented as: n.s. if $P >= 0.05$, or * if $P < 0.05$) in t-test, conducted using numbers of events performed by each male.
IR = irradiated, Non-IR = non-irradiated.

demonstrated that partially sterile males irradiated with doses close to 3.5 Krad may actually be more effective in reducing overall reproductive potential of females (measured by number of non-hatched eggs) than totally sterile males irradiated with higher doses (Favret et al. 1995).

In the past, when only bisexual medfly strains were available and used, applying a high radiation dose which would guarantee total sterility of both sexes was necessary to ensure the efficiency of SIT treatment, as well for the regulatory reasons. Our results revealed, however, that such an irradiation strategy partially incapacitates the mass-reared males, which is substantially reducing their performance and increasing overall costs of the SIT operations. Since "male-only" strains are now available, the irradiation strategy should now be re-considered. Partially sterile but highly competitive males could be released

and may prove more effective than the totally sterile, but less competitive males produced at the moment.

Several improvements in the irradiation treatments have been suggested in the past (Hooper 1971a, Ohinata et al. 1977, Zumreoglu et al. 1979) which might reduce the damage caused by the sterilization process. However, since the irradiation process was widely considered to cause only insignificant damage to the sterilized males, in most mass rearing facilities, such improvements, though well known, have not been applied, and presentations of our early results (Lux et al. 1996b, Lux et al. 1997) were received with skepticism. To corroborate our earlier results, several additional independent experiments (reported in this paper) were conducted in Argentina and Mexico. The consistent results obtained not only confirm the significance of the negative effects of

TABLE 4. THE EFFECT OF IRRADIATION ON FEMALE RESPONSES DURING COURTSHIP.

Stage of courtship and female response	Seibersdorf G-47 (90 min observation)			Moscamed (30 min observation)		
	IR	Non-IR	P[a]	IR	Non-IR	P[a]
Wing fanning (vibration)						
a. touching male with front leg/legs	0.62	0.93	*	0.11	0.11	n.s.
b. touching male him with head	0.21	0.14	n.s.	0.06	0.08	n.s.
c. sudden jump towards male	0.20	0.14	n.s.	0.01	0.08	*
d. vibrating wings (very short)	0.03	0.22	*	0.00	0.00	n.s.
e. lowering wings	0.00	0.00	n.s.	0.00	0.00	n.s.
Wing buzzing						
a. touching male with front leg/legs	3.94	3.53	n.s.	3.38	3.00	n.s.
b. touching male him with head	0.96	0.69	n.s.	0.71	0.96	n.s.
c. sudden jump towards male	0.38	0.45	n.s.	0.52	0.82	n.s.
d. vibrating wings	0.25	0.83	*	0.00	0.03	n.s.
e. lowering wings	0.10	0.00	*	0.00	0.00	n.s.

[a]Significance level (presented as: n.s. if $P >= 0.05$ or * if $P < 0.05$) in t-test, conducted using numbers of events performed by each female.
IR = irradiated, Non-IR = non-irradiated.

TABLE 5. THE EFFECT OF IRRADIATION ON MALE BEHAVIOUR DURING ADVANCED STAGE OF COURTSHIP (BUZZING).

Buzzing	Seibersdorf G-47 (90 min observation)			Moscamed (30 min observation)		
	IR	Non-IR	P^a	IR	Non-IR	P^a
Male "patience" during buzzing:						
a. remains still while buzzing	56%	64%	0.28	53%	68%	0.026
b. approaches and/ or pushes female	44%	36%		47%	32%	

[a]Significance level (presented in as the exact probability) in one-sided chi^2 contingency test, conducted using the actual frequencies of events. IR = irradiated, Non-IR = non-irradiated.

TABLE 6. FIELD CAGE TEST: EFFECT OF IRRADIATION DOSE ON MALE MATING PERFORMANCE (MENDOZA STRAIN).

Radiation dose (rad)	No. of matings	Mating success (%)	Results of Chi^2 test
0	71	41 a	
3500	69	40 a	Chi^2 = 0.04, P = 0.841
7000	29	16 b	Chi^2 = 26, P < 0.0001
14000	5	3 c	Chi^2 = 87, P < 0.0001

Numbers followed by the same letter are not significantly different.

TABLE 7. EFFECT OF IRRADIATION DOSE ON SELECTION OF MATING SITES WITHIN THE FIELD CAGE (MENDOZA STRAIN).

Radiation dose [rad]	Screen	Tree	Results of Chi^2 test
0	18	53 a	
3500	22	47 a	
7000	13	16 a	Chi^2 = 3.64, P = 0.16
14000	4	1 b	Chi^2 = 8.78, P = 0.032

Numbers followed by the same letter are not significantly different.

the irradiation process and re-emphasize the critical need for using hypoxia or a nitrogen atmosphere during irradiation, but have also triggered the process of re-investigating the sterilization strategy and methods.

The most recent findings (Rendon 1999) indicate that the detrimental effects of the same dose of irradiation were much more severe in the case of mass-reared males as compared to wild males. This suggests that reduced male tolerance to irradiation and the consequent increased severity of the damage caused by the irradiation process, may depend on the number of generations of a strain under mass rearing. If confirmed, this would add one more reason for periodically changing the mass-reared strains and maintaining strict quality control regimes in the rearing process.

It is being recognized that ensuring a high level of mating competitiveness of mass-reared irradiated males and, at the same time, maintaining economic efficiency in the mass rearing process, presents an enormous challenge. Ultimately, however, the increased rearing or processing costs are likely to be offset by benefits from the increased efficiency of SIT operations, if the same results can be achieved by releasing smaller numbers of more competitive insects.

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CONSISTENCY IN COURTSHIP PATTERN AMONG POPULATIONS OF MEDFLY (DIPTERA: TEPHRITIDAE): COMPARISONS AMONG WILD STRAINS AND STRAINS MASS REARED FOR SIT OPERATIONS

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ABSTRACT

The objective of the study was to compare courtship behavior of various wild and mass reared medfly strains, in order to document the degree of diversity in courtship behavior among medfly populations and to assess its implications for strategy of application of the Sterile Insect Technique. Recordings of medfly courtship behavior were collected from several locations world-wide using a standard protocol. Qualitative and quantitative analysis of the collected behavioral materials was conducted. No major differences were found among the strains both in male and female behavioral repertoire, which indicates general lack of behavioral incompatibility among the strains studied. However, the analysis revealed several qualitative and quantitative differences in courtship details among locations. The females from Madeira strain were more "choosy" then those from other strains, rejecting male courtship most frequently in spite of the fact that the males from this strain displayed their courtship activities in the most expressed manner. It has been suggested, therefore, that development of an efficient strain for world-wide application shall be based on the most competitive strains (such as Madeira strain), and only individuals with the most pronounced pattern of male courtship should be selected as founders.

Key Words: *Ceratitis capitata*, Mediterranean fruit fly, medfly, mating behavior, courtship, strain comparisons

RESUMEN

El objetivo de este estudio fue de comparar el comportamiento en el cortejo de varias razas de mosca del Mediterráneo salvajes y criadas en masas, de manera de determinar el grado de diversidad en el comportamiento del cortejo entre poblaciones de moscas del Mediterráneo y para poder analizar sus implicaciones para la estrategia de aplicación de la técnica del insecto estéril. Información con respecto al comportamiento del cortejo con la mosca del Mediterráneo fue recolectada de varias localidades alrededor del mundo utilizando un protocolo estándar. Análisis cualitativos y cuantitativos de los materiales de comportamiento recolectados fueron conducidos. No se encontraron mayores diferencias entre las razas, ambas con un repertorio de comportamiento entre machos y hembras, lo cual indica una carencia general de incompatibilidad de comportamiento entre las razas estudiadas. Sin embargo, el análisis reveló varias diferencias cualitativas y cuantitativas en cuanto a detalles en el cortejo entre las localidades. Las hembras de la raza proveniente de Madeira fueron más selectivas

que aquellas de otras razas, rechazando el cortejo de los machos con mayor frecuencia a pesar de que los machos de esta raza desplegaron sus actividades de cortejo de la manera más expresiva. Se ha sugerido, por lo tanto que el desarrollo de una raza eficiente para la aplicación a nivel mundial se debe basar en las razas más competitivas tal como la raza de Madeira), y solamente individuos con el patrón más pronunciado de cortejo por parte del macho deben seleccionarse como fundadores.

The Mediterranean fruit fly (medfly), *Ceratitis capitata*, is a pest of African origin which, over the last hundred years, has expanded beyond its original area and colonized substantial part of other tropical and sub-tropical regions world-wide. Such extensive expansion necessitated adaptations to new ecological conditions and host ranges. Usually, such processes result in diversification within the expanding species, which may progress till separation into geographical races. Such races may differ in many respects, among others, in the pattern of reproductive behavior or composition of the signals used in intra-specific communication. In more extreme scenario, it may ultimately result in partial or complete sexual incompatibility among the most isolated sub-populations. Numerous examples of such processes, already accomplished by other expanding insect species, are well documented. In the case of medfly, however, in spite of its importance, the question of the degree of behavioral homogeneity among its populations or possible existence of behavioral geographical races, has not been addressed before.

Currently, medfly control is largely based on application of the Sterile Insect Technique (SIT). The SIT relies on reducing the reproductive potential of wild females by induced egg sterility caused by the release of sterile males. This can be achieved only if the released sterile males substantially participate in mating with wild females. In the past, the SIT was usually based on local wild medfly populations adapted to the mass rearing conditions. In such cases, the question of possible geographical differences and mating incompatibility among strains was of no practical relevance.

Recently, however, the SIT is increasingly based on mono-sexual strains artificially created by genetic selection and manipulation. Development of such strains is complicated and expensive. At the same time, spectacular successes of SIT operations resulted in increased number of requests for its application in many regions all over the world. Building separate mass rearing facilities in each of these regions and creating mono-sexual strains based on local populations would drastically increase costs of SIT. However, using insects produced in already established facilities in any part of the world would be possible, if behavioral homogeneity of world medfly populations and lack of behaviorally incompatible races were confirmed. Technologies already exist which allow sending insects among so distant locations as from Guatemala to Israel, without substantial loss in their quality (Taylor et al. in press) Relatively inexpensive and reliable global air transport systems make such approach economically and technically viable. Hence, the questions of behavioral homogeneity of world medfly populations and the degree of their mating compatibility have recently gained enormous practical importance.

Presented research was focused on comparison of typical patterns of male courtship behavior among the strains studied, when the males interacted with females from the same strain. The study was designed to support extensive field cage evaluations of inter-strain mating compatibility among various wild medfly strains and the strains mass reared for SIT operations.

MATERIAL AND METHODS

Biological Material

The experiments were conducted using wild medfly from various locations and several medfly strains mass-reared for SIT operations or maintained in laboratory colonies. The wild medflies used in the experiments originated from the following locations: Argentina (Patagonia, collected from peaches), Greece (Crete, collected from oranges), Guatemala (collected from coffee), Israel (collected from oranges), Kenya (collected from coffee near Ruiru, Central Province), Madeira (collected from oranges) and Reunion (collected from guava). The wild insects were reared from fruit and, after reaching sexual maturity, were used for video-recording. The mass reared medfly strains used for the experiments originated from the following mass rearing facilities and laboratories: Madeira in Portugal, Mendoza in Argentina, Moscamed in Guatemala, Metapa in Mexico, Seibersdorf in Austria, and from experimental colonies maintained in Tel Aviv, Israel and at IC-IPE in Nairobi, Kenya. Details about the strains used in the study are given below:

– Seibersdorf: the G-47 temperature sensitive lethal, *tsl,* strain, created and produced in the mass-rearing facility of FAO/IAEA Joint Division, Seibersdorf, Austria, was used. At the time of the experiments, the strain was mass-produced for a pilot SIT demonstration program in Tunisia and, for this study, samples of routinely produced and irradiated flies were

used. Later on maintenance of the G-47 strain was discontinued. Courtship behavior was video-recorded for 90 min.

– Argentina: the wild type Mendoza strain adapted to mass-rearing conditions was used, which was produced in the mass-rearing facility in Mendoza, Argentina for large-scale medfly control/eradication program executed in Patagonia. For logistic reasons, a sample of flies from Mendoza facility was transferred to the laboratory in Buenos Aires and reared for one generation following Teran's (1977) method as described by Calcagno et al. (1996). Pupae were kept in a chamber at 23-25ºC with a 12:12 L:D photoperiod. Every day emerged adults were sexed, to ensure virginity of both males and females. Adult flies were aged up to 10-12 days to reach sexual maturity before the experiment. The courtship behavior was video-recorded for 30 min.

– Mexico: a bisexual strain was used which was mass-produced in Moscamed facility in Metapa, Mexico for routine medfly suppression operations along Mexican-Guatemalan border. The insects were taken directly from the mass rearing facility. Courtship behavior was video-recorded for 30 min.

– Madeira: only wild flies were used, collected as pupae from the field, shipped to Seibersdorf, Austria where wild adults were tested.

– Greece: the laboratory strain used in the experiments was SEIB 6-95, a genetic sexing strain mass-reared in the University of Crete and carrying a white pupae, *wp*, mutation. Wild flies used were collected locally as pupae from oranges.

– Guatemala: Vienna-42/Guatemala (also called Toliman-*tsl*) genetic sexing strain, mass reared in El Pino facility, and carrying both a *wp* and a *tsl* mutations with a Guatemalan genetical background, was used in the experiments. Wild flies were collected as pupae from coffee.

• Israel: the laboratory strain used was maintained under small-scale rearing conditions in Bet Dagan for several years, and "refreshed" by adding wild insects every two years. Wild flies were collected as pupae from citrus fruits.

• Reunion: an established laboratory colony was used as laboratory flies and compared with wild flies collected as pupae from fruits.

• Kenya: a laboratory colony was established using medflies reared from coffee collected in Ruiru, near Nairobi, Kenya. The insects were maintained in laboratory in Plexiglas cages (about $50 \times 50 \times 50$ cm) at rather low density. The colony was maintained for about two years, and was renewed on a regular basis by frequent adding wild-collected insects.

In all the experiments, males were paired with females originating from the same strain. All insects were virgin and mature, 7-14 days old.

Data Collecting and Analysis

To ensure unbiased data collection as well as comparability of the results, the video recording experiments were conducted by independent researchers from each location following a standard protocol, while the ethological analysis of the recorded material was conducted by the same team at ICIPE using consistent methodological approach.

Video Recording. The recordings were conducted using the methodology and set-up proposed by Lux (1994). A sound-proof room was maintained at approximately 23-26°C. The equipment consisted of a Sony Hi 8 video camera (Model CCD-TR805, Japan) with a Novoflex (Germany) macro lens, a color TV, a Hi 8 videocassette recorder, and a microphone (Sennheiser, Model K6P/MKE102, Germany).

Cylindrical mating cages (70 mm × 85 mm diameter) were used, made from acrylic pipe. The top of the cage was covered with a Petri dish and the open base was placed on a 2 mm thick transparent glass plate. The video recording was carried out through the glass from below. To simulate natural field conditions, a fresh lemon or coffee leaf was placed inside the cage and fixed to its top cover and, as it usually happens in the field, the males tended to establish their territories on the underside of the leaves. A microphone for recording sound signals was inserted through a lateral hole in the cage. Another lateral hole permitted the release of flies into the cage.

In the experiments with the Seibersdorf strain, the males used for video recording were selected at random from the general population. In the case of other strains, the males were randomly pre-selected. Each morning, several mating cages were prepared. Approximately 30-60 min prior to the start of the recording, a male was gently placed into each mating cage and allowed to calm down and establish a territory. The first male that began to call (i.e., release pheromone from the abdomen) continuously for 5 min was chosen for the first recording.

Five min after releasing a male into the cage, a female was gently released into the same cage. Immediately following release of the female, behavior of the male and his interactions with the female were recorded for 30 min, (except the Seibersdorf strain, where male behavior was recorded for 90 min). The video recordings were carried out between 10 AM and 2 PM, which was the period of the highest diurnal mating activity rate. About 30-40 recordings were conducted for each strain and treatment. A male was considered successful if he copulated within the observation period.

Data analysis. Male behavior was categorized into the following activities: calling, fanning (wing vibration), wing buzzing, mating attempt and copulation. The analysis of the recorded material was performed in three steps: (1) description of general structure of courtship sequence and pattern of component behaviors, (2) quantitative description of male behavior during observation, and (3) quantification of male-female interactions when both were close to each other.

In the case of some strains, departures from the standard recording protocol made it difficult or impossible to perform quantitative analysis, and in such cases the analysis was restricted only to the first step; description of general pattern of courtship sequence and its component behaviors. In particular, non-continuous recording sessions (recording only when interaction between male and female were noticed) made it impossible to perform the second step of analysis. Insufficient magnification or inadequate following the two individuals and adjusting magnification to keep continuously both in the field of view, made the third step of analysis difficult.

Whenever quality of the recordings allowed, each strain, both wild and mass reared ones, was analyzed as a whole respective sample, and than various parts of the recordings of each strain were selected for additional analysis: successful males, unsuccessful males, successful males—5 min before copulation. Quantitative ethological analysis was performed using QuantEtho software (Lux 1989). The program selected a specified part of each observation and calculated:

- mean duration of each activity (and its variability and SE),

- number of occurrences and total time spent on each activity (and their variability and SE)

- probability of passing from other activities to the given one (input chances),

- probability of passing from the given activity to others (output chances), and

- ratio of time spent for each activity.

For each analyzed part of observation, a chart of time budget was prepared showing a percentage of time spent on each activity during analyzed part of the observation.

Each time, when the two observed individuals (a male and a female) were closer than 3 cm to each other and the magnification rate and quality of the recordings was sufficient, responses of both a male and a female as well as interactions between them, were quantified. When necessary, the frame-by-frame function of the time-lapse video recorder was used, which allowed 1/30 second reviewing resolution and analysis of very short-lasting responses, such as touching a male by front legs of a female.

The analysis of all recordings produced a very large body of numerical data and charts and only a very small part of it is presented in the paper.

RESULTS AND DISCUSSION

In medfly, the males collectively attract females by forming leks. The courtship is initiated whenever a female approaches one of males from the lek (Arita & Kaneshiro 1985, Calkins 1987, Prokopy & Hendrichs 1979, Whittier & Kaneshiro 1991 and 1995, Whittier et al. 1994). Typical sequence of a successful male courtship was described by Feron (1962) and comprises of the following main steps: calling, fanning (wing vibration), wing buzzing, mating attempt and copulation. The collected recordings and quantitative material allowed comparisons among strains taking various behavioral aspects and parts of the behavioral spectrum into consideration.

Non-reproductive Activities and Transition into Reproductive Behaviors: Calling and Courtship

The strains differed in general level of activity (time spent active versus time spent passive) and average duration of the component behaviors (Tables 1 and 2). They could be broadly characterized into two groups. The first group, represented by the strains from Seibersdorf and Argentina (both reared and wild) had rather short active and pas-

TABLE 1. CHARACTERISTICS OF LABORATORY INSECTS.

Laboratory insects	Israel	Mendoza	Kenya (ICIPE colony)	Moscamed	Seibersdorf G-47
Passive—average duration of a single act	97.04	23.66	38.78	25.08	18.86
Active—average duration of a single act	30.11	13.7	35.57	34.55	10.18
Activity index (time spent active/passive)	0.35	0.74	0.57	1.58	0.51
No. of fights with a female/observation	0.28	0.10	0.13	0.88	0.72
Transition to calling	20.9%	16.8%	5.6%	13.0%	2.0%
Transition to courtship	25.4%	26.7%	7.1%	18.0%	3.5%

TABLE 2. CHARACTERISTICS OF WILD INSECTS.

Wild insects	Israel	Patagonia	Kenya
Passive—average duration of a single act	35.92	19.09	41.4
Active—average duration of a single act	28.62	13.55	36.86
Activity index (time spent active/passive)	0.79	0.87	1.00
No. of fights with a female/observation	0.10	2.14	0
Transition to calling	14.2%	18.4%	4.5%
Transition to courtship	16.4%	22.6%	4.5%

sive events (lasting about 20 and 10 s respectively), with frequent switches between each other. The second group, represented by the strains from Israel, Mexico and Kenya (both reared and wild) had longer active and passive events (lasting over 30 and about 35 s respectively) and, consequently, less frequent switches between the two. The strains also differed in the level of aggressiveness in male-female interactions (frequency of fights) and probability of progressing from the non-reproductive activities to reproductive ones, i.e. initiating calling and courtship, which seems to indicate variation in the level of sexual motivation. Generally, though less active, the mass reared insects were "more cooperative" and, while placed in a small video-recording chamber, they easier initiated calling and progressed into courtship, as compared to the wild insects newly transferred to the laboratory (Tables 1 and 2).

In the phase of non-reproductive activities, insects have to respond to fluctuating environmental conditions and this phase tends to be rather flexible and loosely organized, with its characteristics being largely modified by environmental factors. It has to be emphasized also, that the data were collected in the process of multi-location studies conducted by several independent observers, using insects from different climatic conditions, collected from various host plants (fruit species). Therefore, apparently substantial differences in these aspects of behavior may, at least to large extent, be attributed to variation in insect nutrition, stage of maturity, level of sexual deprivation, level of habituation to the confined laboratory conditions and crowding, and differences in handling procedures before the experiments. Though indeed, they may also indicate differences in general quality among the strains. In spite of largely standardized methodology being used, separating true inherent differences among strains from those influenced by the climatic or nutritional conditions or various insect treatment and handling procedures, presents a tremendous challenge. Certainly, generally easier initiation of courtship by the mass reared insects shall not be interpreted as an indication of their higher vitality. It simply reveals the effects of preselection during colonization period. As a result,

the mass reared insects seem to be less demanding in terms of the conditions required to initiate lek forming, calling and courtship, such as light intensity, temperature, humidity and environmental attributes, like canopy of the right plant species and accompanying volatiles.

Elaborate additional research would be required to separate and apportion contributions of environmental and inherent factors. However, even if confirmed to be partially inherent, differences in the characteristics of non-reproductive behaviors and general level of activity will be of limited relevance to the problem of possible mating incompatibility among strains.

Organization of the Courtship Sequence
and General Pattern of Reproductive Activities
(calling, courtship, and mating)

Remarkably, no major qualitative differences in the reproductive activities were found among the strains tested. In all cases (strains from; Argentina, Austria, Greece, Guatemala, Israel, Kenya, Madeira, Mexico, Reunion), the reproductive activities were composed of the same major behavioral steps. Within the sequence, the component activities (calling, wing fanning, buzzing, mating attempt and copulation) were organized in the same manner. In addition, each activity, if occurred, was performed in a largely similar pattern. The reared males from all the mass rearing facilities were generally able to display calling and courtship and their courtship did not differ from that of the wild males.

However, intra-specific communication in medfly relies not only on a sequence of behaviors (gestures), but also on several other modalities, such as chemical (pheromone) communication among males during lek formation, and a combination of chemical and acoustic signals exchanged between males and females during courtship. Differences among strains in terms of qualities of these signals were not studied, therefore, drawing definite conclusions may be premature.

Nevertheless, remarkable uniformity in the pattern of the activities and organization of the courtship sequence seem to indicate lack of mating incompatibility among various wild and mass

reared medfly strains. Indeed, results of parallel studies on direct mating compatibility among strains conducted in field cages generally supported such conclusion (Cayol 2000).

Detail Pattern of Main Courtship Activities

No differences in the detail pattern of calling, attempt to copulate and copulation were noticed among the strains. Also wing fanning was conducted in a similar manner and with similar frequency. However, several quantitative differences among strains were found (Tables 3 and 4) in average duration of calling (both passive and active), wing fanning and buzzing. Alike in the case of non-reproductive activities, the strains from Seibersdorf and Argentina (both reared and wild) had rather short calling events, both active and passive, with frequent switches between each other. The second group, represented by the strains from Israel, Mexico and Kenya (both reared and wild) had longer active and passive calling events and, consequently, less frequent switches between the two. The strains also differed in average duration of wing fanning (vibration). In the case of Kenyan strain (both wild and from lab colony), fanning lasted about twice as long as compared to the strains from Israel, Argentina and Mexico, while those from Seibersdorf G-47 strain was about three times shorter. The differences in male persistence in calling and courtship appear to be linked to the probability of mating success, and may likely indicate differences in mating competitiveness among strains (Lux et al. 1996). However, fanning and buzzing is influenced also by female responses to the courting male, and differences in their duration may also indicate variation in female sexual motivation or maturity due to substantial variability among strains in the period necessary for reaching sexual maturation. Such differences, however, are unlikely to contribute to possible mating incompatibility among strains.

Remarkably, substantial differences were documented in the pattern of buzzing. In general, buzzing is a vigorous display of both wings, presented during advanced stages of courtship. It is composed of several, rhythmically repeated, broad wing movements in horizontal plane (towards the back and towards the front) combined with wing vibration. Slow motion analysis revealed that the buzzing was actually composed of three autonomous elements, occurring in a sequence: vigorous wing movement with their vibration (0.12-0.16 s) followed by short break, though with continued wing vibration (0.04 s), and then head rocking (0.8-0.12 s).

In the case of some strains (especially in the case of flies from Madeira), the buzzing was preceded by brief burst of intense head rocking. Substantial differences in the intensity of head rocking were noticed among strains, though, in the experimental conditions (recording with a camera in vertical, bottom-up position), precise measurement of the angle or amplitude of head rocking was impossible. To accomplish it, video recording conducted in horizontal plane (head-on position) would be required. Nevertheless, the intensity of head rocking was broadly graded into three categories (intense, weak and none) and frequencies of courtships with intense or no head rocking were compared among strains (Table 5). Most frequently, intense head rocking was observed in the case of strains from Argentina (both wild and reared), Madeira and Greece (wild) (37%-73% of courtships). However, the most spectacular head rocking was observed in the case of males from Madeira, though it occurred less frequently then in the case of Argentina strains. Intensity of head rocking was not consistent among all individuals from these strains, some displayed less intense (weak) head rocking and quite a number did not display it at all (20%-63% of courtships). In the case of strains from Mexico and Guatemala, intense head rocking was observed rather seldom (9%-16% of courtships). No head rocking at all was observed in the case of strains from Israel (both wild and reared) and Seibersdorf (reared).

In general, advanced stages of courtship sequence tend to be generally more rigid, in terms of the pattern of the activities involved and in terms of their quantitative characteristics, with a trend of progressive reduction in variability within the subsequent steps. For example, variability index of average duration of wing vibration ranged between 1.13 and 1.6 for all the strains (wild and reared inclusive), while that of the next step in sequence (buzzing) ranged between 0.54 and 0.92, respectively. While differences in pre-reproductive activi-

TABLE 3. AVERAGE DURATION OF BEHAVIORS IN LABORATORY INSECTS.

Laboratory insects	Israel	Mendoza	Kenya (ICIPE colony)	Moscamed	Seibersdorf G-47
Pas call	94.45	37.53	85.71	58.81	20.46
Act call	16.07	9.87	14.80	17.28	5.58
Fanning (vibrat)	19.63	21.64	41.55	17.87	5.94
Buzzing	6.90	5.53	8.00	12.09	3.97

TABLE 4. AVERAGE DURATION OF BEHAVIORS IN WILD INSECTS.

Wild insects	Israel	Patagonia	Kenya
Pas call	116.26	33.59	121.28
Act call	16.95	7.12	19.91
Fanning (vibrat)	22.30	20.10	57.88
Buzzing	6.58	5.61	15.18

ties should be interpreted with extreme caution, differences in the advanced courtship activities, such as wing vibration, head rocking and wing buzzing, are likely to indicate genuine differences among strains. In contrast to the pre-reproductive activities, even relatively minor differences in the pattern of courtship may have profound consequences on mating competitiveness and, in extreme cases, may lead to a degree of incompatibility and reproductive isolation among strains.

Male-Female Interactions

Medfly courtship is of a "dialogue type" with intense signal exchange between sexes (Lux & Gaggl 1995, Lux et al. 2002). Slow motion analysis revealed rich repertoire of female responses to calling males e.g. touching a male with her head or front legs, jumping towards male, short wing vibrations, stretching wings just after mounting etc. Most of these behaviors were nearly non-perceptible during direct observation due to their short duration (range: 0.04-0.16 s), hence, to quantify them, a "frame by frame" analysis had to be applied.

Females from all the strains tested, both reared and wild, presented the same repertoire of responses to courting males. Among the wild strains, the highest average number of meetings per pair

was noticed in this strain where the male courtship activities were most expressed and where the females were the most "choosy". During the observation, the males from Madeira met females nearly twice as frequently as those from the other strains (Table 6). Consequently, the number of initiated courtships (wing fanning) was highest in this strain (Table 7). At that stage, however, only 5% of Madeira males elicited friendly response from the courted females, as opposed to 8-22% in the case of other strains. For all the strains tested, only 43 to 70% of wing fanning progressed to the next step of courtship (buzzing). Again, the highest rate of rejections at the stage of wing fanning was noticed in the Madeira strain. While buzzing, however, the males from this strain stopped courtship at the first signs of female disinterest (78%, as opposed to 20-45% in the case of other strains). The ability of a male to respond correctly to the signals given by the courted female; suspend the courtship when the female indicated lack of interest and resume it again at the right moment, has been reported to be one of important traits of successful males (Lux et al. 1996). Only 10% of Madeira females responded friendly during buzzing, as opposed to 16-44% in the case of other strains (Table 8). Even when buzzing progressed to mating attempt, 87% of females vigorously objected, rejecting the male (48-81% of rejections in the case of other wild strains) (Table 9).

TABLE 5. PREVALENCE AND INTENSITY OF HEAD ROCKING DURING VIBRATION.

Origin of the strain	No. of courtships observed	intensity of head rocking			% of head rocking	
		intense	weak	none	intense	none
Wild males						
Patagonia	112	60	17	35	54%	31%
Madeira	137	52	7	78	38%	57%
Greece	60	22	0	38	37%	63%
Guatemala	56	9	3	44	16%	79%
Israel	45	0	0	45	0%	100%
Reared males						
Mendoza	49	36	3	10	73%	20%
Moscamed	66	6	1	59	9%	89%
Seibersdorf	92	0	0	92	0%	100%
Israel	30	0	0	30	0%	100%

TABLE 6. MALE-FEMALE INTERACTIONS DURING MEETING.*

Meeting (male & female are closer than 3 cm)	Wild strains					Reared strains	
	Patagonia	Greece	Guatemala	Madeira	Mendoza	Seibersdorf G-47**	Moscamed
No. pairs observed	36	25	62	31	37	23	29
Meetings—total	177	96	228	255	138	161	179
Meetings/pair	4.92	3.84	3.68	8.23	3.73	7.00	6.17
Calling (just before the meeting)	84%	97%	83%	78%	99%	73%	80%
Vigor and reactiveness							
a. passive	65%	89%	85%	72%	60%	57%	79%
b. active	35%	11%	15%	28%	40%	43%	20%
Signalling (wing signals)	41%	31%	48%	55%	32%	92%	23%
a. female only	55%	67%	66%	77%	73%	19%	36%
b. male only	21%	17%	22%	14%	7%	39%	55%
c. sign. exchange	25%	17%	13%	9%	20%	45%	12%
Who approaches:							
a. female	95%	96%	88%	90%	94%	83%	84%
b. male	5%	4%	11%	10%	6%	16%	16%
Adverse reactions during the meeting	31%	6%	16%	22%	5%	22%	29%
a. chasing by male	11%	33%	22%	30%	0%	6%	13%
b. chasing by female	24%	50%	11%	19%	29%	78%	51%
c. fighting	65%	17%	73%	52%	71%	17%	36%

*Some observations were not included in the analysis due to poor quality of the recording, lack of zoomimg (poor magnification rate) or other technical reasons.
**In the case of Seibersdorf strain, the observation and recording session lasted 90 min, in contrast to all other strain where a period of 30 min was used.

TABLE 7. MALE-FEMALE INTERACTIONS MALE-FEMALE INTERACTIONS DURING EARLY STAGE OF COURTSHIP (WING VIBRATION).

Fanning (vibration)	Wild strains					Reared strains	
	Patagonia	Greece	Guatemala	Madeira	Mendoza	Seibersdorf G-47	Moscamed
Vibrations/male	3.75	3.32	2.27	5.23	3.65	4.74	3.90
Vibrations/meeting	0.76	0.86	0.62	0.67	0.98	0.68	0.63
Distance to female at start of vibration (in the male body lengths)	3.19	2.76	2.35	2.14	3.94	2.74	2.47
Female reaction during vibrating:							
—friendly	22%	8%	21%	5%	16%	36%	3%
a. she touches him with front leg/legs	0.64	0.12	0.50	0.13	0.43	0.93	0.00
b. she touches him with head	0.05	0.00	0.13	0.01	0.01	0.14	0.00
c. "friendly" jump	0.04	0.06	0.06	0.04	0.06	0.14	0.03
d. vibrating wings (very short)	0.13	0.00	0.00	0.00	0.01	0.22	0.00
e. lowering wings	0.00	0.00	0.00	0.01	0.00	0.00	0.00
—neutral (remains still)	73%	90%	75%	96%	83%	56%	97%
—adverse	4%	1%	1%	3%	2%	6%	6%
a. chasing	0.40	1.00	0.50	0.60	0.33	0.29	0.00
b. fighting	0.60	0.00	0.50	0.40	0.67	0.71	1.00
Male reaction during vibration:							
a. remains still	94%	99%	87%	103%	96%	77%	104%
b. approaching the female	2%	1%	11%	0%	3%	22%	2%
c. "pushing" the female	3%	0%	0%	4%	1%	1%	0%

TABLE 8. MALE-FEMALE INTERACTIONS MALE-FEMALE INTERACTIONS DURING ADVANCED STAGE OF COURTSHIP (BUZZING).

Buzzing	Wild strains					Reared strains	
	Patagonia	Greece	Guatemala	Madeira	Mendoza	Seibersdorf G-47	Moscamed
Buzzing/male	2.61	1.72	1.11	2.32	3.19	4.26	2.62
Buzzing/vibration	0.70	0.52	0.50	0.43	0.87	0.90	0.64
Buzzing/meeting	0.53	0.45	0.30	0.28	0.86	0.61	0.42
Distance to female at start of buzzing (in the male body lengths)	0.61	0.59	0.72	0.57	0.76	0.52	0.74
Female reaction during buzzing:							
—friendly	44%	16%	41%	10%	36%	59%	26%
a. she touches him with front leg/legs	7.41	5.29	1.96	3.71	6.05	3.53	2.25
b. she touches him with head	0.15	1.29	0.46	0.43	0.16	0.69	0.70
c. "friendly" jump	0.10	0.14	0.14	0.29	0.07	0.45	1.00
d. vibrating wings	0.00	0.00	0.00	0.00	0.09	0.83	0.00
e. lowering wings	0.20	0.00	0.07	0.57	0.00	0.00	0.00
—neutral (remains still)	55%	84%	58%	89%	62%	37%	75%
—adverse	0%	0%	0%	1%	2%	6%	0%
a. chasing				0%	50%	67%	
b. fighting				100%	50%	33%	
Male reaction during buzzing:							
a. remains still	48%	33%	52%	32%	66%	64%	46%
b. approaching the female	50%	60%	43%	67%	31%	32%	55%
c. "pushing" the female	4%	19%	10%	1%	2%	4%	4%
Female shows lack of interest and the male stops buzzing when the female is oriented:	29%	23%	49%	32%	38%	51%	33%
a. front 0-45°	56%	20%	56%	78%	16%	36%	56%
b. side 45-135°	22%	20%	6%	0%	16%	26%	16%
c. back 135-180°	0%	10%	12%	4%	11%	12%	4%
d. female is leaving	11%	50%	32%	17%	51%	26%	28%
e. female is chasing him away	15%	0%	3%	0%	9%	4%	0%

TABLE 9. MALE-FEMALE INTERACTIONS DURING MATING ATTEMPT.

Mating attempt	Wild strains					Reared strains	
	Patagonia	Greece	Guatemala	Madeira	Mendoza	Seibersdorf G-47	Moscamed
Attempt/meeting	38%	35%	15%	19%	55%	35%	31%
a. no attempt	62%	64%	85%	81%	44%	65%	70%
b. failed jump	8%	2%	2%	1%	11%	4%	10%
c. successful attempt	30%	33%	13%	18%	44%	32%	21%
Female reaction just after jump during mating attempt							
a. calm and passive (wings "lateral")	2%	0%	10%	0%	2%	10%	0%
b. calm and passive (wings "normal")	21%	3%	34%	2%	26%	24%	3%
c. peacefully objects	4%	16%	7%	11%	3%	8%	5%
d. vigorously objects	74%	81%	48%	87%	70%	67%	89%
Mating							
Mating/attempt	21%	18%	44%	12%	25%	25%	5%
Mating/meeting	8%	6%	7%	2%	14%	9%	2%
Mating/male	39%	24%	24%	19%	51%	61%	10%

CONCLUSIONS

In the wild strains, more pronounced and spectacular pattern of male courtship activities (in particular intense head rocking before buzzing as in the case of Madeira strain) may have evolved as a result of more choosy females forcing more harsh competition among males. Indeed, the females were very "choosy" and frequently rejected courting males, which could happen at any stage of the courtship sequence. For all the strains tested (wild and reared), an average rejection rate of courting males (combined for all its stages) was within the range of 79-97%. In other words, only between 3 to 21 percent of initiated courtships culminated in successful mating.

Through their complex interactions with courting males, females stimulated and moderated male behaviors. Therefore, female responses may be indicative of courtship quality and are likely to act as a major selection force shaping male courtship activities in terms of their pattern and level of expression. In medfly reproductive process, uniformity among strains and mutual compatibility of male and female contributions to the "courtship dialog" are of fundamental importance. Lack of major differences among strains both in male and female behavioral repertoire indicate general lack of behavioral incompatibility among the strains studied.

Though females from all the strains responded in the same standard manner, but it appears that those from some strains tended to respond only to more expressed male behaviors, ignoring or rejecting "less impressive" courtships. Indeed, females from some strains were more "choosy" then those from other strains. Interestingly, in spite that the males from Madeira displayed the courtship in the most expressed manner, Madeira females accepted only 4% of courtships, which differed significantly from other wild strains, accepting 7-11% of courtships.

This is likely to result in asymmetrical mating competitiveness among the Madeira strain and some others, especially those where the intensity of head rocking is largely reduced or not present (Israeli strain). One could speculate that, while competing for Madeira females, the males from Israeli strain would be less competitive as compared to the Madeira males. However, in the case of competition for Israeli females, the difference might be less pronounced, though some advantage of the Madeira males still should be expected. Such differences in male competitiveness may result in partial reproductive isolation between strains with the most emphasized male behaviors and those where male behaviors are less expressed.

The above may have important implications for the strategies of SIT development and implementation. All medfly strains tested seem to be generally compatible, which implies that mass reared insects, if of high quality, may be used for SIT operations world-wide. However, development of an efficient strain for world-wide application shall be based on the most competitive strains, and only individuals with the most pronounced pattern of male courtship should be selected as founders. Our results suggest that well selected individuals from Madeira strain are likely to be good candidates for founding a strain with a potential for world-wide application.

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MATING AND REMATING OF MEDFLIES (DIPTERA: TEPHRITIDAE) IN GUATEMALA: INDIVIDUAL FLY MARKING IN FIELD CAGES

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Abstract

The sterile insect technique (SIT) depends critically upon the ability of sterilized, released males to locate and mate with wild females. The overall efficiency of the method also depends upon the relative frequencies of remating by wild females following first matings to laboratory or wild males. Using a newly devised technique that individually marks the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), a field cage study was undertaken in a Guatemala coffee orchard to record individual fly mating behaviors between each of several laboratory strain and coffee-reared wild flies. Five laboratory strains were tested- a genetic sexing strain examined in sex ratios between 50%-100% sterile males, two standard bisexual strains, and two F1 hybrid strains. The marking technique revealed a substantial amount of information on individual fly mating and remating. Wild male flies significantly outcompeted each of the lab strains in the first matings with both wild and lab females. Approx. 22% and 3% of wild males and females, respectively, remated in the field cages during two consecutive morning observation periods, while 4-8% of lab males, and 2-8% of lab females remated, respectively. Male flies from each lab strain averaged significantly shorter copulation times than wild males. Female flies, either lab or wild, tended to remate more often if they first mated to a lab male, but the differences were not statistically significant. An index was devised to provide a measure of relative male mating quality. Wild males tended to have higher individual index values than lab strain males. Average values of the latter ranged from ca. half to roughly equal that of wild males.

Key Words: sterile insect technique, wild flies, courtship behavior, copulation, sperm transfer, refractory period

Resumen

La técnica del insecto estéril (SIT) depende críticamente de la habilidad de los machos estériles liberados de localizar y aparearse con las hembras salvajes. La eficiencia general del método también depende de las frecuencias relativas de re-apareamiento por parte de las hembras salvajes siguiendo los primeros apareamientos en el laboratorio o con los machos salvajes. Utilizando la creación de una nueva técnica que individualmente marca a las moscas del Mediterráneo, *Ceratitis capitata* (Wiedemann), un estudio en jaulas de campo se condujo en un cultivo de café en Guatemala para registrar comportamientos individuales del apareamiento de las moscas entre cada una de las diferentes razas del laboratorio y de las moscas salvajes criadas en café. Cinco razas de laboratorio fueron probadas- una raza genéticamente sexada examinada en rangos de sexo entre el 50% y el 100% de machos estériles, dos razas bisexuales estándares, y dos razas híbridas F1. La técnica del marcaje reveló una cantidad substancial de información relativa al apareamiento individual de las moscas y su re-apareamiento. Los machos salvajes significativamente superaron a cada una de las razas del laboratorio en los primeros apareamientos con hembras tanto salvajes como de laboratorio. Aproximadamente 22% y 3% de los machos y hembras salvajes, respectivamente, se re-aparearon en las jaulas de campo durante las observaciones en dos dias consecutivos durante la mañana, mientras que del 4 al 8% de los machos del laboratorio, y del 2 al 8% de las hembras del laboratorio se re-aparearon, respectivamente. Moscas macho de cada una de las razas del laboratorio promediaron tiempos de copulación significativamente menores que los machos salvajes. Moscas hembras, tanto del laboratorio como salvajes, tendieron a re-aparearse con mayor frecuencia si inicialmente se aparearon con un macho del laboratorio, pero las diferencias no fueron estadísticamente significativas. Un índice fue creado para proveer una medida de calidad relativa de apareamiento de los machos. Los machos salvajes tuvieron la tendencia de tener valores individuales de este índice más alto que las razas de machos de laboratorio. Los valores promedio los machos de laboratorio oscilaron desde la mitad hasta aproximadamente un valor igual al de los machos salvajes.

The efficiency of the sterile insect technique (SIT) depends critically upon the ability of sterilized, released males to locate and successfully mate with wild females. In addition, sterile males ideally should be able to keep wild females from remating or, if remating does occur, possess seminal products that compete on equal terms with those of wild males (Jang et al. 1998). A number of field studies have compared laboratory and wild medfly strains in terms of courtship behavior (Prokopy & Hendrichs 1979, Lance et al. in review) and mating competitiveness (McInnis et al. 1996, Cayol et al. 1999). Further, the comparative ability of sterile males to switch the behavior of virgin wild females, from that of seeking mates to seeking ovipositional sources after mating, has been examined in a laboratory wind tunnel (Jang et al. 1998), and more recently in outdoor field cages (Jang et al. in press). However, in spite of evidence that some wild females remate under field conditions (McInnis 1993, Yuval et al. 1996), only recently has some attention shifted to studies of multiple mating behavior in the medfly, in particular the crucial level of remating in wild females (Vera et al. in review).

The present study was conducted in outdoor field cages in Guatemala during February, 1998. Several laboratory reared medfly strains were compared to wild flies with respect to overall mating and remating tendencies of both males and females, using a newly devised technique that individually marked each fly.

MATERIALS AND METHODS

Insect Sources

Experiments were conducted in a coffee farm, Finca San Augustin, near Petapa, about 15 km outside Guatemala City, Guatemala, between February 18-28, 1998. Laboratory reared flies were obtained from the El Pino medfly mass-production facility located roughly 40 km east of the capital. Larvae were reared on a sugarcane bagasse diet, then pupae were irradiated at a sterilizing dose of gamma rays at 145 Grays about 2 d prior to adult emergence. Adult flies were separated by sex within 24 h of emergence and held in 1 liter plastic containers (50 flies/cup) with food (3:1 mixture of sucrose: yeast hydrolysate) and water. Flies were held at $25 \pm 3°C$, 60-80% RH, and a photoperiod of 10:14 L:D for 5-7 d before testing. Laboratory strains tested were of varied ages after colonization: Petapa (Guatemala)—15 yr; Toliman *tsl* (largely Guatemala background)—7 yr; Vienna-42 (Y-chromosome sexing system, Austria); Antigua (Guatemala)—1 yr. The two F1 hybrids evaluated, Toliman *tsl* × Petapa and Petapa × Antigua, were produced by making the 2 reciprocal crosses (ca. 500 per sex) for each hybrid, then mixing the F1 progeny of each hybrid's reciprocal cross.

Wild flies were reared from coffee fruit collected near Retalhuleu, in southwestern Guatemala. Pupae were sifted from sand every 1-2 days at a field station, then shipped to Petapa and held under the same laboratory conditions as were the laboratory strains. Slower maturing wild flies were field tested at 10-12 days of age to be sure they were reproductively mature.

Fly Marking

All laboratory strain flies and wild flies were marked with individual labels. Several days prior to each experiment, flies were anesthetized by exposure to cold temperatures (-5 to -10°C) for about 2 min. Then, with careful handling using soft forceps and a fine point brush, a spot of white acrylic paint was dabbed onto the dorsal mesonotum of each fly. This procedure was followed immediately by the addition of a colored letter or number on paper (font size #3, Arial, 0.5 mm × 1.0 mm) directly onto the spot of paint, which upon drying, sealed the printed label to the body of the fly. Transfer of the printed paper was accomplished easily with a probe tipped with a spot of wax. The above procedure can be accomplished by keeping flies constantly anesthetized with cool temperatures, then placing the insects on 'blue-ice' packs, or by working in a walk-in cold room. Wild flies were coded with black numbers, 0-9, and extra flies, used to replace dead flies at the start of each test, with black letters A-E. Laboratory flies were coded with red, blue, or green letters (A through Z, omitting O). Prior experience under field cage conditions indicated that labels on flies could be clearly identified from a distance of 0.5-1 m.

Test Procedures

Fifteen field cages (3 m diam. × 2.5 m high) were each set up in the coffee plantation over a rooted, single coffee plant, *Coffea arabica* L., about 2 m high. A list of the various treatments is shown in Table 1. Treatments 1-10 involved the Toliman *tsl* genetic sexing strain, Vienna 4/Tol-94, mixed at various sex ratios, ranging from 50% males (as in a normal strain) up to 100%. Treatments 12-15 involved other laboratory strains, one each per treatment, with wild flies, including the standard, bisexual Petapa (Trt. 12), the recently colonized Antigua (Trt. 13), and two F1 hybrids, Petapa × Antigua, and Toliman *tsl* × Petapa. Treatment 11 was the control, in which only wild flies were released. As noted in Table 1, Treatments 1 and 12-15 each involved 25 laboratory and 10 wild flies per sex. Treatments 2 through 10 involved increasing or decreasing numbers of Toliman *tsl* males and females, respectively, with a constant total of 50 sterile insects released into each cage. A constant 10 wild flies per sex was released into each of these cages,

TABLE 1. FLY STRAINS AND NUMBERS OF FLIES PER SEX TESTED IN OUTDOOR FIELD CAGES IN A GUATEMALAN COFFEE FARM (GUATEMALA, 1998).

Treatment	Flies tested*			
	Lab males	Lab females	Wild males	Wild females
1. Toli-*tsl*: 50% males	25	25	10	10
2. Toli-*tsl*: 60% males	30	20	10	10
3. Toli-*tsl*: 70% males	35	15	10	10
4. Toli-*tsl*: 80% males	40	10	10	10
5. Toli-*tsl*: 90% males	45	5	10	10
6. Toli-*tsl*: 92% males	46	4	10	10
7. Toli-*tsl*: 94% males	47	3	10	10
8. Toli-*tsl*: 96% males	48	2	10	10
9. Toli-*tsl*: 98% males	49	1	10	10
10. Toli-*tsl*: 100% males	50	0	10	10
11. Control (Wild)	—	—	35	35
12. Petapa	25	25	10	10
13. Antigua	25	25	10	10
14. (Pet. × Ant.) F1	25	25	10	10
15. (Toli. × Pet.) F1	25	25	10	10

*Note: 5:1 wild fly ratio; N = 5 test replications, Lab strain pupae sterilized at 145 Gy, 2 days before emergence.

while in the control cage (Treatment 11), 35 wild flies per sex were released.

On each test morning, personnel gathered at the test site and received an assigned fly treatment and field cage, both of which had been assigned at random. Flies of each of the lab and wild strains were distributed according to the particular treatment. In addition, weather-recording equipment (for temperature, relative humidity, and light intensity) were provided to each observer. Promptly at 7:15 AM, male flies were released into each cage, followed by female flies 15 min later. Dead flies were replaced as needed to reach the full compliment for each sex and strain. Mating pairs were collected in snap cap, clear plastic vials as they formed. The individual label of each fly was recorded along with the start time of the copulation. Small magnifying glasses were provided for occasional use in distinguishing certain difficult letters or numbers. Each vial was then placed in a shaded area at the base of the tree, until the end of the copulation. Every 5-10 min, the vials were examined to record the end of any mating pair copulation. Flies that separated were promptly re-released from near the base of the tree. Observations and recordings continued in this manner for 6 h (until 1:30 PM), through the principal period for medfly mating in the field. Additional observations were made on the succeeding day (8 AM-12 NOON) for 5 of the treatments involving the Toliman *tsl* strain— Treatments 1,3,5,7, and 9. Flies were left in the cages overnight after the first day, and a small amount of food (standard sugar/protein mixture) and water was provided to enhance survival. Five complete replications of the above test procedure were carried out over a 2-week period.

Laboratory strains were compared to each other, and to wild flies, for proportions mating zero times, once, twice, etc. Observed numbers were compared with expected numbers, the latter based on random mating given the starting numbers for each sex and strain. Average times in copula for each lab strain and sex were compared for flies mating once or multiple times, and for laboratory vs. wild flies. In several instances, lab or wild fly data were pooled to increase sample size, or to simplify comparisons, e.g. the 10 Toliman *tsl* treatments (1-10), the non-*tsl* bisexual strain treatments (12-15), or all treatments with lab flies (1-10, 12-15). Female flies were compared by strain for deviations from the numbers of pairs expected based on random mating to the lab and wild males, on either the first or subsequent matings. Female flies were also compared by strain with respect to the proportion remating, depending on the type of male involved in the first mating. Male flies were compared by strain for the proportions observed mating zero times, once, twice, etc., compared to the numbers expected from random mating. In particular, male strains were compared regarding the proportion of multiple maters (mated 2 or more times). A new index was devised, called the Male Quality Index, MQI, which considers male flies that mated 2 or more times, i.e. the most active males during the test. Because the index involves having at least one male refractory period (time between consecutive matings), only multiple mating males were considered. All female mates of a multiply-mated

male were considered, i.e. females did not have to remate. If no remating occurred, the time from the end of the mating until the end of the test was taken as the refractory period, albeit a minimum for that particular mating. An individual males index is then defined as follows:

$$\text{MQI} = (\#\text{ matings}) \times \frac{(\text{Avg. FRP}),}{(\text{Avg. MRP})}$$

where,

MQI = Male Quality Index,

matings = number of copulations observed for male X in the 1-2 day period,

Avg. FRP = average refractory period of females mated to individual X,

Avg. MRP = average refractory period of male X.

Observed and expected values in each tested comparison were analyzed by Chi-square procedures, while analyses of variance were carried out using the ANOVA procedure (SAS Institute Inc., 1998) to compare MQI or copulation time treatment means.

RESULTS AND DISCUSSION

The individual laboratory or wild strains, along with the numbers of flies tested per sex, are shown in Table 1. As can be noted, except for the control cage (Treatment 11), the sterile:wild male fly ratio was 5:1, counting both sexes of sterile flies.

Table 2 presents the results for the numbers of mating pairs collected per treatment and mating type, mating indices, and estimated fly competitiveness. Numbers of mating pairs varied widely across treatments, with numbers of pairs for lab females (L × L and W × L) declining for Treatments 1-10, as expected, as the proportion of males among the sterile flies increased. In the treatments involving equal numbers of sterile females, Treatments 1, and 12-15, a strong deficiency of the desired lab male × wild female matings were observed. Two indices were used to evaluate the numbers of mating pairs- the Relative Isolation Index (RII), and the Relative Sterility Index (RSI), (McInnis et al. 1996). The former measures the relative compatibility of the two strains, in comparison to random mating expectations (index = 1.0). Values above 1.0 indicate positive assortative mating (inbreeding), while values less than 1.0 indicate negative assortative mating (outcrossing). Obtaining zero mating pairs for L × W or W × L leads to an undefined index, as occurred for Treatments 9 and 10. In the event of undefined values for the RII, one can use a similar mating index provided by Cayol and co-workers (Cayol et al. 1999). The listed values of RII vary from 1.28-6.75, indicating near random mating to moderate levels of positive assortative

mating. The lowest value was obtained by the Toliman × Petapa F1 hybrid. The second index, RSI, measures the proportion of wild females that mated with sterile males. These values ranged from a low 0.17 for the Petapa × Antigua F1 hybrid, to a high of 0.47 for the other F1 hybrid, Toliman × Petapa. With the ratio of the number of sterile males: wild males ranging from 2.5 to 5.0 among the treatments, one can calculate the expected RSI based on random mating, as shown in Table 2 (see formula in footnotes). An estimate of sterile male competitiveness follows from the quotient, RSI observed/RSI expected, as shown in the table. Competitiveness values range from a low of 0.24, again for the Petapa × Antigua F1 hybrid, to a high of 0.66 for the Toliman × Petapa F1 hybrid. Most values are near 0.50, indicating that the sterile males were roughly half as competitive as wild males.

The proportions of flies mating one or more times, with respect to strain and sex, are presented in Table 3. Wild males, from combined data for Treatments 1,12,13,14, and 15, mated significantly more often, 72.1%, than any of the lab strains, 24.0-36% (Tukey's HSD, $P < 0.05$). On the other hand, the laboratory strains and wild females all mated to the same degree, 42.4-58.4%, though the two hybrids produced the highest averages. Regarding multiple maters, wild males again were the highest, at 20.6% remated over two mornings of observation. This value was significantly higher than the proportions that remated for all of the lab strains, except for the Toliman × Petapa F1 hybrid (8.1% remated, Tukey's HSD tests, $P < 0.05$), see Table 3 and Fig. 4. Female multiple maters varied between 2.0% (wild) to 8.0% (Petapa) with no significant differences among the strains. Consistently, however, lab females tended to remate more than did wild females (Table 3).

Based on the total number of mating pairs in each cage, an expected number of matings per type of cross can be calculated, assuming random mating conditions. Figures 1 (for all Toliman *tsl* vs. wild) and 2 (for all other lab strains vs. wild) show the observed and expected numbers of mating pairs for each of the four mating types ($\male \times \female$)- LL, LW, WL, and WW, where L = lab and W = wild. As can be noted, for both lab fly groups, there was a deficiency of observed compared to expected matings involving lab males, LL and LW, while there was an excess of wild males mating, WL and WW. The departures from random mating were highly significant in both cases (χ^2 tests, df = 3, $P < 0.01$). Female percent remating in relation to mating type is shown in Figure 3, combining data for all treatments involving 1:1 sex ratios of lab and wild flies (i.e., for Toliman *tsl*, Trt. 1 only). The data for each lab strain were pooled since each strain when tested alone showed homogenous, non-significant differences (individual paired

TABLE 2. NUMBERS OF MATING PAIRS COLLECTED IN FIELD CAGES BY TREATMENT AND MATING TYPE, MATING INDICES, AND ESTIMATED FLY COMPETITIVENESS (GUATEMALA, 1998).

Treatment	# Mating pairs[a]				RII[c]	RSI[d]	Exp. RSI[e]	Compet[f]
	L × L[b]	L × W	W × L	W × W				
1	24	8	24	16	2.00	0.33	0.71	0.46
2	19	11	9	19	3.35	0.37	0.75	0.49
3	15	14	10	20	2.14	0.41	0.78	0.53
4	8	12	7	15	1.43	0.44	0.80	0.55
5	8	13	2	21	6.46	0.38	0.82	0.46
6	3	8	3	19	2.38	0.30	0.82	0.37
7	3	8	1	18	6.75	0.31	0.82	0.34
8	1	10	1	15	1.50	0.40	0.83	0.48
9	2	12	0	16	(und.)	0.43	0.83	0.52
10	—	8	—	10	(und.)	0.44	0.84	0.52
11	—	—	—	104	—	—	—	—
12	34	7	27	20	3.60	0.26	0.71	0.37
13	25	7	27	12	1.59	0.37	0.71	0.52
14	42	5	32	24	6.30	0.17	0.71	0.24
15	41	16	36	18	1.28	0.47	0.71	0.66

[a]Based on totals of 5 replications.
[b]Mating pair type: (L or W) × (L or W); L = Lab sterile; W = Wild.
[c]Relative Isolation Index = # pairs for (L*L)(W*W) / (W*L)(L*W).
[d]Relative Sterility Index = # pairs for (L*W) / (L*W) + (W*W).
[e]Expected index based on random mating = released L / (L + W).
[f]Competitiveness = RSI (observed) / Expected RSI (w/ random mating).

t-tests, P < 0.05). As seen in the figure (that differs from Table 3 by excluding females that did not mate at all), laboratory females (9.3%) tended to remate more than wild females (5.4%). However, a paired t-test was non-significant (P > 0.05). Also, females mated first to lab males, LL (11.7%) and LW (9.9%), tended to remate more than if the first mating was with a wild male, WL (7.0%) and WW (3.7%), though the differences were not statistically significant (paired t-test, P > 0.05).

TABLE 3. PROPORTION OF INDIVIDUALLY MARKED FLIES MATING ONE OR MORE TIMES, AMONG SEVERAL LABORATORY AND WILD STRAINS (GUATEMALA, FEB. 1998).

	Strain	Sex	Prop. Mating	Prop. multiply mating (2 or more times)
1.	Toliman	Male	0.240 b	0.040 b
		Female	0.424 A	0.016 A
2.	Petapa	Male	0.336 b	0.041 b
		Female	0.488 A	0.080 A
3.	Antigua	Male	0.248 b	0.040 b
		Female	0.424 A	0.040 A
4.	Petapa × Antigua (F1 Hybrid)	Male	0.352 b	0.048 b
		Female	0.584 A	0.048 A
5.	Toliman × Petapa (F1 Hybrid)	Male	0.360 b	0.081 ab
		Female	0.560 A	0.048 A
6.	Guate. Wild	Male	0.721 a	0.206 a
		Female	0.496 A	0.020 A

*Note: Means for each sex followed by the same letter within a column are not significantly different at the P = 0.05 level by Tukey's HSD test.

All TSL 1st Mating x Wild
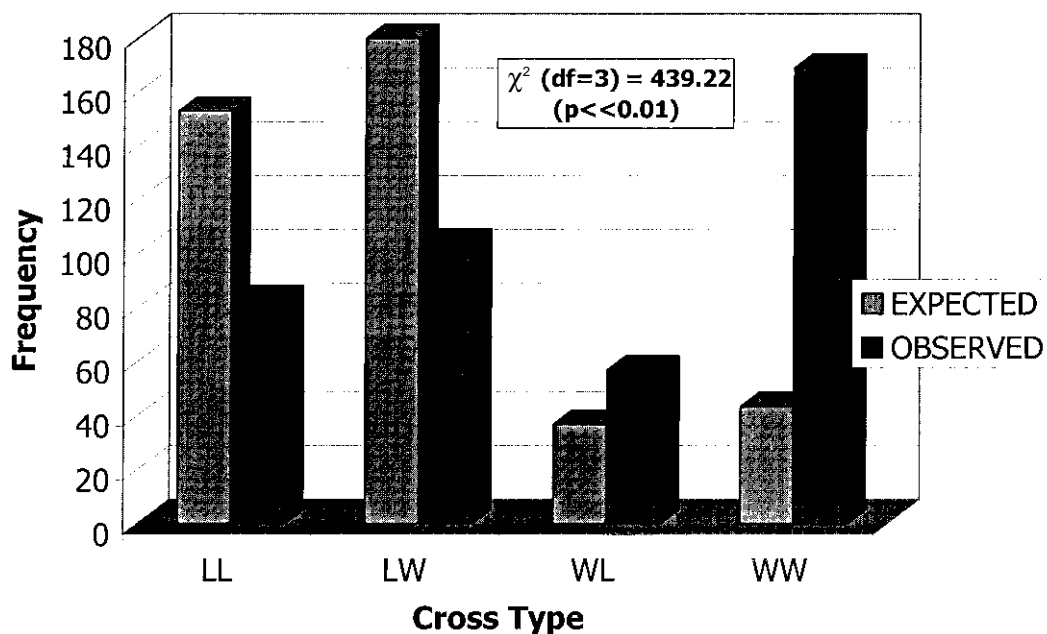
Fig. 1. Numbers of expected and observed first mating pairs for the medfly Toliman tsl and wild strains in outdoor field cages (Guatemala 1998). L = lab, W = wild, LL = lab male × lab female, etc.

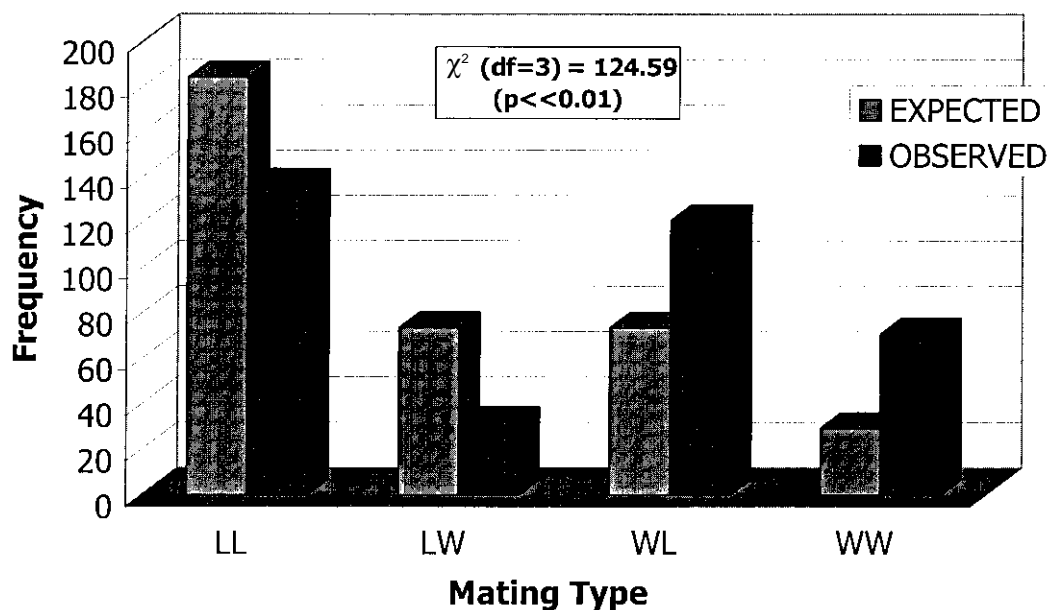## Antigua, Petapa, 2 F1 Hybrids (combined, 1st mating) x Wild

Fig. 2. Numbers of expected and observed first mating pairs for the medfly Antigua, Petapa, and 2 F1 Hybrid strains in outdoor field cages (Guatemala 1998) (Labels as in Fig. 1).
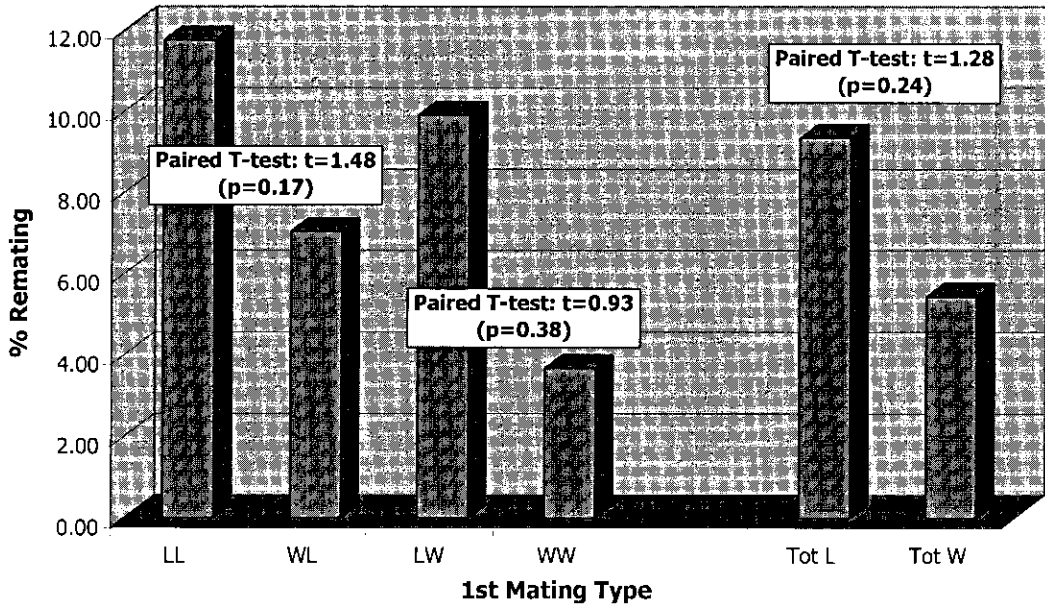
Fig. 3. Percentage of medfly females remating for all lab and wild strains after first mating with one of 4 mating types in outdoor field cages (Guatemala 1998). Paired T-tests results are shown for lab or wild females mating according to either male type separately or combined.

Fig. 4. Proportion of medfly males remating for each of the lab strains compared to wild flies in outdoor field cages (Guatemala 1998). Wild flies had significantly higher remating in each of the 5 comparisons (ANOVA F-statistic, $P < 0.001$).

Table 4 presents the mean copulation time by sex for mating pairs of the various laboratory strains tested, including single vs. multiply mated flies, and lab vs. wild. Copulation times averaged ca. 100 min in length and were generally longer for wild flies and for single maters. Laboratory male flies copulated for significantly shorter times, on average, than wild flies for all 5 lab strains, while for lab females, only the Toliman *tsl*

strain had significantly shorter copulations than their wild counterparts (ANOVA F-statistics, $P < 0.05$). With respect to single vs. multiple maters, and lab and wild flies combined for each sex, there was no effect on copulation time between first and multiple maters for any of the treatments. However, for females, multiple maters averaged shorter copulations compared to single maters in all 5 treatment cages with 1:1 sex-ratios (wild fe-

TABLE 4. COPULA DURATION FOR MATING PAIRS OF THE DIFFERENT LAB STRAINS BY SEX FOR SINGLE VS. MULTIPLY MATED FLIES, AND FOR LAB VS. WILD FLIES (GUATEMALA, 1998).

Strain	Sex	Mating class	n	Time in copula (min)	ANOVA Prob. > F
1. Antigua	Female	mated once	67	110.93	0.847
		mated twice+	4	101.50	
		Lab	52	118.79	0.496
		Wild	19	107.33	
	Male	mated once	51	114.63	0.759
		mated twice+	12	124.08	
		Lab	30	90.23	0.0013*
		Wild	33	140.24	
2. Petapa	Female	mated once	75	74.93	0.023*
		mated twice+	12	46.83	
		Lab	60	66.17	0.457
		Wild	27	81.93	
	Male	mated once	63	73.63	0.797
		mated twice+	16	83.38	
		Lab	41	56.78	0.001*
		Wild	38	95.92	
3. Toliman *tsl*	Female	mated once	381	99.74	0.0095*
		mated twice+	32	70.03	
		Lab	140	83.56	0.001*
		Wild	273	101.55	
	Male	mated once	354	95.12	0.343
		mated twice+	42	107.57	
		Lab	189	83.73	0.0001*
		Wild	207	108.05	
4. Petapa × Antigua F1	Female	mated once	97	89.93	0.026*
		mated twice+	6	52.00	
		Lab	74	84.87	0.216
		Wild	29	95.00	
	Male	mated once	66	89.42	0.666
		mated twice+	21	102.10	
		Lab	44	77.09	0.0001*
		Wild	43	108.23	
5. Toliman *tsl* × Petapa F1	Female	mated once	88	90.69	0.178
		mated twice+	11	69.18	
		Lab	71	88.44	0.966
		Wild	28	87.96	
	Male	mated once	66	97.15	0.0832
		mated twice+	20	75.70	
		Lab	45	71.44	0.0001*
		Wild	41	114.90	

males included), and in 3 of these the differences were significant (ANOVA F-statistic, $P < 0.05$). Shorter copulations of lab medfly strains have been recorded previously (Briceno et al. 1996, Field & Yuval 1999). In the present experiment, this occurred for males, but not for females, in the outdoor field cages. Presumably, shorter copulations are a consequence of normally crowded lab conditions, and this effect apparently carried over to the larger and much less crowded field cages, at least for lab males. As for the copulation times for single vs. multiply mating females, the effect of significantly shorter second copulations in several instances may be a consequence, at least in part, of the so-called "behavioral switch" from mating to oviposition (Jang et al. 1999). A shorter remating copulation time may be one indication of a female mating refractory period. Such an effect of copulation duration might therefore logically be observed in females, not in males.

Female percent remating, following an initial mating with either virgin or mated males for each lab strain, is shown in Figure 5. No statistically significant effect was noted in any case (all χ^2 test P-values > 0.05, df = 1). In some strains, females averaged higher remating frequencies after mat-

ing to virgin males, while in other cases after mating to mated males.

The index of male mating quality, MQI, was calculated for all lab or wild males mating 2 or more times during the 2 morning observation periods. Results are shown in Figure 6. Lab flies averaged 3.68 and wild flies 4.91—a difference of 33%. As can be noted, most of the higher values above 7.0 are from wild males. Curiously, however, by far the highest value came from a Petapa male with an index of 19.92. That male mated 4 times, the most of any male in the entire test. Figure 7 compares the MQIs for each of the lab and wild strains. As shown, the Antigua strain recorded the highest index, 5.29, just above the wild male value of 4.91. Excluding the outlier value of 19.2, Petapa males averaged 2.48, the lowest value of any strain. With respect to remating, because the MQI is determined solely from flies that remated, no independent determination can be made of a possible effect of MQI on female remating tendency.

Though the mean MQI values were not significantly different statistically (Tukey's HSD, $P > 0.05$), the absolute differences suggest a possible negative correlation between age of each labora-
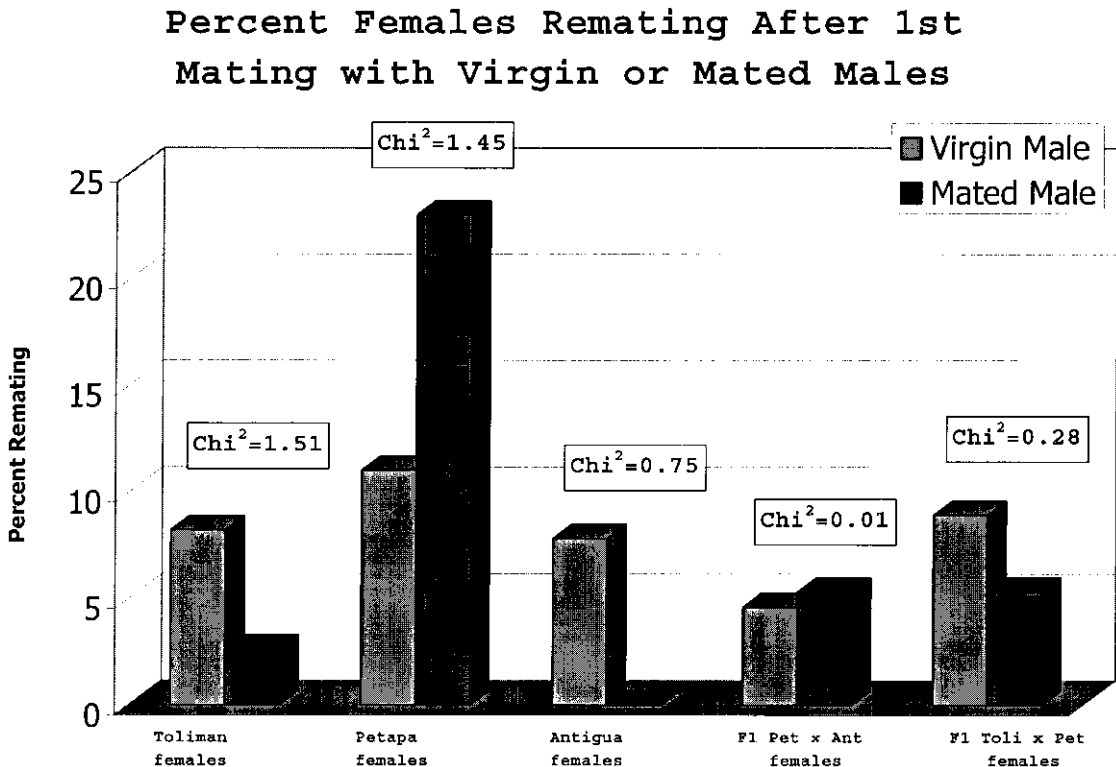
Fig. 5. Percentage of medfly females remating after first mating with either virgin or mated males in outdoor field cages (Guatemala 1998). Chi² test results (df = 1) are shown for each female strain (all tests non-significant, $P > 0.05$).
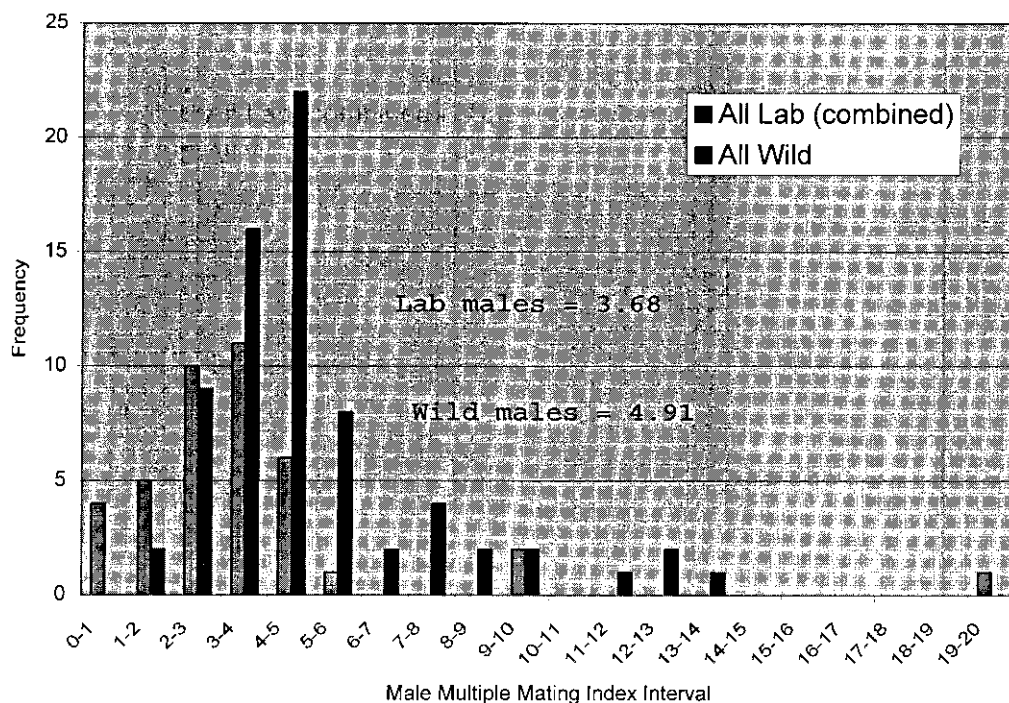
Male Quality Index

Fig. 6. Male Quality Index, MQI, for individual medfly males of lab strains (all data combined) and wild flies from outdoor field cage studies (Guatemala 1998). Only males mating 2 or more times are considered.
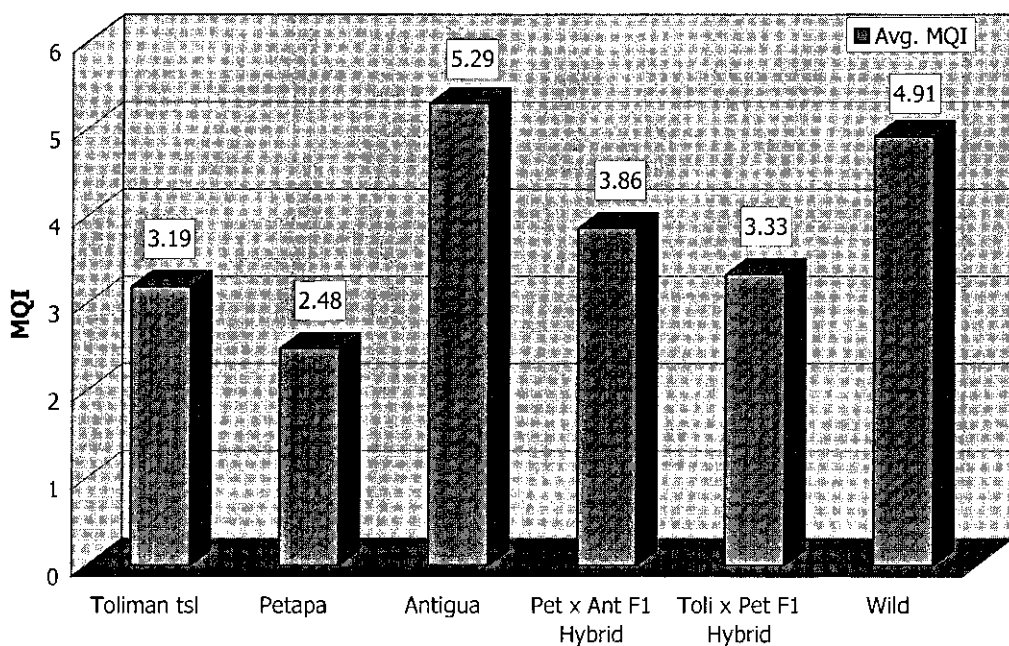
Male Quality Index (MQI), by Strain

Fig. 7. Male Quality Index, MQI, averaged for each of the lab and wild strains from outdoor field cage studies (Guatemala 1998).

tory strain (colonization age) and the MQI. For the two hybrid strains, colonization age was calculated by simply taking the arithmetic mean of the ages of the two parental strains. This relationship is presented in Figure 8 for all six strains of this study, including wild flies (colonization age = zero). As can be noted, there is a steady decline in the MQI vs. strain colonization age in years. The statistical correlation is highly significant (r = -0.940, df = 4, P < 0.01). If the two hybrid values were excluded due to the arbitrary manner in which they were calculated, the result is just barely significant (r = -0.950, df = 2, P = 0.05). The observed decline in MQI over time serves to graphically illustrate the apparent need to replace mass-production strains regularly, perhaps every several years, to avoid loss of sterile male mating vigor.

The development and practice of the individual medfly marking technique has provided relatively useful information on medfly mating behavior. Some of this information would otherwise remain hidden behind a cloak of fly anonymity. The present cage study has revealed a relatively high level of multiple mating by wild males compared to lab males, and relatively low remating among females, with no significant differences among strains. Though the differences were not statistically significant, both laboratory and wild females tended to remate less if their first mating was with a wild male; and, in general, lab females tended to remate more than wild females. These results have been corroborated in a more recent laboratory study by Vera et al. (2002) using a very similar individual fly marking technique, wild flies from Guatemala, and one of the same laboratory strains, Petapa.

Male quality was expressed in terms of a new index that considers mating frequency and the refractory periods for males and females. Though only multiply mated males are considered for index purposes, the results clearly show an advantage for wild males over most laboratory strain males. Indeed, the advantage of wild males was directly proportional to the age since colonization of the various laboratory strains.

Clearly, more studies are needed that delve deeply into the field mating behavior of the medfly, including aspects relating to initial mating and remating propensities or abilities among various laboratory and wild strains.

MQI vs. Colonization Age
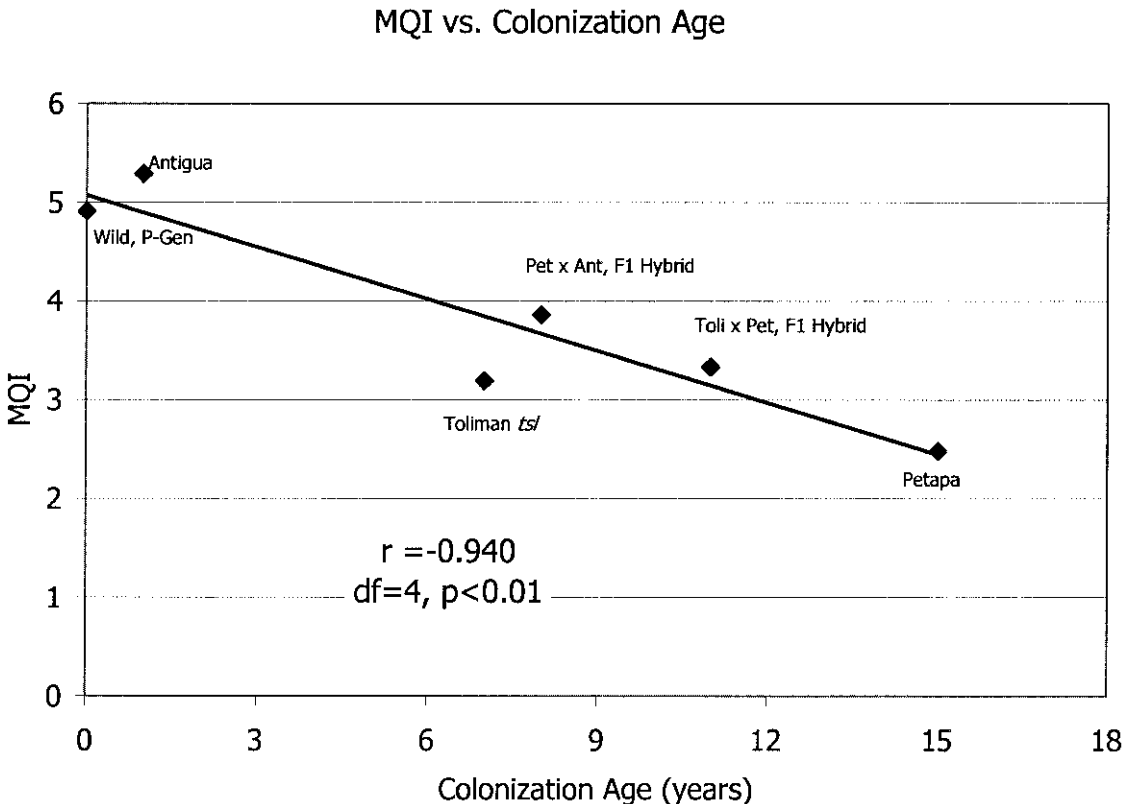
Fig. 8. Male Quality Index (MQI) vs. Colonization Age (years) for each of the six lab or wild strains tested in outdoor field cages (Guatemala 1998). A correlation (r) test result and linear fit to the data are shown (df = 4, P < 0.01).

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COMPARATIVE STUDIES OF COURTSHIP BEHAVIOR OF *CERATITIS* SPP. (DIPTERA: TEPHRITIDAE) IN REUNION ISLAND

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Abstract

Three species of *Ceratitis* (Diptera: Tephritidae) are damaging fruit crops in the French island of La Réunion, in the Indian Ocean. A comparison is given on the status of knowledge of mating behavior in these three species. Aspects examined include male pheromone calling (behavioral sequences, circadian rhythm, factors influencing calling), lek formation and pre-copulatory behavior. While *Ceratitis capitata* courtship behavior has been heavily studied in many countries, research data are still scarce for *Ceratitis rosa* and are only preliminary for *Ceratitis catoirii*.

Key Words: *Ceratitis*, fruit flies, mating behavior, pheromone calling, lek

Resumen

Tres especies de *Ceratitis* (Diptera: Tephritidae) se encuentran dañando cultivos frutícolas en la isla francesa de La Reunión, en el Océano Indico. Se presenta una comparación con respecto al grado de conocimiento que se tiene del comportamiento de apareamiento en estas tres especies. Los aspectos examinados incluyen llamado a través de feromonas del macho (secuencias de comportamiento, ritmo circadiano, factores que influyen en el llamado), formación de áreas específicas para el cortejo y comportamiento pre-copulatorio. Mientras el comportamiento de cortejo en *Ceratitis capitata* se ha estudiado considerablemente en muchos países, la información obtenida a través de investigaciones es todavía escasa para el caso de *Ceratitis rosa* y apenas se está iniciando para *Ceratitis catoirii*.

The Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann), belongs to a large genus currently comprising some 70 species, with main subgenera *Ceratitis* (9 spp.), *Ceratalaspis* (29 spp.), *Pardalaspis* (10 spp.) and *Pterandrus* (24 spp.) (Hancock 1984, Freidberg 1991, Meyer 1996, Hancock & White 1997).

In La Réunion, a French island situated in the Indian Ocean, 200 km north-east of Mauritius, 3 *Ceratitis* species are damaging a large variety of fruit crops. The Natal Fruit Fly, *Ceratitis* (*Pterandrus*) *rosa* Karsch is by far the main one in terms of economic importance. This is due to its large geographical distribution throughout the island, from sea-level up to an altitude of 1500 m, and its wide polyphagy (Etienne 1982, Quilici 1989). It largely dominates the other *Ceratitis* spp. in interspecific competition in most areas of the island *C. capitata* is found in the lowland and mid-altitude areas, and is particularly abundant in the lee-ward, drier side of the island. The third species, *Ceratitis* (*Ceratitis*) *catoirii* Guérin-Mèneville, may be found at low population densities in eastern and southern lowlands areas.

While *C. capitata* has a nearly worldwide distribution, *C. rosa* is present in many African countries, particularly in the eastern and southern parts of the continent. *C. catoirii* is an endemic species from the Mascarenes, found in Mauritius and La Réunion.

In the last twenty years, a large amount of research has been conducted on the sexual behavior of *C. capitata*. This has recently been reviewed by Eberhard (2000). One of the main objectives of this research was to improve the quality of mass-reared males used in SIT (Sterile Insect Technique) programs against this pest around the world. Comparatively very little knowledge is available on the sexual behavior of *C. rosa*, despite the economic importance of this species in many african countries and recent projects for implementing SIT against it in South Africa. The sexual behavior of *C. catoirii*, as is most of its biology, is too date, totally unknown.

Most of the research done in CIRAD Réunion on sexual behavior of *C. rosa* or *C. catoirii* is unpublished, and in this paper we summarize the main results obtained. This will provide a preliminary comparison of sexual behavior characteristics in these 3 *Ceratitis* species.

Material and Methods

Pheromone Calling

For the study of circadian rhythm and factors influencing male calling in *C. rosa*, we used wild flies collected as larvae in infested fruits of guava, *Psidium guayava* L. or Chinese guava, *Psidium cattleyanum* Sabine. Freshly emerged males are

placed in cages ($30 \times 20 \times 34$ cm) by groups of 10 males per cage. The cages are set in a room exposed to natural light, so that the adults may perceive the decrease of light at dusk. Climatic conditions within the rearing room are measured with a luxmeter (Bioblock Scientific Lx-101 K 32 523) and a thermo-hygrograph (Jules Richard, France). For the study of rhythm, before and during the experiments, males receive a complete food regime consisting of sugar, protein hydrolysate and water. The experiment is conducted during two days (from 0600 h to 1815 h) when males are 13 and 15 days old, with 5 replicates (5 cages). The number of calling males, as well as light intensity, are recorded every half an hour.

For the study on the influence of food regime on *C. rosa* male calling, males are subjected to 4 different food regimes: (1): complete food regime (water, sugar, and yeast), (2): water and sugar, (3): water and yeast, (4): water only. The methodology is similar to that of the previous experiment, except that calling males are recorded every half an hour during the period of maximum calling (from 1600 h to 1800 h). For the study of the influence of food shortage on *C. rosa* male calling, males receive a complete food regime until they are 20 days old. They are then subjected to periods of food shortage of 0, 4, 8, 12 and 28 h, with 4 replicates for each treatment.

The circadian rhythm of male calling in *C. catoirii* was studied in a small cubic cage (a = 30 cm) where a recent lab colony is maintained in our laboratory. The cage, containing adults of both sexes, is placed in a laboratory room exposed to natural light, and calling males are recorded every half an hour during the whole photophase, with 3 replicates (3 days).

To study the behavioral sequences of pheromone calling and pre-copulatory behavior of the 3 *Ceratitis* spp. we used the standard methodology for video recordings, that was agreed upon within the FAO-IAEA Co-ordinated Research Program. Adults used for the experiments were of wild origin (collected as larvae in infested fruits), except for the rather rare *C. catoirii*, for which adults from a recent lab-rearing were used. Adults had reached sexual maturity when used for the video recordings.

Lek Formation

For the study of lek behavior we used wild *C. rosa*, collected as larvae in infested fruits from various hosts-plants. For *C. capitata*, that was more difficult to obtain from the wild in the study period, we used flies from a recent lab-rearing. Males are separated from females the day following emergence and maintained in small cubic cages (a = 30 cm) until the experiment. Experiments are conducted in a screenhouse insectarium ($4 \times 4 \times 3$ m), receiving only the natural light and submitted to climatic conditions resembling those of outdoors: temperature varying from 17 to 23°C during the night and 20-30°C during the day, R.H: 45-90% and photoperiod close to L11: D13. Thirty potted Citrus plants are evenly distributed on the ground in order to provide a more natural environment.

To study lek formation, 300 males of a given species are released in the insectarium. The number of males calling on each Citrus plant is then recorded every 15 min. from 1000 h to 1600 h for *C. capitata*, and from 1600 h to 1815 h for *C. rosa*. Two replicates are done for each species.

RESULTS AND DISCUSSION

Pheromone Calling

Behavioral sequences. The release of a pheromone by males of *C. capitata*, attracting females, was shown in the early sixties by Feron (1962). Later, various authors studied the courtship behavior in this species (Prokopy & Hendrichs 1979, Sivinski et al. 1989, Shelly et al. 1993). The basic sequences of pheromone calling have been described by Feron (1962):

– in stage I, the male exerts an anal ampulla, with the abdomen bent dorsally, and emits a pheromone. The lateral pleura of the abdomen are also strongly inflated.

– in stage II, the male initiates a continuous wing vibration (fanning), while bending its abdomen tip ventrally. Abdominal pleura are still strongly inflated. This stage is initiated by the visual perception of another fly in the immediate surroundings.

The first data on the courtship behavior of *C. rosa* were given by Myburgh (1962), who mentioned the rectal ampulla (thought to be the aedeagus), wing fanning, and erection of tibial hair. More recently, field-cage studies and video recordings allowed us to precisely describe the courtship sequences of *C. rosa* males (S. Q., unpublished data). As the two first stages broadly correspond to what is observed in *C. capitata*, the nomenclature of Feron (1962) may be used for their description:

– in stage I, the *C. rosa* male exhibits an anal ampulla bent dorsally. Video recordings clearly show that this ampulla is tri-lobed, while it appears more or less spherical in medfly. The abdominal pleural distension is as conspicuous as in *C. capitata*. Another characteristic of the calling male is the erection of black hair present on male mid-tibias in this species (Myburgh 1962). Wings are maintained perpendicular to the body axis.

– the initiation of stage II is very similar to what is observed for the medfly male: at the approach of another adult, the male faces it

and initiates wing fanning while the abdomen is bent downwards. If the intruder is another male, the calling male then returns to stage I.

Experiments in an insectarium allowed us to confirm that calling males of *C. rosa* attract conspecific females but not those of *C. capitata*, while calling males of *C. capitata* attract conspecific females but not those of *C. rosa* (S. Q., unpublished data). However it is not yet known if this is due to pheromone specificity or to circadian rhythms of female receptivity.

Lab cage observations, macrophotographs and video recordings have recently given us a preliminary description of pheromone calling in *C. catoirii* (S. Q., unpublished data):

– stage I appears very similar to what is observed in medfly male. The exerted anal ampulla is more or less spherical, and appears at the tip of the abdomen, bent dorsally. Abdominal pleura are also strongly inflated.

– stage II also appears similar to the corresponding stage in medfly: male initiates wing fanning while bending its ampulla downwards. During both stages, hair of the male fore-femur are typically erected.

Circadian rhythms. In *C. capitata*, various authors have shown that males congregate in leks in the morning and early afternoon, depending on climatic and experimental conditions (Prokopy & Hendrichs 1979, Arita & Kaneshiro 1989, Whittier et al. 1992).

Myburgh (1962) showed that the sexual activity of *C. rosa* occurred at the end of the day, as in many tephritid species. Most of the matings he observed were in periods when the light intensity was between 0 and 20 foot candles. The results of our study in laboratory cages (Fig. 1), but also studies in field cages and observation in orchards confirmed this pattern in *C. rosa* (S. Q., unpublished data).

Recent lab-cage observations showed that the pheromone calling of *C. catoirii* occurred during the morning, with a maximum at 10.00 h (Fig. 2) (S. Q., unpublished data), confirming that sexual behavior in this species is very similar to that of the medfly.

Factors influencing male calling. The age at which adults of *C. capitata* become sexually mature varies with environmental conditions. Lab-reared males may reach their sexual maturity as early as 3 days old (Feron 1962). The availability, quantity, and quality of food resources also influence pheromone production in tephritids (Nation 1989).

In lab studies of *C. rosa*, calling was shown to be initiated some 10 days after adult emergence. The percentage of calling males then increases quickly in subsequent days and remains more or less stable until males are at least 35 days old (S. Q., unpublished data).

In lab studies, food quality also had a marked influence on the calling propensity of male *C. rosa*. When food consisted of sugar or yeast only, a decrease was observed in the maximum calling rate (20%) compared with adults fed with sugar + yeast (80%). For males previously fed for 20 days with a complete regime (sugar + yeast), food deprivation periods of 4, 8, 12 or 28 h had no effect on the calling rate of males (S. Q., unpublished data).
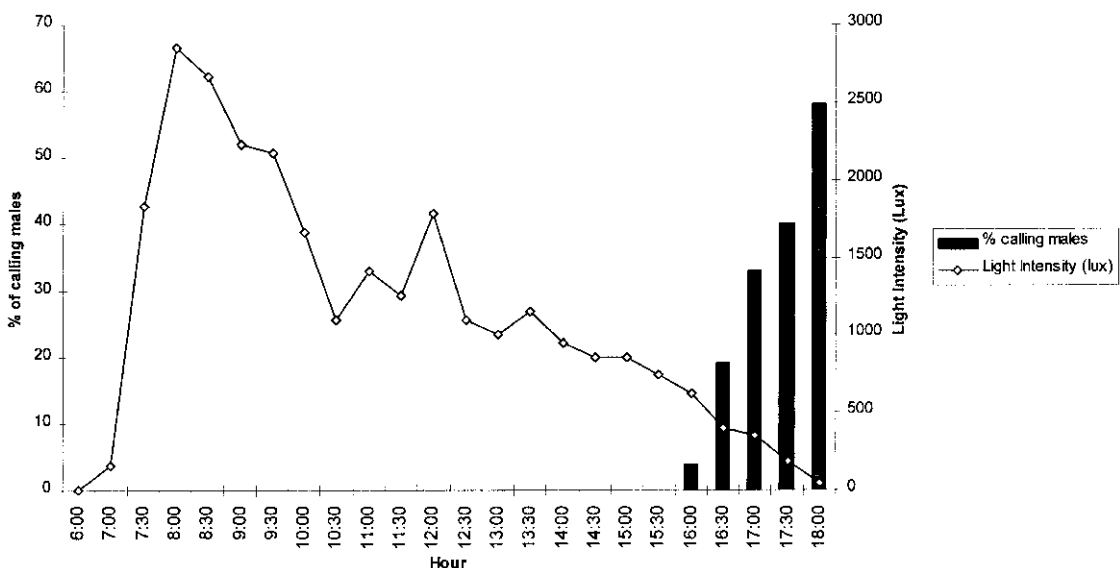

Fig. 1. Circadian rhythm of pheromone calling of male *Ceratitis rosa* in a lab cage, in relation with light intensity (males 15 days-old; mean of 5 replicates).
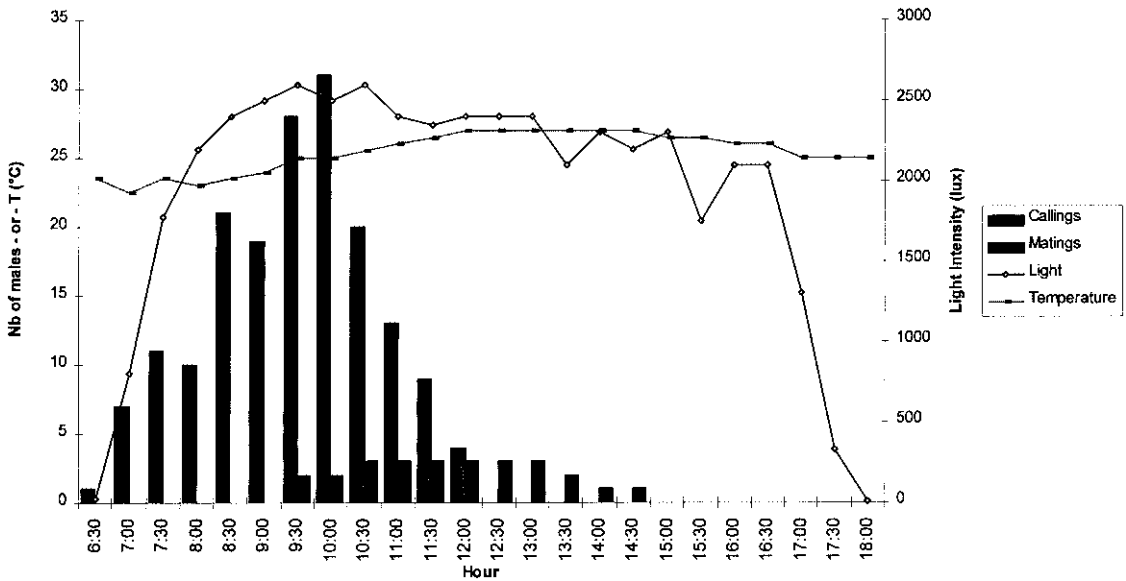
Fig. 2. Circadian rhythm of pheromone calling and mating of *Ceratitis catoirii* in a lab cage containing both sexes, in relation with abiotic factors (2nd replicate; males older than 12 days; N = 53 males).

Lek Formation

Since the first study by Prokopy & Hendrichs (1979), many authors have described the lekking behavior of *C. capitata* (Arita & Kaneshiro 1985, 1989, Whittier et al. 1992, Shelly et al. 1993).

Preliminary field-cage observations showed that males of *C. rosa* also aggregate in leks (S. Q., unpublished data). Our insectarium experiments with wild flies in the presence of potted citrus plants showed that males began aggregating and calling around 1715 h. A sharp increase in calling was observed, with 20% of released males calling at 1800 h, then this percentage dropped to 10% at 1815 h (S. Q., unpublished data). The mean number of calling males per plant reached a maximum of only 1,5, with the biggest aggregation observed including 10 males. Within a lek, males were situated on the underside of leaves, occupied and defended territories, as described previously in *C. capitata*.

More recently, observations were conducted in a citrus orchard during the harvest period. On two successive days, *C. rosa* leks were observed on different trees. Nearly all calling males occupied the underside of a fruit. Interestingly, a study of the circadian rhythm of daily activities of both sexes in this orchard showed that peak female oviposition activity takes place late in the afternoon (S. Q., unpublished data). In certain periods of the year, the situation of calling sites on fruits, and the temporal coincidence of sexual activities with the preferred oviposition period of the females, may constitute an advantage for maximizing the probability of males encountering females. Interestingly, citrus volatiles are known to be strongly attractive in the selection of oviposition sites by females of *C. rosa* (S. Q., unpublished data). Moreover, in artificial rearing conditions, the number of eggs laid by *C. rosa* females is strongly increased when adding pieces of citrus fruit, or citrus juice, into oviposition devices.

Precopulatory Behavior

In *C. capitata*, the final sequences of mating behavior preceding the mating attempt (close range interactions between male and female) have been studied by various authors (Briceno et al. 1996, Eberhard 2000), as well as the factors influencing male mating success (Whittier et al. 1994).

Feron (1962) defined as stage III, the large movements of wings, moved rhythmically forwards and backwards while still vibrating. This stage, also called "approach song" (Sivinski et al. 1989), "buzzing", or "intermittent wing buzzing" (Eberhard 2000), frequently precedes a mating attempt. It is associated with movements of the head ("head-rocking") performed in bursts (Eberhard 2000).

A limited number of video recordings have provided us preliminary data on close-range interactions between males and females of *C. rosa*. They failed to show any type of "buzzing" or "head-rocking" during this stage. Though this is not visible on all sequences, it appears that the male throws its mid-legs forward just before mounting.

In *C. capitata*, the sexually dimorphic capitate bristles on the anterior surface of the male's head may be displayed visually to the female during

"head-rocking" (Eberhard 2000). As such bristles are not present in *C. rosa*, it may be hypothesized that the conspicuous black hair of male mid-tibia could play a similar role in this species. Close-range interactions between sexes of *C. rosa* appear to follow a simpler scheme, and to have a shorter duration than in medfly.

Similar preliminary video recordings of interactions between males and females of *C. catoirii* showed that males of this species display some "buzzing" but very little, if any, "head-rocking". The capitate bristles of *C. catoirii* are white colored, which may limit their possible role in a visual display.

In future studies, it would be worth enlarging such comparisons to various african species of *Ceratitis*. In species within the subgenus *Pterandrus*, the males present more or less developed black hair on the mid-tibias and/or mid-femurs. The males of species within the subgenus *Ceratitis*, such as *Ceratitis malgassa* Munro, also possess capitate bristles as in *C. capitata* and *C. catoirii*.

More video recordings studies will be necessary in order to better understand and quantify the sequences of courtship behavior of *C. rosa* and *C. catoirii*. Additional observations of leks in orchards and experiments in field-cages will also be necessary to understand the factors that are involved in male mating success in these species.

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MORPHOMETRIC TRAITS AND SEXUAL SELECTION IN MEDFLY (DIPTERA: TEPHRITIDAE) UNDER FIELD CAGE CONDITIONS

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ABSTRACT

The effects of male size and other morphometric traits as determinants of male mating success were evaluated under field cage conditions. Males of the laboratory Seib6-96 strain were released into field cages with males and females of a wild population from the Patagonian region. Mating pairs were classified as 'successful', while unmated flies were labeled as 'unsuccessful'. Five morphometric traits were measured in a sample of 141 unsuccessful and 149 successful males: eye length (EL), head width (HW), thorax length (TL), face width (FW), and wing length (WL). An exploratory non-parametric Spearman's rank correlation test indicated that mated males were in average larger for all traits ($P < 0.01$) except FW, indicating that with the exception of FW all traits are positively correlated with mating success. Step-wise multiple regression and principal component analysis + logistic regression indicated that the most likely targets of selection were TL, EL, and FW. The two former are positively correlated, while FW is negatively correlated with the fitness component analyzed here (male mating success). In previous studies where male-male interaction had been removed experimentally, EL was shown to be associated with female choice and no effect relative to mating success was detected on TL. On the basis of that study and the present results it is tempting to suggest that body size (TL) might be important in intra-sexual selection. However, size was found to be strain dependent and flies from the wild were on average bigger than laboratory ones. Size selection might then be correlated with copulatory success, as a side effect due to selection of wild males over lab males due to differential sexual activity or other causes. The potential importance of both intra-sexual selection (male-male interactions) and inter-sexual selection (mate choice) on the morphology of *Ceratitis capitata* is discussed on the basis of the results presented here and previous works.

Key Words: male-male competition, female choice, morphometric traits, genetic sexing strain

RESUMEN

Se evaluaron los efectos del tamaño y otros rasgos morfométricos del macho como determinantes del éxito copulatorio en condiciones de jaulas de campo. Se liberaron en jaulas de campo machos de la línea de laboratorio Seib6-96 junto con machos y hembras de una población salvaje de la región Patagónica. Las parejas en cópula se clasificaron como "exitosas", mientras que las moscas que no se aparearon se marcaron como "no exitosas". En una muestra de 141 machos no exitosos y 149 exitosos se midieron 5 rasgos morfométricos: longitud del ojo (LO), ancho de la cabeza (ACb), longitud del tórax (LT), ancho de la cara (ACr) y largo del ala (LA). Un análisis exploratorio no paramétrico utilizando la correlación de rangos de Spearman indicó que los machos apareados eran en promedio más grandes para todos los rasgos ($P < 0.01$), salvo para ACr, indicando que, con la excepción de ACr, todos los rasgos están positivamente correlacionados con el éxito copulatorio. Análisis de regresión múltiple de a pasos ("step-wise") y análisis de componentes principales (ACP) seguido de regresión logística indicaron que los blancos más probables de la selección eran LT, LO y ACr. Los dos primeros están correlacionados positivamente, mientras que ACr está correlacionado negativamente con el componente de aptitud analizado en este trabajo (éxito copulatorio de los machos). En estudios previos donde la interacción entre machos había sido removida experimentalmente, LO había mostrado asociación con la elección de la hembra, mientras que no se habían detectado efectos de LT sobre el éxito copulatorio. Sobre la base de aquel y del presente estudio es tentador sugerir que el tamaño corporal (LT) podría ser importante en la selección intrasexual. Sin embargo, se comprobó que el tamaño es dependiente de la línea

y que las moscas salvajes eran en promedio más grandes que las de laboratorio. La selección del tamaño podría, por lo tanto, estar correlacionada con el éxito copulatorio como un efecto colateral, debido a la ventaja de los machos salvajes sobre los de laboratorio, por una actividad sexual diferencial o alguna otra causa. La importancia potencial tanto de la selección intrasexual (interacciones entre machos) como de la selección intersexual (elección de la pareja) sobre la morfología de *Ceratitis capitata* se discute sobre la base de los resultados presentados aquí y de trabajos previos.

Sexual selection was proposed by Darwin (1859, 1871) to explain extraordinary sexually dimorphic characters troublesome to his concept of natural selection. Two different kinds of processes could account for the evolution of such traits (Thornhill & Alcock 1983, Alcock & Gwynne 1991). Male-male competition for mates, or intra-sexual selection, is where those males with the most exaggerated trait are supposed to be able to fight and win fights over other males for access to mates. The second process is active choice of individuals of one sex by individuals of the other, usually female choice of mates, namely inter-sexual selection. Again, males with the most exaggerated (Moller & Pomiankowski 1993), most symmetric (Moller 1990), or most attractive traits are assumed to be at a selective advantage because they are more likely to be chosen by a female and they should therefore experience higher mating success. For some species, finding the direct targets of sexual selection can be a difficult task and may frequently lead to misinterpretations. Moreover, the relative role of male-male competition and female choice in sexual selection has been assessed in only few cases, but there is little doubt that while male combat is of overwhelming importance in some species, mate choice predominates in others (Bradbury & Davies 1987).

A good knowledge of sexual selection mechanisms is required for the successful implementation of the sterile insect technique (SIT) developed by Knipling (1955) to control insect pest populations (Burk & Calkins 1983). Sexually selected traits are usually a good reflection of male fitness and are thus a representation of both its genotype and phenotype. Mass-rearing conditions can be controlled to improve male quality in order to promote the occurrence of sexually selected phenotypes in a high frequency. Moreover, directed selection events may be used to select genotypes with high mating success.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) has a well-established lek mating system (Prokopy & Hendrichs 1979, Arita & Kaneshiro 1989, Hendrichs & Hendrichs 1990, Whittier et al. 1992). Leks generally take place on the underside of leaves of trees, where males establish territories and release a pheromone to attract females (Féron 1962, Prokopy & Hendrichs 1979, Arita & Kaneshiro 1989). After the arrival of a female, the male begins courtship (Féron 1962) and finally jumps onto the female and attempts to copulate. If unreceptive, the female either leaves before the male jumps or drops from the leaf when the male has already jumped and is trying to copulate (Whittier & Kaneshiro 1995). Females discriminate among potential mates at a lek and reject most courtships (Whittier et al. 1992, Whittier et al. 1994), leading to differential copulatory success within males. The common finding that copulatory success is highly variable among lekking males has led to the predominant view that mate choice is of great importance for sexual selection in such mating systems (Whittier et al. 1992, Whittier et al. 1994, Norry et al. 1999). Despite this disparity of male mating success within leks, male-male interactions may result in the males sorting themselves by territory location or position in a dominance hierarchy as reported for other dipterans (Shelly 1987, Sivinski 1989). As a consequence many traits could be correlated with copulatory success without being mate-choice cues. Male mating activity and aggressive behavior are examples of intra-sexually selected traits in many lek-mating species. According to Whittier et al. (1992) and Whittier et al. (1994) *C. capitata* males defend their territories very weakly, and there does not appear to be any relationship between territory location and mating success. However, male-male interactions are commonly observed both in the laboratory and in the field (though probably not very well documented).

Mating success in *C. capitata* males has been associated with nutritional level (Blay & Yuval 1997); decreasing fluctuating asymmetry of male supra-orbital bristles (Hunt et al. 1998); body size (Churchill-Stanland 1986, Orozco & Lopez 1993); eye length and mating activity (Norry et al. 1999). However, as yet there is still no unifying theory. In particular some studies have found an important role of male size (Orozco & Lopez 1993, Blay & Yuval 1997) while others have shown that body size itself is not a direct target of sexual selection (Hunt et al. 1998, Norry et al. 1999). Body size has been associated with male mating success in many studies of sexual selection (Davies & Halliday 1979, Berven 1981), particularly in some Diptera (Ewing 1961, Borgia 1981, Partridge et al 1987), but this association might be explained by male-male competition (Partridge et al. 1987) and even to differential survival associated with size (Hasson et al. 1993).

Most of the studies on sexual selection of *C. capitata* have been done under laboratory conditions or with laboratory adapted strains, so the potential role of male-male interactions and female

mate choice in the field is still poorly understood. The aim of the present work was to examine sexual selection based on morphometric traits of males of *C. capitata* from both a genetic sexing strain sterilized with irradiation and a wild population controlled with SIT under field cage conditions.

MATERIALS AND METHODS

Biological Materials

The laboratory strain used in this work was the Seib 6-96. This genetic sexing strain carries a white pupae (*wp*) mutation (Rössler 1979). A translocation T(Y:5) 2-22 (Franz et al. 1994) produces females with a white puparium and wild type males, enabling sorting of the sexes at this stage. Currently this strain is being reared at the Bioplanta Km8 (Mendoza, Argentina) for its use in a SIT program. Pupae were air-shipped to the testing area after irradiation. Wild flies were obtained from infested figs and peaches from the Alto Valle Region, Patagonia, Argentina. Fruits collected in the field and yards were taken to the laboratory and placed in trays on sand litter. Sand was checked periodically to collect wild pupae and land-shipped to the testing area. Once at the testing site pupae were placed in flasks until emergence. Virgin adults were aspirated from the flask within 24 h. after emergence in order to separate the sexes. Once sexed, the flies were kept in separate rooms until they reached sexual maturity at the age of 6-8 days for lab flies and 8-10 days for wild flies.

Field Cage Tests

The test was carried out at Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina. Field cages were used to analyze both sexual compatibility between strains and sexual selection based on male morphology. For sexual compatibility test results and procedures see Cayol et al. (1999). Outdoor cylindrical field cages (2.0 m high and 2.9 m diameter, saran screen 20 by 20 mesh) with a young host tree (*Citrus* sp.) inside were used to score male mating success under a mass selection experiment. Individuals from different strains were identified with water based paint labels painted on their notothorax. Each test consisted of the release of 30 wild males and 30 Seib 6-96 sterile males at dawn, about 7:00 AM. Half an hour later, 30 wild females were released into the cage. During a 7-h observation period, mating pairs were scored and gently removed from the cage as they formed with the aid of a vial. Male strain was determined and couples were placed in the shade until the end of copulation. Mated males were labeled as 'successful' while those males which were not able to copulate during the test were labeled as 'unsuccessful'.

Morphometric Analyses

Five body size related traits were measured in a sample of 141 unsuccessful and 149 successful males: eye length (EL), head width (HW), thorax length (TL), face width (FW), and wing length (WL) (Fig. 1). Measurements were performed with a binocular microscope fitted with an ocular micrometer. More details can be found in Norry et al. (1999).

Data Analysis

In the current study we use the term fitness to mean copulatory success. Successful individual fitness was coded 1, while unsuccessful individual fitness was 0. All morphometric measures were standardized to have mean zero and unit variance before the analyses were carried out. In a first exploratory approach, non-parametric Spearman's rank correlation tests were performed to estimate the correlation of male origin and fitness with each trait. As the results obtained from such analysis should be taken with caution due to possible correlation among traits, two statistical alternative approaches were applied: Step-wise multiple regression and principal component analysis + logistic regression. Principal Component Analyses (PCA) was used to identify major factors of variation within traits. To maximize the explained variance and obtain orthogonal variables, the factors (PCs) were rotated using VARIMAX method as done in previous works (Norry & Vilardi 1996, Norry et al. 1999).

RESULTS

Approximately 50% of the flies mated in each cage. Descriptive statistics for the traits measured are given on Table 1. It can be seen that for
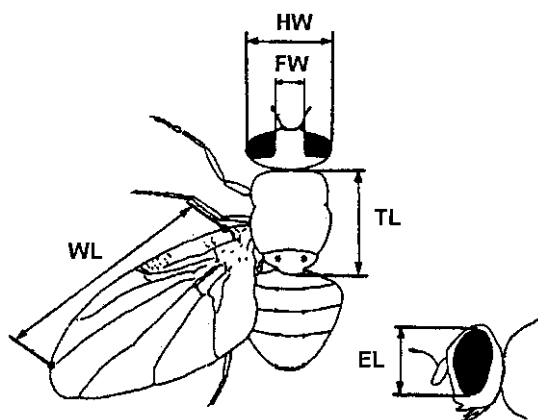

Fig. 1. Description of measured traits. WL, wing length; TL, thorax length; HW, head width; FW, face width; EL, eye length.

TABLE 1. DESCRIPTIVE STATISTICS (IN MM) FOR THE MORPHOMETRIC TRAITS ANALYZED IN MATED AND UNMATED SEIB6-96 (LAB) AND WILD MALES.

	Patagonia				Seib 6-96			
	Successful (N = 114)		Unsuccessful (N = 59)		Successful (N = 34)		Unsuccessful (N = 83)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Eye length	0.950	0.038	0.928	0.047	0.933	0.035	0.919	0.044
Face width	0.544	0.023	0.550	0.027	0.540	0.021	0.543	0.020
Head width	1.715	0.050	1.716	0.056	1.679	0.047	1.670	0.049
Thorax length	2.244	0.103	2.217	0.109	2.121	0.096	2.089	0.083
Wing length	3.571	0.111	3.559	0.112	3.415	0.115	3.387	0.111

all traits Patagonia males were larger than Seib 6-96 males. Moreover, in both strains successful males showed higher average values than unsuccessful males for all traits except FW.

In order to test the observed trends statistically, Spearman's rank correlations were estimated between 1) morphological traits and strain origin and 2) morphological traits and fitness (Table 2). Significant differences were observed between strains for all traits but FW. This analysis also showed that all traits except FW were positively and significantly correlated with fitness.

The identification of the actual target of sexual selection requires the possible correlation among traits to be removed. This was done by means of a Step-wise multiple regression analysis (Table 3). The three variables that showed a high association ($P < 0.01$) with fitness were TL, FW and EL (enumerated according to the relevance order), while the effect of WL was non-significant ($P = 0.086$). According to these results, the apparent lack of effect of FW on fitness in the original

Spearman's correlation might be a consequence of its correlation with the other size related traits.

In order to obtain completely uncorrelated (orthogonal) variables a principal component analysis (PCA) was performed with the morphometric traits analyzed. Three PCs were obtained from the factor analyses, which account for 90% of the total variance, and are explained respectively by TL and WL (PC1), FW (PC2), and EL (PC3). Factor loadings and eigen values are detailed in Table 4.

Since in the current experiment fitness was a dichotomic variable, a logistic (instead of a linear) regression analysis of fitness on the three axes from the PCA was performed. All the obtained axes showed a highly significant association with fitness (probability of mating in Table 4). It should be noted that the logistic regression coefficient for FW was negative, suggesting again that those males with smaller traits have the highest mating success.

DISCUSSION

In the present study head traits, eye length (EL) and face width (FW), and male size, thorax length (TL), have been revealed as targets of sexual selection. EL had previously been reported as a predictor of mating success under laboratory conditions with two different strains and in outdoors field cage tests with a long established laboratory strain (Norry et al. 1999). These authors have shown the occurrence of sexual selection on head morphology even in the absence of male competition, suggesting female choice. Results from the present study performed under a different testing protocol (wild females and outdoors field cages) are highly consistent. Also, EL appeared to be associated with mating success for both wild and Seib 6-96 males when each strain was analyzed separately (data not shown). These results can be interpreted as further evidence of the importance of mate choice over male-male competition in the sexual selection process acting on EL, and probably other head associated traits

TABLE 2. SPEARMAN'S RANK ORDER CORRELATION OF MORPHOMETRIC TRAITS WITH FITNESS AND STRAIN. IN ALL CASES r ESTIMATES ARE BASED ON 290 INDIVIDUALS. *P*: SIGNIFICANCE.

	Spearman R	N	*P*
Fitness			
Eye length	0.254	290	0.000
Face width	-0.068	290	0.248
Head width	0.157	290	0.007
Thorax length	0.332	290	0.000
Wing length	0.294	290	0.000
Male strain			
Eye length	0.219	290	0.000
Face width	0.086	290	0.143
Head width	0.399	290	0.000
Thorax length	0.588	290	0.000
Wing length	0.611	290	0.000

TABLE 3. STEP-WISE REGRESSION.

Variable	R²	Variable order	β	SE	P
Thorax length	0.541	1	0.248	0.078	0.002
Face width	0.249	2	-0.331	0.061	0.000
Eye length	0.318	3	0.232	0.064	0.000
Wing length	0.542	4	0.134	0.078	0.086

as well. Discrimination among potential mates on the basis of the male head morphology probably takes place when the female approaches the wing-fanning and head rocking male (Féron 1962, Calcagno et al. 1999) face to face (Norry et al. 1999). Hunt et al. (1998) have reported an association between male supra-orbital bristle fluctuating asymmetry and mating success, another example of head traits also identified as targets of sexual selection.

The finding that TL was associated with mating success suggests that male size may be another trait subjected to sexual selection. Pupal weight, as an adult size related trait, has been reported as a determinant of mating success in field cage studies of sexual selection with laboratory irradiated and wild flies (Orozco & Lopez 1993). Additionally pupal weight has been proved as a key determinant of both male and female mating activity (Churchill-Stanland et al. 1986), though these results account more for assortative mating rather than sexual selection towards male size. Blay & Yuval (1997) using wing length as a determinant of male size have found that for matings taking place within the first 1.5 h after release of females, variation in copulatory success could be explained by variation in wing length for both protein-fed and protein deprived males. When whole day observation data was pooled this effect disappeared for protein-fed males but remained for protein deprived males. Wing length can only be environmentally influenced at the larval stage and not after imaginal molt, and the experimental design followed by these authors determined the difference among protein-fed and protein-deprived males at the adult stage. It is thus tempting to conclude that the variation in mating success of these males is probably a consequence of differential mating activity, and not necessarily due to any possible effect of male size. Male size might be indirectly associated with mating activity as a consequence of nutritional reserves passed from the larvae to the adult, bigger adults being best nourished at the larval stage than the smaller ones.

In the case of the present study TL seems to be associated directly with copulatory success. This view is supported by the step-wise regression analysis (Table 3) and the significant correlation of PC1, which accounted for body size only, with probability of mating (Table 4). The non-significant effect of wing length (WL) in the step-wise regression (Table 3) indicates that wing size even when included on PC1 (Table 4) is a trait associated with mating success only as a side effect of its high correlation with TL. This is a clear example of the power of the combination of step-wise regression plus factor analysis coupled with regression analysis (either logistic or linear). These approximations succeed in removing WL as a target under selective pressure contrary to the first exploratory Spearman's rank correlation analysis (Table 2).

On the contrary, Whittier et al. (1994) found no correlation between male body weight and copulatory success, but they did detect a high correlation for number of attempted copulations, i.e., mating activity. Similar results have been found by Norry et al. (1999) in relation to WL and TL and by Hunt et al. (1998) for WL. These studies have been done under laboratory conditions, and the field cage test results reported by Norry et al. (1999) where no male size advantage was detected was carried out with a laboratory strain (approximately 30 generations under lab conditions). To summarize, the work by Orozco & Lopez (1993) was the only evidence to date of sexual selection favoring male size of lab males mated to wild females under field cage conditions. No evidence had been detected when females belonged to a lab strain even when tests were performed in outdoor field cages (Norry et al. in press), or when flies were tested under laboratory conditions

TABLE 4. FACTOR ANALYSES COUPLED WITH LOGISTIC REGRESSION ANALYSES.

Variable	PC1	PC2	PC3
Eye length	0.265	0.229	**0.935**
Face width	0.178	**0.948**	0.172
Head width	0.586	0.633	0.332
Thorax length	**0.876**	0.194	0.201
Wing length	**0.877**	0.204	0.202
Eigenvalue			
% Variance	39.6	28.6	21.9
Cumulative %	39.6	68.2	90.1
Probability of mating			
$\chi^2_{(1)}$	34.07	10.82	16.78
P	0.000	0.001	0.000
β	0.732	-0.398	0.501

(Hunt et al. 1998, Blay & Yuval 1997, for the case of pooled data, Norry et al. 1999). A close look at this apparent contradiction reveals that for the indoor and lab strain tests percentages of mating were very high (ranging from 69 to 86%) compared with percentages of mating found for wild females (50%, the present work). Such percentages imply that highly receptive yet low discriminating lab females may be responsible for hiding the effect of male size or size related traits on sexual selection. Rearing conditions may also represent a different environment where lek formation might not be so important as in nature.

Another possible cause for the lack of detectable size effects on sexual selection is that size can account for nutritional level only in those cases where food quality has been the same and any size variation is only a consequence of lesser consumption. Arita & Kaneshiro (1988) have found that flies emerging from coffee beans copulated more frequently even being significantly smaller than flies emerging from cherry beans. Orozco & Lopez (1993) also reported smaller size for flies emerging from coffee berries but no effect on mating activity was detected. In the current study, the advantage of bigger males was observed in both strains (the interaction fitness × origin was non significant), and the wild males, which were shown to have higher success (Cayol et al. 1999), were on average bigger than their mass-reared counterparts.

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EFFECT OF ADULT DIET ON SIGNALING ACTIVITY, MATE ATTRACTION, AND MATING SUCCESS IN MALE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Field experiments were performed to examine the effect of adult diet on calling activity, female attraction, and mating success in male Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann). In all tests, comparisons were drawn between males fed sugar only ("protein-deprived" males) and males fed a protein-sugar mixture ("protein-fed" males). In tests of long-distance attraction, aggregations consisting of protein-deprived males exclusively or protein-fed males exclusively were established in a coffee field, and females were released from a central release point. Protein-fed and protein-deprived males displayed similar calling levels, but approximately twice as many female sightings were recorded at groups of protein-fed males than at groups of protein-deprived males. A second test of female attraction compared single groups of protein-deprived and protein-fed males within the canopy of a field-caged host plant. As before, calling activity did not vary with diet, and in this case numbers of female sightings were also similar between aggregations of protein-fed vs. protein-deprived males. In mating trials conducted on field-caged host plants, protein-fed males achieved significantly more matings than protein-deprived males. These results are compared with other recent studies on the nutritional ecology of male Mediterranean fruit flies.

Key Words: medfly, *Ceratitis capitata*, adult diet, mate attraction, signaling

RESUMEN

Se llevaron a cabo experimentos de campos para examinar el efecto de la dieta de los adultos sobre la actividad de llamado, atracción de la hembra, y éxito de apareamiento en machos de la mosca del Mediterráneo, *Ceratitis capitata* (Wiedemann). En todas las pruebas se establecieron comparaciones entre machos alimentados solamente con azúcar (machos "privado de proteína") y machos alimentados con una mezcla de azúcar y proteína (machos "alimentados con proteínas"). En pruebas de atracción a largas distancias, grupos que consistían de machos privados de proteínas exclusivamente o de machos alimentados con proteínas exclusivamente fueron establecidos en un cultivo de café, y se liberaron hembras desde un punto central de liberación. Machos alimentados con proteínas y machos privados de proteínas desplegaron niveles de llamado similares, pero aproximadamente el doble de observaciones hacia los machos por parte de la hembra fueron registradas en los grupos de machos alimentados con proteínas comparados con el grupo de machos privados de proteínas. Una segunda prueba de atracción de las hembras comparó grupos individuales de machos privados de proteínas y grupos de machos alimentados con proteínas dentro del área foliar de la planta hospedera de la jaula en campo. Como se determinó anteriormente la actividad de llamado no varió con la dieta, y en este caso el número de observaciones hacia los machos por parte de las hembras fueron también similares entre los grupos de machos alimentados con proteínas vs. machos privados de proteínas. En las pruebas de apareamientos conducidas en plantas hospederas dentro de jaulas en el campo, los machos alimentados con proteínas lograron significativamente mayor número de apareamiento que los machos privados de proteínas. Estos resultados son comparados con otros estudios recientes sobre la ecología nutricional de los machos de la moscas del Mediterráneo.

In many insects, nutritional status may affect the ability of males to attract females and obtain matings. The association between nutrition and male reproductive behavior is manifest in 2 major ways. First, sexual activities, such as production of advertisement and courtship signals (Burk 1988, Landolt & Sivinski 1992, Epsky & Heath 1993, Droney 1996) and defense of calling sites or territories (Marden & Waage 1990, Plaistow & Siva-Jothy 1996, Hack 1997), are often energetically expensive, and male reproductive success may depend on the maintenance of sufficient fuel reserves

to adequately perform these behaviors. In addition to offsetting behavioral costs, males may use nutrients to synthesize material products needed for reproduction, i.e., pheromones (Edgar et al. 1974, Lofstedt et al. 1989, Nishida et al. 1997) or substances transferred during copulation, such as nuptial gifts (Gwynne 1990, Simmons et al. 1992) or sperm (Pitnick & Markow 1994).

In a series of provocative papers, Yuval and his colleagues (Warburg & Yuval 1996, 1997, Blay & Yuval 1997, Yuval et al. 1998, Field & Yuval 1999) investigated the effects of adult diet on male reproductive behavior in the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann. This species exhibits a lek mating system in which males defend individual leaves on host trees as mating territories and attract females to their perch via production of a sex pheromone (Prokopy & Hendrichs 1979, Arita & Kaneshiro 1989, Whittier et al. 1992). Yuval and his associates drew the following important conclusions: (a) in the field, lekking males were heavier and contained greater (mass-specific) amounts of sugar, protein, and protein than resting (non-lekking) males; (b) with the exception of sugar, nutrient levels declined through the day for field-collected, lekking males, suggesting a energetic cost of lekking; (c) under experimental dietary regimes, protein-fed males sustained longer bouts of pheromone-calling and courted more often than protein-deprived males; (d) in no-choice, laboratory trials, females mated more readily with protein-fed than protein-deprived males; and (e) females first mated to protein-deprived males were more likely to remate than females first mated to protein-fed males. Collectively, these results strongly suggest that adult diet has an important effect on the ability of *C. capitata* males to meet the energy costs associated with both courtship behavior and pheromone and ejaculate production.

The purpose of the present paper is to investigate further the effect of adult diet on the signaling behavior and mating success of *C. capitata* males. Three field experiments were conducted that compared protein-fed and protein-deprived males with respect to (1) calling level and female attraction between host-plants, (2) calling level and female attraction within the canopy of a single host plant, and (3) mating success under competitive (female choice) conditions.

MATERIALS AND METHODS

Female Attraction: between Host Plants

Because of the limited availability of wild individuals, the flies used in the between-plant, attraction trials were from a 6-year old strain mass-produced at the USDA-APHIS Fruit Fly Rearing Facility, Waimanalo, Oahu. This strain (known as "Maui-Med") originated from adults reared from coffee (*Coffea arabica* L.) collected on Maui. Non-irradiated pupae were obtained 2 days prior to eclosion, and adults were separated within 24 h of emergence and held in plastic buckets covered with nylon screening (volume 5 liters; 50-60 flies per bucket). Males were separated into 2 dietary regimes: "protein-deprived" males were given only sugar (sucrose) plus water, and "protein-fed" males were given a 3:1 mixture (by volume) of sugar and protein hydrolysate plus water. All females were given the sugar-protein mixture plus water.

Trials were conducted within a 500-ha coffee field 8 km south of Haleiwa, Oahu (elevation 300 m). Prior detection efforts involving fruit collections as well as trimedlure-baited traps revealed that the wild population of *C. capitata* was very low in the study area. Rows contained approximately 5 plants per 10 m, and adjacent rows were separated by 2 m of bare ground. Coffee plants were 2.0-2.5 m tall and bore no fruit during the study.

During a given trial, we monitored male pheromone calling and female sightings for groups of protein-deprived vs. protein-fed males. Four aggregations (or leks) were established at individual coffee plants, with 2 plants containing protein-deprived males exclusively and 2 plants containing protein-fed males exclusively. Groups of 6 males (7-13 days old) were placed in transparent plastic cups (volume 400 ml) that were covered on both ends with wire mesh. Cups were hung horizontally with wire (i.e., with the long axis parallel to the ground), with strips of masking tape placed on the upper surface to provide shaded, "leaf-like" perch sites. A total of 4 cups was used per aggregation, i.e., each lek consisted of 24 males of a given dietary type. Cups on a given plant were placed in the same portion of the canopy (usually within 15 cm of one another) at 1.0-1.5 m above ground.

The 4 test plants were located in 2 rows separated by a central row that contained the plant at which females were released. The resulting spatial arrangement was a rectangle, with the test plants located at the corners and the release point located at the center. Dimensions of this rectangle were 20 m (distance between test plants in the same row) by 6 m (distance between test plants in different rows). Lek sites at diagonal positions were composed of the same male type (i.e., protein-deprived or protein-fed). Ten minutes following placement of the males (between 0815-0830 h), 400 females (8-14 days old) were released at the base of the designated release plant. Starting 10 min after the release of the females, we recorded the numbers of calling males and perching females at each aggregation at 10 min intervals over the next 90 min (i.e., a total of 10 observations per replicate). Females were counted if they perched directly on or within 15 cm of a cup. Because females were not marked, the number of female sightings represents a composite measure

that included both arrivals to and retention near a male aggregation. Trials were conducted under sunny or partly cloudy skies at air temperatures of 22-25°C. The same 4 coffee plants served as lek sites over all replicates, and for a given plant, the type of male present—protein-deprived vs. protein-fed—was alternated between successive trials. A total of 16 replicates was conducted with a minimum of 2 days separating successive replicates to allow female dispersal from the study area.

Female Attraction: within a Host Plant

Tests on within-plant, attraction were performed using wild flies reared from fruits of Jerusalem cherry (*Solanum pseudocapsicum* L.) collected in Hawaii Volcanoes National Park, Hawaii. Larval development proceeded *in situ*, and pupation occurred in vermiculite. Emerging wild adults were handled in the same manner described above, with males separated into protein-deprived or protein-fed groups.

Trials were conducted using a field-caged guava tree (*Psidium guajava* L.) at the University of Hawaii Agricultural Station, Waimanalo, Oahu. The tree was 2.4 m tall, and the canopy occupied most of the upper half of the screen-mesh tent (height—2.4 m; diameter—3 m). For each replicate, we placed 2 cups (same type as described above), 1 containing 5 protein-deprived males and 1 containing 5 protein-fed males, in the eastern part of the canopy. The cups, separated by a distance of 1 m, were placed at a height of 1.7 m in areas having similar leaf densities. When tested, males were 10-18 days old.

Cups were placed on the tree between 0830-0845 h, and 10 min later 30 unmarked females (12-19 days old) were released at the base of the tree. Starting 10 min after female release, we recorded the numbers of calling males and perching females (on or within 15 cm of a cup) for the 2 cups at 2.5 min intervals over the next 90 min (i.e., 37 observation per replicate). At the end of a replicate, females were captured and removed from the tent. All tests were conducted during conditions of full or nearly full sunlight. The cups were suspended in the same locations over all replicates, with the type of male present, protein-deprived vs. protein-fed, alternated between successive tests. A total of 12 replicates were conducted.

Mating Competitiveness

Flies used in the mating trials were reared directly from fruits of *S. pseudocapsicum* as described above or from a stock started with 400-500 wild adults (collected from *S. pseudeocapsicum* as well) and maintained for 3 generations in the laboratory. Emerging adults were handled in the

same manner described above, with males separated into protein-deprived or protein-fed groups. For the purpose of identification, males of a given diet type were marked 1 day prior to testing by first cooling them for 2-3 min and then applying a small dot of enamel paint on the thorax. This procedure has no adverse effects, and flies resume normal activities within minutes of handling.

Trials were conducted at the aforementioned Agricultural Station using 2 field-caged guava trees similar to that described above. For each replicate, we placed 75 protein-fed males, 75 protein-deprived males, and 75 (protein-fed) females into a cage between 0800-0830 h, and mating pairs were collected over the next 5 h. When tested, males were 10-16 days old, and females were 12-18 days old. A total of 13 replicates was conducted.

Statistical Analyses

In both mate attraction experiments, male calling levels and female sightings for protein-deprived and protein-fed males were compared using the nonparametric Mann-Whitney test. Also, in both experiments, the relationship between male calling and female sightings among aggregations of a given diet type was described using simple linear regression. Significance of the regression was tested using ANOVA. Inter-dietary comparisons of regression lines (slopes and y-intercepts) were made using the Students t test.

For the mating trials, deviations from random mating were assessed in 3 ways. First, the sign test was performed over all replicates, testing whether the numbers of replicates in which protein-fed or protein-deprived males achieved the higher number of matings differed from that expected by chance (50% of the replicates for each male type). The binomial test was performed for individual replicates to compare the observed numbers of matings by protein-fed and protein-deprived males against those expected by chance (i.e., 50% of the matings by each male type). Third, the binomial test was run with data pooled over all replicates. The normal approximation (test statistic Z) to the binomial distribution was used for samples exceeding 25. All statistical procedures followed Zar (1996).

RESULTS

Female Attraction: between Host Plants

Protein-deprived and protein-fed males displayed similar levels of pheromone-calling. Within aggregations, an average of 7.2 (SD = 2.2) protein-fed males were pheromone-calling per observation compared to 7.0 (SD = 1.9) protein-deprived males (n = 32 for both groups; T = 1067.0; $P > 0.05$). In contrast, the numbers of female

sightings differed significantly between the 2 dietary types. For individual aggregations, the average total number of female sightings per replicate was 6.6 (SD = 3.9) for protein-fed males compared to only 3.6 (SD = 2.6) for protein-deprived males (n = 32 for both groups; T = 1223.0; $P < 0.02$).

For both diets, a significant positive relationship existed between female sightings and male calling level among aggregations (Fig. 1). Consistent with the above results, the slope observed for protein-fed males was greater than that found for protein-deprived males (1.7 vs. 1.3, respectively), although this difference was not significant (t = 1.1; df = 60; $P > 0.05$). Similarly, elevations did not differ significantly between the two diet types (t = 0.2; df = 61; $P > 0.05$).

Female Attraction: within a Host Plant

As in the previous experiment, the incidence of pheromone-calling was independent of male diet. For individual aggregations, 2.3 (SD = 0.8) protein-fed males were pheromone-calling per observation, on average, compared to 2.1 (SD = 0.8) protein-deprived males (n = 12 for both groups; T = 140.5; $P > 0.05$). Similarly, the average total number of female sightings at aggregations did not differ significantly between aggregations of protein-fed (x = 38.7; SD = 13.8) and protein-deprived (x = 35.6; SD = 18.9) males (T = 144.0; $P > 0.05$).

For both diets, a significant positive relationship existed between female sightings and male calling level among aggregations (Fig. 2). Regression lines did not differ significantly between pro-
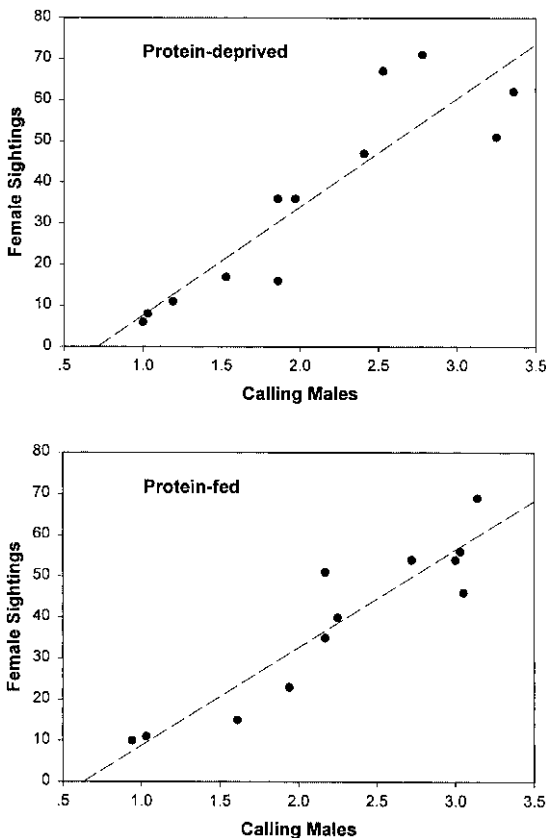
Fig. 1. Relationship between female sightings and male calling activity for protein-deprived and protein-fed males occurring on different host plants. Each point represents an individual aggregation; the ordinate represents total number of female sightings per replicate, and the abscissa represents the average number of calling males per observation for a given replicate. The regression equations were: protein-deprived males - Y = (1.3)X - 5.7 (r^2 = 0.52; F = 31.6; df = 1, 30; $P < 0.001$); protein-fed males - Y = (1.7)X - 5.7 (r^2 = 0.60; F = 44.6; df = 1, 30; $P < 0.001$).
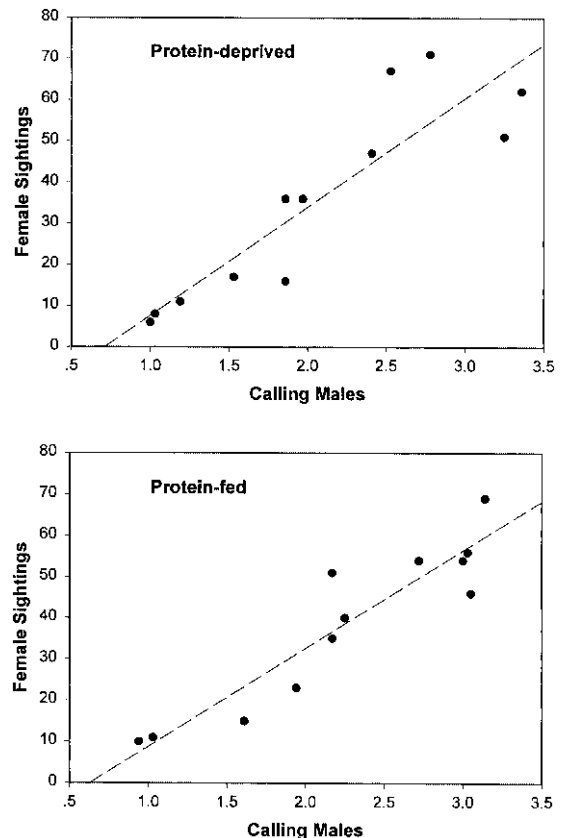
Fig. 2. Relationship between female sightings and male calling activity for protein-fed and protein-deprived males occurring on the same host plant. Each point represents an individual aggregation; the ordinate represents total number of female sightings per replicate, and the abscissa represents the average number of calling males per observation for a given replicate. The regression equations were: protein-fed males - Y = (23.9)X - 15.1 (r^2 = 0.86; F = 63.8; df = 1, 8; $P < 0.001$); protein-deprived males - Y = (26.1)X - 18.1 (r^2 = 0.79; F = 38.3; df = 1, 8; $P < 0.001$).

tein-fed and protein-deprived males in slope (t = 0.4; df = 16; $P > 0.05$) or y-intercept (t = 0.1; df = 17; $P > 0.05$).

Mating Competitiveness

An average of 24 matings (32% of the possible maximum; SD = 6.1; range = 16-41) was observed per replicate. The 3 analyses used to assess mating competitiveness yielded the same result: adult diet had a significant effect on male mating success. Protein-fed males obtained more matings than protein-deprived males in all 10 replicates for which a difference between male types was observed ($P < 0.001$; sign test; in 3 replicates the 2 male types had equal numbers of matings). Binomial tests on the individual replicates detected non-random mating in 4 of the 13 trials; in these cases, protein-fed males accounted for 71-83% of the matings ($P < 0.05$ in all instances; binomial test). Consistent with these results, a binomial test based on data from all replicates revealed a significant deviation from random mating, with protein-fed males achieving 60% (186/312) of all matings compared to 40% (126/312) for protein-deprived males (Z = 3.4; $P < 0.01$).

Although mating frequency varied with diet, the timing of mating activity was similar between protein-fed and protein-deprived males. Based on data from all replicates, we found no significant difference in the hourly distributions of matings for the 2 male types ($\chi^2 = 2.4$; $P > 0.05$). Over 50% of the matings occurred within the first 2 h of the tests for both protein-fed (110/186 = 59%) and protein-deprived (70/126 = 55%), and less than 10% of the matings were observed during the final hour for both types of males.

DISCUSSION

Previous studies on *C. capitata* males have demonstrated associations between diet and activity level, activity level and mating success, or among all 3 of these parameters. Warburg & Yuval (1996) found that protein-fed males expended more energy during courtship (i.e., courted more vigorously) than protein-deprived males, although they did not examine the relationship between courtship vigor and success. Conversely, Whittier et al. (1994) observed that mating frequency was positively associated with calling and courting frequency but did not examine the effects of adult diet on male activity. Prior to the present study, only Field & Yuval (1999) compared the activity level and mating success (or, more accurately, an index of mating success) of *C. capitata* males reared under differing dietary regimes. They reported that protein-fed *C. capitata* males exhibited longer calling bouts and more frequent courtships (the small number of observed matings precluded statistical analy-

sis) than protein-deprived males. Similar results have been reported for other Diptera. In *Drosophila grimshawii* Oldenberg, males maintained on a high protein diet displayed more vigorous courtship displays and obtained more matings than males fed a low protein diet (Droney 1996). Similarly, liver-fed males of the black blowfly, *Phormia regina* (Meigen), displayed a higher level of sexual activity (as indicated by attempted mountings) and inseminated more females than sugar-fed males (Stoffolano et al. 1995).

In the present study, diet-related differences in male calling activity were not evident in either the between- or within-plant, female attraction experiments. However, it is possible that this outcome reflected the short duration of our tests. For example, Field & Yuval (1999) reported diet-based, behavioral differences based on observations made over 7 h intervals, whereas we measured calling activity for periods of only 90 min. If calling involves substantial energy expenditure and if the presence of protein in the diet affects a male's ability to meet these costs, differences in calling level between protein-fed and protein-deprived males may increase with time and be evident only after sustained periods of activity. Although this possibility can not be ruled out, the finding that the temporal distributions of matings were similar between protein-fed and protein-deprived males strongly suggests that adult diet had a minor impact, if any, on the overall level of male sexual activity.

Despite the apparent similarity in activity levels, protein-fed males enjoyed a mating advantage over protein-deprived males. It is not known what factor(s) was responsible for this mating differential. The finding that females were sighted more frequently near protein-fed than protein-deprived males in the between-plant, attraction experiment indicates that diet affected the attractiveness of the pheromonal signal. Thus, the mating advantage of protein-fed males may have reflected directly their enhanced ability to attract potential mates to their territory. While plausible, the within-plant, attraction experiment failed to detect a diet-related difference in male attractiveness, suggesting that potential diet effects on signal quality may be manifest over large (>5 m) but not small (<2 m) distances. Alternatively, the differential mating success may have reflected differences in the courtship behavior of protein-fed and protein-deprived males. As noted above, adult diet affected the incidence of particular courtship displays in *Drosophila* males (Droney 1996), and a similar phenomenon may occur in *C. capitata* as well. Evaluation of this explanation requires detailed analysis of the videotaped courtship sequences of protein-fed and protein-deprived males.

Regardless of the underlying cause, the relationship reported here between adult diet and

mating success has important implications for the procedures used in the release of sterile males in suppression or eradication programs against the Mediterranean fruit fly. In ongoing programs, newly emerged sterile flies are typically held for several days prior to release and given only sugar and water for nutrition. Our data, along with those of Blay & Yuval (1997), suggest that the addition of a protein source to the holding cages might enhance the mating competitiveness of the sterile males. To examine this possibility, we are currently conducting experiments that compare the mating success of protein-fed vs. protein-deprived sterile males in competition with wild males.

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FACTORS AFFECTING FEMALE REMATING FREQUENCY IN THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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Abstract

Mating and remating of two laboratory strains (Petapa and Guate), one wild population (Antigua) of *Ceratitis capitata* (Wiedemann) and one of the hybrids between them were studied under laboratory conditions. No evidence of sexual isolation at first mating was found among them. Remating frequency was higher under crowded conditions for the two laboratory strains. The probability of Petapa females remating depended more on the origin of the male and was negatively associated with the duration of the first mating, but these variables had no effect on remating tendency of Guate females. Matings by Petapa males were significantly less prolonged than those of Guate or hybrid males. With respect to remating, Petapa non-virgin females preferred Petapa to Guate males.

Key Words: remating behavior, mating duration, medfly, *Ceratitis capitata*

Resumen

Apareamiento y re-apareamiento de dos razas de laboratorio (Petapa y Guate), una formada por una población salvaje (Antigua) de *Ceratitis capitata* (Wiedemann) y una de híbridos entre ellos, fueron estudiadas bajo condiciones de laboratorio. No se encontró evidencia de aislamiento sexual durante el primer apareamiento entre ellas. La frecuencia de re-apareamiento fue mayor bajo condiciones de hacinamiento para las dos razas de laboratorio. La probabilidad de que las hembras de Petapa se re-aparearan dependió más del origen del macho y estuvo negativamente asociada con la duración del primer apareamiento, pero estas variables no tuvieron ningún efecto en la tendencia de re-apareamiento en las hembras de Guate. Los apareamientos por parte de los machos de Petapa fueron significativamente menos prolongados que los de Guate o que los machos híbridos. Con respecto al re-apareamiento, las hembras no vírgenes de Petapa prefirieron machos de Petapa que machos de Guate.

The Mediterranean fruit fly (medfly) *Ceratitis capitata*, (Wiedemann) is a highly destructive pest, infesting more than 200 species of fruits and vegetables (Christenson & Foote 1960) and creating a serious impact on the economy of many countries. It is largely controlled using the Sterile Insect Technique (SIT) (Cunningham et al. 1980, Klassen et al. 1994, Hendrichs et al. 1995), which relies on the release of mass-reared sterile males into a target wild population with matings between sterile males and wild females failing to result in the production of viable offspring (Knipling 1955). The fact that a female may mate more than once is significant for the SIT since it increases the chances that the female will encounter and mate with at least one fertile male (Bloem et al. 1993).

Studies on the reproductive biology of the medfly have reported that both sperm and accessory gland fluids may inhibit female remating to some extent (Delrio & Cavalloro 1979, Miyatake et al. 1999). Besides, after the first mating females

switch from mate searching towards oviposition-site searching (Jang 1995, Jang et al. 1998). These mechanisms are insufficient, however, to restrict remating entirely. Multiple matings have been reported both under laboratory conditions (Katiyar & Ramirez 1970, Nakagawa et al. 1971, Bloem et al. 1993) and in the field (McInnis 1993, Yuval et al. 1996). McInnis (1993) found that wild females trapped in a sterile-male release area contained sperm from both irradiated and wild males, but unfortunately no field data are available regarding which male mated first. Remating has been associated with the duration of the first mating (Farias et al. 1972, Saul et al. 1988) and also with the nutritional status (protein fed or deprived) of the male (Blay & Yuval 1997). Females mated to sterile males with inactive sperm have a higher remating frequency than those mated to normal males (Cavalloro & Delrio 1970, Katiyar & Ramirez 1970, Bloem et al. 1993). On top of lacking fertile sperm, sterile males may differ from their wild counterparts in a variety of as-

pects as a result of inadvertent yet strong selection on flies to adapt to artificial factory conditions (Briceño & Eberhard 1998). The effect of such selected changes on remating is unknown. Hence any information comparing the remating frequency of females mated either with laboratory-adapted or wild males is potentially of importance for the success of the SIT.

The aim of the present work is to study remating frequency in medfly females from strains with different colonization histories. Their responses when first mated to a male from the same or from a different strain was investigated using a continuous observation design and individual labels for the flies.

MATERIALS AND METHODS

Biological Material

Three strains of medflies were used in the study: Petapa, Guate and Wild. The Petapa strain was a subculture from the mass rearing strain established at the Moscamed factory in Guatemala in 1984 for SIT programs (Rendon 1996). It originated from pupae collected from infested coffee beans near Lake Atitlán, Guatemala. The Guate strain originated also from coffee collected in southwestern Guatemala near Retalhuleu and established as a colony at the School of Biological Sciences, Manchester University, UK, seven months before the study. Wild flies emerging from pupae from coffee beans near Antigua City, Guatemala, were also used. An inter-strain hybrid was produced by crossing wild males with Petapa females. This Hybrid strain was investigated only in its first generation.

Rearing Procedures

Flies were reared at $25 \pm 1°C$, $70 \pm 5\%$ relative humidity and a photoperiod of 12:12 (L:D). The adult diet consisted of a yeast:sucrose mix (1:3) and larvae were maintained on a carrot-based diet (Busch-Petersen & Wood 1986). Experimental flies were kept virgin until tests began. Virgin adults were collected within 24 h of emergence and immobilized (one minute under -20°C) in order to separate the sexes. Once sexed, the flies were kept under the same conditions until they reached sexual maturity at the age of 5-7 days old. Wild flies were tested at the age of 7-9 days.

Re-mating Trials

Mating cages were established to assess remating frequency. Each cage consisted of a clear plastic box, $30 \times 20 \times 20$ cm., having one side fitted with a sleeve to allow the collection of mating couples. In every trial 25 males of each of the two strains being compared and 25 females of the same two strains, resulting in a total number of 100 flies, were released into a mating cage. Released flies were individually labeled one day prior to testing. They were immobilized by rapid cooling and a small printed letter (Arial, font size 3) was glued to its thorax with a dot of paint (McInnis et al. 2002). Different colors of paper were used to identify strains. Once labeled, males were released into the mating cage and females were released into a separate cage. All cages contained adult diet and water. Both cages were then transferred to the testing room ($24 \pm 1°C$, $64 \pm 1\%$ RH, and 12:12 L:D).

On testing day 1, females were released into the mating cage with the males, half an hour after room lights were turned on. Every copulating pair was gently removed from the cage and both male and female were identified. Mating duration was timed, and each pair released back into the mating cage after separation. Water and food were provided throughout the test. After eight h of observation the females were removed from the mating cage, and transferred into a separate cage with food and water. Flies were left overnight in the testing room. On the following day (testing day 2) the females were released back into the mating cage half an h after the lights were turned on. Couples were collected and scored during a six-h observation period, after which the females were again removed. The procedure was daily repeated during four consecutive days, with six and four-h observation periods on days three and four. Flies were constantly observed except for occasional breaks of no more than half an h (during these breaks it is highly unlikely that any successful copulations occurred because copula duration averaged more than two h).

A parallel set of tests was performed with lower fly density. In this situation each cage contained only 20 flies (5 females of two strains and 5 males of the same strains). Otherwise, these tests followed the same procedure as described above. The two types of tests will be referred to as crowded (100 flies/cage) and relaxed (20 flies/cage) conditions.

Under crowded conditions, 3 tests were performed: Wild and Petapa (1 replicate), Guate and Petapa (7 replicates) and Hybrid, Guate and Petapa (1 replicate). In this last case, Hybrid males were used in the place of Guate males. Under relaxed conditions, two tests were performed: Guate and Petapa (15 replicates) and Hybrid and Petapa (5 replicates).

Statistical Analyses

The percentage of mated females was calculated in order to determine whether the conditions were optimal for mating tests. A χ^2 test of homogeneity was calculated to assess if the strains mated assortatively. Relaxed cages were

pooled to provide an adequate sample size and crowded cages were analyzed separately The effect of density on remating rate was determined by evaluating the Guate × Petapa crowded cages against the relaxed ones by means of a χ^2 test. Remating rate was calculated for every female strain in each cage grouped by the origin of the first male they mated with.

To investigate the effect of different aspects of the first mating on the probability of remating of Guate and Petapa females, a logistic regression analysis was performed. All the mated females were assigned a value of 0 if they mated only once, or a value of 1 if they remated during the test. Remating condition (0 for non-rematers or 1 for rematers) was used as the dependent variable. The origin and copulatory status (virgin or non-virgin) of the first male, the duration of the first mating and the total number of copulations that the first male achieved during the test (# matings) were computed as the independent variables. Significance levels were determined using log-likelihood ratio χ^2 tests. The presence of correlation between variables was analyzed. Females that died during the experiment were removed from the remating analysis. Given that only one cage was run with wild females and that both wild and Hybrid females hardly remate at all, females from these two strains were not considered in the analysis.

The duration of mating for each type of cross was analyzed by ANOVA and Tukey's HSD tests. For females that mated more than once, their preference in the selection of the second partner was investigated by means of a χ^2 test of homogeneity. All statistical analyses were performed using Statistica for Windows (Statistica 5.1, StatSoft, Inc. 1996).

<center>RESULTS</center>

General Mating Conditions and Mate Selection

For crowded cages, the percentage of mating was high and there was no evidence of sexual isolation among strains (Table 1). Good mating conditions and lack of assortative mating were also shown for relaxed cages (Table 2). The high percentage of matings achieved both in relaxed as well as in crowded conditions indicates that environmental and biological (nutritional level and age of flies) conditions were adequate. However, wild males did not mate readily in laboratory cages, and never with their own females, Cage 1. The low number of matings achieved by them could probably be explained by a lack of sexual maturation.

Factors affecting remating rate by Guate and Petapa females

The proportion of females that mated more than once was significantly higher under crowded conditions compared to relaxed conditions for both female strains (Table 3-A). Consequently all subsequent analyses were performed for each density separately. However when the results are broken down according to the type of male, the effect of density was significant only for Petapa x Petapa matings (Tables 3-B and 3-C). Mean remating rates (number of rematers/number of mated females in each cage) were higher for crowded than for relaxed conditions for all crosses (Fig. 1), but no statistical differences were found (Mann-Whitney test, $P > 0.05$), probably due to small sample size in relaxed cages.

The logistic regression analysis revealed that other variables apart from fly density in the cage affected remating tendency (Table 4). The previous mating history of the first male was significantly associated with the remating rate of Guate females under both conditions, with non-virgin males being more successful at inhibiting remating (Guate females, Table 4). In contrast male mating status revealed no significant effect on Petapa females.

The number of matings achieved by each male during the test showed no association with remating probability for both female strains.

The origin of the first male significantly affected the remating rate of Petapa females under both density conditions. Petapa females showed a higher remating rate if first mated to Petapa males than Guate or Hybrid males (Fig. 1-A). Under relaxed conditions remating rate was lower if the female mated to Hybrid males instead of Petapa males (Mann-Whitney test, Z = 2.12, $P = 0.034$). Moreover, of the 4 Petapa females that mated to Wild males none remated during the experiment. In contrast Guate females showed no sensitivity to male origin (Fig. 1-B). It should be noted that Petapa females were exposed to Guate, Petapa, Wild and Hybrid males, whereas Guate females had access only to Guate and Petapa males.

The duration of the first mating was negatively associated with the likelihood of remating of Petapa females under relaxed conditions, and the same tendency, close to significance, was found under crowded conditions. Guate females, on the contrary, showed no differences in remating tendency in association with mating duration.

The duration of copulation of males from different strains differed consistently irrespective of which type of female they mated with (Table 5). Matings involving Petapa males were shorter than those involving Hybrid or Guate males (Tukey's HSD test; $P < 0.01$). Mating duration was highly correlated with male origin ($r^2 = 0.137$, $P < 0.001$) but not with female origin ($P > 0.05$).

Second Mating of Guate and Petapa Females

Under crowded conditions, there was evidence of non-random mating (Table 6). Petapa females showed a strong preference to remate with Petapa

TABLE 1. PERCENTAGE OF MATING, NUMBER OF COUPLES OBTAINED FOR EACH MATING COMBINATION AND MATING COMPATIBILITY BETWEEN STRAINS IN CROWDED CAGES.

Cage number	Strains	% Mating	Mating combination[a]				χ^2 P value[c]
			W × W[b]	W × P	P × W	P × P	
1	Wild/Petapa	75%	0	4	3	8	
			G × G	G × P	P × G	P × P	
2	Guate/Petapa	100%	14	10	11	14	ns
3	Guate/Petapa	88%	13	9	13	11	ns
4	Guate/Petapa	91%	13	6	6	11	0.0469
5	Guate/Petapa	100%	5	15	7	9	ns
6	Guate/Petapa	90%	9	9	15	11	ns
7	Guate/Petapa	85%	13	6	7	7	ns
8	Guate/Petapa	94%	9	12	15	11	ns
	Guate/Petapa[d]	92.57%	76	67	74	74	
			H × G	H × P	P × G	P × P	
9	Guate/Petapa/Hybrid	86%	8	9	14	12	ns

[a]Only the first mating was considered for each female.
[b]Male strain is listed first, with G for Guate, H for Hybrid, W for Wild and P for Petapa strains.
[c]Significance of homogeneity χ^2 value.
[d]Mean percentage of mating for Guate × Petapa 7 replicates.

TABLE 2. MEAN PERCENTAGE OF MATING ACHIEVED FOR ALL CAGES, TOTAL NUMBER OF COUPLES OBTAINED FOR EACH MATING COMBINATION AND MATING COMPATIBILITY BETWEEN STRAINS UNDER RELAXED CONDITIONS.

Strains	% Mating	Mating combination[a]				χ^{2c}
		$G \times G$[b]	$G \times P$	$P \times G$	$P \times P$	
Guate/Petapa	90.3%	39	23	35	30	ns
		$H \times H$	$H \times P$	$P \times H$	$P \times P$	
Hybrid/Petapa	85.5%	11	12	7	8	ns

[a]Only the first mating was considered for each female.
[b]Male strain is listed first, with G for Guate, H for Hybrid, and P for Petapa strains.
[c]Significance of homogeneity χ^2 value.

males over Guate males (χ^2 = 9.25; P = 0.002). This tendency was stronger if the female had originally mated with a Petapa male, although the difference was not significant (χ^2 = 3.62; P = 0.057, data not shown). Guate females showed no preference for males of a particular strain for either their first or second mate. Under relaxed conditions, no evidence of assortative mating was detected (χ^2 = 0.61; P = 0.434), although Guate females showed a just significant preference for Petapa males (χ^2 = 3.88; P = 0.049).

DISCUSSION

Some interesting observations on remating frequency of *Ceratitis capitata* females from different strains, particularly on the influence of density of flies and variables of the first mating have been shown in the present report.

The observed effect of density on remating (Table 3) reinforced the importance of test design and underlined the limitation of comparisons between different studies and extrapolations from them (see also Fowler & Partridge 1989). Higher remating rates under crowded conditions are probably due to prolonged exposure to courting males and not enough space for females to escape from them. Laboratory-adapted flies probably have higher remating rates compared to flies from the wild, though more information on remating rate of wild females is needed to support this hypothesis.

Despite the effect of density on remating frequency, the present work suggests that other variables influence female remating (Table 4). Male

TABLE 3. TOTAL NUMBER OF FEMALES THAT MATED ONCE OR MORE THAN ONCE ACCORDING TO THE DENSITY OF FLIES IN THE TESTING CAGE.

	Petapa females		Guate females	
	Crowded	Relaxed	Crowded	Relaxed
A. All males[a]				
Mated once	66	37	51	35
Remated	68	16	95	34
Total	134	53	146	69
χ^2	6.49*		4.87*	
B. Petapa males[b]				
Mated once	28	19	24	15
Remated	41	11	48	18
χ^2	4.34*		1.42	
C. Guate males[c]				
Mated once	36	18	27	20
Remated	27	5	47	16
χ^2	3.22		3.60	

[a]All males irrespective of their origin.
[b]Only matings involving Petapa males.
[c]Only matings involving Guate males.
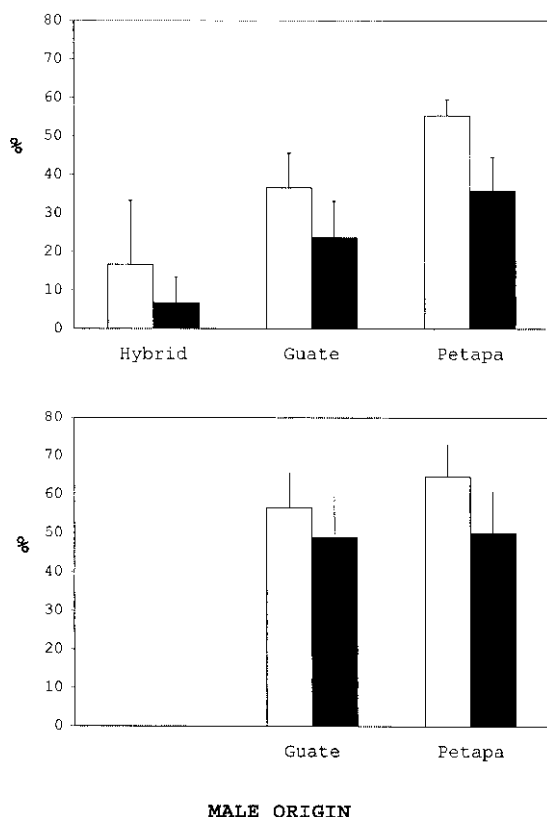Crowded: 100 flies/cage.
Relaxed: 20 flies/cage.
*P < 0.05.

Fig. 1. Mean percentage (±SE) of remating for Petapa and Guate females according to male origin and fly density: (□) crowded, (■) relaxed conditions.

reproductive status has been reported to have no effect on remating of female medfly (Bloem et al. 1993). But in the present case, mated males seemed better at inhibiting remating than virgin males (Table 4, status). It should be considered that as days went by during the tests, there was a growing deficit of virgin males and a reduction in the time available for females to remate (i.e., to show their remating tendency). When females that mated on the first day of the trial were the only ones analyzed, no significant effect of male status was revealed (data not shown), which reinforced the idea that the apparent effect of male status on remating could be due to a design artifact. With respect to the lack of association between number of matings achieved by the male and remating (Table 4, # matings), it may be suggested that males with a higher mating success do not necessarily induce a higher refractory period. Again the test design could interfere in the interpretation of the results. Very successful males found virgin females hard to encounter, so they mated several times with rematers and thus were not taken into account in the logistic regression. To determine whether mating success and post-

copulatory success are correlated or not, a different test design should be used.

The tendency revealed of higher rates of remating in Petapa females first mated to Petapa males than those mated to Hybrid or Guate males have remained under both densities (Fig. 1). This result suggests that aspects of the first mating that are strain dependent may affect female tendency to remate regardless of fly density. The present study showed that remating rate of Petapa females was determined by the male strain under crowded conditions and by both male strain and mating duration under relaxed conditions.

Saul et al. (1988) has reported mating duration as a determinant of the refractory period and remating rate. Remating propensity may be associated with insufficient sperm load (Farias et al. 1972). The present study showed differences between Guate and Petapa males mean copula duration times. On this basis, the observed effect of male strain on Petapa females remating tendency (Table 4, male origin) could be attributed to the difference in the mean time of copula duration. If mating duration and origin of the male are analyzed together in a two-variable logistic regression to obtain the predicted values for remating tendency, some effect of male strain, apart from mating duration, is shown to be still present in the determination of Petapa females remating tendency (Fig. 2). Interestingly, even though remating is higher for Petapa females mated to Petapa males than other males, the shape of the regression is very similar for the three male strains. This suggests that as mating duration increases, remating probability decreases at a similar rate for the three types of male. Besides this, it could be postulated that there is another factor determining remating that is independent of mating duration.

No association was found between remating and mating duration on Guate females (Table 4), possibly due to the fact that Guate females were not mated with any strain whose males lasted (on average) more than their own. However, a higher remating probability for Guate females mated to Petapa males compared to Guate × Guate matings, as a consequence of shorter copula, would be expected. The reason why Guate females did not show such a tendency is unclear.

The shorter duration of matings involving Petapa males compared to Guate or Hybrid males provides evidence that males from long-established strains have shorter mating times than recently colonized or wild ones. These results are in agreement with the study of Cayol et al. (1999) in which mass-reared males copulated for less time than wild males, with either mass-reared or wild females. Shorter copula duration as well as a shorter time spent during courtship of laboratory males to avoid interruptions from other males reported by Briceño & Eberhard (1998) appear to be examples of laboratory induced changes.

TABLE 4. LOGISTIC REGRESSION ANALYSIS OF THE EFFECTS OF DIFFERENT ASPECTS OF THE FIRST MATING ON THE PROBABILITY OF REMATING.

| | Petapa females | | | | Guate females | | | |
| | Crowded | | Relaxed | | Crowded | | Relaxed | |
Independent variable	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Status of the male	0.05	0.825	3.32	0.067	4.02	0.045	5.10	0.024
# matings	0.24	0.622	1.81	0.178	2.04	0.153	0.00	0.954
Origin of the male	4.31	0.038	4.98	0.026	0.16	0.689	0.70	0.401
Duration	3.27	0.071	9.73	0.002	0.07	0.795	0.33	0.568

For Petapa females all cages were considered, for Guate females only the Guate × Petapa cages were used (df = 1).

TABLE 5. MEAN MATING DURATION (H: MM) FOR EACH MATING COMBINATION.

| | Origin of females | | | | | | | |
| | Wild | | Hybrid | | Guate | | Petapa | |
Origin of males	Mean	N	Mean	N	Mean	N	Mean	N
Hybrid	—		2:31	(4)	3:01	(6)	3:00 a	(15)
Guate	—		—		2:34 a	(98)	2:37 a	(61)
Petapa	1:46	(3)	2:21	(6)	2:02 b	(99)	2:08 b	(80)

Only matings involving virgin flies were computed. Means are shown in bold followed by different letters identifying significance groups according to Tukey's HSD test with $P < 0.01$; mating combinations involving more than 10 cases were the only included in this analysis.

TABLE 6. NUMBER OF COUPLES FOR EACH MATING COMBINATION FOR THE FIRST (VIRGIN) AND SECOND (NON-VIRGIN) MATINGS.

	Mating combination[a]				
Crowded cages	G × G	G × P	P × G	P × P	$\chi^{2\,b}$
First mating	76	67	74	74	0.29
Second mating	48	17	48	50	9.98**
Relaxed cages	G × G	G × P	P × G	P × P	
First mating	39	23	35	30	1.07
Second mating	11	7	23	9	0.61

[a]Male strain is listed first, with G for Guate and P for Petapa strains.
[b]Homogeneity χ^2 value, df = 1.
**$P < 0.01$.
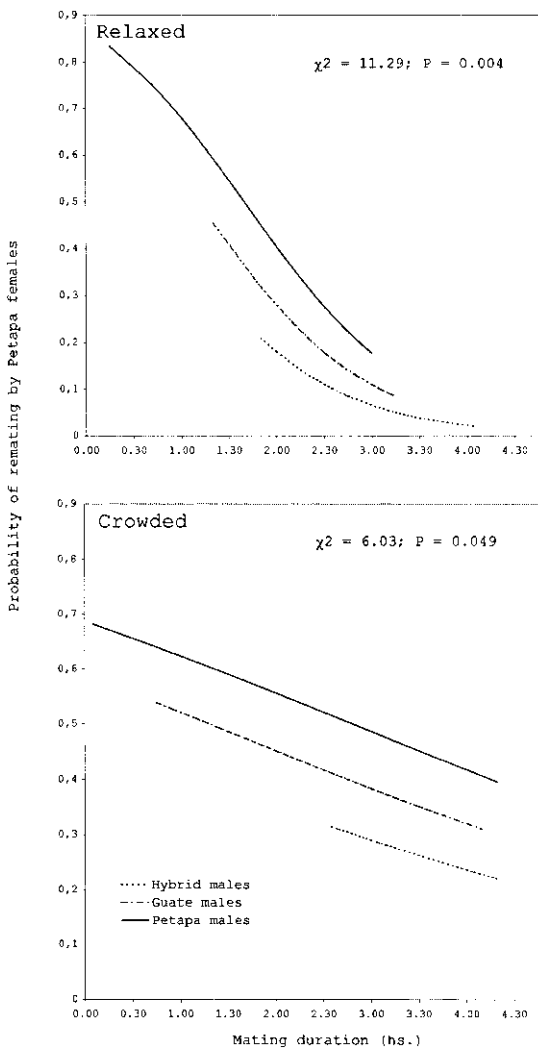
Fig. 2. Effect of male strain and copula duration on probability of remating for Petapa females for relaxed and crowded conditions. Log-likelihood ratio determines the significance of the logistic regression.

Few factors were found to influence the remating rate of Guate females. However, Guate females only encountered males from their own strain and Petapa males. Trials with Wild or Hybrid males may have revealed more associations.

The strong preference showed by non-virgin Petapa females for Petapa males suggests assortative mating after the first mating. This phenomenon has not been reported in medfly females before and might suggest some kind of post-copulatory selection (Eberhard & Cordero 1995). The way by which Petapa females discriminate between males is unknown although male courtship should be considered.

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EFFECTS OF POST-TENERAL NUTRITION ON REPRODUCTIVE SUCCESS OF MALE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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Abstract

To realize their reproductive potential, male Mediterranean fruit flies must run a gauntlet of behavioral challenges during which they may be edged out by rivals, or fail the acid test of female choice. Milestones on this perilous road include: 1. showing up at a lek site, 2. emitting pheromone, 3. performing courtship, 4. Copulation, 5. sperm transfer and storage, 6. fertilization of eggs, 7. preventing or delaying female remating. In a number of recent studies focused on each of these steps we tested the hypothesis that post-teneral male nutrition affects male sexual performance. Both field and laboratory data indicate that protein nutrition increases a male's probability of emitting pheromone in a lek. Field cage data show that protein fed males are also more likely to engage in critical elements of close-range courtship, and evidence from several studies indicate that protein fed males are more likely to copulate than sugar-fed or starved flies. As to sperm transfer and storage, we find that the context of the experiment and the source of flies used affect the outcome, suggesting that diet alone cannot explain the variability in the probability of sperm being transferred, and in the amount of sperm transferred. To date we have not studied effects of male diet on fertilization. Nevertheless, we have shown that male diet significantly affects female receptivity: females whose first mate was protein-deprived, remate sooner than females whose first mate was protein-fed.

Key Words: *Ceratitis*, nutrition, sexual behavior, reproductive success, SIT

Resumen

Para realizar su potencial reproductivo, los machos de la mosca mediterránea deben aceptar el reto en cuanto a los desafíos de comportamiento durante los cuales ellos podrían ser eliminados por rivales, o fallar la prueba critica de la selección de la hembra. Los puntos de referencia en este peligroso camino incluyen: 1. presencia en el lugar designado para el cortejo, 2. emisión de feromonas, 3. realización del cortejo, 4. copulación, 5. transferencia de esperma y almacenamiento, 6. fertilización de los huevos, 7. prevención o retraso del re-apareamiento de la hembra. En estudios recientes enfocados a cada uno de estos pasos, se probó la hipótesis de que la nutrición posterior a la muda en los machos afecta la actividad sexual del macho. Tanto la información del campo como la de laboratorio indican que la nutrición con proteínas aumenta la probabilidad del macho de emitir feromonas en el lugar del cortejo. La información de las jaulas de campo demuestran que los machos alimentados con proteínas también son más probables que se envuelvan en elementos críticos del cortejo a corta distancia, y evidencia proveniente de varios estudios indican que los machos alimentados con proteínas son mas dados a copular que las moscas alimentadas con azúcar o no alimentadas. Con respecto a la transferencia de esperma y almacenamiento se ha conseguido que el contexto del experimento y la fuente de las moscas utilizadas afectan el resultado, sugiriendo que la dieta por si sola no puede explicar la variabilidad en la probabilidad que el esperma se transfiere, y en la cantidad de esperma transferida. Hasta la fecha no hemos estudiado los efectos de la dieta del macho sobre la fertilización. No obstante, se ha demostrado que la dieta del macho afecta significativamente la receptividad de la hembra: las hembras cuyo primer apareamiento fue con machos privados de proteínas, se re-aparearon con mayor rapidez que las hembras cuyo primer apareamiento fue con machos alimentos con proteínas.

For male Mediterranean fruit flies, *Ceratitis capitata*, (Diptera: Tephritidae), as indeed for males in many insect species (Thornhill & Alcock 1983), the rewards of investing resources in sexual displays and courtship are not reaped equally by all investors (Whittier et al. 1994). The sterile insect technique (SIT) (Knipling 1955, Krafsur 1998), is being used in many parts of the world to control medfly populations or prevent their establishment in new regions (Hendrichs et al. 1994, 1995, and references therein). As the success of this technique hinges on the ability of released sterile males to copulate and inseminate females in the field, much of the applied research on the sexual biology of this insect is aimed at determining precisely what it is that makes a successful male, (Calkins 1984, Cayol et al. 1999, Shelly & Whittier 1996).

In insect species that acquire and store reproductive resources during the larval period (such as ephemeropterans, most trichopterans and many lepidopterans), male reproductive success

hinges greatly on how well they did as immatures. Thus it is common to find a correlation between male size and copulatory success in these species (Bisoondath & Wiklund 1997, Flecker et al. 1988.). Individuals that develop in sub-optimal environments become small adults and cannot do much to improve their lot. Conversely, the reproductive success of numerous other species hinges on their ability, as adults, to forage efficiently for various nutritional resources (see Droney & Hock 1998, Yuval et al. 1994). Adults in these species must decide when to forage for nutritional resources, when to invest time in reproduction, and when to desist altogether. These decisions are frequently triggered by physiological thresholds that dictate or regulate the expression of a discrete behavior. In these species the handicap of a poor larval environment may be later overcome by foraging successfully. Though the host in which larvae develop greatly affects adult qualities such as size, energetic reserves and fecundity (Hendrichs et al. 1991, Krainacker et al. 1987), the activity patterns of adult medflies in the field suggest that they belong to the latter group, as both males and females spend a considerable amount of time foraging for and feeding on various sources of carbohydrates and proteins (Hendrichs & Hendrichs 1990, Hendrichs et al. 1991, Warburg & Yuval 1997a, Warburg & Yuval 1997b, Yuval & Hendrichs 1999).

The mating system of the medfly, based on leks, is relatively complex, offering females many opportunities for choice (Shelly & Whittier 1997, Yuval & Hendrichs 1999). The sequence of events culminating in fertilization of a females eggs by the sperm of a particular male may be seen, from the males perspective, as an obstacle race, where the height of each obstacle is determined by nutritional thresholds, intra-sexual competition or female choice. Being able to overcome one such barrier, though a step in the right direction, does not guarantee success in the subsequent suite of challenges (see Eberhard 1996).

These hurdles can broadly be designated as follows: copulation, insemination and finally, fertilization. However, the more we learn about the medfly mating system, more specific milestones on the road to reproductive success can be identified. These are:

1. joining a lek
2. pheromone emission
3. performing courtship
4. copulation
5. sperm transfer and storage
6. fertilization of eggs
7. preventing or delaying female remating

We have investigated the relationship between post teneral nutrition of males and their success at most of these discrete steps. In the present paper, we briefly review our main findings on the subject.

LEK JOINING AND PHEROMONE EMISSION

The relationship between male nutritional status and participation in leks was studied both in the field and in field-cages.

Field Studies.

We studied lekking males on pitanga (*Eugenia uniflora*) trees located near Rehovot, in the central plain of Israel. We compared the nutritional status of males participating in leks to that of others resting nearby. Males were collected, their behavior (lekking or resting), recorded, and they were immediately chilled. Subsequently, biochemical analyses were performed to determine the levels of glycogen, sugar, lipids, and protein in each male. For full details of procedure, see Yuval et al. (1998). There were no significant size differences between resting and lekking males, indicating that size does not determine whether a male will join a lek. However, lekking males were significantly heavier than resting males, and this difference was reflected in the nutrients they contained. Males in both groups contained similar amounts of lipid, on average 21.06 µg/mg for lekking males (n = 183) vs. 20.9 µg/mg for resting males (n = 148). Similarly, glycogen did not vary between behavioral categories, 2.21 µg/mg in lekking males vs. 2.43 in resting males. However, lekking males contained significantly more protein and sugar than resting males. Lekking males averaged 5.85 µg/mg protein vs. 5.00 in resting males. Similarly, sugar in lekking males averaged 53.4 µg/mg compared to 42.4 in resting males (P = 0.01). We concluded that leks are exclusive and that males must first forage successfully before they can join (Yuval et al. 1998).

Field Cage Experiments.

We tested the above conclusion in field cage experiments. We carried out two series of experiments in two field cages (1.85 × 1.85 × 1.80m, covered with a shade cloth on top), located on the campus of the Hebrew University in Rehovot. Citrus trees (*Citrus* sp.) with canopies of approximately 0.5m in diameter and 1.5 m in height, were placed inside the field cages as a lek site (Prokopy & Hendrichs 1979, Kaspi & Yuval 1999). Wild flies, reared from infested guava, were used in all experiments. After emergence, flies were segregated by sex, cloistered in 5-liter plastic containers, at densities of 60-80 per container, and were given one of two diet regimes: 'protein-fed' or 'sugar-fed'. Protein-fed flies had *ad libitum* access to water, dry sucrose and protein hydrolysate, sugar-fed flies had access to water and 20% sucrose solution.

When males were 9-10 days old, they were released in the field cage, and their propensity to lek and ability to copulate sexually mature virgin

females was monitored throughout the day of release. protein-fed males were more likely to emit pheromone in leks, and consequently, were more likely to copulate than sugar-fed males. Furthermore, protein-fed males tended to start calling earlier than their nutritionally deprived competitors. Though size was not related to initiation of lek behavior, large males were significantly more likely to copulate than small males. Amongst protein-fed males, large individuals tended to mate earlier than smaller ones (Kaspi et al. 2000). Independent experiments by other researchers, albeit conducted in small cages in the laboratory, found similar effects of male nutrition on pheromone emission (Papadopoulos et al. 1998). Recently, Shelly et al. (2002), determined in the field that leks of protein fed males are more attractive to females than leks of protein deprived males.

COURTSHIP

We analyzed the effect of nutrition on the various elements of courtship, by observing the behavior of lekking males towards virgin females on trees in a field cage. Within lek visits, the most frequently observed behavior was non-calling, while the most frequent mate-attraction behavior was calling. The only behavior to show a significant tendency to covary in frequency with diet was *court*, (defined as "orient toward a female and perform close-range courtship behavior" (Briceno et al. 1996)), which was more frequently performed by protein fed males (S. Field unpublished observations).

COPULATION

There is compelling evidence from several laboratory strains and from wild males that protein fed males are more likely to copulate. Flies of the long established "Vienna" strain copulated much more frequently (and faster) in the laboratory than did their sugar fed brethren (Blay & Yuval 1997), as did males of the much younger strain "Sade" (Taylor & Yuval 1999). Wild males that emerged in the laboratory and were tested for copulations in a field cage also succeeded significantly better than sugar fed males (see 1 & 2 above, Kaspi et al. 2000).

Copula duration is also affected by male diet (Field & Yuval 1999). However, this part of the sexual encounter comprises many conflicting and coinciding interests of both males and females, and deserves a more detailed analysis in the future (Taylor & Yuval 1999, Field et al. 1999).

SPERM TRANSFER & STORAGE

Probability of Sperm Transfer.

Not all copulations culminate in sperm storage by females (Seo et al. 1990). We conducted laboratory experiments with the "Sade" laboratory strain, in 5 liter cages, We found sperm in the spermathecae of 94% of 178 females who had copulated. Females were significantly less likely to store sperm of protein deprived males. Interestingly, effects of diet were limited to small males, and large males were comparatively unaffected (Taylor & Yuval 1999). However, when we dissected wild females who had copulated with wild males in a field cage, there was no evidence for a diet effect on sperm transfer (Taylor et al. 2000).

Number of Sperm Stored.

In the study of laboratory flies copulating in small cages, diet also significantly affected the amount of sperm stored. Females who copulated with protein-fed males stored more sperm in their spermathecae (on average, 3693 sperm cells) than did females who copulated with protein-deprived males (3037 on average; Taylor & Yuval 1999). When this study was repeated with wild males copulating with virgin females in a field cage, we found that the total number of sperm stored by females varied between testing days and increased with female size but was not influenced by male size or diet (Taylor et al. 2000). It appears that the context of the experiment and the source of flies used affect the outcome, suggesting that diet alone cannot explain the variability in the probability of sperm being transferred, and in the amount of sperm transferred.

FERTILIZATION

For a number of insect species, evidence is accumulating that when males of differing quality copulate with a female, this difference in quality is reflected in paternity patterns, irrespective of the copulation sequence. This may be the result of competition between males extended to the female reproductive tract (sperm competition), or of female ability to manipulate ejaculates and favor the sperm of a preferred male (Bisoondath & Wilkund 1997, LaMunyon & Eisner 1993, Eberhard 1996, Ward 1993). It is not known whether multiply mated medfly females will preferentially use the sperm of better nourished males over that of undernourished males. However, the non-random patterns of sperm allocation between the two spermathecae (Yuval et al., Taylor & Yuval 1999, Taylor et al. 2000) suggest that females may be able to exert some control over fertilization.

If females can control fertilization by manipulating ejaculates, then one way for males to preempt them (or avoid male-driven sperm competition), is to limit or postpone the receptivity of the females they copulate. As detailed below, the nutritional status of a female's first mate does affect her tendency to remate.

Renewal of Female Receptivity

We studied the effect of male diet on subsequent female receptivity by allowing virgin females to copulate with protein fed or protein deprived males (Blay & Yuval 1997). On the day following copulation, females were confined with a fresh set of virgin, protein fed males. Females whose first mate was protein deprived were significantly more likely to remate than females whose first mate was protein fed: 76% vs 61% (n = 235, x^2 = 5.4; P < 0.05). Furthermore, the latency to remating of females whose first mate was protein deprived was significantly shorter (Blay & Yuval 1997). We are still in the dark as to how this refractoriness is mediated. Recent studies with spermless males indicate that sperm has a short-term effect on female receptivity (Miyatake et al. 1999). The fact that protein-fed males are simultaneously superior at both inseminating (Taylor & Yuval 1999) and inducing non-receptivity in mates (Blay & Yuval 1997) points to an involvement of sperm or some associated quality. However, even poorly nourished males transfer an abundance of sperm to females, enough to fertilize most (if not all) of its eggs (Blay & Yuval 1999). Therefore additional factors associated with male nutrition, such as quality of accessory gland secretions (Jang 1995, Jang et al. 1998), weight or execution of copulatory courtship routines (Eberhard 1991), may influence female receptivity to further copulations.

Male Nutrition and The Sterile Insect Technique

Millions of sterile male medflies are released in SIT operations every week, in many locations throughout the world where the medfly is a pest or a threat. For SIT to succeed, these males must be able to join the leks of wild males or establish leks of their own, attract wild females, court, copulate and inseminate them, and inhibit them from remating for as long as possible. Managers of fly rearing facilities and program agencies as well as biologists at research institutions share a common interest in identifying male attributes and developing handling techniques that will contribute to the optimal performance of released males (Calkins 1984, Calkins et al. 1994, Cayol et al. 1999, FAO/IAEA 1998, McInnis et al. 1996, Shelly 1999, Shelly & Whittier 1996).

The significant effect of protein nutrition on the tendency of males to join leks, copulate and delay female remating suggests that adding protein to the diet of sterile males prior to their release will improve their performance in the field. To test this hypothesis, we examined how post-teneral nutrition during the first 4-8 days after emergence affects performance and copulatory success in leks of mass-reared sterile (*tsl* strain

Vienna 4/Tol-94) males (Kaspi & Yuval 2000). We found that males fed both protein and sugar were significantly more likely to emit pheromone in leks, and more likely to copulate than males fed only sugar. Sterile males, who had access to water and apples following four days feeding on protein and/or sugar were significantly more likely to copulate than their starved competitors who had access to water only. These results alone would encourage us to recommend a high protein diet for sterile males prior to release. However, we also found that after 24 hours of starvation, four day old protein-fed males suffered higher mortality than sugar-fed males (Kaspi & Yuval 2000). To date, there is no information on the foraging behavior of released males in the field. If they are capable of finding and exploiting sources of protein nutrition in the field, pre-release feeding on protein will not affect their survival and may greatly enhance their competitiveness. Conversely, if they are unable to forage efficiently, protein feeding prior to release may hasten their demise (see also Carey et al. 1998, Jacome et al. 1999). Thus more research is needed to determine the optimal diet that will balance sexual prowess and longevity of released sterile flies.

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RECENT FINDINGS ON MEDFLY SEXUAL BEHAVIOR: IMPLICATIONS FOR SIT

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ABSTRACT

The preceding papers presented in this issue represent some of the activities of a group of researchers working on fruit fly mating behavior as it relates to the use of the Sterile Insect Technique (SIT). The group was co-ordinated and partially funded by the FAO/IAEA Joint Division of Nuclear Techniques in Food and Agriculture, Vienna Austria. A variety of approaches were used to examine lekking and courtship behavior of wild and mass-reared fruit flies including video analysis, morphometrics, physiological status, geographical variation and field cage evaluation. No major qualitative differences could be demonstrated between mass-reared males and wild males, although there were some specific changes related to mass rearing and irradiation. Many studies were carried out using field-caged host trees and the results of this work have led to the establishment of a standardized protocol that is now followed by all fruit fly programs using the SIT. Using these cage studies it was shown that there are no barriers to mating between medfly populations from many parts of the world. This information is of major relevance as it permits sterile flies to be shipped from one program to another. It also has some significance for the eventual commercialization of the SIT. The use of various compounds to improve the mating success of mass reared males could have a major impact on the efficiency of SIT programs. However, the initial experiments reported here will need to be expanded before this approach can be integrated into operational programs.

Key Words: medfly, *Ceratitis capitata*, sterile insect technique, genetic sexing strain, behavior, lek

RESUMEN

Los trabajos anteriores presentados en esta edición representan algunas de las actividades de un grupo de investigadores que trabajan en el comportamiento de apareamiento en mosca de la fruta y su relación con el uso de la técnica del insecto estéril (TIE). El grupo fue coordinado y parcialmente financiado por la Division Conjunta de Técnicas Nucleares en la Alimentación y la Agricultura de la FAO/IAEA, Viena Austria. Una variedad de métodos fueron utilizados para examinar el cortejo y el comportamiento de apareamiento de moscas de la fruta salvajes y criadas en forma masiva incluyendo análisis por video, morfometría, estatus fisiológico, variación geográfica y evaluación en jaulas de campo. No se pudieron demostrar diferencias cualitativas mayores entre machos criados masivamente y machos salvajes, aunque hubo algunos cambios específicos relacionados con la cria masiva y la irradiación. Muchos estudios fueron llevados a cabo utilizando árboles hospederos en jaulas de campo y los resultados de estos trabajos nos han dirigido al establecimiento de un protocolo estandarizado que actualmente es utilizado por todos los programas de moscas de la fruta que usan la TIE. Al utilizar estos estudios en jaula se demostró que no existen barreras a el apareamiento entre poblaciones de la mosca del Mediterráneo provenientes de muchas partes del mundo. Esta información es de gran relevancia ya que permite que moscas estériles puedan ser enviadas de un programa a otro. También tiene algún significado debido a la eventual comercialización de la TIE. El uso de varios compuestos para mejorar la eficiencia de apareamiento de los machos criados en masas podría tener un gran impacto en la eficiencia de los programas TIE. No obstante los experimentos iniciales reportados aquí necesitaran extenderse antes de que este método pueda ser integrado en programas operacionales.

A successful medfly Sterile Insect Technique (SIT) program requires that released sterile males locate a lekking site (Whittier et al. 1992), perform a courtship (Féron 1962), attract wild females (Whittier & Kaneshiro 1995), inseminate them and elicit the appropriate behavioral response from their mates (Jang et al. 1998). Consequently, mass reared sterilized medfly males

must be able to deliver a behavioral repertoire that approximates as closely as possible that of their wild counterparts. Any departure from the "wild" behavior, or lack of field competitiveness could cause the failure of an SIT program. The female medfly is the final arbiter of male success or failure and this component cannot be influenced by any activities of the program. All strategies for maintaining a high level of mating between the sterile male and the wild female have to be directed towards the mass reared and sterilized male. An improved knowledge of what makes a successful, sexually competitive and attractive sterile male can contribute to increasing the efficiency of SIT field programs.

Sterile males are the key players in the SIT and competitiveness is usually discussed in terms of this sex and their ability to mate with field females (Hooper 1972, Tsubaki & Bunroogsook 1990, Shelly & Whittier 1996a). It is quantified as a function of the mating success of the sterile male compared to that of the field male (Orozco & Lopez 1993). Competitiveness of the sterile male in the field represents the cumulative effects of rearing, sterilization and release procedures of that particular cohort of insects as well as the "behavioral history" of the colony from which the cohort was reared. These two components of competitiveness can exert quite different effects on the insect. The history of the strain in terms of its behavioral adaptation to mass rearing conditions will tend to set the upper limit of field competitiveness (Cayol 2000) while the rearing, handling and sterilization of a particular cohort will determine the actual competitiveness of that cohort in the field. Both of these components are subject to a large degree of variability. Each strain has a different colonization history and is continually evolving and new rearing procedures, such as the Filter Rearing System (FRS) (Fisher & Caceres 2000), contribute to the behavioral makeup of a strain. Experimental approaches that would tease apart the historical and contemporary components and assign values to their overall contribution to field competitiveness would be very useful for SIT programs.

The preceding papers in this volume are an attempt to do this by 1) improving our understanding of the different components of fruit fly mating behavior; 2) evaluating how mass rearing and sterilization influence these components and 3) assessing how changes impact on the competitiveness of the flies in the field. Two questions can be posed in this last chapter, can the results of these different approaches be assimilated into a unified description of male mating success in medfly and can the results at one level (e.g. the laboratory) predict outcomes at a second level (e.g. the field)? An affirmative answer to the first question could enable mass rearing strategies to be developed which preserve male mating "success" and an affirmative answer to the second could enable QC protocols to be revised so as to improve their predictive value for the field effectiveness of sterile males.

A reading of the papers in this issue indicates that these questions cannot yet be answered fully although there is now a much better understanding of which components are crucial and perhaps, more importantly, which are not. In this paper the implications for the SIT of the methods and findings presented in the preceding papers are discussed.

What Makes a Medfly Male Successful?

Lekking Behavior

To be successful medfly males first need to join a lek (Prokopy & Hendrichs 1979). Field et al. (2002) discuss in detail lek formation in medfly and examine the different theoretical concepts that have been used to explain the origin of lek formation in different species. Although there is no doubt that medfly males do form a lek to which females are attracted, the functional basis of lek formation in this species has still to be fully elucidated. In medfly there are probably several interacting components which taken together can explain why medfly males form leks. Field cage and field observations of mass reared medflies have repeatedly shown that they do indeed participate in lek formation (Zapien et al. 1983, Cayol et al. 1999) and appear to do so in numbers which suggest that this behavioral component is not changed following mass rearing. Their preferences among foliage types on which to establish leks is also the same as in wild males (Katsoyannos et al. 1999). This is surprising as all the requirements for lekking behavior are removed during mass rearing and it suggests that there is little genetic variance for this particular character for selection to act on. Despite the extensive knowledge of medfly mating behavior in the field, key questions still remain to be answered. What proportion of males in the population take part in mating? Do males change their "position" in the lek as leks dissolve and re-form? If wild males change their position in different leks over time do mass reared flies do the same?

Participation in a lek is the first step that released males must successfully complete in order to have a chance to participate in mating. This can be partially studied in a field cage but confinement in a cage does not truly mimic lekking behavior in the open field. Some important spatial and temporal components of lek formation are absent in the field cage. Field cage tests are generally carried out during a single day using a single tree and they cannot monitor the behavior of the released males over time and space as they attempt to participate in new lek formations. An-

other restriction of field cage tests is that generally a constant number of flies are used whereas in the field, fly densities vary and leks of different size will be formed. Are mass-reared males capable of participating in leks of different size both in terms of the number of flies and the physical distribution of the lek within the leaf canopy? These observations are very difficult, if not impossible, to make in the open field and reliance will still have to be placed on results coming from field cages. In conclusion mass reared medfly males appear to participate fully in lek formation under field cage conditions, but it is not known if there are preferential positions within the lek and, if there are, whether mass reared medflies can identify them.

Lek behavior in other economically important *Ceratitis* spp was also analysed (Quilici et al. 2002). In general a similar picture emerged but there were species specific differences in the timing of lek formation as well as trend towards a more "simplified" courtship behavior.

Morphometric Traits

Does a female medfly see anything in a male that makes him attractive as a mate? This question was addressed by several authors (Hasson & Rossler, Hunt et al., Rodriguero et al. 2002) focusing on male morphometric traits. Hasson & Rossler (2002) addressed the question of fluctuating asymmetry (FA) of sexual characters that is known to play a role in sexual selection with symmetric males having a slight advantage in the mating arena. In medfly, there is a positive correlation between mating success and the symmetry of the superior frontal orbital setae (SFO) (Hunt et al. 1998). It is also known that stress during development can lead to an increase in asymmetry as measured by the size of bilaterally produced structures. The question can then be asked concerning the effects of mass rearing stress on the physical attractiveness of sterile medfly males to wild females. Hasson & Rossler (2002) have attempted to answer the fundamental question as to whether developmental homeostasis operates at the level of the individual or is regulated independently for each set of characters. By generating differently sized adults using different larval densities, measurements were made on both sexually dimorphic and shared bilateral characters. No effect of larval density could be demonstrated on the FA of any of these characters and even with thorax length, the best quality indicator, the association was weak. However, FA was highly correlated with the size of the character. The authors conclude that for medfly, character specific homeostasis plays a major role in FA but that whole body regulation is also implicated. The question of the relationship between the FA of sexually dimorphic characters and quality remains contentious. It is also likely that many other factors will over-ride any small effects that FA will have on the quality of flies that are released in an SIT program. Whatever its cause, asymmetry is present in the SFO bristles and as indicated above males symmetrical for this character are more successful in obtaining mates than are males showing asymmetry for this trait (but see Rodriguero et al. 2002). Mendez et al. (1999) showed that the removal of the two male bristles affects sexual competitiveness, even though it does not affect courtship behavior. Hunt et al (2002) examined in more detail the role of asymmetry in these bristles for male mating success. They artificially induced the ultimate asymmetry by surgically removing either one or two bristles and examining the effect this has on male mating success. The authors showed that the loss of one bristle did not have an effect on mating success as compared to a two bristle male. However a one-bristle male was more successful than a male with no bristles. These, in some way contradictory, results do not provide many more clues as to the real function of the SFO bristles in relation to male mating success.

The question of whether or not large males are sexually more competitive and attractive for females than small males has very often been raised (Partridge & Farguhar 1983), especially in the light of mass rearing (Burke & Webb 1983, Churchill-Stanland et al. 1986). Blay & Yuval (1999) showed that male size correlated positively with hatch rate in later stages of female reproductive cycle, however, in a recent study, Taylor et al. (2001) showed that there was no correlation between male size, copula duration and insemination success. Rodriguero et al. (2002) concluded that larger males are more sexually competitive than smaller ones. This was already demonstrated by several other authors (Burk & Webb 1983, Churchill-Stanland et al. 1986, Krainacker et al. 1989, Bloem et al. 1993, Orozco & Lopez 1993) who showed that larger male tephritids, in addition to being more sexually competitive, are stronger fliers and live longer than smaller flies. Pupal size is therefore an essential component of the fruit fly QC protocol (FAO/IAEA/USDA 2002).

Physiological Status

Liedo et al. (2002) examined the effect of the age of the fly on its propensity to mate in field cages, both laboratory and wild flies were evaluated. The authors showed that the optimal age for mating ranged between 7 to 13 days for wild flies against 3 to 5 days for the mass reared flies. This work was used to standardize the age of flies used in field cage tests to enable results between different laboratories and with different strains to be compared. They also showed that when sexes are held separately, insects are more prone to mate. This finding

supports the use of all male strains (Hendrichs et al. 1995, Rendon et al. 2000) since sterile males will be virgin by the time of the release. Releasing 2 day-old sexually immature flies, as is still the case (Pereira et al. 1997, Cayol & Zarai 1999, Barbosa et al. 2000), wastes resources since most of the sterile insects will be eaten by predators before they can mate (Hendrichs et al. 1993). Male only releases can be improved by holding emerged flies longer (providing them food and water) and releasing 4 day old sexually mature virgin males.

Feeding status is another physiological character that can have direct implication for SIT. Shelly et al. (2002) showed that when fed with protein, medfly males attracted more females when calling in leks. Protein-fed males also achieved more matings than protein–deprived but sugar-fed males. This finding confirms the work by Blay & Yuval (1997), and would suggest that sterile males should be fed with a mixed diet of protein and sugar before being released into the field, rather than sugar-only as is the case in current SIT projects. However, even though Yuval et al. (2002) confirmed that protein-fed males are sexually more attractive and competitive that sugar-fed or starved males, Kaspi & Yuval (2000) found that, after 24 hours of starvation, 4 day-old protein-fed males suffered higher mortality than sugar-fed ones. The benefits, or not, of feeding mass reared sterile males with protein prior to their release will need further investigation.

Shelly (1994, 1995) reported that when fed with methyl-eugenol, a male attractant of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), irradiated males of this species were more sexually competitive. Shelly & Whittier (1996b) showed that medfly males exposed (not fed with) to trimedlure exhibited higher level of pheromone calling and were more successful in mating than not exposed males. This advantage, though of limited duration (24 h), could theoretically, be put into practice as a preliminary treatment before male release. Recently it was also determined that exposure to wounded orange peel substances conferred to males a mating advantage over unexposed males and this advantage lasted at least ten days following exposure (Papadopoulos et al. 2001). Other recent developments in the same field (Shelly, 2001) show that, medfly males fed on ginger oil are sexually more competitive than normal males. These findings, though under development, would represent a major step forward in increasing SIT effectiveness.

CAN WILD FEMALE REMATING BE LIMITED AND/OR CONTROLLED?

The probability that a wild female will remate after mating with a sterile male is highly relevant to the effectiveness of SIT and a successful male should ensure that the female he mates with uses only his sperm to fertilize all her eggs. Males must be able to transfer sufficient sperm and accessory fluid during mating in order to change the behavior of the female from mate seeking to oviposition and so prevent remating. *Drosophila melanogaster* males have elegantly solved the problem of female remating by transferring a peptide in the accessory fluid that changes the behavior of the female in two ways. It makes her refractory to mating and initiates oviposition behavior (Fowler 1973, Clark et al. 1999). A similar behavioral switch does exist in medfly (Jang et al. 1998). Accessory gland fluid in medflies has been shown to contain biologically active compounds which cause a switch in wild female behavior from attraction to pheromone to attraction to fruit odors implying a shift from mating to oviposition (Jang 2002). Interestingly, laboratory females did not show this switch but irradiated laboratory males were equally good at causing this switch as wild males, when mated to wild females. It appears that laboratory colonization does not lead to any change in the production of these compounds even though its behavioral requirement in the female is probably completely suppressed under the laboratory conditions. This might explain why laboratory females fail to exhibit this behavioral switch. The fact that mass reared males elicit the correct response from wild females would suggest that this is not a major component of reduced effectiveness of sterile males in the field.

Multiple mating of wild medfly females seems to occur in field populations (Prokopy & Hendrichs 1979, Saul et al. 1988, Saul & McCombs 1993a, 1993b) and it is thought to enhance female fitness (Whittier & Shelly 1993). Two studies, one using linear measurements of sterile and fertile sperm (McInnis 1993) and the other using the numbers of sperm in the spermatheca (Yuval et al. 1996), provided the early evidence that wild medfly females engage in multiple mating. The lack of data on this point is not surprising, as it is a very difficult parameter to approach experimentally. However molecular analysis of progeny from field collected females has provided the first preliminary data on the level of remating in wild populations (Gasperi, pers. comm.). Measurement of rematings in field cages (Hendrichs et al. 1993) indicated that wild females, initially mated with laboratory males, tended to remate more frequently than the wild females initially mated with wild males. Other field cage studies (McInnis et al. 2002) used individually marked flies so that both male and female remating could be followed for a wild and a mass reared strain. The authors could show no statistically significant difference in the tendency of females to remate depending on which male they mated with first. The authors also concluded that wild males remated more frequently than wild females. In laboratory studies, Whittier & Kaneshiro (1995) demonstrated

a similar non-randomness in the mating frequency of medfly males and females with ca. 27% of males not mating at all.

In the laboratory, female multiple mating in medfly is the rule as shown by the detailed studies on mating frequency (Vera et al. 2002). There is however the possibility that this is a laboratory artifact and of limited relevance to field populations. However, interesting differences were revealed in the study which, although compounded by the laboratory environment, will probably be of wider relevance. Females initially mated with laboratory males tended to remate more frequently than the females initially mated with wild males and there was a negative correlation between duration of mating and the chance of remating. Under laboratory conditions the wild flies performed very poorly and the experimental conditions of the test were in some way designed so that multiple mating was encouraged. Other factors were also identified which affect this parameter such as fly density, and male status. These studies confirmed that long term rearing in the laboratory significantly shortens male mating duration which indirectly increases the chances of female remating.

The whole subject of the relevance of female multiple mating to the effectiveness of the SIT through the part played by released sterile males will only be satisfactorily answered when more is known of this phenomenon in a wild medfly population using the molecular approaches indicated above.

IMPROVING THE SIT

Mass Rearing

Under "traditional" mass rearing conditions the requirements for appropriate mating "behavior" are removed as cost effective production processes demand that important compromises be made in the environmental arena presented to the fly for mating. The most important biotic change relating to mating is probably adult density in the production cages. Here, fly density, in terms of unit volume and area, is orders of magnitude higher than in the field. This single change initiates a cascade of responses in the fly which leads to a degeneration in most aspects of the normal mating behavior that is observed in the field (Cayol 2000). The critical question concerns the long term effects of this distorted mating "behavior" on the effectiveness of a male fly when it is released into the field. In other words, how hardwired is the courtship behavior of medflies?

Major abiotic changes are also made, including constant light and temperature regimes and the provision of an artificial diet for adults and larvae, that is much richer in proteins than the available diets in nature. These changes also impact directly on mating behavior. All in all, it would not

be surprising if continuous rearing under these totally artificial conditions for a species such as medfly, which has a complex and refined mating behavior, led to permanent and irreversible changes in mating behavior. Briceno & Eberhard (2002) confirmed their previous conclusion (Briceno & Eberhard 1998) that, due to the crowded conditions in mass rearing cages, a shorter courtship of males evolved in an "effort" to avoid interruption. Mass reared females co-evolve and accept, more than wild females, males that exhibit shortened courtship duration. Briceno et al. (2002) found no consistent differences between the courtship of wild and mass reared flies from various origins, but a tendency for interruptions in the buzzing phase of the courtship to occur more frequently in mass reared flies. In return, such an interruption is more likely to result in a rejection by wild females. These modifications in medfly courtship behavior are more likely with flies originating from a strain with a long history in mass rearing. Calcagno et al. (2002) reports an increased aggressiveness from mass reared males, thought to be due to the crowded conditions that prevail in mass rearing. These results suggest that changes in mass-rearing protocols may lead to improvements in the quality of males.

The Filter Rearing System (FRS) as described by Fisher & Caceres (2000) represents a major step forward in insect mass rearing philosophy and can perhaps be used to improve insect quality. At the heart of the FRS is a small colony that is maintained under relaxed rearing conditions and optimized environmental conditions and from which eggs are harvested on a regular basis. The eggs are the starting point for colony development and following 2 or 3 generations of mass rearing sufficient flies are produced for irradiation and release. In this way no behavioral changes induced by mass rearing are accumulated over time as all the insects that are mass reared are released and are not returned to the FRS. The size of the FRS will depend on the number of flies required for release and the reproductive potential of the strain. Care must be taken to maintain those adaptations, such as oviposition behavior or appropriate mating behavior, in order for large production lines to function efficiently and without changes in field fitness of the flies (Fisher & Caceres 2000). The FRS provides a unique opportunity to evaluate the use of "field-like" conditions in the small colony, introducing a host tree in a large room (no cage), natural light, selection for predator avoidance etc. The FRS is already in place in rearing facilities Guatemala (Caceres et al. 2000), Argentina, Chile, Madeira, South Africa and Australia.

Reducing the negative effects of irradiation

The negative effects of irradiation on sexual competitiveness of fruit flies are well documented

(Holbrook & Fujimoto 1970, Hooper 1972, Rossler 1975, Wong et al. 1983, Kanmiya et al. 1987, Moreno et al. 1991, 2002). Lux et al. (2002a) described in detail these negative effects on courtship behavior of mass reared medflies. The authors recommend re-evaluating the sterilization strategy and irradiation doses for males used in SIT programs. One way to approach this is to irradiate flies in nitrogen. The protective effects of nitrogen on the induction of somatic damage are well known and have been shown to increase the competitiveness of the sterile male (Fisher 1997). In addition, increasing the irradiation dose beyond the 99% egg sterilizing dose greatly reduces the mating competitiveness of males (Fisher 1997). However program managers still demand doses of radiation giving 100% egg sterility resulting in reduced quality of the males for a marginal gain in sterility.

One Strain for all Programs

The current geographical distribution of medfly probably represents its dispersal, from eastern Africa, over the last 150-200 years as a result of man's agricultural and commercial activities. Such a short evolutionary time would be unlikely to lead to significant pre or post-mating isolation barriers. Genetic analysis has also shown limited divergence of medfly populations at the protein and DNA levels (Gasparich et al. 1997). Against this background, field cage studies have confirmed that there are no significant pre-mating isolation barriers when populations from different parts of the world are confined in field cages. The only current exception to this is the population from Madeira, Portugal and of course not all populations over the whole distribution of the fly have been evaluated. Cayol et al. (2002) and Lux et al. (2002 b) summarize these studies and reach the same conclusions. The lack of any mating barrier between the tested populations has major implications for the SIT in that a particular mass reared strain can be used at any location, as well as to deal with outbreaks where the medfly population to be eradicated is of unknown origin. This has already enabled the large medfly rearing facility in El Pino, Guatemala to provide sterile male medflies, from a genetic sexing strain, to California, Florida, South Africa and Israel. Despite these developments, many program managers still request that a strain with the genetic background of their particular geographical area be developed for mass rearing and release. Their appears to be very little, if any, scientific justification for this type of request. The lack of pre-mating isolation barriers in medfly has encouraged the development and use of generic genetic sexing strains such that a particular strain can be used in any country. Any future commercialization of the SIT will depend on this principle being widely accepted.

Field Cage Test: a Regularly Requested QC Test

Assessing the behavioral quality of mass reared fruit flies under small cage conditions in the laboratory is of little, if any, relevance to SIT programs (Chambers et al. 1983). Field cages were used for the first time by Boller et al. (1977) to assess the mating activity of the European cherry fruit fly, *Rhagoletis cerasi*. Since then, the protocol for field cage tests has evolved considerably and, through the work done by the authors of this volume, has resulted in the inclusion of a field cage test in the FAO/IAEA/USDA (2002) QC manual. Field cage tests have also been proved to be useful to compare the mating behavior of wild and sterile flies of other fruit flies of economic importance such as *Anastrepha* spp. (Aluja et al. 1993, Moreno et al. 1981), *Bactrocera* spp. (Jackson & Long 1997, Samoeri et al. 1980) and other Ceratitis spp. (Quilici et al. 2002).

The research group agreed on 3 main conclusions concerning the use of field cage tests. 1) Field cage tests with host trees are the best compromise between laboratory conditions and costly and impractical field observations to assess fruit fly mating behavior under semi natural conditions. 2) Improved standardization is required e.g. nutritional status, sex ratio and density of flies in relation to available canopy surface in the field cage. 3) Wild flies remaining on the tree canopy reflect adequate environmental conditions within a field cage. Based on these observations the FAO/IAEA/USDA (2002) QC manual has already been modified.

This series of studies did not reveal all the intricacies of medfly courtship behavior as it relates to the SIT, nevertheless, the following conclusions were reached:

- There is a need to devise improved rearing systems (the benefits of the Filter Rearing System is now recognized worldwide) which can maintain critical aspects related to male courtship,

- It was demonstrated that medfly has not yet evolved pre-mating isolation barriers between different geographical populations enabling transboundary and intercontinental shipment of sterile flies to be carried out,

- The lack of pre-mating isolation barriers among medfly populations enables the development and use of generic genetic sexing strains in medfly SIT programs,

- Field cage tests are now included in the routine quality control test for SIT programs.

CONCLUSIONS

An SIT program can only be successful if wild females are mated with sterile males in a propor-

TABLE 1. THE MAJOR CONCLUSIONS OF THE CRP REGARDING THE DIFFERENT ASPECTS OF MEDFLY MATING BEHAVIOR.

Type of study	Conclusions of the CRP
Courtship behavior	• Wild and mass reared flies from different origins exhibit similar courtship behavior patterns. • A high variability is noticed in the quantitative aspect of the behavior of mass reared and wild flies. • Mechanisms determining female acceptance of a mate are complex and not yet fully understood. • Mass rearing conditions do affect the quantitative aspects of the courtship behavior. • Irradiation has a negative effect on the behavior of mass-reared males.
Lekking behavior	• Compared to other stages in the reproductive sequence of the medfly, leks are poorly understood. • Lek behavior is common to all medfly populations studied. • Mass reared, sterile males appear to have maintained the ability to find, join and participate in leks. • Leks appear at 2 different scales: the large scale, i.e. at the individual tree level and the small scale, i.e. at the microhabitat level within a tree canopy. • Predation and female preference appear to be of primary importance in driving and regulating lek behavior, • Laboratory cages are unsuitable for studying lek behavior.
Morphometric studies	• Multiple measures of body size are more reliable than single measures to determine levels of mating compatibility and competitiveness. • There are contradictory data on the importance of male body size on mating success. • The absence or abnormalities of SFO bristles can have a negative effect on male mating success. • Mass-reared males occasionally lack SFO bristles.
Nutritional aspects in relation to sexual behavior	• Studies on fruit flies indicate that ingestion of specific precursors affects pheromone production and male reproductive success. • Post teneral nutrition enhances reproductive success of male medflies by affecting: a) the ability to emit pheromone in leks, b) the copulatory success, c) the renewal of female receptivity.
Remating studies	• Remating in wild medfly females is common in nature. • Sterile males are less able than wild males to suppress remating in wild females. This effect is increased in males that have been colonized for a longer time. • Mass reared males have reduced copulation times when compared with wild males. These reduced copulation times are associated with increased female tendency to remate. • There is a strong evidence that male accessory gland fluids influence female receptivity and olfactory behavior. However, the mechanisms and variables involved are not well understood.
Compatibility and sexual isolation	• Wild strains from different geographic origins show a high degree of compatibility. • Mass reared strains originating from any wild population can potentially be used worldwide. • There is evidence that some mass reared strains of medfly exhibit a degree of incompatibility when tested with different wild populations. • Regular field cage monitoring of mass reared strains is required to assess the compatibility with the local target population. • In case of incompatibility, the role of visual, sexual pheromone and acoustic signalling is unknown.
Mating test on field caged host trees	• Field cage tests with host trees are the best compromise between laboratory conditions and costly and impractical field observations to assess medfly mating behavior under semi-natural conditions. • Results of field cage studies are still not fully comparable due to insufficient standardization of test protocols. • The nutritional status, sex ratio and density of flies in relation to available canopy surface in the field cage influences test results.

TABLE 1 (CONTINUED). THE MAJOR CONCLUSIONS OF THE CRP REGARDING THE DIFFERENT ASPECTS OF MEDFLY MATING BEHAVIOR.

Type of study	Conclusions of the CRP
Mating test on field caged host trees	• Adequate environmental conditions within a field cage are reflected by wild flies remaining on the tree canopy.
Field evaluation of sexual competitiveness	• Field cages are the closest approximation to open field evaluation. However, results obtained under these conditions may not always fully represent field performance. • Given the variability in control programme environment, field evaluation becomes a necessity for major field programmes. • Currently field evaluations are based primarily on trapping, fruit infestation, and dispersal and survival studies. • Field evaluations are used to determine effectiveness of mass-reared strains in inducing sterility in target populations.
Comparative approaches to sexual behavior in Tephritidae	• Courtship behavior and morphology studies in related Tephritid species have provided valuable information to understand medfly courtship behavior. • The phylogenetic relationships of members of the Ceratitini tribe is poorly understood at the present time.

tion that will cause an irreversible decline in the size of the wild population. It is therefore imperative that protocols are in place to monitor any change in the ability of the released male to successfully mate with wild females. The major outcome of this research network has been the provision of a standardized field cage test to be used by all medfly SIT programs. This test serves two purposes; 1) it enables a program to monitor male quality over time and also to assess the effects of any change in the production process and 2) it enables comparisons to be made between the quality of males being mass-reared in different programs. The inclusion of the test in the FAO/IAEA/USDA (2002) QC manual is a testament to its relevance for SIT programs.

The detailed video analysis of courtship behavior of mass reared and wild type flies revealed relatively few major qualitative differences. Most of the component behaviors of the wild males could be identified in the mass reared males. However, it played a major role in developing a deeper understanding of courtship and female choice decisions and certain new behavioral components were identified. In addition quantitative differences were identified between different populations.

The nutritional and physiological state of released sterile males is an area of growing interest in relation to their effectiveness in the field. Exciting work on increasing the mating success of fruit flies by feeding or exposure to compounds related to pheromones may have a major impact on the SIT efficiency. The use of these compounds could even compensate for the negative effects associated with mass-rearing and radiation. It is also possible that the nutritional status of flies could be improved by a better understanding of the role that gut microflora play in the nutrition of fruit flies.

Despite the significant expansion in our knowledge of fruit fly mating behavior as documented in this issue (Table 1), many questions remain unanswered. Continuing improvements in the application of the SIT will surely come from a better understanding of fruit fly mating behavior.

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