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MASS REARED FOR SIT OPERATIONS**

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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” than 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 23-25°C 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, *w<sub>p</sub>*, 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 *w<sub>p</sub>* 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 × 50 × 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 pre-selection 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 than 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 & Gagli 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 high 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
<b>Wild males</b>						
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%
<b>Reared males</b>						
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 reactivity							
a. passive	65%	89%	85%	72%	60%	57%	79%
b. active	35%	11%	15%	28%	40%	43%	20%
Signalling (wing signals)							
a. female only	41%	31%	48%	55%	32%	92%	23%
b. male only	55%	67%	66%	77%	73%	19%	36%
c. sign. exchange	21%	17%	22%	14%	7%	39%	55%
Who approaches:							
a. female	25%	17%	13%	9%	20%	45%	12%
b. male	95%	96%	88%	90%	94%	83%	84%
Adverse reactions during the meeting							
a. chasing by male	5%	4%	11%	10%	6%	16%	16%
b. chasing by female	31%	6%	16%	22%	5%	22%	29%
c. fighting	11%	33%	22%	30%	0%	6%	13%
	24%	50%	11%	19%	29%	78%	51%
	65%	17%	73%	52%	71%	17%	36%

\*Some observations were not included in the analysis due to poor quality of the recording, lack of zooming (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).

	Wild strains					Reared strains		
	Patagonia	Greece	Guatemala	Madeira	Mendoza	Seibersdorf G-47	Moscamed	
Fanning (vibration)								
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 DURING ADVANCED STAGE OF COURTSHIP (BUZZING).

	Wild strains					Rearred strains		
	Patagonia	Greece	Guatemala	Madeira	Mendoza	Seibersdorf G-47	Moscamed	
Buzzing								
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					Rearred 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" than 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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