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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<sup>+</sup>/X)sw<sup>x</sup>] 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<sup>+</sup>/X)sw<sup>x</sup>], 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, videgrabaciones 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, *Ceratitidis 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<sup>x</sup> (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<sup>x</sup> (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  $9 \pm 1$  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  $\times$  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  $11 \pm 1$  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 <sub>L</sub>                                 | the female remains still, close (<3 cm) to the laboratory male  |
|                   |                   | S <sub>w</sub>                                 | the same as previous but refers to the wild male  |
|                   | male-female fight | Fi→m <sub>L</sub>                              | the female attacks the laboratory male or there is mutual aggression                                  |
|                   |                   | Fi→m <sub>w</sub>                              | the female attacks the wild male or there is mutual aggression  |
|                   |                   | Fi←m <sub>L</sub>                              | the laboratory male attacks (fights with) the female  |
| Fi←m <sub>w</sub> |                   | the wild male attacks (fights with) the female |   |
| Male-male         | male-male fanning | Fa→m   | the male under observation is displaying fanning as a response to the proximity of another male       |
|                   | male-male buzzing | B→m  | the same as the former but referred to buzzing  |
|                   | male-male attempt | A→m  | the male under observation attempts copulation with the other male                                    |
|                   |                   | A←m  | the other male attempts to copulate with the male under observation                                   |
|                   | male-male fight   | Fi→m   | the male under observation attacks (fights) the other male or there is mutual aggression              |
|                   |                   | Fi←m   | the male under observation is attacked by the other male, or there is mutual aggression               |
|                   | head-to-head      | H↔H  | the males confront each other, head-to-head, and remain in this attitude immobile for several seconds |
|                   | Female            | Mobile   | M   |
| 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 ( $P =$

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

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 <sup>-6</sup> |

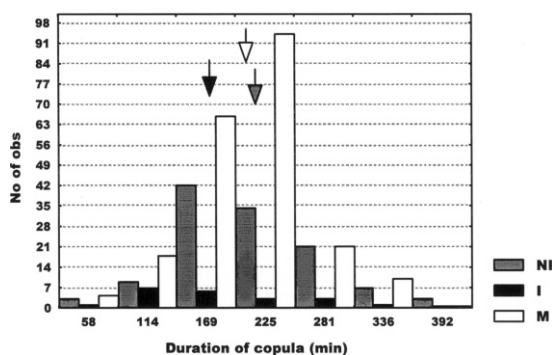


Fig. 1. Duration of copula (min) for males of the Mendoza (M) strain and non-irradiated (NI) and irradiated (I) males of the T228 strain. The arrows indicate the mean value for each group.

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 spermatozooids 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  | L vs W | 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  | S vs U                             | L vs W  | S vs U   | L vs W   | S vs U   | L vs W |
| S    | 38.5           | 348.7 | 193.6  | 19.9   | 271.9 | 145.9  | -2.868**                           | -0.203  | -2.868** | 1.381    | 2.667**  |        |
| M    | 34.0           | 266.7 | 150.35 | 66.3   | 312.8 | 189.55 | -3.758**                           | -1.392  | -3.758** | 2.581*   | 2.802    |        |
| CS   | 623.0          | 837.8 | 730.4  | 481.9  | 973.7 | 727.8  | -1.818                             | -0.789  | -1.818   | 0.588    | 1.940    |        |
| CM   | 107.4          | 85.4  | 96.4   | 42.7   | 95.2  | 68.95  | -0.672                             | 0.593   | -0.672   | -0.750   | 1.621    |        |
| Fa   | 1.1            | 104.2 | 52.65  | 5.0    | 21.9  | 13.45  | -1.876                             | 0.999   | -1.876   | 2.136*   | 0.366    |        |
| Fa→f | 77.1           | 75.2  | 76.15  | 15.3   | 60.2  | 37.75  | 1.825                              | 0.000   | 1.825    | -1.714   | -0.677   |        |
| Fa→m | 8.1            | 49.5  | 28.8   | 0.6    | 27.6  | 14.1   | -1.914                             | 1.521   | -1.914   | 1.074    | 2.112*   |        |
| B→f  | 16.4           | 5.9   | 11.15  | 9.4    | 9.2   | 9.3    | 4.147**                            | 0.337   | 4.147**  | -3.041** | -2.769** |        |
| B→m  | 2.7            | 3.5   | 3.1    | 0.0    | 0.3   | 0.15   | -0.717                             | 3.531** | -0.717   | 0.594    | 0.660    |        |
| PA   | 34.9           | 0.0   | 17.45  | 12.4   | 0.1   | 6.25   | 8.591**                            | -0.141  | 8.591**  | -6.207** | -5.884** |        |
| VA   | 0.6            | 0.8   | 0.7    | 0.0    | 1.2   | 0.6    | -0.928                             | 0.000   | -0.928   | 0.115    | 1.203    |        |
| A→m  | 0.4            | 8.2   | 4.3    | 0.0    | 0.1   | 0.05   | -0.325                             | 3.311** | -0.325   | 0.308    | 0.461    |        |
| A←m  | 0.0            | 0.1   | 0.05   | 0.9    | 8.0   | 4.45   | -2.556*                            | -2.556* | -2.556*  | 0.660    | 0.632    |        |
| Fi→m | 1.1            | 3.3   | 2.2    | 0.4    | 2.3   | 1.35   | -2.256*                            | 1.542   | -2.256*  | 1.885    | 1.132    |        |
| Fi→f | 0.8            | 0.8   | 0.8    | 0.6    | 1.2   | 0.9    | -0.625                             | -0.185  | -0.625   | -0.251   | 1.208    |        |
| Fi←m | 0.0            | 0.3   | 0.15   | 0.0    | 2.3   | 1.15   | -2.916**                           | -3.420  | -2.916** | 1.317    | 2.772**  |        |
| Fi←f | 0.0            | 0.2   | 0.1    | 0.6    | 0.7   | 0.65   | -0.698                             | -1.640  | -0.698   | 0.818    | 0.280    |        |
| H↔H  | 0.9            | 3.6   | 2.25   | 0.0    | 6.5   | 3.25   | -0.297                             | -0.412  | -0.297   | -0.662   | 0.957    |        |
| C    | 851.4          | 2.8   | 427.1  | 1143.7 | 0.0   | 571.85 | 0.155                              | 0.155   | 0.155    | -0.062   | 0.755    |        |
| WS   | 1.4            | 2.8   | 2.1    | 0.4    | 4.7   | 2.55   | -1.163                             | -1.163  | -1.163   | 0.818    | 0.461    |        |
| MJ   | 0.0            | 0.0   | 0      | 0.0    | 0.2   | 0.1    | -0.939                             | 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 <sub>L</sub>    | 11.0  | 14.7    | -0.740  |
| S <sub>w</sub>    | 5.4   | 20.6    | 0.807   |
| Fi→m <sub>L</sub> | 0.2   | 1.0     | 1.048   |
| Fi→m <sub>w</sub> | 0.0   | 2.7     | 3.104** |
| Fi←m <sub>L</sub> | 0.4   | 0.3     | -0.543  |
| Fi←m <sub>w</sub> | 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.

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