

## 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 aparearon en las jaulas de campo durante las observaciones en dos días 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  $25 \pm 3^\circ\text{C}$ , 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  $-10^\circ\text{C}$ ) 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- <i>tsl</i> : 50% males	25	25	10	10
2. Toli- <i>tsl</i> : 60% males	30	20	10	10
3. Toli- <i>tsl</i> : 70% males	35	15	10	10
4. Toli- <i>tsl</i> : 80% males	40	10	10	10
5. Toli- <i>tsl</i> : 90% males	45	5	10	10
6. Toli- <i>tsl</i> : 92% males	46	4	10	10
7. Toli- <i>tsl</i> : 94% males	47	3	10	10
8. Toli- <i>tsl</i> : 96% males	48	2	10	10
9. Toli- <i>tsl</i> : 98% males	49	1	10	10
10. Toli- <i>tsl</i> : 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 complement 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:

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

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 ( $\delta \times \text{♀}$ )-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 ( $\chi^2$  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 homogeneous, 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 <sup>a</sup>				RII <sup>c</sup>	RSI <sup>d</sup>	Exp. RSI <sup>e</sup>	Compet <sup>f</sup>
	L × L <sup>b</sup>	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

<sup>a</sup>Based on totals of 5 replications.

<sup>b</sup>Mating pair type: (L or W) × (L or W); L = Lab sterile; W = Wild.

<sup>c</sup>Relative Isolation Index = # pairs for (L\*L)(W\*W) / (W\*L)(L\*W).

<sup>d</sup>Relative Sterility Index = # pairs for (L\*W) / (L\*W) + (W\*W).

<sup>e</sup>Expected index based on random mating = released L / (L + W).

<sup>f</sup>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**

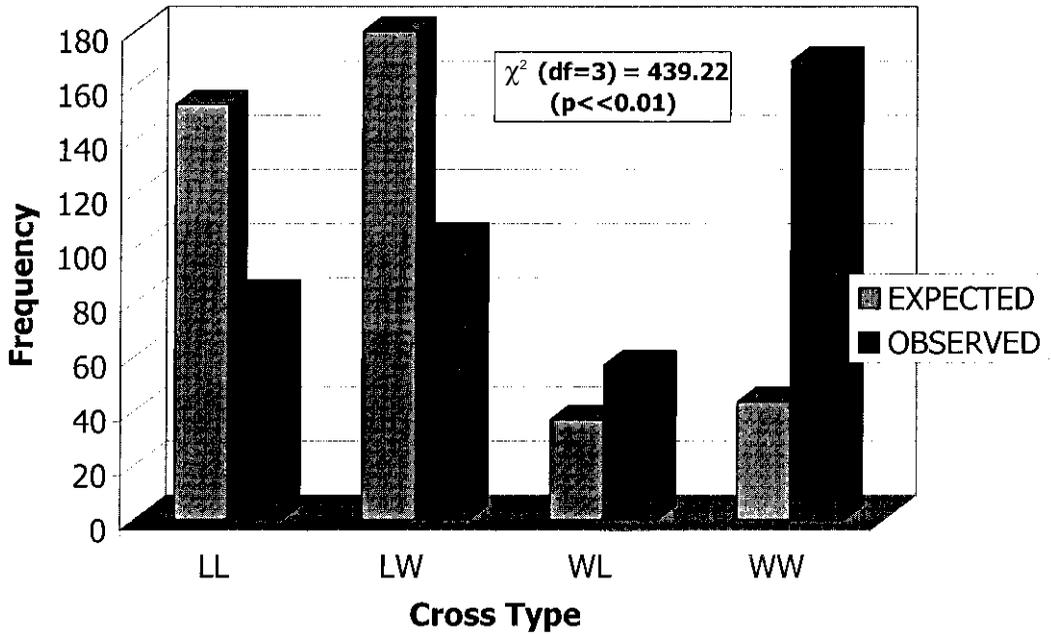


Fig. 1. Numbers of expected and observed first mating pairs for the medfly Toliman tsl and wild strains in outdoor field cages (Guatemala 1998). L = lab, W = wild, LL = lab male x lab female, etc.

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

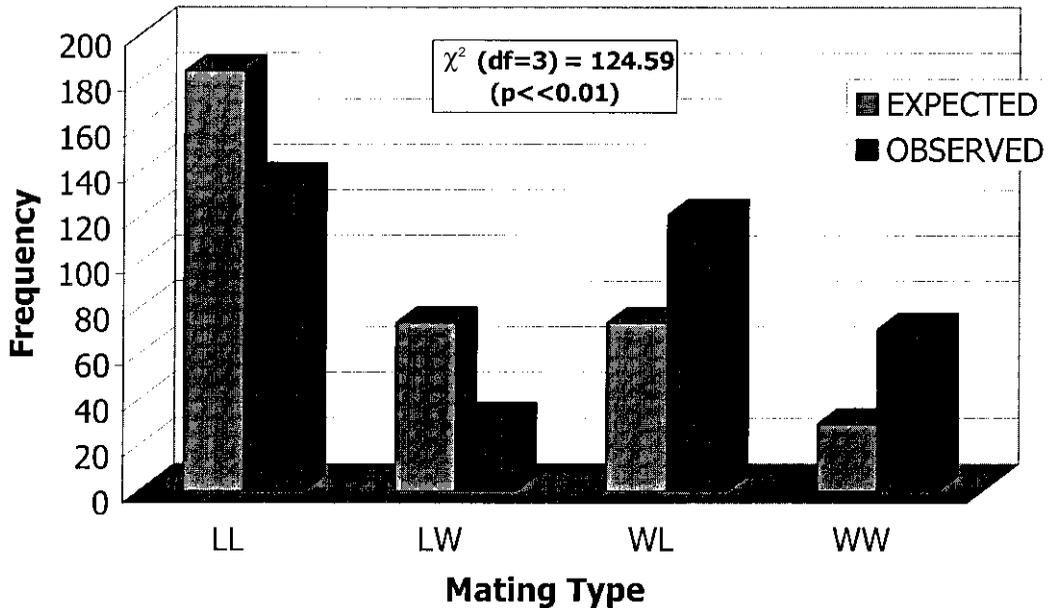


Fig. 2. Numbers of expected and observed first mating pairs for the medfly Antigua, Petapa, and 2 F1 Hybrid strains in outdoor field cages (Guatemala 1998) (Labels as in Fig. 1).

**Female Remating: TSL (Treatment #1), Antigua, Petapa, and 2 F1 hybrids (combined); Wild**

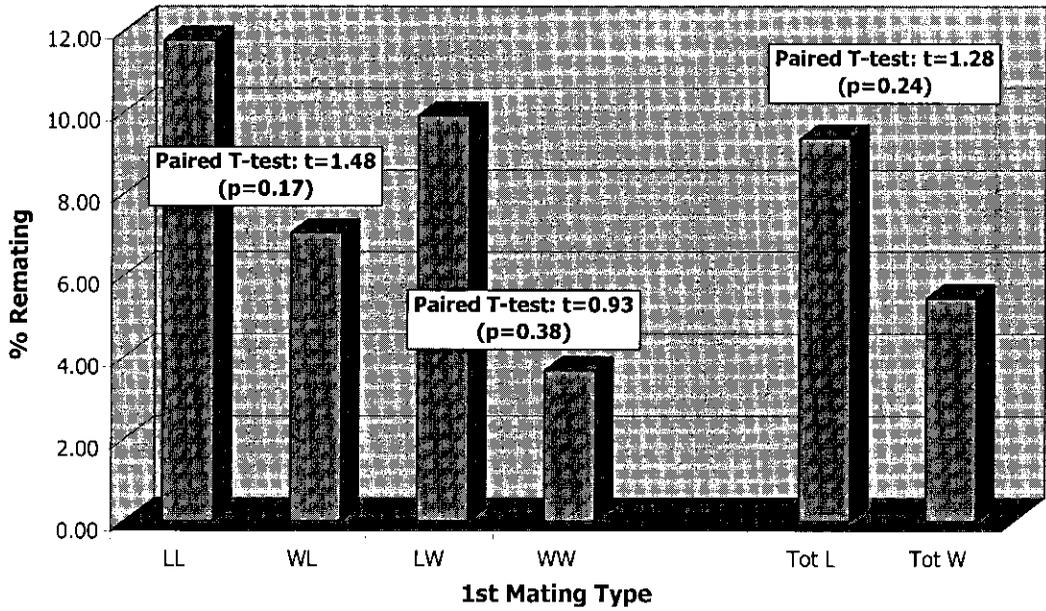


Fig. 3. Percentage of medfly females remating for all lab and wild strains after first mating with one of 4 mating types in outdoor field cages (Guatemala 1998). Paired T-tests results are shown for lab or wild females mating according to either male type separately or combined.

**Male Remating: TSL, Antigua, Petapa, 2 F1 hybrids; Wild**

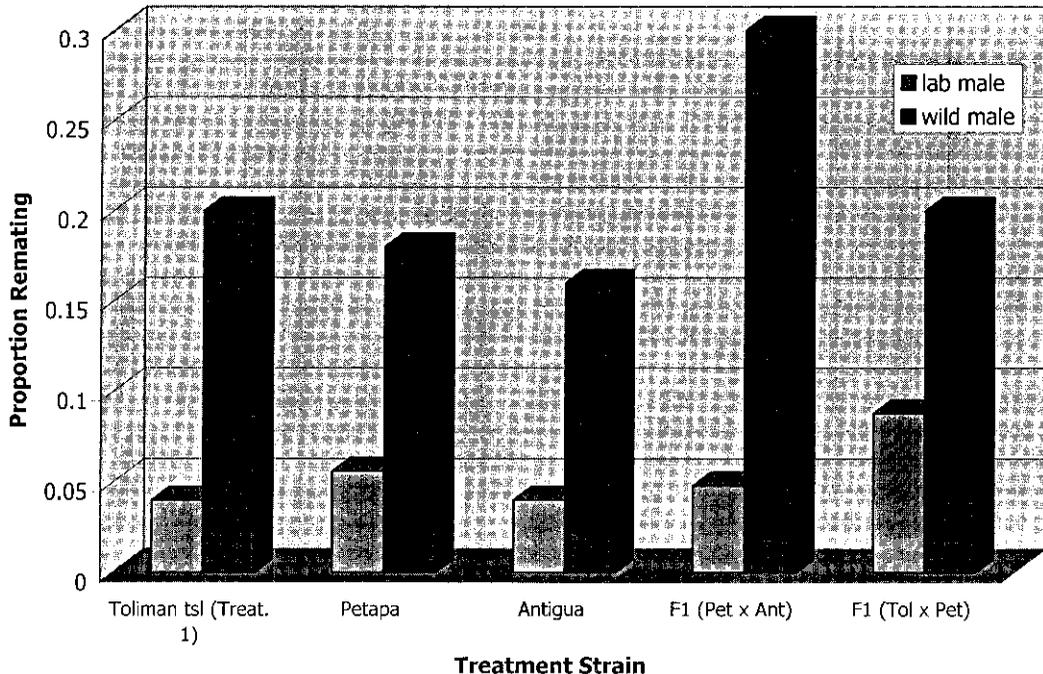


Fig. 4. Proportion of medfly males remating for each of the lab strains compared to wild flies in outdoor field cages (Guatemala 1998). Wild flies had significantly higher remating in each of the 5 comparisons (ANOVA F-statistic,  $P < 0.001$ ).

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	
		Wild	19	107.33	
	Male	mated once	51	114.63	0.759
		mated twice+	12	124.08	
		Lab	30	90.23	
		Wild	33	140.24	
2. Petapa	Female	mated once	75	74.93	0.023*
		mated twice+	12	46.83	
		Lab	60	66.17	
		Wild	27	81.93	
	Male	mated once	63	73.63	0.797
		mated twice+	16	83.38	
		Lab	41	56.78	
		Wild	38	95.92	
3. Toliman <i>tsl</i>	Female	mated once	381	99.74	0.0095*
		mated twice+	32	70.03	
		Lab	140	83.56	
		Wild	273	101.55	
	Male	mated once	354	95.12	0.343
		mated twice+	42	107.57	
		Lab	189	83.73	
		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	
		Wild	29	95.00	
	Male	mated once	66	89.42	0.666
		mated twice+	21	102.10	
		Lab	44	77.09	
		Wild	43	108.23	
5. Toliman <i>tsl</i> × Petapa F1	Female	mated once	88	90.69	0.178
		mated twice+	11	69.18	
		Lab	71	88.44	
		Wild	28	87.96	
	Male	mated once	66	97.15	0.0832
		mated twice+	20	75.70	
		Lab	45	71.44	
		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  $\chi^2$  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-

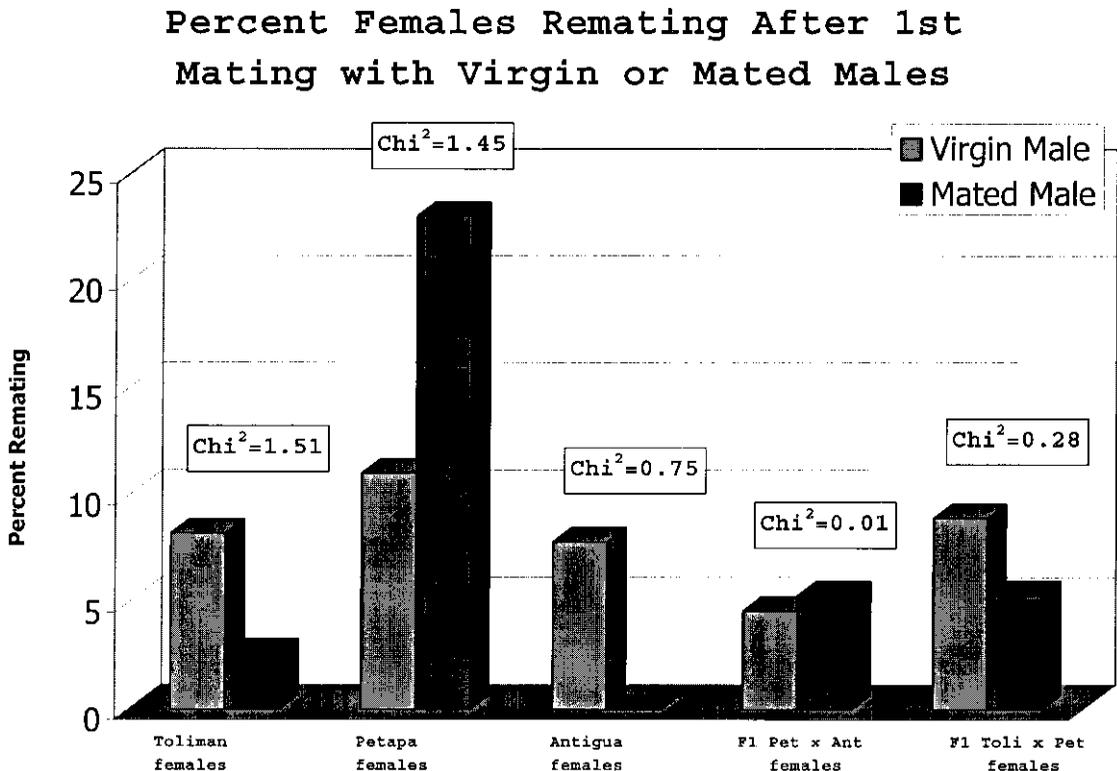


Fig. 5. Percentage of medfly females remating after first mating with either virgin or mated males in outdoor field cages (Guatemala 1998).  $\chi^2$  test results ( $df = 1$ ) are shown for each female strain (all tests non-significant,  $P > 0.05$ ).

### Male Quality Index

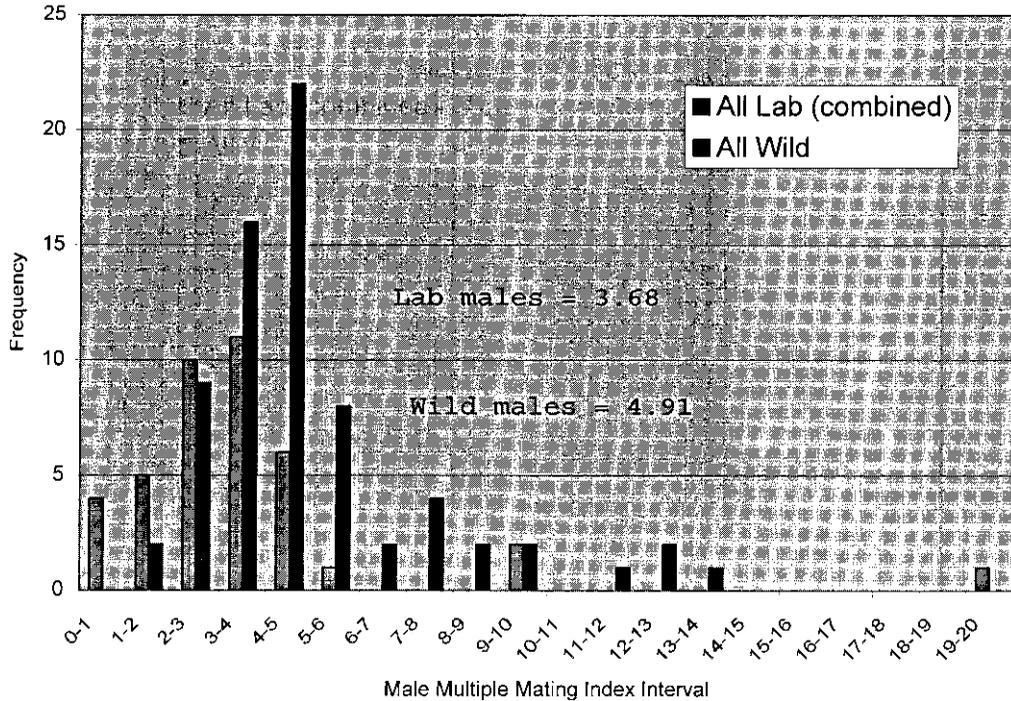


Fig. 6. Male Quality Index, MQI, for individual medfly males of lab strains (all data combined) and wild flies from outdoor field cage studies (Guatemala 1998). Only males mating 2 or more times are considered.

### Male Quality Index (MQI), by Strain

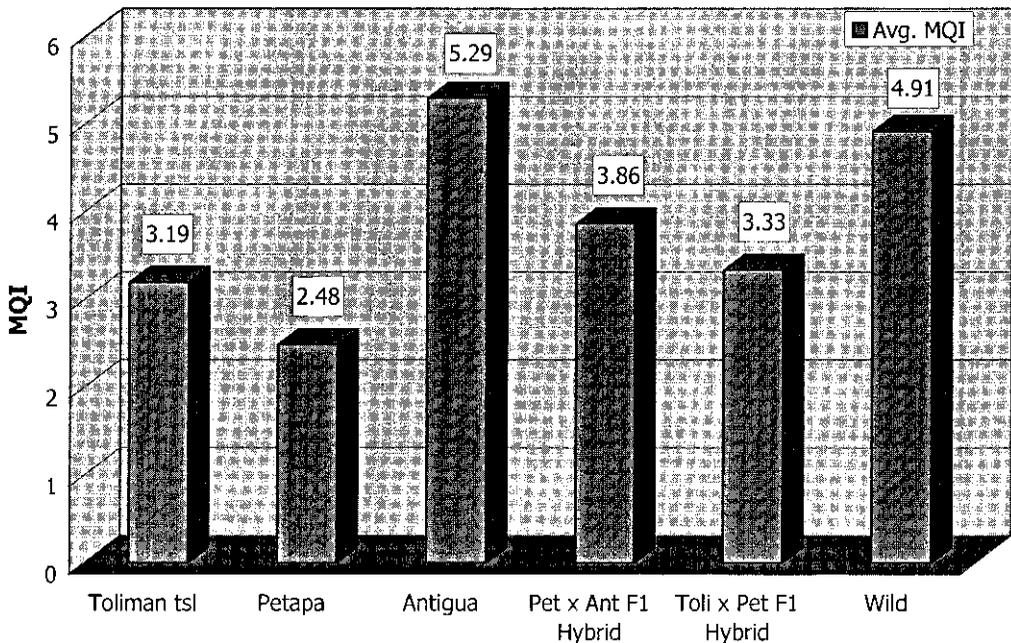


Fig. 7. Male Quality Index, MQI, averaged for each of the lab and wild strains from outdoor field cage studies (Guatemala 1998).

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.

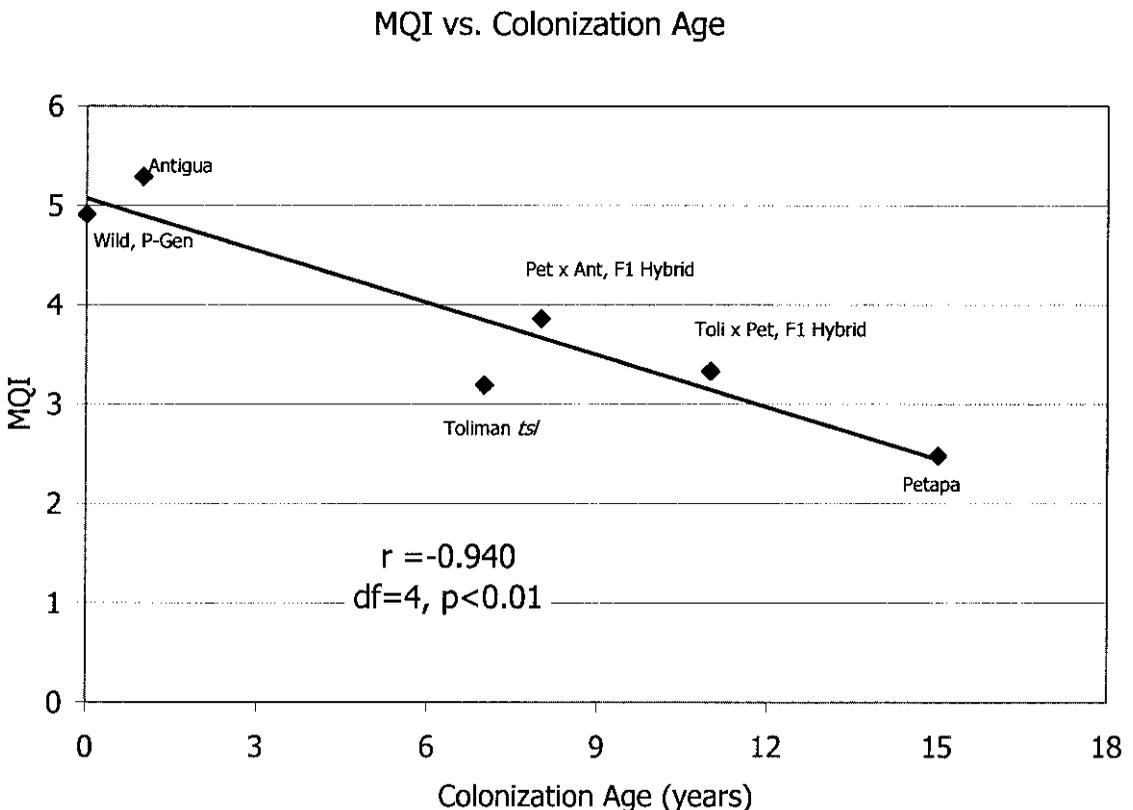


Fig. 8. Male Quality Index (MQI) vs. Colonization Age (years) for each of the six lab or wild strains tested in outdoor field cages (Guatemala 1998). A correlation ( $r$ ) test result and linear fit to the data are shown ( $df = 4$ ,  $P < 0.01$ ).

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