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FACTORS AFFECTING FEMALE REMATING FREQUENCY IN THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Mating and remating of two laboratory strains (Petapa and Guate), one wild population (Antigua) of *Ceratitis capitata* (Wiedemann) and one of the hybrids between them were studied under laboratory conditions. No evidence of sexual isolation at first mating was found among them. Remating frequency was higher under crowded conditions for the two laboratory strains. The probability of Petapa females remating depended more on the origin of the male and was negatively associated with the duration of the first mating, but these variables had no effect on remating tendency of Guate females. Matings by Petapa males were significantly less prolonged than those of Guate or hybrid males. With respect to remating, Petapa non-virgin females preferred Petapa to Guate males.

Key Words: remating behavior, mating duration, medfly, *Ceratitis capitata*

RESUMEN

Apareamiento y re-apareamiento de dos razas de laboratorio (Petapa y Guate), una formada por una población salvaje (Antigua) de *Ceratitis capitata* (Wiedemann) y una de híbridos entre ellos, fueron estudiadas bajo condiciones de laboratorio. No se encontró evidencia de aislamiento sexual durante el primer apareamiento entre ellas. La frecuencia de re-apareamiento fue mayor bajo condiciones de hacinamiento para las dos razas de laboratorio. La probabilidad de que las hembras de Petapa se re-aparearan dependió más del origen del macho y estuvo negativamente asociada con la duración del primer apareamiento, pero estas variables no tuvieron ningún efecto en la tendencia de re-apareamiento en las hembras de Guate. Los apareamientos por parte de los machos de Petapa fueron significativamente menos prolongados que los de Guate o que los machos híbridos. Con respecto al re-apareamiento, las hembras no vírgenes de Petapa prefirieron machos de Petapa que machos de Guate.

The Mediterranean fruit fly (medfly) *Ceratitis capitata*, (Wiedemann) is a highly destructive pest, infesting more than 200 species of fruits and vegetables (Christenson & Foote 1960) and creating a serious impact on the economy of many countries. It is largely controlled using the Sterile Insect Technique (SIT) (Cunningham et al. 1980, Klassen et al. 1994, Hendrichs et al. 1995), which relies on the release of mass-reared sterile males into a target wild population with matings between sterile males and wild females failing to result in the production of viable offspring (Knipling 1955). The fact that a female may mate more than once is significant for the SIT since it increases the chances that the female will encounter and mate with at least one fertile male (Bloem et al. 1993).

Studies on the reproductive biology of the medfly have reported that both sperm and accessory gland fluids may inhibit female remating to some extent (Delrio & Cavalloro 1979, Miyatake et al. 1999). Besides, after the first mating females switch from mate searching towards oviposition-site searching (Jang 1995, Jang et al. 1998). These mechanisms are insufficient, however, to restrict remating entirely. Multiple matings have been reported both under laboratory conditions (Katiyar & Ramirez 1970, Nakagawa et al. 1971, Bloem et al. 1993) and in the field (McInnis 1993, Yuval et al. 1996). McInnis (1993) found that wild females trapped in a sterile-male release area contained sperm from both irradiated and wild males, but unfortunately no field data are available regarding which male mated first. Remating has been associated with the duration of the first mating (Farias et al. 1972, Saul et al. 1988) and also with the nutritional status (protein fed or deprived) of the male (Blay & Yuval 1997). Females mated to sterile males with inactive sperm have a higher remating frequency than those mated to normal males (Cavalloro & Delrio 1970, Katiyar & Ramirez 1970, Bloem et al. 1993). On top of lacking fertile sperm, sterile males may differ from their wild counterparts in a variety of as-

pects as a result of inadvertent yet strong selection on flies to adapt to artificial factory conditions (Briceño & Eberhard 1998). The effect of such selected changes on remating is unknown. Hence any information comparing the remating frequency of females mated either with laboratory-adapted or wild males is potentially of importance for the success of the SIT.

The aim of the present work is to study remating frequency in medfly females from strains with different colonization histories. Their responses when first mated to a male from the same or from a different strain was investigated using a continuous observation design and individual labels for the flies.

Materials and Methods

Biological Material

Three strains of medflies were used in the study: Petapa, Guate and Wild. The Petapa strain was a subculture from the mass rearing strain established at the Moscamed factory in Guatemala in 1984 for SIT programs (Rendon 1996). It originated from pupae collected from infested coffee beans near Lake Atitlán, Guatemala. The Guate strain originated also from coffee collected in southwestern Guatemala near Retalhuleu and established as a colony at the School of Biological Sciences, Manchester University, UK, seven months before the study. Wild flies emerging from pupae from coffee beans near Antigua City, Guatemala, were also used. An inter-strain hybrid was produced by crossing wild males with Petapa females. This Hybrid strain was investigated only in its first generation.

Rearing Procedures

Flies were reared at 25 ± 1°C, 70 ± 5% relative humidity and a photoperiod of 12:12 (L:D). The adult diet consisted of a yeast:sucrose mix (1:3) and larvae were maintained on a carrot-based diet (Busch-Petersen & Wood 1986). Experimental flies were kept virgin until tests began. Virgin adults were collected within 24 h of emergence and immobilized (one minute under -20°C) in order to separate the sexes. Once sexed, the flies were kept under the same conditions until they reached sexual maturity at the age of 5-7 days old. Wild flies were tested at the age of 7-9 days.

Re-mating Trials

Mating cages were established to assess remating frequency. Each cage consisted of a clear plastic box, $30 \times 20 \times 20$ cm., having one side fitted with a sleeve to allow the collection of mating couples. In every trial 25 males of each of the two strains being compared and 25 females of the same two strains, resulting in a total number of 100 flies, were released into a mating cage. Released flies were individually labeled one day prior to testing. They were immobilized by rapid cooling and a small printed letter (Arial, font size 3) was glued to its thorax with a dot of paint (McInnis et al. 2002). Different colors of paper were used to identify strains. Once labeled, males were released into the mating cage and females were released into a separate cage. All cages contained adult diet and water. Both cages were then transferred to the testing room (24 ± 1°C, 64 ± 1% RH, and 12:12 L:D).

On testing day 1, females were released into the mating cage with the males, half an hour after room lights were turned on. Every copulating pair was gently removed from the cage and both male and female were identified. Mating duration was timed, and each pair released back into the mating cage after separation. Water and food were provided throughout the test. After eight h of observation the females were removed from the mating cage, and transferred into a separate cage with food and water. Flies were left overnight in the testing room. On the following day (testing day 2) the females were released back into the mating cage half an h after the lights were turned on. Couples were collected and scored during a six-h observation period, after which the females were again removed. The procedure was daily repeated during four consecutive days, with six and four-h observation periods on days three and four. Flies were constantly observed except for occasional breaks of no more than half an h (during these breaks it is highly unlikely that any successful copulations occurred because copula duration averaged more than two h).

A parallel set of tests was performed with lower fly density. In this situation each cage contained only 20 flies (5 females of two strains and 5 males of the same strains). Otherwise, these tests followed the same procedure as described above. The two types of tests will be referred to as crowded (100 flies/cage) and relaxed (20 flies/cage) conditions.

Under crowded conditions, 3 tests were performed: Wild and Petapa (1 replicate), Guate and Petapa (7 replicates) and Hybrid, Guate and Petapa (1 replicate). In this last case, Hybrid males were used in the place of Guate males. Under relaxed conditions, two tests were performed: Guate and Petapa (15 replicates) and Hybrid and Petapa (5 replicates).

Statistical Analyses

The percentage of mated females was calculated in order to determine whether the conditions were optimal for mating tests. A χ^2 test of homogeneity was calculated to assess if the strains mated assortatively. Relaxed cages were

pooled to provide an adequate sample size and crowded cages were analyzed separately The effect of density on remating rate was determined by evaluating the Guate × Petapa crowded cages against the relaxed ones by means of a χ^2 test. Remating rate was calculated for every female strain in each cage grouped by the origin of the first male they mated with.

To investigate the effect of different aspects of the first mating on the probability of remating of Guate and Petapa females, a logistic regression analysis was performed. All the mated females were assigned a value of 0 if they mated only once, or a value of 1 if they remated during the test. Remating condition (0 for non-rematers or 1 for rematers) was used as the dependent variable. The origin and copulatory status (virgin or non-virgin) of the first male, the duration of the first mating and the total number of copulations that the first male achieved during the test (# matings) were computed as the independent variables. Significance levels were determined using log-likelihood ratio χ^2 tests. The presence of correlation between variables was analyzed. Females that died during the experiment were removed from the remating analysis. Given that only one cage was run with wild females and that both wild and Hybrid females hardly remate at all, females from these two strains were not considered in the analysis.

The duration of mating for each type of cross was analyzed by ANOVA and Tukey's HSD tests. For females that mated more than once, their preference in the selection of the second partner was investigated by means of a χ^2 test of homogeneity. All statistical analyses were performed using Statistica for Windows (Statistica 5.1, StatSoft, Inc. 1996).

RESULTS

General Mating Conditions and Mate Selection

For crowded cages, the percentage of mating was high and there was no evidence of sexual isolation among strains (Table 1). Good mating conditions and lack of assortative mating were also shown for relaxed cages (Table 2). The high percentage of matings achieved both in relaxed as well as in crowded conditions indicates that environmental and biological (nutritional level and age of flies) conditions were adequate. However, wild males did not mate readily in laboratory cages, and never with their own females, Cage 1. The low number of matings achieved by them could probably be explained by a lack of sexual maturation.

Factors affecting remating rate by Guate and Petapa females

The proportion of females that mated more than once was significantly higher under crowded conditions compared to relaxed conditions for both female strains (Table 3-A). Consequently all subsequent analyses were performed for each density separately. However when the results are broken down according to the type of male, the effect of density was significant only for Petapa x Petapa matings (Tables 3-B and 3-C). Mean remating rates (number of rematers/number of mated females in each cage) were higher for crowded than for relaxed conditions for all crosses (Fig. 1), but no statistical differences were found (Mann-Whitney test, $P > 0.05$), probably due to small sample size in relaxed cages.

The logistic regression analysis revealed that other variables apart from fly density in the cage affected remating tendency (Table 4). The previous mating history of the first male was significantly associated with the remating rate of Guate females under both conditions, with non-virgin males being more successful at inhibiting remating (Guate females, Table 4). In contrast male mating status revealed no significant effect on Petapa females.

The number of matings achieved by each male during the test showed no association with remating probability for both female strains.

The origin of the first male significantly affected the remating rate of Petapa females under both density conditions. Petapa females showed a higher remating rate if first mated to Petapa males than Guate or Hybrid males (Fig. 1-A). Under relaxed conditions remating rate was lower if the female mated to Hybrid males instead of Petapa males (Mann-Whitney test, $Z = 2.12$, $P = 0.034$). Moreover, of the 4 Petapa females that mated to Wild males none remated during the experiment. In contrast Guate females showed no sensitivity to male origin (Fig. 1-B). It should be noted that Petapa females were exposed to Guate, Petapa, Wild and Hybrid males, whereas Guate females had access only to Guate and Petapa males.

The duration of the first mating was negatively associated with the likelihood of remating of Petapa females under relaxed conditions, and the same tendency, close to significance, was found under crowded conditions. Guate females, on the contrary, showed no differences in remating tendency in association with mating duration.

The duration of copulation of males from different strains differed consistently irrespective of which type of female they mated with (Table 5). Matings involving Petapa males were shorter than those involving Hybrid or Guate males (Tukey's HSD test; $P < 0.01$). Mating duration was highly correlated with male origin ($r^2 = 0.137$, $P < 0.001$) but not with female origin ($P > 0.05$).

Second Mating of Guate and Petapa Females

Under crowded conditions, there was evidence of non-random mating (Table 6). Petapa females showed a strong preference to remate with Petapa

TABLE 1. PERCENTAGE OF MATING, NUMBER OF COUPLES OBTAINED FOR EACH MATING COMBINATION AND MATING COMPATIBILITY BETWEEN STRAINS IN CROWDED CAGES.

Cage number	Strains	% Mating	Mating combination[a]				χ^2 P value[c]
			W × W[b]	W × P	P × W	P × P	
1	Wild/Petapa	75%	0	4	3	8	
			G × G	G × P	P × G	P × P	
2	Guate/Petapa	100%	14	10	11	14	ns
3	Guate/Petapa	88%	13	9	13	11	ns
4	Guate/Petapa	91%	13	6	6	11	0.0469
5	Guate/Petapa	100%	5	15	7	9	ns
6	Guate/Petapa	90%	9	9	15	11	ns
7	Guate/Petapa	85%	13	6	7	7	ns
8	Guate/Petapa	94%	9	12	15	11	ns
	Guate/Petapa[d]	92.57%	76	67	74	74	ns
			H × G	H × P	P × G	P × P	
9	Guate/Petapa/Hybrid	86%	8	9	14	12	ns

[a]Only the first mating was considered for each female.
[b]Male strain is listed first, with G for Guate, H for Hybrid, W for Wild and P for Petapa strains.
[c]Significance of homogeneity χ^2 value.
[d]Mean percentage of mating for Guate × Petapa 7 replicates.

TABLE 2. MEAN PERCENTAGE OF MATING ACHIEVED FOR ALL CAGES, TOTAL NUMBER OF COUPLES OBTAINED FOR EACH MATING COMBINATION AND MATING COMPATIBILITY BETWEEN STRAINS UNDER RELAXED CONDITIONS.

		Mating combination[a]				
Strains	% Mating	G × G[b]	G × P	P × G	P × P	χ^{2c}
Guate/Petapa	90.3%	39	23	35	30	ns
		H × H	H × P	P × H	P × P	
Hybrid/Petapa	85.5%	11	12	7	8	ns

[a]Only the first mating was considered for each female.
[b]Male strain is listed first, with G for Guate, H for Hybrid, and P for Petapa strains.
[c]Significance of homogeneity χ^2 value.

males over Guate males (χ^2 = 9.25; P = 0.002). This tendency was stronger if the female had originally mated with a Petapa male, although the difference was not significant (χ^2 = 3.62; P = 0.057, data not shown). Guate females showed no preference for males of a particular strain for either their first or second mate. Under relaxed conditions, no evidence of assortative mating was detected (χ^2 = 0.61; P = 0.434), although Guate females showed a just significant preference for Petapa males (χ^2 = 3.88; P = 0.049).

DISCUSSION

Some interesting observations on remating frequency of *Ceratitis capitata* females from different strains, particularly on the influence of density of flies and variables of the first mating have been shown in the present report.

The observed effect of density on remating (Table 3) reinforced the importance of test design and underlined the limitation of comparisons between different studies and extrapolations from them (see also Fowler & Partridge 1989). Higher remating rates under crowded conditions are probably due to prolonged exposure to courting males and not enough space for females to escape from them. Laboratory-adapted flies probably have higher remating rates compared to flies from the wild, though more information on remating rate of wild females is needed to support this hypothesis.

Despite the effect of density on remating frequency, the present work suggests that other variables influence female remating (Table 4). Male

TABLE 3. TOTAL NUMBER OF FEMALES THAT MATED ONCE OR MORE THAN ONCE ACCORDING TO THE DENSITY OF FLIES IN THE TESTING CAGE.

	Petapa females		Guate females	
	Crowded	Relaxed	Crowded	Relaxed
A. All males[a]				
Mated once	66	37	51	35
Remated	68	16	95	34
Total	134	53	146	69
χ^2	6.49*		4.87*	
B. Petapa males[b]				
Mated once	28	19	24	15
Remated	41	11	48	18
χ^2	4.34*		1.42	
C. Guate males[c]				
Mated once	36	18	27	20
Remated	27	5	47	16
χ^2	3.22		3.60	

[a]All males irrespective of their origin.
[b]Only matings involving Petapa males.
[c]Only matings involving Guate males.
Crowded: 100 flies/cage.
Relaxed: 20 flies/cage.
*P < 0.05.

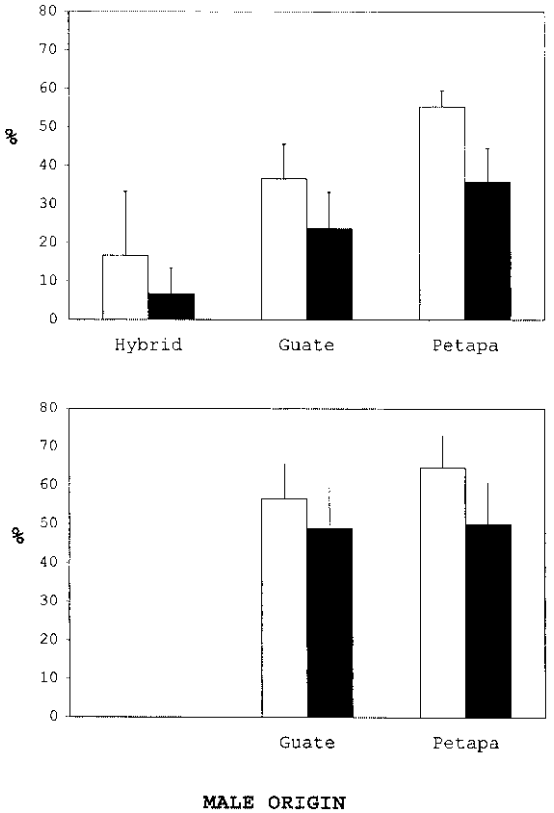

Fig. 1. Mean percentage (±SE) of remating for Petapa and Guate females according to male origin and fly density: (□) crowded, (■) relaxed conditions.

reproductive status has been reported to have no effect on remating of female medfly (Bloem et al. 1993). But in the present case, mated males seemed better at inhibiting remating than virgin males (Table 4, status). It should be considered that as days went by during the tests, there was a growing deficit of virgin males and a reduction in the time available for females to remate (i.e., to show their remating tendency). When females that mated on the first day of the trial were the only ones analyzed, no significant effect of male status was revealed (data not shown), which reinforced the idea that the apparent effect of male status on remating could be due to a design artifact. With respect to the lack of association between number of matings achieved by the male and remating (Table 4, # matings), it may be suggested that males with a higher mating success do not necessarily induce a higher refractory period. Again the test design could interfere in the interpretation of the results. Very successful males found virgin females hard to encounter, so they mated several times with rematers and thus were not taken into account in the logistic regression. To determine whether mating success and postcopulatory success are correlated or not, a different test design should be used.

The tendency revealed of higher rates of remating in Petapa females first mated to Petapa males than those mated to Hybrid or Guate males have remained under both densities (Fig. 1). This result suggests that aspects of the first mating that are strain dependent may affect female tendency to remate regardless of fly density. The present study showed that remating rate of Petapa females was determined by the male strain under crowded conditions and by both male strain and mating duration under relaxed conditions.

Saul et al. (1988) has reported mating duration as a determinant of the refractory period and remating rate. Remating propensity may be associated with insufficient sperm load (Farias et al. 1972). The present study showed differences between Guate and Petapa males mean copula duration times. On this basis, the observed effect of male strain on Petapa females remating tendency (Table 4, male origin) could be attributed to the difference in the mean time of copula duration. If mating duration and origin of the male are analyzed together in a two-variable logistic regression to obtain the predicted values for remating tendency, some effect of male strain, apart from mating duration, is shown to be still present in the determination of Petapa females remating tendency (Fig. 2). Interestingly, even though remating is higher for Petapa females mated to Petapa males than other males, the shape of the regression is very similar for the three male strains. This suggests that as mating duration increases, remating probability decreases at a similar rate for the three types of male. Besides this, it could be postulated that there is another factor determining remating that is independent of mating duration.

No association was found between remating and mating duration on Guate females (Table 4), possibly due to the fact that Guate females were not mated with any strain whose males lasted (on average) more than their own. However, a higher remating probability for Guate females mated to Petapa males compared to Guate × Guate matings, as a consequence of shorter copula, would be expected. The reason why Guate females did not show such a tendency is unclear.

The shorter duration of matings involving Petapa males compared to Guate or Hybrid males provides evidence that males from long-established strains have shorter mating times than recently colonized or wild ones. These results are in agreement with the study of Cayol et al. (1999) in which mass-reared males copulated for less time than wild males, with either mass-reared or wild females. Shorter copula duration as well as a shorter time spent during courtship of laboratory males to avoid interruptions from other males reported by Briceño & Eberhard (1998) appear to be examples of laboratory induced changes.

TABLE 4. LOGISTIC REGRESSION ANALYSIS OF THE EFFECTS OF DIFFERENT ASPECTS OF THE FIRST MATING ON THE PROBABILITY OF REMATING.

	Petapa females				Guate females			
	Crowded		Relaxed		Crowded		Relaxed	
Independent variable	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Status of the male	0.05	0.825	3.32	0.067	4.02	0.045	5.10	0.024
# matings	0.24	0.622	1.81	0.178	2.04	0.153	0.00	0.954
Origin of the male	4.31	0.038	4.98	0.026	0.16	0.689	0.70	0.401
Duration	3.27	0.071	9.73	0.002	0.07	0.795	0.33	0.568

For Petapa females all cages were considered, for Guate females only the Guate × Petapa cages were used (df = 1).

TABLE 5. MEAN MATING DURATION (H: MM) FOR EACH MATING COMBINATION.

	Origin of females							
	Wild		Hybrid		Guate		Petapa	
Origin of males	Mean	N	Mean	N	Mean	N	Mean	N
Hybrid	—		2:31	(4)	3:01	(6)	3:00 a	(15)
Guate	—		—		2:34 a	(98)	2:37 a	(61)
Petapa	1:46	(3)	2:21	(6)	2:02 b	(99)	2:08 b	(80)

Only matings involving virgin flies were computed. Means are shown in bold followed by different letters identifying significance groups according to Tukey's HSD test with $P < 0.01$; mating combinations involving more than 10 cases were the only included in this analysis.

TABLE 6. NUMBER OF COUPLES FOR EACH MATING COMBINATION FOR THE FIRST (VIRGIN) AND SECOND (NON-VIRGIN) MATINGS.

	Mating combination[a]				
Crowded cages	G × G	G × P	P × G	P × P	χ^{2} [b]
First mating	76	67	74	74	0.29
Second mating	48	17	48	50	9.98**
Relaxed cages	G × G	G × P	P × G	P × P	
First mating	39	23	35	30	1.07
Second mating	11	7	23	9	0.61

[a]Male strain is listed first, with G for Guate and P for Petapa strains.
[b]Homogeneity χ^{2} value, df = 1.
**$P < 0.01$.

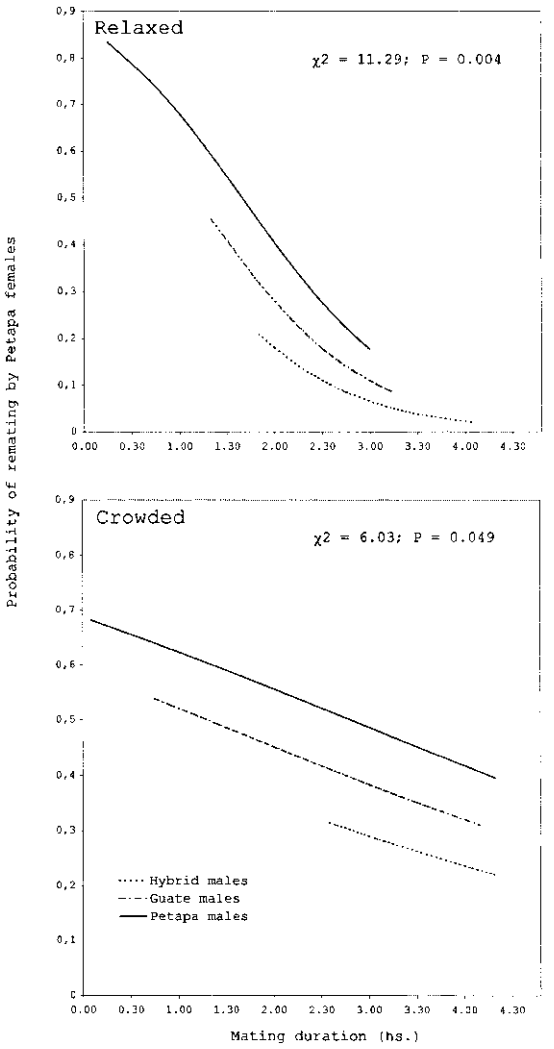

Fig. 2. Effect of male strain and copula duration on probability of remating for Petapa females for relaxed and crowded conditions. Log-likelihood ratio determines the significance of the logistic regression.

Few factors were found to influence the remating rate of Guate females. However, Guate females only encountered males from their own strain and Petapa males. Trials with Wild or Hybrid males may have revealed more associations.

The strong preference showed by non-virgin Petapa females for Petapa males suggests assortative mating after the first mating. This phenomenon has not been reported in medfly females before and might suggest some kind of post-copulatory selection (Eberhard & Cordero 1995). The way by which Petapa females discriminate between males is unknown although male courtship should be considered.

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