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INFLUENCE OF MALE DIET ON MALE MATING SUCCESS AND LONGEVITY AND FEMALE REMATING IN THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT

The purpose of the present study was to investigate the effect of dietary protein on the mating behavior and survival of male Mediterranean fruit flies (medflies), *Ceratitis capitata* (Wied.), as a means of enhancing the effectiveness of mass-reared males in sterile release programs to suppress wild populations. Conducted in the laboratory, our study addressed three main questions: 1) Does the inclusion of protein in the adult diet affect mating success of wild and mass-reared males? 2) Are copulation duration and remating tendency of wild females affected by the strain (wild versus mass-reared) and diet (protein-fed versus protein-deprived) of their initial mating partner? 3) Does the inclusion of protein in the adult diet affect the longevity of mass-reared males? In mating trials involving wild flies, protein-fed males had a mating advantage over protein-deprived males. However, the addition of protein to the diet did not boost the mating success of mass-reared males in competition with wild or mass-reared males for wild females. The inclusion of protein in the male diet had no apparent effect on female remating tendency, copulation duration, or male longevity. Independent of male diet, we found no difference between wild and mass-reared males in the duration of copulations with wild females, and wild females mated initially to wild and mass-reared males displayed similar remating propensity. The implications of these findings for SIT are discussed.

RESUMEN

El propósito del presente estudio fué para investigar el efecto de la proteina dietética en el comportamiento del apareamiento y la sobrevivencia de machos de la mosca mediterranea, *Ceratitis capitata* (Wied.), como una manera de mejorar la eficacia de la producción en masa de los machos en programas de liberaciones de machos esteriles para suprimir las poblaciones silvestres. Nuestro estudio, conducido en el laboratorio, se enfocó en tres preguntas principales: 1) Si la inclusión de proteina en la dieta del adulto afecta el éxito en el apareamiento de los machos silvestres y de los machos criados en masa? 2) Si la duración de la cópula y la tendencia de reaparearse las hembras silvestres con la pareja inicial son afectadas por la raza (silvestres versus criados en masa) y la dieta (alimentadas con proteina versus privadas de proteina)? 3) Si la inclusión de proteina en la dieta del adulto afecta la longevidad de los machos criados en masa?
En pruebas de apareamiento con moscas silvestres, los machos alimentados con proteina tuvieron una ventaja de apareamiento sobre los machos privados de proteina. Sin embargo, la adición de proteina a la dieta no aumentó el éxito de aparearse en los machos criados en masa en competencia con machos silvestres con las hembras silvestres. La inclusión de proteina en la dieta del macho no tuvo un efecto aparente sobre la tendencia de las hembras para reaparearse, la duración de la cópula, o la longevidad del macho. Independientemente de la dieta del macho, no encontramos una diferencia en la duración de la cópula entre machos silvestres y machos criados en masa al aparearse con hembras silvestres, y las hembras silvestres apareadas inicialmente con machos silvestres o con machos criados en masa mostraron una tendencia similar para aparearse de nuevo. Se discuten las implicaciones de estos resultados para técnica del insecto estéril (SIT).

The sterile insect technique (SIT) is widely used in suppression or eradication programs against the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (Hendrichs et al. 1995). To a large degree, the success of SIT depends on the ability of mass-reared, sterile males to compete successfully against wild males in obtaining copulations with wild females (unless otherwise indicated, males derived from large-scale, production facilities are hereafter referred to as 'mass-reared' regardless of whether they were irradiated (sterilized) prior to study or release). Unfortunately, the mass-rearing environment, and, in particular, the high density at which adults are held, imposes strong selection factors that may alter courtship behavior and subsequently lessen

the competitive ability of sterile males in the wild (Leppla & Ozaki 1991; Cayol 2000). For example, Briceno and Eberhard (1998) observed that mass-reared males displayed shorter courtships than wild males and suggested that accelerated courtship evolved in response to frequent disturbances resulting from dense crowding.

Genetically based changes in sexual behavior and life history traits appear to be an inherent consequence of mass-rearing and are therefore difficult (if not impossible) to avoid or mitigate. Because of this, there is a persistent need to develop procedures that boost the performance of mass-reared males via simple, easily incorporated, and inexpensive modifications to the standard mass-rearing protocol. For example, a recent study on the medfly (Shelly & McInnis 2001) demonstrated that exposure to the odor of ginger root oil dramatically enhanced the mating success of mass-reared males. In the absence of chemical exposure, wild males outcompeted mass-reared males and obtained 74% of all matings. However, following exposure to ginger root oil, the mating frequencies were reversed, and mass-reared males achieved 75% of all matings.

Modification of the pre-release, adult diet may represent another simple approach to increase the effectiveness of male medflies in SIT. Current programs (e.g., California, Israel) feed newly emerged adult medflies with sucrose-containing agar exclusively. However, recent studies showed that the addition of protein hydrolysate to the diet resulted in a significant increase in male mating success. In noncompetitive conditions, mass-reared male medflies fed a protein-sugar mixture mated more frequently than males fed sugar only (Blay & Yuval 1997). Likewise, in direct competition for females, mass-reared males given the sugar-protein diet signaled (pheromone-called) and mated more frequently than mass-reared males that were protein-deprived (Kaspi & Yuval 2000; Taylor & Yuval 1999). Similar findings were reported for tests involving wild medflies as well (Kaspi et al. 2000; see also Papadopoulos et al. [1998]).

Although these findings clearly support the addition of protein to the pre-release diet, this modification may have costs in terms of reduced male survivorship after release. Following 24 h of starvation on the fifth day of life, mass-reared males fed a protein-containing diet for the preceding 4 d were far more likely to die than were males fed sugar only during the first 4 d of life (Kaspi & Yuval 2000). Thus, development of a pre-release, protein-containing diet that optimizes male effectiveness in SIT programs requires additional information on the balance between heightened sexual competitiveness and reduced survivorship.

The purpose of the present study was to investigate further the effect of dietary protein on the mating behavior and survival of male medflies. Based on laboratory observations, our study addressed three main questions: 1) Does the inclusion of protein in the adult diet affect mating success of wild and mass-reared males? 2) Are copulation duration and remating tendency of wild females affected by the strain (wild versus mass-reared) and diet (protein-fed versus protein-deprived) of their initial mating partner? 3) Does the inclusion of protein in the adult diet affect the longevity of mass-reared males? Although field cages would have provided a more natural environment, we chose to conduct this study in the laboratory, because we were able to measure copulation duration, female remating and male longevity with greater accuracy and obtain larger sample sizes than possible from field tests.

Materials and Methods

Mating Experiments

Wild flies were reared from the fruits of Jerusalem Cherry (*Solanum capsicum* L.) collected in the Hawaii Volcanoes National Park, HI. Fruits were held over vermiculite, and larval development proceeded *in situ*. Pupae were sifted from the vermiculite 7-9 d after fruit collection, and adults were separated by sex within 2 d of eclosion, well before reaching sexual maturity (T. E. S., unpublished data). Mass-reared males were obtained from the Hawaii Fruit Fly Rearing Facility, Waimanalo, HI. Pupae were exposed in air to 150 Gy of gamma radiation from a ^{137}Cs source 2 d before eclosion and then delivered to our laboratory. Males were collected within 12 h of eclosion. Both wild and mass-reared adults were held in plastic buckets covered with nylon screening (volume 5 liters; 100-200 flies per bucket). Room temperature was maintained at 22-25°C and relative humidity at 65-90%, and flies were exposed to natural and artificial light in an approximately 12: 12 h light:dark cycle. Wild and mass-reared males were separated into 2 dietary regimes: "protein-deprived" males were given only sugar (sucrose) plus water, and "protein-fed" males were given a 3:1 mixture (by volume) of sugar and protein hydrolysate plus water. Wild females were given the sugar-protein mixture plus water. Samples (n = 30 individuals) of wild and mass-reared males maintained on the two diets and wild females were weighed (wet weight) to the nearest 0.1 mg with an electronic balance. In all cases, adults were weighed when 5 d old.

Three experiments were performed that compared the mating frequency of 1) protein-fed versus protein-deprived wild males, 2) protein-fed versus protein-deprived mass-reared males, and 3) protein-fed or protein-deprived mass-reared males versus protein-fed wild males. Wild females were used in all cases. Mating tests were conducted in the same manner for all experiments. Sixty males of each diet type (or strain type for ex-

periment 3) and 60 females were placed in transparent plexiglass cages (30 × 30 × 40 cm with screen-covered openings on the top and sides). Although this density far exceeds natural levels (Shelly et al. 1994), females nonetheless had ample space for movement and were clearly capable of moving away from (i.e., rejecting) courting males. Wild flies were used at 10-14 d of age, and mass-reared males were 6-9 d old when tested (the different ages reflect differing maturation rates between wild and mass-reared males). Males were marked 1 d before testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no adverse effects, and males resumed normal activities within minutes of handling.

Flies were placed in the cages between 0730-0800 hours and monitored continuously for the next 5 h. Cages were placed adjacent to east-facing windows and thus received both natural and artificial light. Mating pairs were collected by gently coaxing couples into vials, which were then labeled and placed on holding trays. Vials were monitored continuously, and times of collection (i.e., mating start) and break-up (i.e., mating stop) were recorded for each pair. When mating ceased, males were removed and discarded, and females were held in plastic buckets for the remating trials (see below; data on copulation duration and female remating were collected only for experiments 1 and 2). All unmated flies were discarded. Two or 3 cages were run on each of 5 days for all experiments. Eleven replicates (cages) were run for each experiment.

Mated females were provided with food and water as above and a perforated, plastic vial (containing a sponge soaked with lemon juice) for oviposition. Two days after their initial mating, groups of females that mated with males of the same diet type were placed in glass cages (30 cm cubes with a cloth sleeve covering one side) with an equal number of protein-fed, wild males between 0730-0800 hours. Cages were monitored for 5 h, and copulation durations were recorded as noted above. Females that remated 2 d after the initial mating were discarded, and the remaining females were held and re-tested in the same manner 4 d after the initial mating. Females that failed to mate at this time were held and tested a final time 7 d after the initial mating. Males used in the different remating trials were 11-19 d old. Female mortality was relatively low and varied independently of the strain or diet of the initial mate. Over all females, 95% survived from the initial mating to the 2-d test, 96% survived from the 2-d test to the 4-d test, and 91% survived from the 4-d to the 7-d test.

Male Longevity

Two experiments were performed to investigate the effect of adult diet on the longevity of mass-reared males. First, the probability of surviving the first week of adult life was compared between protein-fed and protein-deprived males. Emerging males were collected between 0600-0900 hours, and groups of 50 individuals were placed in screen cages (30 cm cubes) and given continuous access to water plus sugar only or the protein-sugar mixture. Dead flies were removed daily. Twenty-one cages were run for each diet type. The second experiment examined the effect of dietary protein on male longevity following food removal. Newly emerged males were protein-fed or protein-deprived for 4 d following eclosion. On the morning of the fifth day, groups of 50 males from a given diet were placed in screen cages with water only, and deaths were recorded every morning over the next 4 d (by which time all individuals in both treatments were dead; see below). Nine cages were run for each diet type.

Statistical Analyses

The Mann-Whitney test (test statistic T) was used for pairwise comparisons involving male weights, the number of matings achieved by competing male types, and the number of surviving males from the two diet groups. With respect to sexual competition, this test does not explicitly test for deviation from random mating (i.e., each male type accounts for 50% of the total matings), so a binomial test (using the normal approximation and Z scores with Yates correction for continuity) was performed with data pooled over all replicates. Remating frequencies were compared using the log-likelihood ratio for contingency tables (test statistic χ^2, where df = (rows-1) X (columns-1)) with Yates correction for continuity. Because sample sizes were relatively small (especially for females initially mated to mass-reared males) and because remating was a relatively rare event, comparisons were made using data pooled over all groups of females initially mated to the same male type. Copulation durations were compared among male types using the Kruskal-Wallis test (test statistic H). Statistical procedures followed Zar (1996).

Results

Adult Body Weight

Among wild males, diet had no apparent effect on weight; protein-fed males had an average weight of 7.9 mg (SD = 1.2; range: 5.4-9.8 mg) compared to 7.5 mg (SD = 1.0; 5.4-9.4 mg) for protein-deprived males ($T = 799$, $P > 0.05$, $n_1 = n_2 = 30$ in this and subsequent weight comparisons). Among mass-reared males, however, protein-fed males ($\bar{x}$ = 7.8 mg; SD = 0.9; range: 6.0-9.7 mg) were significantly heavier, on average, than protein-deprived males ($\bar{x}$ = 7.3; SD = 0.7; range: 6.0-

8.6 mg). Wild and mass-reared males fed protein-containing diets did not differ significantly in body weight ($T = 926.5$, $P > 0.05$), but protein-fed, wild males were significantly heavier than protein-deprived, mass-reared males ($T = 1078.0$, $P < 0.05$). The average weight of wild females was 8.2 mg (SD = 1.3; range: 5.0-10.7), which did not differ significantly from that of protein-fed, wild ($T = 986$; $P > 0.05$) or mass-reared ($T = 999.0$; $P > 0.05$) males.

Male Mating Success

In the experiment using wild males exclusively, diet had a marked effect on male mating success, and protein-fed males obtained an average of 16.5 matings per replicate compared to only 10.2 for protein-deprived males (Table 1). Protein-fed, wild males obtained 61% (182/296) of the total matings observed over all replicates ($Z = 4.0$; $P < 0.001$).

Diet had a lesser effect on the outcome of mating competition involving mass-reared males only (Table 1). On average, protein-fed males obtained a greater number of matings per replicate than protein-deprived males (6.6 versus 4.5, respectively), but this difference was not statistically significant. However, pooling data over all replicates, we found that protein-fed, mass-reared males obtained a disproportionately larger number of matings than expected by chance alone (73/123 = 59%; $Z = 2.2$; $P < 0.05$). Combined over both diet types, the total number of matings recorded per replicate was significantly higher for wild than mass-reared males (26.7 vs. 11.2, respectively; $T = 184.0$; $n_1 = n_2 = 11$; $P < 0.001$).

In the final experiment, where wild and mass-reared males competed directly, diet had no detectable influence on the relative mating success of mass-reared males (Table 1). When both strains were protein-fed, wild males obtained an average of 19.0 matings per replicate compared to 5.4 for the mass-reared males. In this case, wild males obtained 78% (209/269) of the matings over all replicates ($Z = 10.9$, $P < 0.001$). When the mass-reared males were protein-deprived, the wild males obtained an average of 18.4 matings per replicate compared to only 4.1 for the mass-reared males. Wild males obtained 82% (202/247) of the total matings recorded over all replicates ($Z = 13.2$, $P < 0.001$). In competition with protein-fed wild males, there was no significant difference in the proportion of total matings obtained by protein-fed and protein-deprived mass-reared males ($\chi^2 = 1.4$, $P > 0.05$).

Female Remating

In tests involving wild males, female remating tendency was independent of the diet of the mating partner in tests conducted 2, 4, or 7 d after the initial mating. Approximately 10% of the females that mated with protein-fed (18/173) or protein-deprived (11/107) males remated 2 d after the initial mating ($\chi^2 = 0.3$; df = 1 in this and all subsequent pairwise comparisons of frequency; $P > 0.05$). In tests conducted 4 d after the first mating, 4% (6/150) and 2% (2/95) of females mated to protein-fed and protein-deprived males remated, respectively ($\chi^2 = 0.4$; $P > 0.05$). In tests conducted 7 d after the first mating, 7% (9/131) and 6% (5/79) of females mated to protein-fed and protein-deprived males remated, respectively ($\chi^2 = 0.2$; $P > 0.05$). Combining data over diet types, we found significant variation in the incidence of remating over the different intervals tested ($\chi^2 = 10.6$, $P < 0.01$), reflecting primarily the large difference in remating probability between 2 d (29/280 = 10%) and 4 d (8/245 = 3%) after the initial mating.

In tests involving mass-reared males, female remating tendency was also independent of the diet of the mating partner. Remating frequencies 2 d after the initial mating were 3% (2/67) for females that mated with protein-fed males and 6% (3/46) for females that mated with protein-deprived males ($\chi^2 = 0.6$; $P > 0.05$). In tests conducted 4 d after the first mating, none of the females that initially mated to protein-fed or protein-deprived males remated ($\chi^2 = 0$; $P > 0.05$). In tests conducted 7 d after the first mating, 7% (4/

TABLE 1. INFLUENCE OF MALE DIET AND STRAIN ON MATING SUCCESS. MATING VALUES REPRESENT AVERAGE (±1 SD) NUMBER OF MATINGS PER REPLICATE (N = 11 IN ALL CASES).

Experiment	Male strain	Male diet	Matings	T	Significance
1	Wild	Protein-fed	6.5 (3.8)	161.0	$P < 0.05$
	Wild	Protein-deprived	10.2 (3.1)		
2	Mass-reared	Protein-fed	6.6 (2.6)	147.0	$P > 0.05$
	Mass-reared	Protein-deprived	4.5 (2.0)		
3a	Wild	Protein-fed	19.0 (4.2)	187.0	$P < 0.001$
	Mass-reared	Protein-fed	5.4 (2.2)		
3b	Wild	Protein-fed	18.4 (5.2)	189.0	$P < 0.001$
	Mass-reared	Protein-deprived	4.1 (2.2)		

TABLE 2. SURVIVAL AND REMATING OF WILD FEMALE MEDFLIES FIRST MATED TO WILD VERSUS LABORATORY MALES FED PROTEIN-CONTAINING (+) OR PROTEIN-DEPRIVED (-) DIETS.

Female event	Identity of first mate			
	Wild male		Laboratory male	
	Protei +	Protein -	Protei +	Protein -
Mate initially	182	114	73	50
Die prior 2 d	9	7	6	4
Test at 2 d	173	107	67	46
Mate at 2 d	18	11	2	3
Die prior 4 d	5	1	6	3
Test at 4 d	150	95	59	40
Mate at 4 d	6	2	0	0
Die prior 7 d	13	14	4	1

55) and 5% (2/39) of females mated to protein-fed and protein-deprived males remated, respectively (χ^2 = 0.2; P > 0.05). Combining data over diet types, we found that the probability of female remating varied significantly over the intervals tested (χ^2 = 9.0; P < 0.05), owing chiefly to the absence of any remating in tests conducted 4 d after the initial mating.

Combining data over diet types, we found no difference in remating frequency between females mated to wild or mass-reared males for any time interval tested (2 d: wild - 29/280, mass-reared - 5/113, χ^2 = 3.4; P > 0.05; 4 d: wild - 8/245, mass-reared - 0/99, χ^2 = 3.0; P > 0.05; 7 d: wild - 14/210, mass-reared -6/94, χ^2 = 0.2, P > 0.05). Using data combined across male diet and male strain, we found significant variation in the incidence of female remating over the intervals tested (χ^2 = 14.8, P < 0.001). Over all females, remating frequencies were 9% (34/393) at 2 d, 2% (8/344) at 4 d, and 6% (20/304) at 7 d after the initial mating.

Copulation Duration

For initial matings, copulation duration varied independently of male strain and diet (H = 6.1; df = 3; P > 0.05; Fig. 1). Among wild males, mating times averaged 144.3 min (SD = 40.8; range: 31 – 275) and 141.5 min (SD = 46.3; range: 21 - 239) for the protein-fed (n = 182) and protein-deprived (n = 114) males, respectively. Among mass-reared males, mating times averaged 135.6 min (SD = 41.5; range: 11 - 256) and 134.4 min (SD = 39.8; range: 67 - 223) for the protein-fed (n = 73) and protein-deprived (n = 50) males, respectively. Independent of diet, there was no significant difference in copulation duration for initial matings with wild ($\bar{x}$ = 143.3 min; SD = 42.3; n = 296) and mass-reared ($\bar{x}$ = 135.1; SD = 40.6; n = 123) males (T = 22,054.0; P > 0.05).

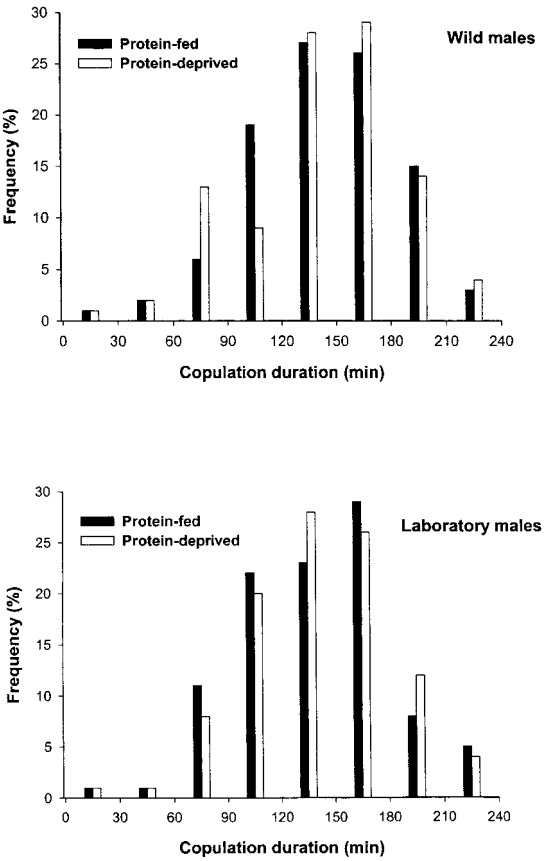

Fig. 1. Frequency distributions of duration of initial copulations between wild females and protein-fed (n = 182) and protein-deprived (n = 114) wild males (top) and protein-fed (n = 73) and protein-deprived (n = 50) mass-reared males (bottom).

For females mated initially with wild males (independent of diet), the duration of second matings did not vary significantly with the number of days elapsed since the initial mating (H = 1.4; n_1 = 29, n_2 = 8, n_3 = 14; df = 3; P > 0.05). Likewise, among females first mated with a mass-reared male (independent of diet), there was no significant difference in the length of rematings that occurred 2 d or 7 d after the initial mating (T = 29.0; n_1 = 5, n_2 = 6; P > 0.05). Rematings ($\bar{x}$ = 114.4 min; SD = 45.1) were significantly shorter than initial matings for females first mated to wild males (T = 6107.0; n_1 = 296, n_2 = 51; P < 0.01). For females first mated to mass-reared males, rematings (x = 121.5; SD = 23.6) were similar in length to initial matings (T = 633.5; n_1 = 123, n_2 = 11; P > 0.05).

Male Longevity

Among mass-reared males, survival probability to 7 d of age was independent of diet type. On average, 38.5 (SD = 4.5) protein-fed males per

cage survived the first week of life compared to 37.4 (SD = 4.0) protein-deprived males (T = 494.0; $n_1 = n_2 = 11$; $P > 0.05$).

Survivorship following food removal was likewise independent of diet type. On average, 45.0 (SD = 3.7) protein-fed males per cage were alive 1 d following food removal compared to 46.7 (SD = 2.2) of the protein-deprived males (T = 97.0; $n_1 = n_2 = 9$; $P > 0.05$). Two days after food removal, an average of 4.4 (SD = 3.6) protein-fed and 5.4 (SD = 5.0) protein-deprived males were alive per cage (T = 89.0; $n_1 = n_2 = 9$; $P > 0.05$). Of the 900 males observed in this experiment, only 4 protein-fed and 3 protein-deprived males survived for 3 d after food removal, and none survived to 4 d in either treatment.

Discussion

Consistent with previously conducted field-cage trials (Shelly et al. 2001), the laboratory data reported here revealed that, among wild male medflies in Hawaii, protein-fed individuals achieved significantly more matings than protein-deprived individuals in direct competition for wild females. In the field-cage trials, we found no difference in levels of pheromone-calling between protein-fed and protein-deprived, wild males (Shelly et al. 2001), and consequently it appears that the difference in mating frequency reported here does not simply reflect a diet-mediated difference in sexual signaling. This situation differs from other studies reporting increased calling and mating for mass-reared (Kaspi & Yuval 2000) and wild (Kaspi et al. 2000) male medflies fed protein as adults. Although diet-mediated variation in signaling activity may not be evident among wild males in Hawaii, the inclusion of protein in the adult diet may have resulted in qualitative differences in the composition, and hence attractiveness, of the male sex pheromone. In comparing female arrivals to artificially established leks in the field, we found that, while signaling activity did not vary noticeably with diet, approximately twice as many female sightings were made at leks composed of protein-fed males than at leks composed of protein-deprived males (Shelly et al. 2001).

In contrast to wild males, mating success was independent of adult diet among mass-reared males whether the competition involved mass-reared males only (experiment 2, although a mating advantage for protein-fed males was suggested when data were pooled over all replicates) or wild and mass-reared males (experiment 3). Although the results are preliminary, ongoing field-cage tests (T. E. S., unpublished data) similarly indicate that, relative to wild males, the inclusion of protein in the adult diet has no influence on the mating success of mass-reared males from a genetic sexing (temperature sensitive lethal, Franz & McInnis 1995) strain. Thus, contrary to other studies (Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi & Yuval 2000), our results do not support the notion that the addition of protein to the pre-release diet would, in general, enhance the mating competitiveness of mass-reared medfly males in SIT programs (but see below).

Male body weight had no obvious impact on the outcome of mating competition in any of the experiments. Among wild males, protein-fed and protein-deprived males were similar in body weight, yet protein-fed males enjoyed a significant mating advantage (experiment 1). Conversely, protein-fed, mass-reared males were heavier, on average, than protein-deprived, mass-reared males, yet there was no difference in mating frequency between them (experiment 2). When wild and mass-reared males competed directly, the protein-fed and protein-deprived, mass-reared males had similar mating success relative to wild males despite the fact that wild males were similar in weight to protein-fed, mass-reared males but significantly heavier than protein-deprived, mass-reared males (experiment 3).

The low level of mating observed in the experiment involving mass-reared males exclusively (experiment 2) suggests that mass-reared males were generally unacceptable to wild females independent of their diet. This interpretation, in turn, suggests that females of *C. capitata* select males largely on the basis of "absolute" criteria and not on a relative or "best-of-n" basis (Janetos 1980). That is, females may accept only males above a certain threshold level for a particular sexual signal(s) and reject other males. If true, *C. capitata* females are not choosing mates on the basis of between-male comparisons made over a sampling interval, and consequently the relative abundance of males of varying quality does not affect female choice. This, in turn, implies that mass-rearing facilities for SIT should concentrate, not simply on increasing production of males, but on maintaining sexual competitiveness of the males as well (Calkins 1984).

The frequency of female remating varied independently of male diet regardless of whether the initial mate was a wild or mass-reared male. This finding differs from that of Blay and Yuval (1997), who, in their study of mass-reared flies, found that females that first mated with a protein-fed male were less likely to remate (on the day following the initial mating) than females that first mated with a protein-deprived male. In that study, protein-fed males were significantly heavier than protein-deprived males, a difference that may have affected renewal of female receptivity (Blay & Yuval 1997; see also Bloem et al. 1993a). Although diet-related differences in male weight were detected in our study, the incidence of female remating was similar following initial mating to males of different sizes (e.g., protein-fed and protein-deprived, mass-reared males).

We also noted that, when data were combined over male diet types, wild females initially mated to wild or mass-reared males remated at approximately the same frequency. To our knowledge, no prior studies have drawn this comparison despite its potential importance for SIT programs. Several studies, however, have examined the effect of irradation per se on female remating, and the results obtained are contrary to our finding. Working exclusively with laboratory flies, Katiyar and Ramirez (1970) and Bloem et al. (1993b) both found that females first mated to irradiated males were more likely to remate than females whose initial mate was non-irradiated.

Within wild and mass-reared strains of the medfly, male diet had no apparent effect on copulation duration. Taylor et al. (2000) likewise found no effect of male diet on copulation duration for matings involving flies from a wild population in Israel. However, male diet has been found to affect copulation duration in matings involving mass-reared flies (Taylor & Yuval 1999; Field & Yuval 1999), with copulations involving protein-fed males being shorter than those involving protein-deprived males. Independent of male diet, we also found no difference in copulation duration between matings (with wild females) involving wild or mass-reared males (see also Orozco and Lopez 1993). Using laboratory flies exclusively, several studies have examined the influence of irradiation on males on copulation duration with inconsistent results: Katiyar and Ramirez (1973) found no effect of irradiation, whereas Seo et al. (1990) reported that copulations involving irradiated males (and non-irradiated females) were shorter than those involving non-irradiated males.

Data from the present study contribute further evidence for global variation in copulation duration in the medfly. For example, copulation duration is similar between wild flies from Hawaii ($\bar{x}$ = 143 min) and Israel (median = 145 min; Taylor et al. 2000) but is appreciably longer for wild flies in Argentina ($\bar{x}$ = 172 min; Cayol et al. 1999). Conversely, copulation duration between mass-reared males and wild females is similar between Hawaii ($\bar{x}$ = 135 min) and Argentina ($\bar{x}$ = 134 min calculated using from Table 1 in Cayol et al. 1999) but is much longer for mass-reared flies in Israel (median = 160 - 180 min; Field et al. 1999; Taylor et al. 2001).

We also failed detect an effect of diet on the longevity of mass-reared males. In contrast, Kaspi and Yuval (2000) found that, after 4 d of feeding, protein-fed males were less likely to survive a 24 h period of starvation (on day 5) than protein-deprived males. Following the same protocol, we found that the number of males surviving the starvation period was nearly identical between protein-fed and protein-deprived males. Survivorship of protein-deprived males was similar between the two studies (approximately 10%), but protein-fed males had much higher mortality in Kaspi and Yuval's (2000) study than in our experiment (approximate mortality after 1-d starvation: 50% versus 10%, respectively). Protein hydrolysate comprised 25% of the protein-containing diet in our study but only 9% in Kaspi and Yuval (2000), but whether this difference was responsible for the differential mortality is unknown.

In conclusion, the finding that dietary protein affected mating frequency in wild males indicates an important role for adult nutrition in mating competition in the Mediterranean fruit fly, a conclusion consistent with other studies (Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi & Yuval 2000; Shelly et al. 2001). The absence of the same effect in Hawaiian mass-reared males might indicate inter-strain differences in the behavioral and physiological responses of males to dietary composition. However, it appears more likely that it derived from the low overall quality of the Hawaiian mass-reared strain tested, which essentially 'overwhelmed' any positive effect resulting from the inclusion of dietary protein. This, in turn, suggests that the potential benefits to SIT of including protein in the pre-release, adult diet will vary with the quality of the mass-reared strain, being greatest for males that compete relatively well against wild males independently of diet composition.

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