MATING PROPENSITIES FROM DIFFERENT RATIOS OF MALE AND FEMALE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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The sterile insect technique (SIT) is used as a preventative measure against the establishment of wild populations of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in California and Florida. It involves the mass-release of sterile flies that mate with wild flies, when present, resulting in nonviable progeny. The replacement of bisexual strains with male-only, genetic sexing strains [e.g., strains carrying a temperature sensitive lethal (*tsl*) mutation] has occurred, or is anticipated, in many sterile release programs, because of their increased effectiveness in suppressing pest populations (Rendon et al. 2000). The release of far fewer females (<1%) than males results in a male-biased sex ratio that could change field mating dynamics. Medflies exhibit a lek mating system, in which males congregate to attract potential mates, and female choice determines male success (Emlen & Oring 1977; Prokopy & Hendrichs 1979; Whittier & Kaneshiro 1995; Eberhard 2000). In a field study in Hawaii, Shelly et al. (1994) found an average of 4 wild males/lek. Our research examined different ratios of male and female flies to determine their effects on the amount of time until, and probability of, mating pair formation.

Male Mediterranean fruit flies used in mating tests were from the Vienna-7/Tol-99 (males-only, *tsl* genetic sexing) strain and were obtained as puparia from the California Department of Food and Agriculture laboratory in Waimanalo, HI. Flies were irradiated 2 d before eclosion at 145 Gy, at the USDA laboratory in Waimanalo. Males were provided with water and sugar. Female flies used in mating tests were obtained from T. Shelly's laboratory (USDA-APHIS, Manoa, HI), and were from a stock of flies in the 6th generation from wild flies collected as larvae from Jerusalem cherry (*Solanum pseudocapsicum* L.). Virgin females, isolated from males less than 24 h after eclosion, were provided water and fed honey, sugar, and protein hydrolysate until they were sexually mature.

Male flies used in mating tests were 6-9 d-old and females were 7-14 d-old, both sexually mature. Tests occurred between 0900 and 1200 in transparent, upside-down, plastic containers (266 ml) that were isolated from each other using paper dividers (18 cm × 25 cm). A total of 80 replicates were completed (March 2002), with one replicate consisting of 5 containers each with a different combination of males (m) and females (f): 1m, 1f; 1m, 2f; 1m, 3f; 2m, 1f; and 3m, 1f. Males were aspirated into containers 10-15 min prior to females. Containers were checked every 3-5 min for mating pairs and when found, the time from female introduction was recorded. Observations were made until 90 min after female introduction.

Logistic regression was used to analyze the proportion of males and females mating and the amount of time prior to mating pair formation was analyzed using an ANOVA with means separated by Fisher's LSD test (SAS Institute 1999).

There were significant fly sex effects on the proportion of flies mating (Likelihood ratio 19.97; df 2; χ^2(prob) < 0.001). Female ratio effects were significant (χ^2 = 17.54, df 1, χ^2(prob) < 0.001), while male ratio effects were not (χ^2 = 3.06, df 1, χ^2(prob) < 0.08). Equal sex ratios resulted in the lowest number of mating pairs, while higher ratios of females to males resulted in a larger proportion of flies mating than comparable ratios of males to females (Fig. 1). There were no overall ratio effects on the time to form a mating pair (F = 2.14; df 4, 273; P = 0.076), but data suggest that skewed ratios (1:2, 1:3, 2:1, and 3:1) resulted in mating pairs forming earlier than for a 1:1 ratio (Fig. 2).

The sequence of male behaviors leading up to copulation are 1) pheromone calling by males, 2) orientation to the female (after the female has arrived), 3) continuous wing vibration, and 4) intermittent wing buzzing/head rocking (Eberhard 2000). Routine inspection of containers indicated that almost all males exhibited precopulatory be-

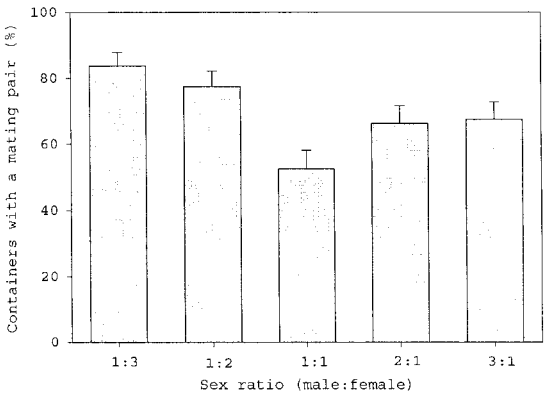

Fig. 1. Frequency of Mating Pair Formation. Percent of containers (mean ± SE) with a mating pair for different sex ratios of Vienna-7 males and 6th generation wild females. (Likelihood ratio 19.97, df 2, χ^2(prob) < 0.001; female ratio effects: χ^2 = 17.54, df 1, χ^2(prob) < 0.001; and male ratio effects: χ^2 = 3.06, df 1, χ^2(prob) < 0.08).

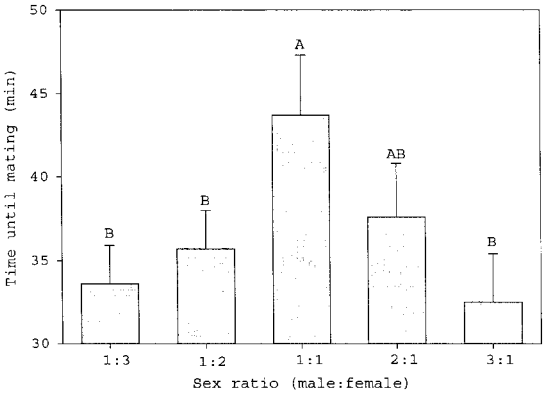

Fig. 2. Time Until Mating Pair Formation (min). Time to form a mating pair (mean ± SE) for different sex ratios of Vienna-7 males and 6th generation wild females. (ANOVA $F = 2.14$; df 4, 273; $P = 0.076$). Means were separated using Fisher's LSD test. Containers with no mating pairs were excluded from the analysis.

havior (i.e., steps 1-4 above). Interpretation of results is in reference to females, because Mediterranean fruit flies use a lek mating system, where females control choice of a mate (Arita & Kaneshiro 1985). When one female was in a container with two or three males, females could assess mate potential by discriminating among several males. In containers with one female and one male, females were less likely, and took longer to mate. This suggests that mate selection by females was slowed and inhibited because of an absence of rival males that could be compared. When two or three females were in a container with only one male, females were likely less selective because of competition with other females, and mated sooner. An alternate explanation is that female acceptance thresholds were constant but variable among individuals and the likelihood of including a female with relatively low acceptance criteria increased with an increasing number of females.

These mating trials were unusual in that they allowed evaluation of precise sex ratios, because in each treatment, one sex was limited to a single individual (or both sexes in the case of the 1:1 ratio). This resulted in a constant sex ratio for each container. In typical experiments with unequal sex ratios, the operational sex ratio changes as mating pairs form (i.e., without fly replacement to keep the sex ratio constant). In addition, sex ratios can vary locally within a field cage. Shortcomings of our study that make it of less direct value to field-based SIT programs include (1) experiments were performed in the laboratory and (2) because of the experimental design, different fly densities were present with different treatments.

Although male-biased ratios should make females more selective for mates, within the protocols of current SIT release programs, females would have a large supply of sterile males from which to choose.

Summary

Increasing the total number of flies in a laboratory container to form skewed ratios of high males or females, resulted in a higher proportion of mating pairs and their formation earlier. There were significant female ratio effects on the proportion of flies forming mating pairs. Male ratio effects were not statistically significant but suggested a trend similar to female effects.

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