Feeding on Papaya Flowers Enhances Mating Competitiveness of Male Oriental Fruit Flies, *Bactrocera dorsalis* (Diptera: Tephritidae)

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Abstract: Males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), are attracted to and feed on methyl eugenol. The goal of the present study was to determine whether feeding on a methyl eugenol-bearing plant, papaya (*Carica papaya* L.) would result in a mating advantage for *B. dorsalis* males. Mating frequencies of males given access to flowers (treated) and flower-deprived males (control) were compared in trials conducted 2 and 7 d after treated males were exposed to the flowers. For both intervals, treated males accounted for a significantly larger number of matings than control males. A second experiment compared female attraction to control and treated males. When at a lek, males display vigorous wing-fanning behavior, presumably to increase dispersal of the sex pheromone. Floral feeding resulted in a significant increase in wing-fanning activity but did not appear to affect the attractiveness of the pheromonal signal per se. A field experiment revealed that male captures in methyl eugenol-baited traps were not reduced by prior feeding on papaya flowers.

Males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), are strongly attracted to methyl eugenol (ME hereafter), a compound found in the leaves, flowers, or fruits of plants in over 10 families (Fletcher 1987, Metcalf 1990). Male attraction is so powerful that ME, when mixed with an insecticide, constitutes an effective suppression tool via 'male annihilation'(Cunningham 1989). This method was, in fact, used successfully to eradicate *B. dorsalis* from entire islands in the western Pacific (Steiner et al. 1965, 1970).

Despite the frequent use of ME in control programs, little attention has been given to the biological function underlying male attraction to this chemical. However, there is increasing evidence that ME has an important role in sexual communication and mating competition. Nishida et al. (1988) demonstrated that *B. dorsalis* males fed pure ME concentrated metabolites of this compound in their rectal glands (the presumed site of sex pheromone production), whereas ME-deprived males did not. Tan and Nishida (1996) confirmed this result and also noted (as had Shelly and Dewire 1994) that ME-fed males had a mating advantage over control (unfed) males (see Hee and Tan 1998 for similar results with *B. papayae* Drew and Hancock). Importantly, enhanced mating success was also noted when *B. dorsalis* males were provided with flowers of *Fagraea berteriana* A. Gray (Loganiaceae; Nishida et al. 1997) and *Cassia fistula* L. (Fabaceae; Shelly 2000a) both natural sources of ME.

While these latter findings suggest a link between ME ingestion and sexual selection in *B. dorsalis*, investigations of additional ME-containing plants are needed to assess more rigorously the strength and generality of this association. The chief goal of the present study therefore was to examine the effects of feeding on the mating success of oriental fruit flies for another ME-containing plant, papaya, *Carica papaya* L. (Howlett 1915), from a third plant family (Caricaceae). Two experiments were conducted in addition to the mating trials. First, female arrivals to fed and unfed males were compared to determine whether plant

feeding affected male attractiveness via possible changes in the quantity or quality of the pheromonal signals produced. Earlier work has yielded inconsistent findings: *B. dorsalis* males that fed on pure ME showed increases in both signaling activity and signal attractiveness (Shelly and Dewire 1994), whereas males that fed on ME-containing flowers showed an increase in signal attractiveness only (Shelly 2000a). Second, a trapping experiment was conducted to determine whether feeding on papaya reduced the capture probability in ME-baited traps. Previous work (Shelly 1994) showed that *B. dorsalis* males that fed on pure ME were less likely to be trapped than unfed control males, suggesting limits on the efficacy of the male annihilation technique in areas with abundant natural sources of ME. However, no reduction in capture probability was detected for males given access to ME-containing flowers of *F. berteriana* or *C. fistula* (Shelly 2000b). The present experiment was undertaken to confirm an unchanged capture probability following male exposure to another ME-containing plant species.

Materials and Methods

Mating behavior of *B. dorsalis*. The following summary derives from observations made of wild flies in the laboratory (Fletcher 1987) or in the field (Shelly and Kaneshiro 1991). *Bactrocera dorsalis* appears to display a lek mating system. Males aggregate on the foliage of host trees approximately 1 h before dusk and defend individual leaves as mating territories. While perching, males engage in vigorous wing fanning (also termed pheromone calling), an activity that both produces an audible buzz and disperses a pheromone attractive to females. Upon detecting a female in their territory, males immediately cease wing fanning and jump on the female. The female then either decamps or copulation ensues. Mating couples remain paired throughout the night and separate at sunrise.

Mating frequency. Flies used in the mating trials were from a laboratory stock started with 200–300 adults reared from field-collected mangoes. The colony was housed in a large screen cage with superabundant food (a mixture of honey and protein hydrolysate) and water. Ripe papayas were provided periodically for oviposition. Room temperature was maintained at 20–22 °C and 65–75% RH; under these conditions, generation time was about 6 weeks. The experiments were performed when the colony was 4 - 6 months old, and correspondingly the flies used were 3–4 generations removed from the wild. Adults used in the trials were separated within 7 d of eclosion, well before reaching sexual maturity at 15–20 d of age (Foote and Carey 1987).

Mating trials were performed in the same manner for all experiments. Eight treated males given prior exposure to flowers of *C. papaya* (as described below) and 8 control males having no exposure to flowers were placed with 8 females into transparent plexiglass cages (30 by 30 by 40 cm) at least 2 h before sunset. The males were marked earlier by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no detectable adverse effects, and males resumed normal activities within minutes of handling. The cages were kept adjacent to a large, west-facing window, and room lights were extinguished at least 2 h before sunset. Identities of mating males were recorded 3 - 4 h after sunset under dim light. Ten cages were observed on a given day; data collected on a particular day constituted a replicate. All flies were used for a single replicate only.

Males were exposed to *C. papaya* using the following protocol. Flowers were collected at the University of Hawaii Agricultural Experiment Station, Waimanalo, and transported immediately to the laboratory. Fifty flowers were placed into each of 2 screen cages (30 cm cubes) between 0900–1000 hrs (about 1 h after collection). Fifty males were then introduced to each cage (along with food and water) and left until 1600 hrs when the flowers were removed. Flower-containing cages were isolated to prevent inadvertent exposure of

floral volatiles to control males. Although systematic observations were not made, feeding was observed throughout the day.

Mating trials were conducted 2 or 7 d following floral exposure to the treated males to examine time-dependent variation in potential behavioral effects. In a given trial, the control and treated males were approximately the same age; treated males were given flowers when 25 - 35 d old. Females in all trials were 27 - 36 d of age. To determine whether exposure to floral volatiles alone would influence mating success, an experiment was performed in which treated males were given flowers covered with screen mesh that prevented direct contact. Mating tests for this experiment were conducted 2 d after exposure only.

Mate attraction. Because males from our laboratory stock were reluctant to wing-fan in the mini-cages (see below), the flies used in this experiment were from a colony maintained by the U.S. Pacific Basin Agricultural Research Center (USDA-ARS), Honolulu, for > 150 generations. Pupae were obtained 2 d before eclosion, and adults were separated by sex within 3 d of emergence (sexual maturity in this stock is attained about 10 d of age; Foote and Carey 1987). The flies were maintained in the same manner described above.

Tests of male attractiveness were conducted on groups of 8 males (4 treated, 4 control) in a large screen cage (1.2 by 0.6 by 0.6 m) that contained three potted plants (*Ficus* sp.). Treated males were exposed to flowers in the manner described above when 12–20 d old and were used 2 d after exposure. In a given trial, control and treated males were the same age. Approximately 3 h before sunset, males were placed singly in mini-cages, which, in turn, were placed at specific locations on the plants. The cylindrical mini-cages (6 cm long by 3 cm diameter) were made of wire screening and were suspended from branches with a wire hook. The mean nearest-neighbor distance between mini-cages was 19 cm (range 15 - 23 cm). Mini-cages were placed at the same locations for each trial, but the type of male (control or treated) at a given location was assigned randomly at the start of a trial. Immediately after the males were in place, 35 females (12 - 20 d old) were released into the cage. The room lights were extinguished, and the large cage, which was near a west-facing window, received only natural light.

Starting 1.5 h before sunset, males were observed continuously for the start of wing fanning by any male. At its onset, males were checked at 1-min intervals for the presence/absence of wing fanning and for the number of females resting on the individual mini-cages. Wing-fanning level was considered to be a direct indicator of pheromone production, a legitimate assumption because pheromone emission occurs only during wing fanning (Ohinata et al. 1982). Data on female visitation reflected numbers of female sightings alone; because females were not marked, neither the number of different females arriving nor the duration of individual visits was known. Observations were made over 5 d (n = 20 males for both control and treated groups) for observation periods of 30 - 45 min (interval between first wing-fanning and sunset).

Capture probablility. Males used in this experiment were from the USDA-ARS colony and were maintained in the manner described above. Treated males were given access to C. papaya flowers following the protocol described earlier. Males were exposed to flowers at ages of 11 - 20 d and were released 2 d later. In a given trial, control males were the same age as treated males upon release. Control and released males were marked with different colors using the protocol noted above.

For a given trial, groups of 100 treated and 100 control males were released between 1100–1200 hrs in an area of the agricultural experiment station that contained mango (*Mangifera indica* L.), orange (*Citrus sinensis* L.), and guava (*Psidium guajava* L.) trees. Males were marked prior to release using the protocol described above (in the Mating Frequency section). Eight Steiner traps were placed singly in trees in a circle (50 m radius) around a central release point. Traps contained a cotton wick to which ME (1 ml) had been

applied and a killing agent (a 2 cm long strip of dog flea collar containing naled). Traps were checked 24 h after release, and flies were examined individually in the laboratory for marks.

Statistical analyses. The Mann-Whitney test (test statistic T) was used to compare the numbers of matings achieved by control and treated males. This test does not test explicitly 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. Data on trap catches were analyzed in the same manner. In the mate attraction experiment, the proportion of the total observation time spent wing-fanning, the total number of female sightings, and the number of female sightings per 10 min of wing-fanning were computed for individual males, and these values were used in Mann-Whitney tests to compare control vs. treated males. All statistical procedures follow Zar (1996).

Results

Mating frequency. In trials conducted 2 d after exposure, treated males obtained an average of 20.1 matings (range: 9 - 33) per replicate compared to only 10.3 matings (range: 4 - 17) for control males ($T = 41.0$; $n_1 = n_2 = 8$; $P < 0.01$). Treated males obtained 66% (161/244) of the total matings observed over all replicates ($Z = 5.2$; $P < 0.001$).

In trials conducted 7 d after exposure, treated males obtained an average of 24.7 matings (range: 18 - 35) per replicate compared to 11.0 (range: 6 - 18) for control males ($T = 36.5$; $n_1 = n_2 = 8$; $P < 0.001$). Treated males obtained 69% (198/286) of the total matings observed over all replicates ($Z = 5.8$; $P < 0.001$).

Exposure to volatiles had no apparent effect on male mating success. Males that were given screen-covered (and hence inaccessible) flowers obtained, on average, 18.7 matings (range: 12 - 21) compared to 15.6 (range: 8 - 33) for control males ($T = 81.5$; $n_1 = n_2 = 8$; $P > 0.05$). Treated males achieved 55% (150/275) of the total matings observed over all replicates, a proportion not significantly different from that expected by chance ($Z = 1.6$, $P > 0.05$).

Mate attraction. Floral feeding had a positive effect on male wing-fanning activity. On average, treated males were fanning for 33% (range: 0– 89%) of the observation period compared to 10% (0–41%) for the control males ($T = 494.5$; $n_1 = n_2 = 20$; $P < 0.05$). Consistent with this difference, more female sightings were recorded, on average, for treated ($x = 9.3$; range: 0–52) than control ($x = 1.3$; range: 0–6) males ($T = 499.5$; $n_1 = n_2 = 20$; $P < 0.05$). Importantly, female visitation differed significantly between control and treated males even when the difference in male activity was taken into account, i.e., when female sightings were compared relative to the amount of time males spent wing-fanning. The average number of female sightings per 10 min of wing fanning was 6.5 (range: 0–20.8) for treated males but only 2.7 (range: 0–6.7) for treated males ($T = 88.0$; $n_1 = 14$, $n_2 = 10$; $P < 0.05$).

Although this latter comparison was made relative to signaling effort, the difference in activity levels between control and treated males nonetheless potentially confounds its interpretation, because treated males frequently wing-fanned in the absence of concurrent wing-fanning by control males while the reverse was not true. Thus, the difference in female sightings per unit time spent wing fanning could have reflected the fact that, during much of the time that treated males were wing-fanning, females had no choice between male types. To examine this possibility, I identified periods exceeding 5 min during which at least 1 male of both control and treated groups were wing-fanning concurrently and compared female visitation during these periods. Over 5 periods (lasting 7–9 min each; 39 min total), the average number of female sightings was 4.0 for treated and only 1.3 for

control males ($T = 31.0$; $n_1 = 10$ treated, $n_2 = 6$ control; $P < 0.05$).

Capture probability. Prior exposure to papaya flowers had no apparent effect on capture probability in ME-baited traps. On average, 19.9 treated (range: 12 - 37) and 21.9 control (range: 12–42) males were captured per replicate ($T = 95.0$; $n_1 = n_2 = 9$; $P > 0.05$). Over all replicates, the proportions of male types captured did not differ significantly from that expected by chance ($Z = 0.9$; $P > 0.05$).

Discussion

The study reported here provides evidence, from a third plant family, that feeding on a natural source of methyl eugenol increases the mating success of male oriental fruit flies. This advantage was evident as long as 7 d after feeding on the papaya flowers. Based on the mate attraction experiments, enhanced mating success apparently reflected both greater signaling activity and production of a more attractive signal by flower-fed males. Pheromonal signals of *B. dorsalis* fed pure methyl eugenol have been shown to be more attractive to females than those of control males (Shelly and Dewire 1994; see also Hee and Tan 1998 for data on *B. papayae*), and the present study (along with Shelly 2000a) provides further evidence of increased signal attractiveness via feeding on a natural source of ME. As shown for methyl eugenol-fed males, flower-fed males presumably ingested the lure and incorporated methyl eugenol derivatives into their sex pheromone, although direct proof of this biochemical pathway is not yet available. Ingestion of pheromonal precursors from natural sources has been demonstrated for adult males of various Lepidoptera (e.g., Krasnoff and Dussourd 1989) and Coleoptera (e.g., Byers 1982).

While females mated preferentially with floral-fed males, the adaptive basis of this preference remains unknown. Mating with methyl eugenol-fed males does not appear to increase female fecundity: egg production during an 8 week period immediately following mating did not differ significantly between females mated to treated (methyl eugenol-fed) males or control (methyl eugenol-deprived) males (Shelly 2000c). Likewise, whether or not a male fed previously on methyl eugenol had no significant effect on the proportion of eggs hatching or post-mating survival of females (Shelly 2000c). In these experiments, males were fed pure methyl eugenol, and it is possible that the combination of methyl eugenol and some other co-occurring compounds may act to increase female fecundity. Experiments using natural sources of methyl eugenol are required to evaluate this possibility.

Alternatively, the presence of methyl eugenol-derived pheromonal components in the male signal may indicate a superior ability to locate natural sources of methyl eugenol in the wild. As such, by selecting males whose pheromones contain methyl eugenol metabolites, females may increase the odds that their sons will have high ability to locate methyl eugenol sources and hence enjoy high mating success. This scenario depicts a case of runaway selection, whereby female choice provides indirect benefits via a trait that confers an advantage to her sons in sexual competition but is arbitrary with respect to offspring viability (Andersson 1994). This explanation still begs the question as to the origin of the female preference. One interesting possibility is that females prefer male pheromone containing metabolites of methyl eugenol because it triggers a strong, pre-existing sensory bias that evolved in a different context (e.g., food searching). Pheromones of other male tephritids have, in fact, been shown to contain certain compounds that mimic food and host odors (Baker et al. 1990, Robacker and Warfield 1993). Thus, methyl eugenol-bearing pheromone may represent a 'sensory trap' (West-Eberhard 1984) or a case of 'sensory exploitation' (Ryan 1990).

In conclusion, several factors, the strong attraction of males to methyl eugenol, the pronounced mating advantage of methyl eugenol-fed males, and the long duration of this ad-

vantage, indicate collectively that methyl eugenol-containing plants have an important influence on the natural mating system of *B. dorsalis*. Aside from anecdotal observations on the diurnal variation in male abundance on ME-bearing flowers (Shelly 2000a), there are no field data on the frequency or duration of male visits to natural ME sources or on the consequences of this feeding on male mating success. However, limited field data (Shelly and Kaneshiro 1991, Stark 1995, Shelly 2000a) suggest that mating aggregations of *B. dorsalis* do not occur on ME-bearing plants and consequently that leks and ME-bearing plants are located independently of one another.

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