# Outcrossing and the Mating Competitiveness of Male Mediterranean Fruit Flies (Diptera: Tephritidae): Results from the World's Oldest Mass-Reared Strain

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**Abstract:** The Hawaiian HI-LAB strain is the oldest mass-reared strain of Mediterranean fruit fly, *Ceratitis capitata* (Wied.), in the world, and recent laboratory and field data show that HI-LAB males perform poorly in mating competition against wild males. The purpose of this study was to determine whether the mating performance of HI-LAB males could be improved by a single outcrossing event with wild flies (HI-LAB females X wild males). Using field-caged host trees, I monitored male mating success with wild females and found that (1) HI-LAB males had low mating success relative to wild males, (2) $F_1$ hybrid males mated with equal frequency as wild males, and (3) $F_{10}$ hybrid males had a reduced mating success similar to that recorded for the HI-LAB males. The implications of the present findings for mass-rearing strategies are discussed.

**Key words:** *Ceratitis capitata*, sterile insect technique, mass rearing, male mating success

## Introduction

The sterile insect technique (SIT) involves the release of a large number of mass-produced, irradiated (sterile) males to achieve matings with wild females, resulting in the production of infertile eggs and the subsequent decline of the target population. As an autocidal, environmentally benign method, SIT is an increasingly important management tool against tephritid fruit fly pests, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (Hendrichs et al. 1995). Despite increased usage, it is widely recognized that SIT has an inherent problem that may often limit its effectiveness, i.e., mass-rearing procedures impose intense artificial selection that alters male courtship behavior, thus greatly reducing the acceptability of mass-reared males to wild females (Rossler 1975; Shelly et al. 1994; Hendrichs et al. 1996; Lance et al. 2000). Calkins (1984), for example, suggested that the dense crowding characteristic of mass-rearing conditions may select for shortened courtship (prior to mounting) to reduce interruptions by other males, a trend subsequently documented for a mass-reared strain in Costa Rica (Briceno and Eberhard 1998).

A change in male courtship behavior has also been documented for the Hawaiian HI-LAB strain, the oldest mass-reared strain of *C. capitata* in the world (see below). Liimatainen et al. (1997) identified differences in the courtship behavior of wild and HI-LAB males using a transitional analysis of behavioral elements. For example, compared to wild males, HI-LAB males were more likely to initiate wing vibration prior to female approach (i.e., prior to directed female response to the male), a behavior that lessened the probability of continued courtship. Liimatainen et al. (1997) also reported low mating success of HI-LAB males with wild females in the laboratory, a result consistent with previous field studies (Shelly et al. 1994; Shelly and Whittier 1996) in which HI-LAB males released in natural populations achieved significantly fewer copulations than expected based on their abundance in the study area. In these field tests, HI-LAB males located and joined natural leks, displayed the same diurnal activity pattern as wild males, and signaled as frequently as wild

males. Additional studies further showed that HI-LAB and wild males were equally attractive to females over long distances (5-10 m; Shelly 1999) and equally successful in aggressive encounters (Shelly 2000). Collectively, these observations strongly support the conclusion that the low competitiveness of the HI-LAB males did not reflect failure to participate in lek formation or engage in sexual advertisement but rather derived from altered courtship behavior favored under mass-rearing conditions.

One possible way to minimize behavioral changes in mass-reared strains of *C. capitata* is to perform periodic outcrosses with wild flies. Although the potential value of such genetic "refreshment" has been posited (Boller 1972; Nunney 1995), I am unaware of any published data that explicitly demonstrate its benefits (but see below). The purpose of the present study was to determine whether the mating success of males from the HI-LAB strain could be improved through a single outcrossing event with wild flies. The protocol followed was to first compare the mating frequencies of HI-LAB and wild males in competition for wild females, mate HI-LAB females with wild males to start two replicate hybrid lines, and make comparisons of mating frequencies using hybrid males from the 1st and 10th generations after outcrossing. Tests using the 1st generation evaluated changes in mating competitiveness that resulted directly from outcrossing, while tests using the 10th generation evaluated changes that occurred within the hybrid lines themselves. The implications of the present findings for mass-rearing strategies are discussed.

## Materials and Methods

**Pre-Hybridization Mating Tests.** In the initial mating trials, males from the world's oldest, mass-reared strain (HI-LAB) competed against wild males for matings with wild females. The HI-LAB strain was established in 1956 with flies reared from fruits collected in Kula, Maui (D. McInnis, personal communication), and maintained using standard rearing procedures (Tanaka et al. 1969) by the USDA-APHIS Hawaii Fruit Fly Rearing Facility until 1996 and the fly rearing facility of the California Department of Food and Agriculture until 1999. [The strain is currently being maintained in low numbers (several thousand per generation) by the USDA-ARS Tropical Fruit, Vegetable, and Ornamental Crop Research Laboratory, Honolulu (D. McInnis, personal communication).] Non-irradiated pupae were obtained 2 d prior to eclosion, and adults were separated within 24 h of eclosion, before reaching sexual maturity at 2–3 d of age. Wild flies were reared from fruits of the Jerusalem Cherry (*Solanum pseudocapsicum* (L.)) collected in Hawaii Volcanoes National Park. Field-collected fruits were placed over vermiculite, and larval development proceeded *in situ*. Pupation occurred in the vermiculite, and adults were separated by sex within 48 h of eclosion, before reaching sexual maturity at 7–10 d of age. Adults of both laboratory and wild strains were held in plastic buckets (5 liter volume; 100-150 individuals per bucket) and given ample water and food (a mixture of sugar and protein hydrolysate). The laboratory was maintained at 22–25 °C with a 65–85% RH and received both artificial and natural light during a natural 12:12 (L:D) photoperiod.

Mating tests were conducted during April-May, 1999, at the Agricultural Experiment Station of the University of Hawaii, Waimanalo, Oahu. Groups of 100 HI-LAB males, 100 wild males, and 100 wild females were released between 0800-0830 hrs into field cages (3 m diameter, 2.5 m high) containing rooted guava trees (*Psidium guajava* L.). Males were marked 1 d prior to 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. Observers monitored the cages continuously for 4 h and collected mating pairs and identified the males. When tested, HI-LAB males were 6–8 d old, and wild flies 11–16 d old. Individual flies were used for only one trial.

**Hybridization.** Following the initial mating tests, two hybrid lines ($H_1$ and $H_2$, respec-

tively) were established by mating HI-LAB females with wild males. Wild males were derived from field-collected Jerusalem cherries as described above. I obtained 250 (unique pair) matings to start each hybrid line; outcrossing was conducted in June and July, 1999, for $H_1$ and $H_2$, respectively. Mated females were placed in screen cages (30 cm cubes) with ample food, water, and an artificial oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were collected 3 times/week and placed on standard larval diet (Tanaka et al. 1969). Adults were separated within 24 h of eclosion and maintained using the above procedures.

**Post-Hybridization Mating Tests.** Mating trials were conducted for the $H_1$ and $H_2$ lines in the manner described above using 100 hybrid males from the $F_1$ generation, 100 wild males, and 100 wild females. Wild flies were once again derived from field-collected Jerusalem cherries. When tested, hybrid males were 7–9 d old, and wild flies were 10–18 d old. Tests were run during July-September, 1999.

Following these trials, stocks of the two hybrid lines were maintained in the laboratory. For both lines, approximately 1000 adults per generation were housed in each of three screen cages (25 cm cubes), and adult maintenance, egg collection, and larval rearing followed the aforementioned procedures.

A second set of mating trials was conducted (following the same protocol) during June-July, 2000, using males from the $F_{10}$ generation of both hybrid lines. Unfortunately, owing to a severe drought, Jerusalem cherries were scarce at this time, and I was unable to collect large numbers of wild *C. capitata* from the original source population. As a result, the wild flies used in these mating trials were reared from a different host and locality (peaches, *Prunus persica* (L.), from Kula, Maui). When tested, hybrid males were 7–8 d old, and wild flies were 12–17 d old.

**Statistical Analysis.** The number of matings obtained by males of the different strains was compared over all replicates using a Mann-Whitney test (test statistic $T$). Because this test does not explicitly test for deviation from random mating (i.e., 50% of the matings by each male type), a binomial test (using the normal approximation with test statistic $Z$) was performed using data pooled over all cages. Relative mating success of HI-LAB and hybrid males was compared between experiments using the log-likelihood ratio (with Yates' correction for continuity; test statistic $G$). All statistical procedures follow Zar (1996).

## Results

In the pre-hybridization tests, wild males obtained, on average, 31.3 matings (range: 19–45) per replicate compared to only 14.6 (range: 5–31) for HI-LAB males ($T = 242.5$; n = 13 replicates; $P < 0.001$). Over all replicates, wild males achieved 68% (407/597) of the total matings ($Z = 9.5$; $P < 0.001$).

In the mating trials involving $F_1$ hybrid males, there was no significant difference in the mating success of wild and hybrid males for either hybrid strain. For $H_{1,}$ wild males achieved an average of 19.9 matings (range: 13–29) per replicate compared to 18.9 matings (range: 12–26) per replicate for hybrid males ($T = 87.0$; n = 9 replicates; $P > 0.05$). Over all replicates, wild males achieved 49% (170/349) of the total matings ($Z = 0.4$; $P > 0.05$). For $H_2$, wild males achieved an average of 17.8 matings (range: 15–23) per replicate compared to 19.1 matings (range: 11–30) per replicate for hybrid males ($T = 73.5$; n = 8 replicates; $P > 0.05$). Over all replicates, wild males achieved 48% (142/295) of the total matings ($Z = 0.6$; $P > 0.05$). For both hybrid lines, the proportion of total matings obtained by hybrid males was significantly greater than that observed for HI-LAB males in competition against wild males ($H_1$: $G = 34.8$; $H_2$: $G = 32.4$; df = 1 and $P < 0.001$ in both cases).

In mating trials involving $F_{10}$ hybrid males, wild males had a distinct competitive advantage over males from the $H_1$ line, but results were less clear-cut for the $H_2$ line. For $H_{1,}$ wild males

achieved an average of 27.9 matings (range: 20–39) per replicate compared to 13.2 matings (range: 9–19) per replicate for hybrid males ($T = 100.0$; n = 8 replicates; $P < 0.001$). Over all replicates, wild males achieved 68% (223/329) of the total matings ($Z = 6.9$; $P < 0.001$). For the $H_1$ line, the proportion of total matings obtained by the $F_{10}$ males was similar to that observed for HI-LAB males in the pre-hybridization tests ($G = 0.1$; df = 1; $P > 0.05$) and significantly lower than that observed for $F_1$ males ($G = 25.4$; df = 1; $P < 0.001$). For $H_2$, wild males achieved an average of 25.5 matings (range: 14–35) per replicate compared to 19.2 matings (range: 12–23) per replicate for hybrid males, a non-significant difference ($T = 79.5$; n = 8 replicates; $P > 0.05$). Over all replicates, wild males achieved 57% (204/358) of the total matings, a proportion that differed significantly from random ($Z = 2.6$; $P < 0.01$). For the $H_2$ line, the proportion of total matings obtained by the $F_{10}$ males was greater than that observed for HI-LAB males in the pre-hybridization tests ($G = 12.0$; df = 1; $P < 0.001$) but significantly lower than that observed for $F_1$ males ($G = 5.0$; df = 1; $P < 0.05$).

The results of all the mating trials are summarized in simple graphical manner in Fig. 1.

## Discussion

Several key findings emerge from this study. First, the present data confirm recent results (Shelly et al., 1994; Shelly and Whittier 1996) that demonstrated the poor mating performance of the HI-LAB males relative to wild males. Interestingly, and unlike other mass-reared strains of *C. capitata* (Rossler 1975; Cayol 2000), the HI-LAB strain apparently maintained a high level of male mating competitiveness for nearly 25 years after initiation. In field-cage tests, Wong et al. (1983) found that HI-LAB males competed equally with wild males, and thus the strain's deterioration appears to have occurred primarily within the past 15 years. More importantly, the improved mating performance of the $F_1$ hybrid males revealed that HI-LAB line's poor quality was not irreversible but, in fact, could be enhanced dramatically by a single outcrossing event. Although the factors responsible were not identified, improved mating success presumably reflected, in part at least, more natural or "wild-like" courtship that was more acceptable to females. The study also documented a substantial decline in male mating competitiveness of the hybrid lines over only 10 generations to the original level of the HI-LAB strain. This decline was more pronounced for $H_1$ than for $H_2$, where the mating success of $F_{10}$ hybrid males decreased to a level similar to that of HI-LAB males in the pre-hybridization tests. In $H_2$, $F_{10}$ hybrid males obtained a greater proportion of matings than HI-LAB males (pre-hybridization) but a smaller proportion than recorded for the $F_1$ hybrid males. Collectively, these observations suggest that, while outcrossing may greatly improve the mating success of mass-reared males, the rapid decline in male mating competitiveness typically observed under laboratory conditions requires that outcrossing, to be effective, be performed at frequent intervals (e.g., annually).

Data from the Moscamed Program in Mexico revealed that strain replacement (with wild flies) may yield slightly more competitive males than hybridization between wild and mass-reared males. In a series of mating trials, Liedo and his colleagues (P. Liedo, personal communication) found that males from a newly established strain obtained 36% of all matings (in competition against wild males for wild females) compared to 28% for males produced via a wild-laboratory strain hybridization. More striking than this difference, however, is the evidence for rapid decay in male competitiveness under mass-rearing conditions. Both the newly established and hybrid lines had been mass-reared for about a year prior to testing, and as noted above, males from both lines accounted for only about 1/3 of all matings. Aside from the potential difficulty of collecting large numbers of wild flies on a regular basis, outcrossing does not present any serious logistical problems for bisexual strains of *C. capitata*. As described by Hendrichs et al. (1995), however, mass-rearing facilities worldwide are shifting toward production of genetic sexing strains, particularly those based on a

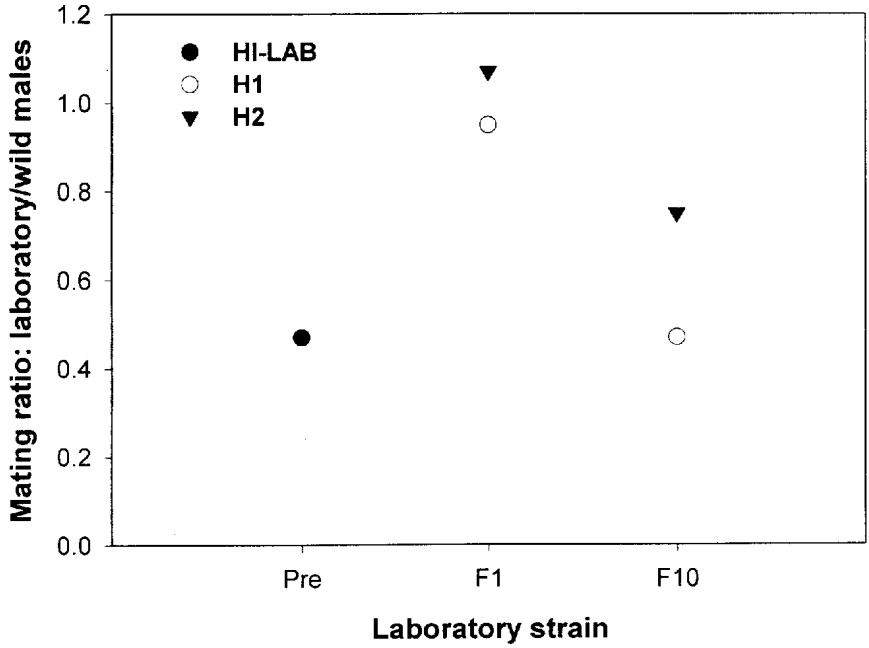


**Figure 1.** Relative mating success of laboratory males versus wild males. Abscissa represents the three times at which mating tests were performed (Pre = pre-hybridization or HI-LAB males; F1 = hybrid males from 1st generation following hybridization; F10 = hybrid males from 10th generation following hybridization). Values along the ordinate represent ratios of average matings per replicate between laboratory (i.e., HI-LAB or hybrid) and wild males, where a value of 1.0 represents equal male mating ability.

mutation inducing temperature sensitive lethality (*tsl*) of females in the egg stage. In *tsl* strains, dominant, wild-type alleles for temperature tolerance and pupal color (brown) are linked via genetic translocation to the Y chromosome, yielding males heterozygous for these two traits. Females, on the other hand, are homozygous for both *tsl* and white pupal color mutations (Franz and McInnis 1995). Incubating the eggs at an elevated temperature (33 $^0$C) is lethal to individuals displaying the mutant phenotype (females), and this difference in thermal sensitivity is used to separate the sexes.

Given this genetic basis, it is apparent that simple outcrossing of *tsl* strains with wild flies would lead to rapid destabilization of the sexing system. Nonetheless, as discussed by Franz et al. (1996), the genetic background of *tsl* strains can be changed via outcrossing separately for the *tsl* (and white pupal color) mutation in females and the Y-autosome translocation in males. Even following a single outcrossing event, however, a mating protocol involving inbreeding and single pair crosses of hybrid progeny is required over several generations to reconstruct the *tsl*-based, genetic sexing mechanism. The time and resources required to complete such a process are obviously substantial, and it may not be feasible to outcross *tsl* strains frequently enough to maintain a consistently high level of male mating competitiveness. Still, to the extent that temporary enhancement of male quality is achieved, outcrossing of *tsl* strains should be performed as frequently as possible.

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