Colonization of a Hybrid Strain to Restore Male *Anastrepha ludens* (Diptera: Tephritidae) Mating Competitiveness for Sterile Insect Technique Programs

**JUAN RULL** and **ABRAHAM BARREDA-LANDA**

Instituto de Ecología, A. C. Km. 2.5 Antigua Carretera a Coatepec, 19070 Xalapa, Veracruz, México

**ABSTRACT**

To restore male mating competitiveness of Mexican fruit flies, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), reared for sterile insect releases by the Mexican Fruit Fly Eradication Campaign, two strain replacement techniques were evaluated. Field cage male competitiveness tests revealed that laboratory males of the Metapa strain mated 3 times less often with wild females than field-collected wild males. A strain developed from the cross of wild males and laboratory females (hybrid strain) was similar to a strain developed from the cross of laboratory males and females (laboratory strain) in that its females produced similar amounts of eggs and the eggs displayed similar levels of hatch and egg-to-pupa transformation in artificial diet. By contrast, a strain developed from the cross of wild males and females (wild strain), forced into artificial rearing, experienced a series of bottlenecks involving reduced egg laying and extremely poor development in diet. The male F1 progeny of the hybrid strain and field-collected wild males outcompeted F1 laboratory males in field cage tests for matings with field-collected wild females. In conclusion, we found that strains developed from the cross of wild males and laboratory females allowed us to restore male mating competitiveness of F1 Mexican fruit flies without compromising mass-rearing production.

**KEY WORDS** *Anastrepha ludens*, sterile insect technique, strain replacement, mating competitiveness

The sterile insect technique (SIT) has been successfully applied to eradicate and suppress several insect species of economic importance in the world (Klassen and Curtis 2005). Examples include eradication of the screwworm, *Cochliomyia hominivorax* (Coquerel), from the southern United States to Panama (Vargas-Terán et al. 2005); Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), from northwestern Mexico (Reyes et al. 2000); melon fly, *Bactrocera cucurbitae* Coquillett, from Japan (Koyama et al. 2004); and the *Bactrocera tryoni* (Froggatt) from western Australia (Fisher 1996). Sterile insect technique application also has been gaining worldwide acceptance, with SIT programs under way in Argentina, Mexico, Guatemala, Canada, and the United States (De Longo et al. 2000, Reyes et al., 2000, Villaseñor et al. 2000, Bloem et al.2005). Despite success and increased application, there is still need for SIT improvement. SIT requires not only the ability to produce large quantities of insects at a reasonable cost but also the ability to mass produce insects displaying a behavioral repertoire allowing sterile males to compete with wild males to transfer sperm to wild females and induce sterility into the target population.

The production–quality dichotomy may result in evolution of mating isolation between laboratory males and wild females (McInnis et al. 1996). Program managers are reluctant to replace strains adapted to mass-rearing conditions, fearing reduced production from a nonlaboratory-adapted strain. Nevertheless, mass-rearing conditions, through artificial selection, affect sterile male mating performance and related traits (Moreno et al. 1991, Cayol 2000, Shelly 2001, Lux et al. 2002, Rull et al. 2005).

Replacing strains to restore male mating competitiveness by forcing wild individuals into mass rearing results in a succession of bottlenecks that affect the genetic composition of the new strain (Parker 2005). Colonization of wild strains also results in a drop of production levels, and it may influence the speed at which reproductive isolation evolves.

An areawide fruit fly eradication campaign was launched in Mexico in 1992 (Rull et al. 1996). The campaign allowed eradication of Mexican fruit fly, a pest of citrus and mangoes, from northwestern Mexico (Reyes et al. 2000), but the campaign is facing technical complications in more ecologically complex areas in the country. Recent evidence highlights the necessity to replace the Mexican fruit fly strain currently in use in Mexico (Rull et al. 2005, Meza and Díaz-Fleisher 2006). However, the need to expand...
areas under SIT and to incorporate new regions to releases of sterile males requires production of large quantities of flies.

Here, we evaluated two strain colonization techniques with the purpose of 1) restoring male mating competitiveness, 2) avoiding population bottlenecks and their potential impact on genetic variability, and 3) avoiding decreased production of Mexican fruit fly.

Materials and Methods

Biological Material. Wild Mexican fruit flies were obtained from naturally infested oranges, *Citrus aurantium* L., in the locality of Apazapan, Veracruz. Pupae were recovered following methods outlined in Aluja et al. (2000) and maintained at 27°C and 65% RH until adult emergence. Nonirradiated laboratory flies were shipped from the Moscafrut mass-rearing facility in Metapa, Chiapa, 2 d before adult emergence and kept under the same environmental conditions as the wild flies. At emergence, wild and laboratory adults were separated by sex and placed according to strain in 30- by 30- by 30-cm Plexiglas cages with free access to water and food (sugar and protein, 3:1).

Mating Compatibility Tests. Twenty-eight sexually mature laboratory males (10–18 d old) and 28 sexually mature field-collected wild males (15–23 d old) were marked on the back of the thorax with a dot of distinctive acrylic paint at age 14 d. Because we failed to obtain sufficient numbers out of laboratory crosses of wild individuals only laboratory × laboratory and wild × laboratory, and eggs were seeded into 1 kg of diet inside a plastic diet tray.

The F1 generation pupae and adults recovered from laboratory crosses as well as a new cohort of field-collected wild individuals were handled as indicated above. Adults of each strain were marked on the back of the thorax with a dot of distinctive acrylic paint at age 14 d. Because we failed to obtain sufficient numbers out of laboratory crosses of wild individuals only laboratory × laboratory and wild × lab males were used in mating performance tests.

Artificial Rearing Tests. Eight days after adult emergence (age at which laboratory females reach sexual maturity), 1) 50 wild males and 50 wild females, 2) 50 laboratory males and 50 laboratory females, and 3) 50 wild males and 50 laboratory females were placed in a 30- by 30- by 30-cm Plexiglas cage with free access to water and food with an artificial oviposition device placed on top of the cage. The artificial oviposition device consisted of a 10-cm-diameter plastic knitting ring fitted with a linen cloth lined with a fine silicone layer to stimulate oviposition. The device was placed on top of the cage with the cloth in contact with the cage mesh and filled with fucelore to prevent egg dehydration.

The total number of egg clutches laid by females in cages (female Mexican fruit fly lay eggs in distinct clusters) was counted, and clutches were removed from oviposition devices daily from day 8 to day 16. Each day, a 30-egg sample per replicate was arranged in a line on a piece black cloth, placed over a moist piece of cotton inside a petri dish, and incubated at 33°C for 4 d. At the end of the incubation period, eggs were observed under a dissecting microscope, and percentage of hatch was recorded. The number of eggs per clutch was counted for two clutches for every replicate every day.

Thirty eggs were seeded after the incubation period on a 2.5-cm layer of standard *A. ludens* diet in a 0.25-liter plastic cup. Plastic cups were then incubated at 33°C and 75% RH for 10 d. At the end of the larval period, pupae were recovered from the diet, and percentage of egg-to-pupa transformation was calculated. The experiment was replicated 10 times.

Field Cage Tests of F1 Mating Performance. To recover adults for field cage tests of mating performance, all remaining eggs recovered from Plexiglas cages were separated by treatment (laboratory × laboratory, wild × wild, and wild × laboratory), and eggs were seeded into 1 kg of diet inside a plastic diet tray.

The F1 generation pupae and adults recovered from laboratory crosses as well as a new cohort of field-collected wild individuals were handled as indicated above. Adults of each strain were marked on the back of the thorax with a dot of distinctive acrylic paint at age 14 d. Because we failed to obtain sufficient numbers out of laboratory crosses of wild individuals only laboratory × laboratory and wild × lab males were used in mating performance tests.

Results

Mating Compatibility Tests. A Wilcoxon matched pair test revealed that wild males mated significantly more often with wild females than laboratory males (*Z* = 2.20, *P* = 0.02; *n* = 6). Wild males copulated on average 15.33 ± 4.03 times per replicate, whereas laboratory males only did so on 5.50 ± 1.97 occasions (Fig. 1A).

A Wilcoxon matched pair test failed to detect a significant difference in average duration of copulations between wild males and laboratory males (*Z* = 1.88, *P* = 0.059; *n* = 6). Laboratory males copulated for slightly longer periods (63.66 ± 16.53 min) than wild males (53.16 ± 9.41 min) (Fig. 1B).

Artificial Rearing Tests. A Kruskal–Wallis test followed by multiple comparisons of mean ranks revealed significant differences among strains in the number of clutches deposited daily in oviposition devices (*H* = 120 = 6.49, *P* < 0.00001). Wild males crossed with wild females laid significantly fewer clutches (15.72 ± 10.73) than wild males crossed with laboratory females (74.25 ± 10.73).
and laboratory males crossed with laboratory females (74.47 ± 36.01) (Fig. 2A).

There were also significant differences among strains in the number of eggs per clutch laid by females (H2,120 = 80.04, P < 0.0001), with wild females crossed with wild males laying smaller clutches (9.25 ± 4.06) than laboratory females crossed with wild males (20.52 ± 3.84) and laboratory females crossed with laboratory males (24.57 ± 6.18) (Fig. 2B).

By contrast, we found no significant differences in the percentage of hatched eggs among strains (H2, 30 = 3.32590; P = 0.1892). The percentage of hatch for wild females (76.71 ± 15.68%) was lower to that observed for laboratory females crossed with wild males (85.63 ± 12.74%) or laboratory males (87.75 ± 10.65%).

Egg-to-pupa transformation in artificial diet was significantly greater for F1 progeny of laboratory males crossed with laboratory females (74.39 ± 7.38%) and wild males crossed with laboratory females (73.90 ± 6.00%) than for the F1 progeny of laboratory-reared wild flies (10.97 ± 13.22%) (H2, 30 = 19.41247; P = 0.0001) (Fig. 3).

Field Cage F1 Mating Performance Tests. A Kruskal–Wallis test revealed significant differences among strains in the number of copulations per replicate (H2, 24 = 10.55; P = 0.0051). Male F1 progeny of wild males crossed with laboratory females obtained more copulations per replicate (6.87 ± 1.12) with field-collected wild females than male F1 progeny of laboratory flies (4.50 ± 0.92) and a similar number of copulations than field-collected wild males (5.75 ± 1.38) (Fig. 4).

A Kruskal–Wallis test failed to reveal significant differences among strains in the duration copulations (H2, 24 = 2.33; P = 0.3115), although consistent with results of mating compatibility tests laboratory males copulated for numerically longer periods (53.3 ± 9.36 min) than field-collected wild males (46.6 ± 7.59 min) and F1 hybrid males (49 ± 8.41 min).

Discussion

Consistent with results by Rull et al. (2005) and Meza and Díaz-Fleisher (2006), laboratory males of the Metapa mass-rearing strain gained significantly
fewer copulations with field-collected wild females than field-collected wild males. During colonization, wild flies forced into artificial rearing underwent several bottlenecks, with wild females ovipositing less and smaller clutches than laboratory females. The F1 progeny of the hybrid strain (wild male/H11003 laboratory female) and the laboratory strain (laboratory male/H11003 laboratory female) gave similar yields in artificial diet and were both vastly superior to the F1 progeny of the wild strain. Importantly, male mating competitiveness was restored for F1 hybrid males competing with field-collected wild males for matings with field-collected wild females.

Genetic changes in laboratory colonies have been cited as the likely causes of shifts in traits such as flight ability, mating age, age at first reproduction, cuticular hydrocarbons, and adult longevity (Lance and McInnis 2005). Bottlenecks have been shown to result in such genetic changes and to lead to reproductive isolation among artificially generated and parental strains (Rice and Hostert 1993, Calkins and Parker 2005).

Here, we found that forcing wild individuals into artificial rearing conditions resulted in a consecutive series of population bottlenecks. Although we did not quantify mating frequency in laboratory cages both Rull et al. (2005) and Meza and Díaz-Fleisher (2006) found that wild A. ludens mate less frequently in Plexiglas cages than laboratory flies, this could perhaps explain why we found a numerically lower proportion of eclosion of eggs deposited by wild flies than those deposited by the hybrid strain and the laboratory control.

We also found a substantial reduction in oviposition, both in clutch number and clutch size by wild females in comparison with laboratory females mated to different types of males. Again, it could be that fewer wild females in our study accepted artificial devices for oviposition than laboratory females, coupled with strong selection for early oviposition of laboratory females.

Although we found relatively high percentages of egg hatch for all strains, a large proportion of eggs of the wild strain seeded into artificial diet failed to reach the pupal stage. Whether this resulted in loss of genetic variability still needs to be shown by means of genetic studies.

For the purpose of mass rearing, colonizing wild strains may result not only in the loss of genetic vari-

Fig. 2. Average ± SD number of egg clutches laid per replicate (A) and number of eggs per clutch per replicate (B) on artificial oviposition devices among wild males crossed with wild females, wild males crossed with laboratory females, and laboratory males crossed with laboratory females. Different letters over columns represent statistical differences at α = 0.05 after a Kruskal-Wallis test followed by comparison of mean ranks.
ability and subsequent modification of genetic composition but also in the loss of traits selected for mass production. Both problems seem to be overcome by using a hybrid strain colonization approach.

Males of the Metapa laboratory strain have been selected for several generations for fast mating with reduced courtship (Rull et al. 2005). Domestication of a wild strain results in mating incompatibility with wild flies. Using a hybrid strain approach allowed us to restore male mating competitiveness for the F1 generation without losing desirable production traits.

It can be argued that the ability to oviposit large clutches of eggs into artificial devices in our experiment reflects adaptation of laboratory females to artificial conditions and that this trait will not necessarily be inherited by F1 hybrid females. Nevertheless, hybrid F1 individuals showed similar levels of adaptation to develop in artificial diet as laboratory adapted individuals, which is an encouraging result.

Regarding mating performance, Shelly (2001) was also able to restore male mating competitiveness by crossing wild male Mediterranean fruit flies, Ceratitis capitata (Wiedemann), with laboratory females of the oldest mass-reared strain in Hawaii. In his experiment, out of two lines, one line returned to reproductive isolation after 10 generations, but the other line did not.

Newly collected field material usually takes several generations to adapt to colony rearing (Calkins and Parker 2005), with the colony stabilizing after about
five generations (Bartlett 1984). Our results seem to overcome this problem, which may result in substantial savings of time and money when strain replacement becomes necessary to maintain the effectiveness of SIT.

We still need to verify that avoiding bottlenecks by hybrid strain colonization results in reincorporating genetic variability into laboratory-adapted strains, and whether such a practice is correlated with renewed male mating success. Artificial rearing performance during the first five generations needs to be evaluated to ascertain that hybrid females retain desirable production traits. Finally, to establish the frequency at which strain refreshment needs to be applied to laboratory strains, performance of successive generations of hybrid males needs to be evaluated with field cage studies of sterility induction.

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