SELECTION FOR NON-RESPONSIVENESS TO METHYL EUGENOL IN MALE ORIENTAL FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

An experiment was conducted to determine whether non-responsiveness of male *Bactrocera dorsalis* (Hendel) to methyl eugenol could be increased via selection. Of four select lines established, males of one line showed a persistent reduction in attraction to the lure over 12 generations in the two assays utilized. Implications of this result for male annihilation programs are discussed.

Key Words: *Bactrocera dorsalis*, methyl eugenol, male response

RESUMEN

Fue llevado a cabo un experimento para determinar si la falta de respuesta de los machos de *Bactrocera dorsalis* (Hendel) al methyl eugenol podria ser aumentada mediante selección. Los machos de una de las cuatro líneas establecidas seleccionadas mostraron una reducción persistente en la atracción por el cebo durante 12 generaciones en los dos ensayos realizados. Son discutidas las implicaciones de este resultado para los programas de aniquilación de machos.

Males of many economically important tephritid species are attracted to particular chemical compounds, termed parapheromones or lures, which either occur naturally in certain plants or are (presumed) synthetic analogues of plant-borne

substances (Metcalf & Metcalf 1992). Because of their powerful attractancy, parapheromones are frequently used in control programs for detecting and monitoring wild populations. In addition, traps baited with a lure-insecticide mixture are often used to eradicate males completely (a technique termed "male annihilation"; Metcalf & Metcalf 1992) or at least greatly reduce male abundance prior to the implementation of the sterile insect technique (SIT).

Although generally effective, prolonged use of lures in a male annihilation program could have a negative effect if it inadvertently selected for non-responsiveness to the lure. Cunningham (1989) reviewed data bearing on this issue and concluded that, although the development of a completely non-responsive strain has never been proven, selection for non-responsiveness is a possibility that should be avoided through quickly implemented and rigorous control methods. Two lines of evidence suggest that non-responsiveness could evolve. In a preliminary study, Ito & Iwahashi (1974) were able to decrease responsiveness of male *Bactrocera dorsalis* (Hendel) to methyl eugenol after only two generations of selection. Although this result could have reflected selection for overall reduction in male mobility (leading to decreased movement to the lure as well), tests were conducted in small cages where travel distances to the lure were negligible. In addition, it appears that a non-responsive strain of *B. dorsalis* may have existed on the remote Ogasawara Islands of Japan. Here, despite the low likelihood of immigration, an intensive two year program of male annihilation failed to eradicate the population (Ito & Iwahashi 1974, Habu et al. 1984).

The purpose of the present study was to determine whether non-responsiveness of *B. dorsalis* males to methyl eugenol could be increased through artificial selection. This work expands upon Ito & Iwahashi's (1974) pilot project by increasing the duration of the experiment (i.e., the number of generations followed) and the number of lines studied. As will be described, responsiveness was monitored in both cage and field tests over at least eight generations for four pairs of control and select lines, and reduced male attraction to the lure was observed in both tests for one of the select lines.

Materials and Methods

The flies used in this experiment were obtained from a laboratory colony established with 200-300 adults of each sex that emerged from mango (*Mangifera indica* L.) collected in Waimanalo, Oahu. At the start of the study, the colony had been maintained in the laboratory for approximately four months or about three generations. The colony was held in a large screen cage (1.2 m by 0.6 m by 0.6 m) and provided with food (protein/honey mixture) and water ad libitum. Ripe papayas (*Carica papaya* L.) were provided frequently for oviposition. Infested papayas were placed in buckets (5 liters volume) containing vermiculite, and larval and pupal development occurred in situ. Sexes were separated within five days of eclosion (well before sexual maturity at 14-21 days of age; T.E.S., unpublished data).

Select lines were initiated by mating males that failed to feed on methyl eugenol in two separate trials. Trials were run as follows. Groups of 15-20 males (21-25 days old) were placed into 10-12 screen cages (45 cm cubes) between 1100-1400 hours, and the cages were placed outside in the shade (26-32 C°). Approximately 10 min later, cotton wicks to which 1.5 ml of pure methyl eugenol had been applied were introduced into the cages. Two observers then monitored the cages continuously for 30 min, and males that landed on the wick were immediately removed and discarded. The remaining males were transferred to a holding container and supplied food and water ad libitum. Then, three days after the first trial, a second trial was conducted following the same procedure. Males that again failed to visit the wick were used as sires for select

lines. To start the lines, sires for control lines and dams (21-28 days old) for control and select lines were taken haphazardly from the colony. For all lines, sires (n = 52-66) and dams (n = 70-85) were placed in screen cages (45 cm cubes) with ample food and water, and papayas were supplied on alternate days for oviposition. Progeny were reared in situ as described above and separated by sex soon after eclosion. Four pairs of control-select lines were examined over the entire study; lines were maintained and tested concurrently.

For all lines, the responsiveness of male progeny (21-27 days old) to methyl eugenol was tested in two ways. First, I ran the double-test method described above to both score male response for all lines and obtain sires for the select lines (see below). Second, other males were used in a field test comparing capture probabilities of control vs. select males at Steiner traps baited with methyl eugenol (3% naled). Groups of 100 control and 100 select males (24-37 days old) were cooled on ice for 60-90 s and then marked by placing enamel paint on the thorax. The males were released the following day between 1000-1100 hours at a large grassy lawn on the campus of the University of Hawaii at Manoa. Ten Steiner traps were placed singly in trees in a circle (50 m radius) around a central point. Traps were checked 72 h after release, and flies were examined individually in the laboratory for markings. Daytime temperatures ranged from 24-33 C° during the releases. Ten releases were conducted per test.

Breeding cages were established as follows. For the select line, males that failed to respond to methyl eugenol in the double exposure test were used as sires for the next generation, and females (21-27 days old) were chosen haphazardly from the select stock. For the control line, sires (22-27 days old) and dams (23-27 days old) were chosen haphazardly from among untested individuals in the control stock. For all lines, sires (n = 55-70) and dams (n = 65-82) were placed in screen cages (45 cm cubes) and provided with unlimited food and water and papayas for oviposition. In all cases, progeny were separated by sex with five days of eclosion.

RESULTS

Results of the cage and field trapping tests are presented in Figs. 1 and 2, respectively. For the cage tests, the frequency of non-responders in control vs. select lines was compared for each generation using the G test with Yates correction (Zar 1974). For the field trapping, the number of captured males from control vs. select lines was compared for each generation using the Mann Whitney test (Zar 1974).

For two of the replicates (1 and 4, respectively), control and select males showed no consistent differences in responsiveness to methyl eugenol in either the cage or the field trapping tests. With only one exception (replicate 1, generation 1, $P < 0.001$), frequencies of non-responders in the cage tests were similar between control and select lines over all generations for both of these replicates ($P > 0.05$ in all cases). Likewise, field trap catches were similar between control and select lines over all generations for both replicates 1 and 4 ($P > 0.05$ in all cases) save one instance (replicate 4, generation 5, $P < 0.05$).

Consistent inter-line differences in responsiveness were, however, evident in the remaining two replicates. In replicate 3, decreased responsiveness of select males was evident in the cage test but not the field trapping test. Here, the mean proportion of non-responders in the cage tests was 24% for the select line compared to 5% for the control line (values based on generations 1-8; $P < 0.001$ in all tests). In contrast, field trap catches for replicate 3 were not statistically different between control and select lines for any generation ($P > 0.05$ in all tests). In replicate 2, select males exhibited reduced responsiveness to methyl eugenol in both cage and field tests. For select males, the proportion of non-responders in the cage test increased rapidly and remained con-

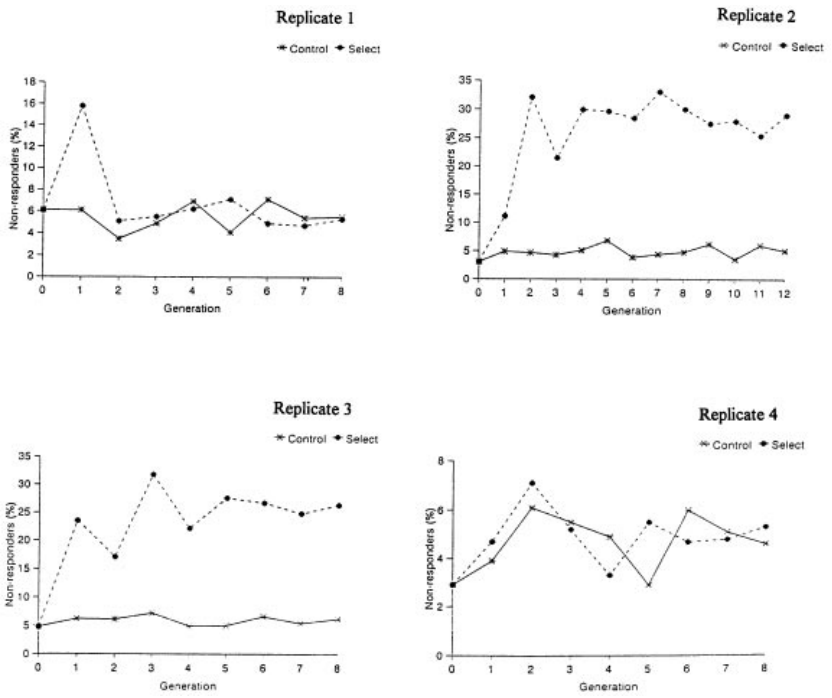

Fig. 1. Proportion of males (n = 602-777 for control, n = 405-1569 for select) that did not land on a methyl eugenol-treated wick during two exposure periods (30 min each) in laboratory cages spaced three days apart plotted against time (generation) since the onset of the experiment. Note differences in the scaling of axes.

sistently high (22%-32%) between generations 2-12 (when the experiment was terminated). In contrast, only a consistently small proportion (3%-6%) of control males failed to respond ($P < 0.001$ for generations 2-12). In the field test, over generations 2-12 an average of 41-54 control males was captured per test compared to only 18-33 select males ($P < 0.001$ in all cases).

Discussion

The present study confirms Ito & Iwahashi's (1974) preliminary data that responsiveness of male *B. dorsalis* to methyl eugenol can be reduced via artificial selection under laboratory conditions. Owing to the relatively large size of the colonies, it is unlikely that the changes observed in responsiveness were the result of genetic drift (Falconer 1981). Reduced responsiveness was not, however, a certain outcome as in only two of the replicates did the select lines differ from control lines. These differences may have reflected the initial presence of rare "non-responder" males in only two of the four select lines (i.e., replicates 2 and 3).

Even between these two replicates, the response to selection was different. In replicate 2, decreased responsiveness was noted in both field and cage tests, whereas in replicate 3 reduced responsiveness was noted in the cage tests only. It is not known why (for this replicate) a lowered response was not observed in the field test as well.

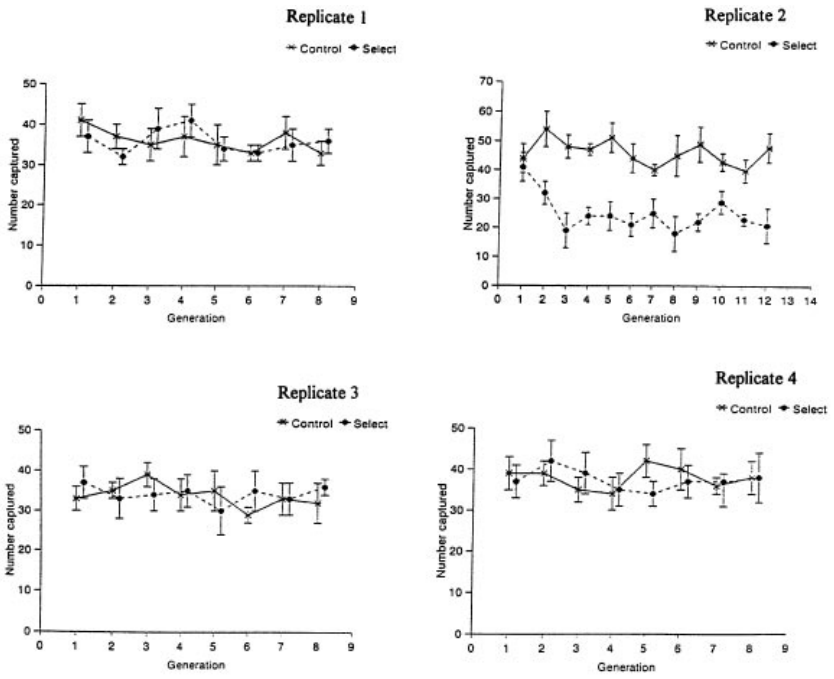

Fig. 2. Number of control and select males captured in Steiner traps baited with methyl eugenol (and naled). Each value represents average of 10 replicates; bar represents ± 1 SD.

It is possible that "responsiveness" to methyl eugenol is a composite trait that involves variable thresholds for physiological and/or behavioral responses with varying distance (concentration) to the lure. Perhaps the selection protocol effectively inhibited the mechanisms associated with close-range attraction in this line without concurrently affecting factors involved with long-range attraction. However, why such differential selection might occur in one select line (replicate 3) but not another (replicate 2) remains unclear.

Even where evident, selection did not result in the complete disappearance of male attraction to methyl eugenol. Although lowered through selection, male responsiveness in replicates 2 and 3 was stable (and not continually decreasing) though 8 and 12 generations, respectively. Still, the rapid (and persistent) response to selection reinforces Cunningham's (1989) recommendation that programs of male annihilation be implemented vigorously and decisively to avoid protracted costs associated with the eradication of unresponsive males. Interestingly, the same recommendation holds for SIT as well, as wild females may evolve "behavioral resistance" to sterile males in protracted release programs (McInnis et al. 1996).

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