

GENETIC TRANSMISSION OF HOST-SEEKING BEHAVIOR IN COLONIZED *Aedes aegypti* (L.)¹L. C. RUTLEDGE,² A. A. KHAN, D. L. SKIDMORE AND H. I. MAIBACHDepartment of Dermatology, University of California School of Medicine,
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ABSTRACT. Laboratory-reared *Aedes aegypti* were selected for responsiveness/nonresponsiveness to the human palm with a glass cylinder divided by a barrier into 2 chambers. Relatively responsive and nonresponsiveness subpopulations of the laboratory stock retained a consistent response differential when re-tested in the same apparatus

over a period of 21 days. The response differential persisted through the gonotrophic cycle and was transmitted from parent to offspring. No genetic gain in either responsiveness or nonresponsiveness was observed in 2 generations of genetic selection.

INTRODUCTION

Numerous workers have documented the principle that mosquito taxa differ in host preference, time of biting and other aspects of host-seeking behavior. Although the inheritance of autogeny (Laven 1967; O'Meara and Craig 1969) and of host preference (Gillies 1964) has been studied, little is presently known of the inheritance of other features of mosquito host-seeking behavior (Mattingly 1967). The present report deals with the genetic transmission of responsiveness to the human palm in *Aedes aegypti* (L.).

MATERIALS AND METHODS

The study was conducted at the University of California Medical Center, San Francisco, during the period January to April, 1974. The strain of *Aedes aegypti* employed was obtained from the Rockefeller Institute in 1964 and has been continuously maintained in our laboratory since that time. The mosquitoes were reared and maintained under continuous incandescent lighting at approximately 27° C and 60% R.H. Guinea pigs were used as the blood-meal source.

All experiments were conducted between 0830 and 1600 hours under ambient laboratory conditions. Temperatures ranged from 21 to 26° C, and the relative humidity ranged from 30 to 40%. Illumination was by overhead fluorescent lamps and window-light.

SELECTION AND TESTING APPARATUS. The selection and testing apparatus consisted of a glass cylinder (3½" in diam. x 21½" high) mounted vertically on a ring-stand. The cylinder was divided into upper and lower chambers with a cardboard ring having an aperture of 2¼" diameter. A barrier to the passage of mosquitoes between the upper and lower chambers was made by gluing 24 nylon bristles to the ring so that each extended radially to the center of the aperture. The upper chamber (8½") was lined with netting to provide footing for the mosquitoes and was closed at the top with fine gauze. The lower chamber (13") was closed at the bottom with fine gauze and a 3½" cardboard disk having a centered rectangular cutout (1½" x 2½").

Mosquitoes placed in the upper chamber respond to an attractive stimulus beneath the apparatus by passing through the barrier into the lower chamber. This is accomplished either by negotiating the barrier in flight or, more often, by landing on it and crawling through. The mosquitoes then fly to the gauze at the bottom of the apparatus and probe actively toward the stimulus. The same apparatus was used both to fractionate mosquito popula-

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tions into relatively responsive and non-responsive subpopulations and (by counting and timing the responses) to measure the responsiveness of particular populations, subpopulations or strains.

SELECTION METHODS. Selection of relatively responsive and nonresponsive subpopulations for testing and propagation was done by introducing 1 to several hundred mosquitoes from the stock into the upper chamber of the selection apparatus and holding the palm of the hand beneath it for 1 hour. Those mosquitoes passing through the barrier into the lower chamber of the apparatus within this period were termed "responsive"; those remaining in the upper chamber were termed "nonresponsive." Either 1, 2 or 4 selections were made from the initial stock. When 2 or 4 selections were made, this was done on consecutive days. After the 1st day, responsive and nonresponsive subpopulations were selected separately, and those mosquitoes that did not behave consistently on consecutive days were eliminated from their respective subpopulations. All selections in the study were made by a single individual to standardize the attractive stimulus.

TESTING METHODS. Corresponding responsive and nonresponsive subpopulations/strains were tested in successive 1-hour periods on either 5 or 10 separate days following selection. The order of testing was alternated on succeeding test days. A sample of 25 mosquitoes from the subpopulation/strain to be tested was introduced into the upper chamber of the testing apparatus, and the attractive stimulus (the palm of the hand or a guinea pig) was placed beneath it. Each mosquito responding within 1 hour was counted and timed as it passed through the barrier into the lower chamber of the testing apparatus. On completion of the test, the 25 mosquitoes were returned to the appropriate holding cage. All tests utilizing the human palm were done by a single individual, and all tests utilizing the guinea pig were done with a single animal.

RESULTS

EFFECTS OF A SINGLE SELECTION. A population of 100 nulliparous female *Aedes aegypti* from the laboratory stock was fractionated into relatively responsive and nonresponsive subpopulations in the manner described above. Each subpopulation was tested on 10 separate days thereafter (Table 1). The response differential ($71.3 - 50.1 = 21.2\%$) was significant at the 1% level (paired t test). Except on day 5, post-selection, the percentage of mosquitoes responding in the responsive subpopulation was always greater than that in the nonresponsive subpopulation. The result obtained on day 5 was one of only 4 such reversals and 2 ties observed in 55 similar comparisons made during the study. This experiment indicated that our laboratory stock could be fractionated into relatively responsive and nonresponsive subpopulations and that such subpopulations would retain a significant response differential for at least 16 days.

REPEATED SELECTION. New subpopulations of responsive and nonresponsive mosquitoes were selected from the laboratory stock by way of 2 selections done on consecutive days as described above.

Table 1. Percent of mosquitoes responding in 10 tests of responsive and non-responsive subpopulations established from laboratory stock with a single selection

Day post-selection	% Responding	
	Non-responsive subpopulation	Responsive subpopulation
0	60	96
1	40	64
2	52	80
5	68	48
6	48	88
7	72	100
8	64	100
12	20	40
14	58	68
16	19	29
Mean	50.1	71.3
Range	19-72	29-100

Table 2. Summary of results obtained in tests of responsive and non-responsive subpopulations established from laboratory stock with 2 selections on successive days.

Item	Non-responsive subpopulation	Responsive subpopulation
No. of Days Tested	10	10
Mean % Responding	28.8	71.6
Range	12-56	60-88

Each subpopulation was tested on 10 days thereafter (Table 2). The response differential ($71.6 - 28.8 = 42.8\%$) was significant at the 1% level (paired t test) and persisted for at least 21 days following selection. These results confirmed those obtained in our preceding experiment.

An apparent effect of repeated selection was to increase the magnitude of the response differential from 21.2% to 42.8% (cf. Tables 1 and 2). This increase was significant at the 5% level (t test). In addition, the (pooled) variance of % responding in the 1-selection experiment (506.9) was significantly greater than that from the 2-selection experiment (146.2), indicating that mosquitoes responding inconsistently could be culled by repeated selection (1% level, F test). For these reasons, subsequent selections from stock were made with repeated selections done on 4 consecutive days.

EFFECT OF A BLOOD-MEAL. Since host-seeking behavior is suppressed during the period between the time of blood-feeding and the time of oviposition, an experiment was conducted to determine whether the response differential observed previously

Table 3. Summary of results obtained in tests of responsive and non-responsive subpopulations selected from laboratory stock before blood-feeding and tested after completion of a gonotrophic cycle (parent generation).

Item	Non-responsive subpopulation	Responsive subpopulation
No. of Days Tested	5	5
Mean % Responding	40.0	77.6
Range	24-60	60-92

would persist through the gonotrophic cycle and be re-asserted after oviposition had occurred. New responsive and nonresponsive subpopulations were selected from the laboratory stock and allowed to feed on a guinea pig and to oviposit prior to testing. Each subpopulation was tested on 5 days (Table 3). The response differential ($77.6 - 40.0 = 37.6\%$) was significant at the 1% level (paired t test), confirming persistence of the response differential through the gonotrophic cycle.

F₁ GENERATION. To test for genetic transmission of the response differential, the progeny of the 2 blood-fed subpopulations (see above) were reared to the adult stage and tested. Each strain was tested at 10 days (Table 4). The response differential ($83.2 - 63.6 = 19.6\%$) was significant at the 5% level (paired t test), indicating that genetic transmission of the response differential had occurred.

It should be pointed out that a degree of reversion was observed in this experiment, since the response differential of the F₁ generation (19.6%, Table 4) was less than that of the P₁ generation (37.6%, Table 3). This result was in accord with expectations, since only the females of the P₁ generation were from selected stock. Since the male mosquito does not exhibit host-seeking behavior, it was not possible to select males for the foundation stock of the responsive and nonresponsive strains.

F₂ GENERATION. In order to confirm our results with regard to genetic transmission of the response differential, the F₁ generations of each strain were then selected and propagated. The F₂ were tested at 10 days against both the human palm and the guinea pig (Table 5). The data were analyzed as a 2 x 2 factorial experiment in

Table 4. Summary of results obtained in tests of responsive and non-responsive strains reared from selected stock (F₁ generation).

Item	Non-responsive strain	Responsive strain
No. of Days Tested	10	10
Mean % Responding	63.6	83.2
Range	12-88	36-100

Table 5. Summary of results obtained in tests of responsive and non-responsive strains reared from selected stock (F_2 generation).

Item	Non-responsive strain	Responsive strain
Human Palm:		
No. of Days Tested	10	10
Mean % Responding	75.6	85.2
Range	40-100	60-100
Guinea Pig:		
No. of Days Tested	10	10
Mean % Responding	29.9	53.0
Range	17-44	32-68

10 blocks, with the mosquito strains (responsive and nonresponsive) and attractive stimuli (human palm and guinea pig) as the 2 factors and the days of testing as the 10 blocks. Main effects and interaction were tested as single degree of freedom comparisons against the partitioned error variance. The response levels of the 2 mosquito strains differed at the 5% level of significance, confirming our previous results with regard to genetic transmission of the response differential. The interaction mean square was not statistically significant, indicating that the magnitude of the response differential was similar in tests against both host species.

Response levels to the 2 host species differed at the 1% level of significance, indicating that the human palm was more attractive than the guinea pig under the conditions of our experiment. Since the interaction mean square was not statistically significant, the magnitude of the host-preference differential was similar in tests of both mosquito strains. The (pooled) variance of % responding was significantly greater in tests against the human palm ($S^2 = 248.0$) than it was in tests against the guinea pig ($S^2 = 105.0$) (5% level, F test).

A degree of reversion was observed in the F_2 generation, since its response differential in tests against the human palm ($85.2 - 75.6 = 9.6\%$, Table 5) was less than that of both the F_1 generation (19.6%, Table 4) and the P_1 generation

(37.6%, Table 3). This result was not in accord with expectations, since there had been 2 generations of selection in the female line and 1 generation of selection in the male line (in the sense that the F_1 males were the progeny of selected females).

SPEED OF RESPONSE. Tables 1-5 record our results in terms of the numbers of mosquitoes responding within a period of 1 hour. An alternate criterion for evaluation of the response level of a given subpopulation or strain is that of the time required for 50% of the mosquitoes to pass through the barrier in the testing apparatus. In Figure 1 the cumulative percentage of mosquitoes responding (probit scale) is plotted against elapsed time (log scale) for the F_2 generations of the responsive and nonresponsive strains in tests against the human palm. The relationship is linear for the time period from 2 to 60 minutes. Each line was calculated as the unweighted linear regression from the totals for the 10 tests done on each strain.

Figure 1 shows that the response differential was established within the first 2 minutes of testing and was maintained essentially unchanged throughout the remaining time of the test. The regression values for $T = 60$ minutes were 76.7% and 86.0% for the nonresponsive and responsive strains, respectively. These figures agree to within 1.1% with the observed values given previously for the 2 strains (Table 5). The median response time was 9.3 minutes for the responsive strain and 16.2 minutes for the nonresponsive strain (Fig. 1). Thus, nearly twice as long a time period was required for 50% of the nonresponsive strain to pass through the barrier as was required for 50% of the responsive strain to pass through.

DISCUSSION

Two prior investigators have obtained results similar to those reported above. Schoenig (quoted by Rai 1967) obtained

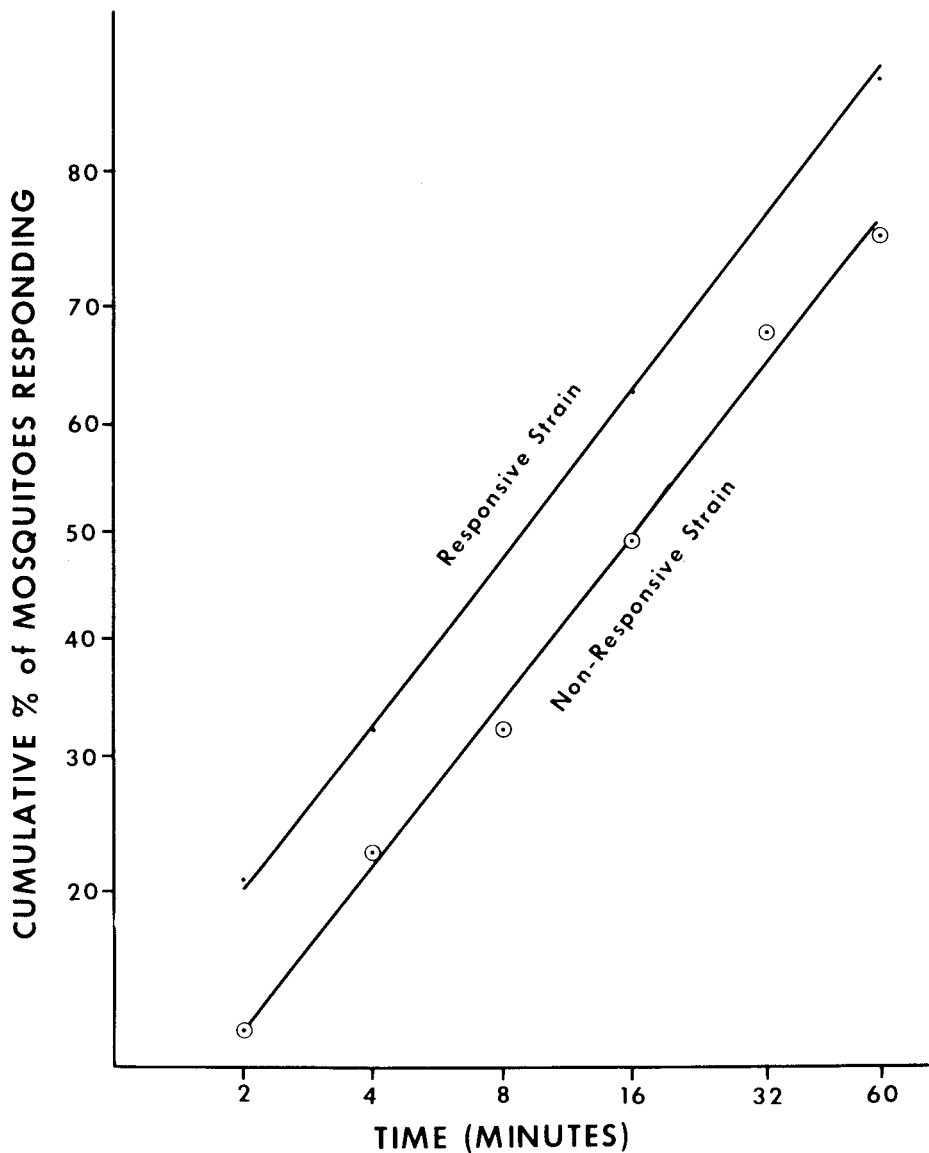


Fig. 1. Relationship of elapsed time (log scale) and cumulative percentage of mosquitoes responding to the human palm, (probit scale) in 10 tests of the F_2 generation of responsive and nonresponsive strains of *Aedes aegypti*.

slow- and fast-moving strains of *A. aegypti* by selection with a series of inter-connected wooden boxes. Gerold (1973) demonstrated a genetic basis for the differential abilities of female *Anopheles gambiae* Giles to escape through a hole when confined over paper impregnated with 4% DDT. To what extent the behavioral traits involved in the 3 studies may have a common genetic basis is not presently known.

In the present study, a statistically significant response differential was transmitted from the P₁ generation to the F₁ and from the F₁ to the F₂. However, the magnitude of this response differential decreased from generation to generation in the face of continued selection for the trait (Tables 3-5). Several genetic mechanisms are known that can produce real or apparent reversions in opposition to applied selection pressure. These include outcrossing effects (as discussed above in connection with the selection of male stock), variable expressivity (in connection with rearing or testing conditions), inadvertent counterselection (as for example in propagating the nonresponsive strain), and epistatic effects (as described by Fuller and Thompson, 1960). The extent to which these or other mechanisms may have contributed to the results obtained in the present study is uncertain, since in longer studies reversions of the kind described frequently turn out to be essentially random short-term fluctuations in the overall trend of the response to selection (see for example Figure 2 of Ward, 1963).

CONCLUSIONS

We conclude that individual females of *A. aegypti* have characteristic, variable thresholds of response to the presence of the host and that these thresholds are, at least in part, genetically determined.

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