Theor. Appl. Genet. 55, 263-268 (1979)

Some Consequences of Selection for Fast and Slow Recovery from the Larval Alarm Reaction in *Aedes aegypti*

R.E. Duhrkopf and S.S.Y. Young
Department of Genetics, The Ohio State University, Columbus, Ohio (USA)

Summary. Replicated divergent selection based upon the time taken to recover from the larval alarm reaction in the mosquito *Aedes aegypti* resulted in lines which recovered faster and slower than the control lines. Estimates of the realized heritability were consistent, ranging from 0.21 to 0.24 in the fast replicates and 0.19 to 0.20 in the slow replicates. After 11 generations of selection an apparent change in the fitness was examined using an application of the path analysis. The relevance of the findings to natural selection is also discussed.

Key words: Selection – *Aedes aegypti*

Introduction

When a mosquito larva is stimulated by a passing shadow or a vibration, it leaves the surface for a period of time. Mellanby (1958) reported differences in patterns of diving behavior among *Aedes aegypti*, *Culex p. molestus*, and *Anopheles maculipennis* larvae. He also found some differences in mean recovery time between species. However, within species the diving behavior is uniform although the lengths of time spent submerged by individual larvae are variable.

Natural populations of *Aedes aegypti* occur in two different larval habitats – standing water in tree holes (feral) and water jars in native huts (domestic) Trpis and Hausermann (1978) suggested that habitat, seasonal and ethological barriers restrict interbreeding between populations in the two habitats, even if they are in close proximity. Dr. G.B. Craig, Jr., (pers. commun.) indicated that it was his impression that larvae in feral habitats dive and return to the surface in a shorter period of time than do larvae in domestic habitats.

Others have studied differences between feral and domestic strains of *Aedes aegypti*. Schlosser and Buffington (1977) found differences in oxygen consumption, development rate, life span, numbers of offspring produced, and intrinsic rates of increase. Crovello and Hacker (1972) and Hacker et al. (1977) found significant differences between life table characteristics, and Trpis and Hausermann (1975, 1978) reported differences in house-entering behavior between different strains.

The aim of the present study is to use selection to estimate the degree of genetic control, if any, over the time spent submerged during the alarm reaction, and to observe any correlated changes in fitness.

Material and Methods

The Selection Experiment

All mosquitoes were reared using standard procedures (200 larvae per 450 ml water in pans; food: ground lab chow, lactalbumin and yeast in equal parts, fed daily) in the laboratory at 27° and 80% relative humidity with a 16:8 light: dark regimen.

The experiment was begun with approximately 1000 eggs from the laboratory colony of *Aedes aegypti* at The Ohio State University. This is a large, random mating population (N ≈ 10,000) begun by Dr. Carl E. Venard. Throughout the years, the viability and fertility have been high.

The original 1000 eggs were hatched and the adults were mated at random. Three groups of 200 larvae were collected at random using a medicine dropper. From each group of 200 larvae, 100 fourth instar larvae were tested. The 30 with the lowest means were selected to begin a fast line and the 30 larvae with the greatest means were selected to begin a slow line. The remaining 40 larvae were discarded. From the remaining 100 untested larvae, 30 fourth instar larvae were chosen at random to begin a control line. Using this scheme, three replicates of the selection experiment were begun. These were designated Fast I, Control I, Slow I, Fast II, Control II, Slow II, Fast III, Control III, and Slow III. These lines were maintained by selection using a similar procedure. That is, 30 larvae were selected from 100 tested larvae in each selection line, and 30 larvae from 100 tested larvae were taken at random for the control line.

The 100 larvae were prepared for testing by putting each indi-

0040-5752/79/0055/0263/$ 1.20

vidual into a 12 X 75 mm test tube with 2 ml of a water-food suspension made by mixing 500 mg of powdered food in 250 ml of aged tap water. Ten tubes were grouped 2.5 cm apart in boards which minimized the amount of the tube which was shadowed in order to avoid any phototaxis in a light gradient. Each group of tubes was separated from neighboring groups by white index cards such that stimulation or diving of individuals in one group could not affect individuals in the next group. The 10 groups of tubes were kept on the laboratory table approximately 1.5 m from an overhead fluorescent light and were allowed to remain undisturbed for 4 hr before testing.

A test of a larva consisted of measuring the amount of time that it remained submerged following stimulation. In testing, 3 larvae were found at the surface. They were stimulated by passing a white index card along the side of the tubes and lightly striking the board. In this way both the visual and tactile stimuli were used. All larvae dived immediately upon stimulus application and the time interval between stimulus application and the return of the larva to the surface was recorded. A larva was considered to be back in contact with the surface when its respiratory siphon was in contact with the surface. Testing continued until all larvae in the group of 10 were tested 3 times. At that point, the next group of 10 larvae was tested.

Selection was based upon the individual means. In each generation, selection differentials were calculated and used in the estimation of realized heritabilities (Falconer 1960). The standard errors of the estimates of realized heritability were calculated using the method developed by Hill (1972).

The Fitness Test

At the end of the selection experiment, the fitness of each of the Fast I, Fast III, Control I, Control III, Slow I, and Slow III lines was tested.

For each line a group of adults was put into a mass rearing cage. Approximately 48 hr after the emergence of the last adult the females were given a blood meal from a restrained rat. Twenty-four hours after the blood meal 30 females were removed and each was put into a small oviposition cage. At the end of one week the strips of eggs were removed. For each strip the number of eggs was recorded. The eggs were hatched and the number of larvae from each strip was counted. The larvae were then put into cups and reared in an amount of water with an amount of food which was proportional with the standard procedures. That is, if there were 100 larvae they were reared in half the amount of water and with half the amount of food as the standard 200 larvae per pan. The number of pupating larvae was recorded, as was the number of adults which emerged from the pupae. In this manner complete records were kept for each female which included the number of eggs laid, the number of larvae that hatched, the number of pupae and the number of adults.

The Fast I, Fast III, Control I, Slow I, and Slow III lines were tested in this manner. The eggs of the Control III line failed to hatch. In addition, a General Control line was tested using eggs taken from the laboratory colony. These represented a population which had undergone no selection, which could then be compared against the Fast I, Fast III, Slow I, and Slow III lines and the Control I line which had undergone systematic reductions in population size.

The data for the number of adults which resulted were analyzed using a standard nested analysis of variance (Snedecor and Cochran 1967). The path analysis (Li 1955; Wright 1968) was used to estimate the relative importance of fitness components in each of the lines.

The model for the path analysis was as follows:

$$Y = \prod_{i=1}^{4} X_i$$

in which Y = the number of adults produced by a single female;
X_1 = the number of eggs laid by that female;
X_2 = the percentage of those eggs that hatch;
X_3 = the percentage of those larvae that pupated, and
X_4 = the percentage of those pupae that emerged as adults.

$$\text{And } \underset{i}{\text{Log}}(Y) = \sum_{i=1}^{4} \text{Log}(X_i)$$

$$\text{or} \qquad y = \sum_{i=1}^{4} x_i$$

where $y = \text{Log}(Y)$ and $x = \text{Log}(X)$

$$\text{Setting} \quad p_i = \frac{\sigma_{x_i}}{\sigma_y}$$

$$\text{and} \quad r_{ij} = \frac{\sigma_{x_i x_j}}{\sigma_{x_i}\sigma_{x_j}}$$

$$\text{hence} \quad \sum_i p_i^2 + 2 \sum_i \sum_j p_i p_j r_{ij} = 1 \quad i < j$$

The values of p_i and r_{ij} can then be used in the evaluation of the relative influence of each of the components to total fitness.

Results

The Selection Experiment

The means for each generation of each replicate are plotted against generation number in Figure 1. It can be seen that the means of the three fast replicates decreased with selection, and that the means of the three slow replicates increased with selection. The mean times of the three control replicates remained relatively constant. It should be noted that all three lines of replicates I and III underwent 11 generations of selection. In replicate II the eggs of the fast and slow lines failed to hatch in the eighth generation. Because of this, all three lines of replicate II were discontinued.

Because of the non-homogeneity of the variances, which was apparently due to a scaling effect, the data from the seventh and eleventh generations were transformed using a logarithmic transformation, and subsequently analyzed by the analysis of variance (Table 1). Both analyses showed significant line and replicate effects ($P < 0.01$). However, in both cases the estimates of the variance components for line effects were approximately twenty times greater than the estimates for replicate effects.

In order to eliminate common environmental effects the mean for each generation of each replicate in each of the selection lines was calculated as a deviation from the

mean of the corresponding control replicate in the corresponding generation. These deviations from control were used to calculate the estimates of realized heritability. These deviations were plotted against cumulative selection differentials and are shown in Figure 2. The estimates of realized heritability and their standard errors are presented in Table 2. The estimates for the three fast lines varied from 0.21 to 0.24, and the estimates for the three slow lines varied from 0.19 to 0.20. All of the 95% confidence intervals overlapped. So, the estimates of realized heritability are consistent between replicates and lines.

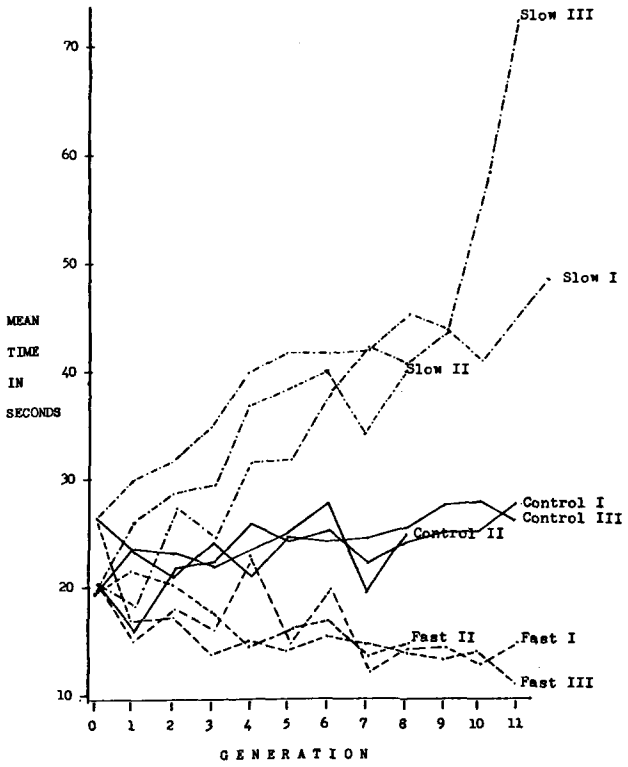

Fig. 1. The mean times of the experimental lines plotted against generation number

Table 1. Analysis of variance for data from the seventh and eleventh generations using a logarithmic transformation

Source	Seventh generation		Eleventh generation	
	D.F.	M.S.	D.F.	M.S.
Lines (L)	2	17.45[a]	2	22.52[a]
Replicates within lines (R)	6	0.25[a]	3	0.38[a]
Larvae within lines and replications	891	0.02	594	0.02
Estimates of variance components				
σ^2_R		0.002		0.004
σ^2_L		0.057		0.074

[a] $P < 0.01$

The Fitness Test

Means and standard errors for each of the components used in the fitness test are presented in Table 3. The results of the nested analysis of variance performed on the number of adults produced are given in Table 4. It can be

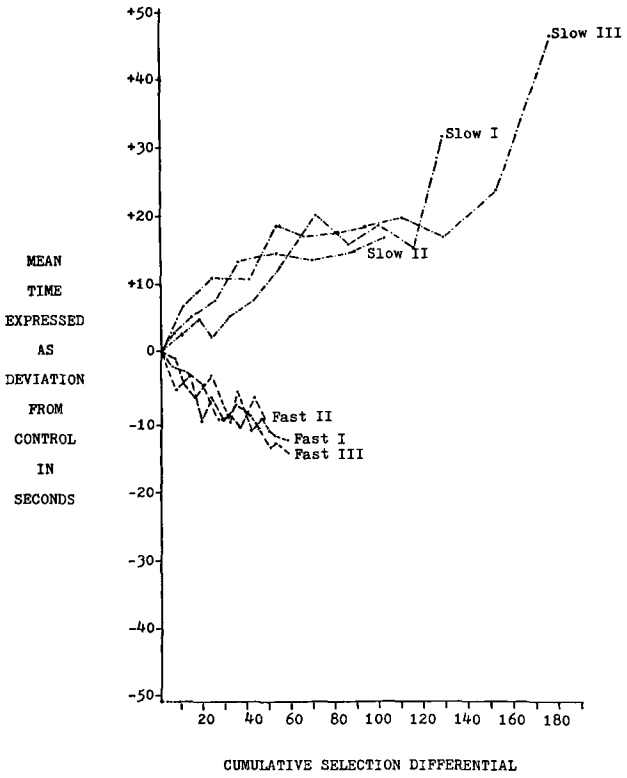

Fig. 2. Mean time expressed as deviation from control plotted against cumulative selection differential

Table 2. Estimates of realized heritability

Line	h^2	S.E.
Fast I	0.21[a]	0.01
Fast II	0.22[a]	0.02
Fast III	0.24[a]	0.01
Slow I	0.20[a]	0.01
Slow II	0.19[a]	0.01
Slow III	0.20[a]	0.01

[a] $P < 0.001$

Table 3. Meanss of fitness components in different lines

Line	No. of eggs		Percent hatch		Percent pupation		Percent emergence		No. of adults per female	
	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.
General Control	82.10	4.65	0.49	0.05	0.54	0.06	0.80	0.05	17.80	3.34
Control I	64.43	6.83	0.21	0.05	0.42	0.07	0.53	0.03	9.57	2.11
Fast I	48.43	6.63	0.17	0.05	0.34	0.07	0.50	0.02	5.43	1.47
Fast III	97.27	7.17	0.44	0.04	0.05	0.02	0.34	0.02	1.70	0.76
Slow I	54.00	6.81	0.49	0.05	0.43	0.06	0.67	0.02	14.53	2.59
Slow III	19.17	5.17	0.10	0.06	0.29	0.06	0.36	0.01	3.47	1.51

Table 4. Analysis of variance for the number of adults per female produced in the fitness test

Source	D.F.	Mean square
Lines	2	1538.01[ns]
Replicates within lines	3	1020.98[a]
Females within lines and replicates	174	138.55

[a] $P < 0.001$

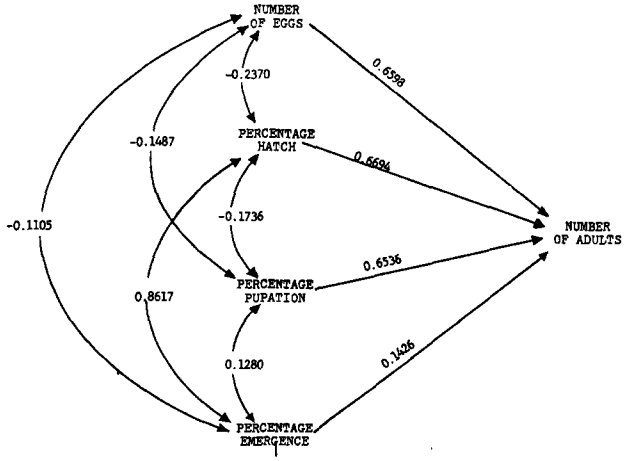

Fig. 3. The path analysis for the General Control line

seen that there were significant differences between replicates within lines ($P < 0.001$), but non-significant differences between lines.

The results of the path analysis are presented in Figure 3 and Table 5. Figure 3 represents the path diagram for the General Control line, and the values for all of the paths in all of the lines are presented in Table 5. The values of the paths varied greatly from line to line and replicate to replicate. One consistent result was the low value for the path for percentage emergence. This indicated that, in every case, the remaining components were relatively more important.

The analysis for the General Control line shows approximately equal influence of the number of eggs and percentages hatch and pupation. Relative to the General Control line, the Control I line shows a slight increase in the importance of the percentage hatch and a slight decrease in the influence of the percentage pupation and number of eggs. However, the high negative correlation between number of eggs and percentage pupation could be a factor in this decrease. In general, the two control lines show only small differences in the influence of the number of eggs, percentage hatch and percentage pupation.

The analysis for the Fast I line shows that there is an increase in the importance of the percentage hatch and a decrease in the importance of the percentage pupation and number of eggs when compared to the control lines. There is still a sizable negative correlation between the number of eggs and the percentage pupation. The Fast III line shows an increase in the importance of the percentage pupation. There is still a large influence due to percentage hatch, and the influence of the number of eggs is again rather low. The increase in the influence of the percentage hatch is no doubt due to the increased negative correlation between hatch and pupation. Thus, in general, the analysis for the fast lines presents the picture of an increase in the joint influence of the percentage hatch and the percentage pupation and a decrease in the influence of the number of eggs as compared with the controls.

The path analysis for the Slow I line shows a prominent influence due to the number of eggs and a lesser influence due to the percentage hatch. The analysis for the Slow III line shows a large influence in the percentage hatch and number of eggs and a further reduction in the importance of the percentage pupation. So, in general, the

Table 5. Path analysis values in the fitness test

Line	P_1	P_2	P_3	P_4	r_{12}	r_{13}	r_{14}	r_{23}	r_{24}	r_{34}
General										
Control	0.66	0.67	0.65	0.14	−0.24	−0.15	−0.11	−0.17	0.86	0.13
Control I	0.48	0.78	0.49	0.13	0.35	−0.54	−0.29	−0.18	0.25	0.28
Fast I	0.40	0.96	0.38	0.08	0.15	−0.41	−0.26	−0.26	−0.19	0.14
Fast III	0.36	0.75	1.12	0.21	0.05	−0.22	0.01	−0.51	−0.01	0.01
Slow I	0.87	0.57	0.65	0.12	0.22	−0.47	−0.24	−0.31	0.05	0.45
Slow III	0.67	0.97	0.29	0.03	−0.40	−0.33	−0.34	0.02	−0.42	0.12

i Trait

1 No. of eggs
2 Percentage hatch
3 Percentage pupation
4 Percentage emergence

analyses for the slow lines present a picture of increased importance of the number of eggs and the percentage hatch and a decreased importance of the percentage pupation.

Discussion

Selection resulted in lines which diverged with respect to the time taken to recover from the larval alarm reaction, and the divergence is symmetrical for the fast and slow lines. At the end of the experiment there were significant differences between replicates and within lines, with the estimated line effects being of the order of 20 times larger than the estimated replicate effects. The estimates of realized heritability are consistent over lines, as all of the 95% confidence intervals overlapped.

The results therefore show that the behavioral phenotype – recovery time – is under partial control of the additive genetic variability of the population.

The fitness test was performed because of an apparent reduction in the number of viable eggs laid during the course of the selection experiment.

The differences in fertility between the selection lines are not significant. However, the path analysis suggests that different lines produced viable offspring via different fitness strategies. In the control lines, the number of eggs, percentage hatch, and percentage pupation were equally important. In the fast lines, the percentage hatch and percentage pupation appear to be more important than the number of eggs. In the slow lines, the number of eggs and percentage hatch appear to be more important than the percentage pupation.

However, our data on the correlated changes in fitness components due to selection must be regarded as preliminary results. This is because of the generally low mean fitness values for the lines.

It is thought that feral larvae spend less time submerged than domestic larvae (Dr. G.B. Craig, Jr., pers. commun.) and several workers have reported differences in life table characteristics between the two strains. Schlosser and Buffington (1977) stated that feral strains tend to be more K-selected and domestic strains tend to be more r-selected. In addition, Crovello and Hacker (1972) and Hacker et al. (1977) suggested that domestic strains have higher reproductive rates than feral strains, and they related this to higher levels of variability in the domestic habitat which is a condition postulated to be important for r-strategists (Wilson and Bossert 1971). Our selection experiment produced lines with fast and slow recovery speeds. The former suggests correspondence to the feral strains. The latter appears to mimic the domestic strains with respect to diving behavior. In the fast lines, survival characteristics were important to fitness. On the other hand, in the slow lines egg number appears to be more important. From this, it is tempting to infer from the present study that selection for larvae that spent more or less time submerged also resulted in selection for mosquitoes with fitness characteristics similar to those in nature.

It must be remembered that, in the fitness test, the fast and slow lines did not show some mean characteristics typical of feral and domestic strains, respectively. Although the slow lines produced more adult offspring than the fast lines, they produced about hafl as many eggs. But, the path analysis demonstrated that, in the fast lines, the number of adults emerging was more strongly correlated with larval survival than egg output, and in the slow lines, the number of adults is more strongly correlated with the number of eggs laid.

It may be argued that diving behavior and survival might be connected in *Aedes aegypti*, since obtaining food and escaping from predators must be the primary reasons for remaining submerged, and food and enemies probably differ between artificial containers and tree holes. One might expect greater amounts of deposited organic material in tree holes. So, larvae would have to spend less time submerged to obtain a specific amount of food. And, feral larvae do not run the risk of being dumped if they surface too quickly after a disturbance, as domestic larvae probably do.

The present analysis suggests the presence of genetic correlation between diving behavior and various fitness components. The path analysis also suggests that correlations between components within lines may be different. However, these should be regarded as tentative findings due to the low fertility of the lines during the fitness test for the reasons discussed above.

Regardless of the genetic correlation between diving behavior and fitness components, the present study clearly demonstrates that a behavioral trait – the recovery from the alarm reaction – is under partial control by a polygenic system.

Acknowledgement

The authors would like to acknowledge the aid of Dr. Walter C. Rothenbuhler during the course of the experiment and in the preparation of the manuscript, and the help of Dr. Woodbridge A. Foster in areas of mosquito biology and in the preparation of the manuscript. We would also like to acknowledge the free computer time provided by The Ohio State University Instructional and Research Computer Center.

Literature

Crovello, T.J.; Hacker, C.S.: Evolutionary strategies in life table characteristics amoung feral and urban strains of *Aedes aegypti*. Evolution **26**, 185-196 (1972)

Falconer, D.S.: Introduction to quantitative genetics. Edinburgh: Oliver and Boyd 1960

Hacker, C.S.; Ling, Wei-Wei; Hsi, B.P.; Crovello, T.J.: An application of mathematical modeling to the study of reproductive adaptations in the yellow fever mosquito, *Aedes aegypti*. J. Med. Ent. **13**, 485-492 (1977)

Hill, W.G.: Estimation of realized heritabilities from selection experiments, I. Divergent selection. Biometrics **28**, 747-765 (1972)

Li, C.C.: Population Genetics. Chicago: University of Chicago Press 1955

Mellanby, K.: The alarm reaction of mosquito larvae. Ent. Exp. and Appl. **1**, 153-160 (1958)

Schlosser, I.J.; Buffington, J.D.: The energetics of r- vs. K-selection in two African strains of *Aedes aegypti*. Ann. Ent. Soc. Am. **70**, 196-202 (1977)

Snedecor, G.W.; Cochran, W.G.: Statistical methods. Ames: Iowa State University Press 1967

Trpis, M.; Hausermann, W.: Demonstration of differential domesticity of *Aedes aegypti* (L.) (Diptera: Culicida) in Africa by mark-release-recapture. Bull. ent. Res. **65**, 199-208 (1975)

Trpis, M.; Hausermann, W.: Genetics of house-entering behavior in East African populations of *Aedes aegypti* (L.) (Diptera: Culicidae) and its relevance to speciation. Bull. ent. Res. **68**, 521-532 (1978)

Wilson, E.O.; Bossert, W.H.: A Primer of Population Biology. Stamford: Sinauer 1971

Wright, S.: Evolution and genetics of populations, Vol. 1. The genetic and biometric foundations. Chicago: University of Chicago Press 1968

Received May 14, 1979
Communicated by R.C. Lewontin

Dr. R.E. Duhrkopf
Laboratories of Medical Entomology
Department of Pathobiology
School of Hygiene and Public Health
The Johns Hopkins University
Baltimore, MD 21205 (USA)

Dr. S.S.Y. Young
Department of Genetics
The Ohio State University
Columbus, Ohio 43210 (USA)