Journal of the American Mosquito Control Association, 10(1):93–100, 1994

STUDIES ON THE INHERITANCE OF REPELLENT TOLERANCES IN *AEDES AEGYPTI*[1]

L. C. RUTLEDGE,[2] R. K. GUPTA,[3] G. N. PIPER[4] AND C. A. LOWE[5]

Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800

ABSTRACT. Two laboratory strains and 6 inbred strains of *Aedes aegypti* were tested against deet, ethyl hexanediol, dimethyl phthalate, and Indalone®. Reciprocal crosses and backcrosses of 2 inbred strains were tested against deet only. Results obtained were compatible with a quantitative genetic model in which the effects of the factors involved were multiplicative. Certain inbred strains differed significantly from cognate laboratory and/or inbred strains in tolerance to one or more test materials. Heritability in the broad sense (H^2) was estimated at 0.05 for deet, 0.22 for ethyl hexanediol, 0.48 for dimethyl phthalate, and 0.51 for Indalone. Partial dominance was observed in the inheritance of tolerance to deet.

INTRODUCTION

Little is currently known of the genetic basis of chemotaxis in insect vectors of disease (Wright and Pal 1967, Steiner et al. 1982). Most studies have been conducted at the organismic level and reported in terms of attractancy, repellency, avoidance, deterrence, sensitivity, irritability, or excito-repellency.

Falk and Atidia (1975) and Tompkins et al. (1979) demonstrated that the genetic factors that determine gustatory responses of the fruit fly, *Drosophila melanogaster* Meigen, to sodium chloride and quinine sulfate are recessive and sex-linked. Fuyama (1976) found that females were more strongly attracted than males to lactic acid, ethyl acetate, ethanol, and butanal, indicating that one or more factors affecting olfactory responses may also be sex-linked. However, factors affecting olfactory responses to ethyl acetate and other esters also occur on chromosome 2 (Fuyama 1978).

Fuyama (1978) reported that olfactory responses of homozygous 2nd chromosome lines of *D. melanogaster* to ethyl acetate, ethyl propionate, 2-butanone, 2-pentanone, and 3-pentanone were negatively correlated with the corresponding responses to lactic acid. This finding indicates that the factors that determine responses to esters and ketones are different from those that determine responses to acids.

Kikuchi (1973) isolated an olfactory mutant of *D. melanogaster* attracted by 18 compounds that repelled the parent strain. The observed alteration of olfactory function was interpreted in terms of a functional group called the "bifunctional unit", which was common to 14 of the compounds. Because low concentrations of repellents are attractant (Mehr et al. 1990), an alternative interpretation could be that the effect of the mutation was to shift the thresholds of response to the test compounds.

Threlkeld (1986) reported increased avoidance of permethrin in 2 strains of *D. melanogaster* derived from the Canton-S strain by mutation with ethylmethanesulfonate. Other studies have demonstrated increased avoidance/irritability in the house mosquito, *Culex pipiens* Linn. (Gaaboub and Dawood 1975), and the house fly, *Musca domestica* Linn. (Fay et al. 1958), in response to selection with organochlorine and organophosphate insecticides. Pluthero and Singh (1984) have provided a useful review of this subject.

Scirocchi and Milita (1986) selected a strain of *M. domestica* tolerant to deet, a commercial repellent, from field-collected material. Becker (1970) selected 2 strains tolerant to deet from wild-type *D. melanogaster*. The deet-tolerant strains reported by Becker (1970) exhibited cross tolerance to a chemically unrelated repellent, oil of citronella (Gramineae: *Cymbopogon nardus*). Crossing experiments indicated that the genetic factors involved were incompletely dominant.

In a prior study, we demonstrated significant differences in tolerance to deet among laboratory strains of the yellow fever mosquito, *Aedes aegypti* (Linn.), and demonstrated that the differences were stable over several generations of laboratory culture (Rutledge et al. 1978). The

[1] The opinions and assertions contained herein are the views of the authors and should not be construed as reflecting the views of the Department of the Army or the Department of Defense. Use of a trade name does not imply official approval or endorsement of the product named.

[2] Current address: 11 Circle Way, Mill Valley, CA 94941-3420.

[3] Current address: Headquarters, U.S. Army Medical Research and Development Command, ATTN: SGRD-PLA, Fort Detrick, Frederick, MD 21701-5012.

[4] Current address: 1202 Glen Oro Drive, Orland, CA 95963-2012.

[5] Current address: Southern Forest Experiment Station, Room T-10210, U.S. Postal Services Building, 701 Loyola Avenue, New Orleans, LA 70113-1912.

Table 1. Significance of tests for goodness of fit of the linear regression model.

Type of test	No. of tests	
	Significant	Not significant
2 parent strains vs. 4 repellents	0	8
6 inbred strains vs. 4 repellents	0	24
6 crosses vs. 1 repellent	0	6
Totals	0	38

purpose of the present study was to demonstrate the heritability of repellent tolerances in *Ae. aegypti* and to investigate the mode of inheritance in laboratory strains of that species.

MATERIALS AND METHODS

Parent strains: Parent strains of *Ae. aegypti* used in the study were obtained from Dr. G. B. Craig, University of Notre Dame (Rutledge and Piper 1984). The MOYO INDOOR strain is a laboratory strain of *Ae. aegypti aegypti* (Linn.) originally collected from a field population in Kenya. The MASAKA strain is a laboratory strain of *Ae. aegypti formosus* Walker originally collected from a field population in Uganda.

Inbred strains: Inbred strains R-3, R-13, R-21, and R-44, derived from the MOYO INDOOR strain, and inbred strains S-15 and S-22, derived from the MASAKA strain, were used in the study. Inbred strains were produced by single-pair, brother–sister mating for 10 generations as described by Rutledge and Piper (1984). The theoretical proportion of heterozygous gene pairs remaining after 10 generations of single-pair, brother–sister mating is 0.114 (Suzuki et al. 1986).

Rearing procedures: Colonies of the parent and inbred strains were maintained as described by Rutledge et al. (1978).

Crosses and backcrosses: Reciprocal crosses and backcrosses of inbred strains R-13 and S-22 were performed. Males and females were separated at approximately 24 h of age to preclude mating of cognates and were subsequently checked for error and combined as needed for the respective crosses and backcrosses. Materials and methods employed for crossing and backcrossing were similar to those described by Rutledge and Piper (1984) for inbreeding.

Test materials: Parent and inbred strains were tested against 4 chemically unrelated repellents: 1) *N,N*-diethyl-1,3-methylbenzamide (deet), technical grade, McLaughlin Gormley King Company, Minneapolis, MN; 2) dimethyl phthalate, technical grade, FMC Corporation, Middleport, NY; 3) 2-ethyl-1,3-hexanediol (ethyl hexanediol), practical grade, Eastman Organic Chemicals, Rochester, NY; and 4) butyl-3,3-dihydro-2,2-dimethyl-4-oxo-2*H*-pyran-6-carboxylate (Indalone®), technical grade, K & K Laboratories, Plainview, NY. The F_1 and backcross generations were tested against deet only.

Test method: Doses of 0.02, 0.04, 0.08, and 0.16 mg/cm^2 of the stated test materials were tested against nulliparous females in the age range of 5–15 days, using the *in vitro* bloodfeeding test method of Rutledge et al. (1978). The test system features an assembly of 5 temperature-controlled feeding cups (3.0 cm diam) that contain blood covered with a natural membrane (goldbeater's skin) when in use. Test materials were diluted in ethanol to provide the stated doses, and 0.025 ml of the solutions and a control (ethanol only) were applied at random to the membranes. After 5 min, the membranes were exposed to 250 mosquitoes by withdrawing a slide in the floor of a 25 × 25 × 25-cm test cage placed over the assembly. Mosquitoes feeding on the 4 treatments and the control were counted at 2-min intervals for 20 min. Feeding counts were totaled over 6 replications for analysis.

Data reduction: Treatment totals were converted to percentage of the control total to express the responses to the treatments in terms of the observed limiting response. The linear regression of the responses (probit transformation) on the corresponding doses (logarithmic transformation) was then calculated. If the coefficient of determination (r^2) in the regression analysis was ≤ 0.95, the data were reanalyzed by the weighted regression method of Finney (1947:185–197) for maximum likelihood analysis of repellency data. If goodness of fit was greater by the weighted regression method, the weighted regression method was adopted in lieu of the simple regression method for that analysis. Goodness of fit was tested by the variance ratio method (Finney 1947).

RESULTS AND DISCUSSION

Goodness of fit: No statistically significant departures from the dose–response regression model were observed in the study (Table 1). Because the data obtained conformed closely with a quantitative model, the variation observed in the study

Table 2. Dose–response data for 2 parent and 6 inbred strains of *Aedes aegypti* in tests against 4 repellents.

Strain	ED_{50}[1]	Coefficient of regression[2]
	Deet	
MOYO INDOOR	0.045 (0.018, 0.079)	−2.5 (−4.2, −0.9)
R-3	0.024 (*.***, *.***)	−3.2 (−7.0, +0.6)
R-13	0.025 (0.007, 0.039)	−2.5 (−3.8, −1.1)
R-21	0.017 (0.011, 0.023)	−2.1 (−2.7, −1.6)
R-44	0.028 (0.017, 0.037)	−2.6 (−3.4, −1.7)
MASAKA	0.023 (0.013, 0.033)	−2.4 (−3.3, −1.6)
S-15	0.026 (0.018, 0.032)	−2.8 (−3.5, −2.1)
S-22	0.008 (0.006, 0.009)	−1.5 (−1.6, −1.3)
	Ethyl hexanediol	
MOYO INDOOR	0.549 (*.***, *.***)	−1.3 (−3.1, +0.5)
R-3	0.199 (0.144, 0.454)	−2.0 (−2.7, −1.4)
R-13	0.077 (0.049, 0.156)	−2.0 (−3.2, −0.9)
R-21	0.226 (*.***, *.***)	−3.2 (−11.8, +5.3)
R-44	0.093 (0.071, 0.140)	−1.8 (−2.5, −1.2)
MASAKA	0.170 (*.***, *.***)	−2.6 (−5.7, +0.5)
S-15	0.306 (0.127, *.***)	−1.0 (−1.7, −0.2)
S-22	0.057 (0.038, 0.084)	−1.8 (−2.6, −1.0)
	Dimethyl phthalate	
MOYO INDOOR	0.037 (0.018, 0.055)	−2.1 (−3.1, −1.0)
R-3	0.073 (0.048, 0.095)	−3.8 (−6.1, −1.5)
R-13	0.111 (0.066, 0.525)	−2.3 (−3.9, −0.8)
R-21	0.027 (*.***, *.***)	−3.3 (−7.1, +0.6)
R-44	0.040 (0.031, 0.050)	−2.9 (−3.7, −2.1)
MASAKA	0.044 (0.033, 0.056)	−2.8 (−3.6, −1.9)
S-15	0.050 (0.030, 0.077)	−2.4 (−3.6, −1.2)
S-22	0.046 (0.015, 0.081)	−2.9 (−5.2, −0.7)
	Indalone	
MOYO INDOOR	0.033 (0.022, 0.044)	−3.2 (−4.3, −2.1)
R-3	0.019 (0.008, 0.030)	−2.3 (−3.3, −1.3)
R-13	0.038 (0.018, 0.058)	−2.1 (−3.3, −0.9)
R-21	0.009 (*.***, *.***)	−2.2 (−8.6, +4.2)
R-44	0.017 (0.001, 0.032)	−2.3 (−3.8, −0.8)
MASAKA	0.019 (0.009, 0.028)	−2.4 (−3.2, −1.5)
S-15	0.020 (0.000, 0.041)	−3.2 (−5.8, −0.5)
S-22	0.001 (*.***, *.***)	−1.0 (−2.2, +0.1)

[1] Median effective dose in mg/cm², 95% confidence limits in parentheses; *.*** indicates no data obtained.
[2] Slope of dose–response regression line, 95% confidence limits in parentheses.

was evidently quantitative (multiple-factor) variation. Because the observed responses to the test materials were proportional to the logarithm of the dose applied, the effects of the factors involved were evidently multiplicative (Brewbaker 1964, Mather and Jincks 1977).

Strain comparisons: Table 2 gives estimates obtained for the median effective doses (ED_{50}s) and coefficients of regression of the test materials in tests against the parent and inbred strains. Estimates obtained for several ED_{50}s were outside the range of doses tested. Such estimates are valid if the regression model used is known to be correct (Finney 1971). The model used in the study was verified by goodness-of-fit tests (Table 1). Low ED_{50}s were obtained primarily in tests against Indalone; high ED_{50}s were obtained primarily in tests against ethyl hexanediol.

On the basis of the confidence intervals of the ED_{50}s, inbred strain S-22 was significantly less tolerant to deet than was its parent (MASAKA) strain, and inbred strain R-13 was significantly more tolerant to dimethyl phthalate than was its parent (MOYO INDOOR) strain. Strain S-15 was more tolerant to deet and ethyl hexanediol than was cognate strain S-22. Strain R-3 was

Table 3. Block (repellent) and treatment (strain) means of ED_{50}s obtained in tests of 4 repellents against 8 strains of *Aedes aegypti*.[1]

Block means		Treatment means	
Deet	0.023a	MOYO INDOOR	0.076a
Ethyl hexanediol	0.166b	R-3	0.049a
Dimethyl phthalate	0.050c	R-13	0.051a
Indalone	0.015a	R-21	0.034a
		R-44	0.038a
		MASAKA	0.042a
		S-15	0.053a
		S-22	0.014b

[1] Antilog of the mean log ED_{50}. Means followed by the same letter do not differ at the 5% level of significance.

more tolerant to ethyl hexanediol than was cognate strain R-44. The statistically significant differences between parent and cognate inbred strains observed in the study can be interpreted as reflecting corresponding differences in the genetic determinants of tolerance to the test materials. Segregants that differ significantly from the parental average have been termed transgressive (Brewbaker 1964).

A two-way analysis of variance of the ED_{50}s of Table 2 (logarithmic transformation) was performed to determine the significance of differences among strain and repellent means. Differences among the strain means were statistically significant ($F = 2.67$, $df = 7,21$, $P < 0.05$). Fisher's (protected) least significant difference (Steel and Torrie 1980) indicated that strain S-22 was significantly less tolerant to the test materials than were all other strains (Table 3). Significant differences in nonspecific tolerance to repellents also occur at the species level (Rutledge et al. 1983).

The significant difference in nonspecific tolerance observed in strain S-22 differed from nonspecific resistance to insecticides ("vigor tolerance") and from the cross tolerance of *D. melanogaster* to deet and oil of citronella reported by Becker (1970) in that strain S-22 was less tolerant, not more tolerant, to the materials involved. Because strain S-22 also exhibited reduced clutch size, prolonged hatching time, and reduced survival in the egg, larval, and pupal stages compared with the parent (MASAKA) strain (Rutledge and Piper 1984), the results obtained can be interpreted as due to inbreeding depression.

Differences among the repellent means also were statistically significant ($F = 23.68$, $df = 3,21$, $P < 0.05$). Fisher's (protected) least significant

Table 4. Analysis of variance within and between 6 inbred strains of *Aedes aegypti* tested against 4 repellents.

Parameter[1]	Deet	Ethyl hexanediol	Dimethyl phthalate	Indalone
		Mean squares		
M_1[2]	0.2445	0.7927	0.5945	1.6537
M_2[3]	0.2007	0.3692	0.1270	0.3182
		Variance components		
$V_G = (M_1 - M_2)/r$[4]	0.0110	0.1059	0.1169	0.3339
$V_E = M_2$	0.2007	0.3692	0.1270	0.3182
		Heritability		
$H^2 = V_G/(V_G + V_E)$[5]	0.0517	0.2229	0.4793	0.5120

[1] Method and notation of Ehrman and Parsons (1976).

[2] Weighted average of the between-strain variance of the MOYO INDOOR strains (R-3, R-13, R-21, and R-44) and the between-strain variance of the MASAKA strains (S-15 and S-22). Variances were computed from the logarithms of the ED_{50}s with adjustment for the number (4) of observations (dose levels) on which the estimates of the ED_{50}s were based.

[3] Average of within-strain variances computed as the square of the reciprocal of the slope of the dose–response regression line (Goldstein 1964).

[4] V_G is estimate of genetic variance; r is number (4) of observations (dose levels) on which the estimates were based.

[5] H^2 is heritability in the broad sense, also known as the degree of genetic determination (Ehrman and Parsons 1976) and the coefficient of intraclass correlation (Steel and Torrie 1980).

Table 5. Dose–response data for parent, cross, and backcross generations of *Aedes aegypti* in tests against deet.

Generation	Abbr.	ED_{50}[1]	Coefficient of regression[2]
S-22	P_1	0.008 (0.006, 0.009)	−1.5 (−1.6, −1.3)
R-13	P_2	0.025 (0.007, 0.039)	−2.5 (−3.8, −1.1)
S-22(M) × R-13(F)	F_{1a}	0.011 (*.***, *.***)	−2.3 (−8.1, +3.5)
F_{1a}(F) × S-22(M)	B_{1a}	0.013 (0.006, 0.020)	−2.2 (−2.8, −1.5)
F_{1a}(M) × R-13(F)	B_{2a}	0.011 (0.002, 0.021)	−1.8 (−2.7, −0.9)
S-22(F) × R-13(M)	F_{1b}	0.024 (0.022, 0.026)	−2.9 (−3.2, −2.7)
F_{1b}(M) × S-22(F)	B_{1b}	0.015 (*.***, *.***)	−3.7 (−7.7, +0.3)
F_{1b}(F) × R-13(M)	B_{2b}	0.007 (*.***, *.***)	−1.2 (−2.8, +0.4)

[1] Median effective dose in mg/cm², 95% confidence limits in parentheses; *.*** indicates no data obtained.
[2] Slope of dose–response regression line, 95% confidence limits in parentheses.

difference (Steel and Torrie 1980) indicated that all differences were statistically significant, except the difference between the means of deet and Indalone (Table 3).

Because the standard deviation of the test population is equal to the reciprocal of the coefficient of regression (Goldstein 1964), Table 2 provides an index of the variability of laboratory and inbred strains in tests against chemically unrelated repellents. A two-way analysis of variance of coefficients of regression was performed to determine the significance of differences among mean coefficients of regression.

The question of whether inbred strains should be less variable because of increased homozygosity or more variable because of decreased homeostasis is controversial. In the present study, differences among mean coefficients of regression of mosquito strains were not statistically significant ($F = 1.08$, df = 7,21, $P > 0.05$), indicating that the inbred strains were neither less variable nor more variable than the parent (laboratory) strains.

Significantly different coefficients of regression imply different mechanisms of action of test materials, whereas equal coefficients of regression imply similar mechanisms of action of test materials (Goldstein 1964). In the present study, differences among mean coefficients of regression of test materials were not statistically significant ($F = 2.63$, df = 3,21, $P > 0.05$). On this basis, it can be concluded that the test materials used in the study acted by similar mechanisms. This conclusion is logically equivalent to that stated earlier, that factors affecting repellent tolerances in strain S-22 were nonspecific.

Table 4 shows the within- and between-strain analysis of variance of inbred strains for each of the repellents tested (Ehrman and Parsons 1976). Heritability in the broad sense (H^2) was least for deet and greatest for Indalone.

Crosses and backcrosses: Table 5 shows estimates obtained for ED_{50}s and coefficients of regression in tests of deet against strains S-22 and R-13 (data repeated from Table 2 for comparison) and their reciprocal crosses and backcrosses. Except for backcross generation B_{2b}, all estimates obtained for cross and backcross generations were intermediate between those obtained for the parent strains. This result is compatible with the quantitative model of inheritance indicated by the goodness-of-fit tests of Table 1.

Comparison of the reciprocal crosses and backcrosses as F_{1a} vs. F_{1b}, B_{1a} vs. B_{1b}, and B_{2a} vs. B_{2b} provides a test for non-autosomal inheritance because subscript "a" refers to the cross S-22(M) × R-13(F) and subscript "b" refers to the cross S-22(F) × R-13(M) (Table 5). The *t*-test of the paired ED_{50}s (logarithmic transformation) was not statistically significant ($t = -0.44$, df = 2, $P > 0.05$), indicating that non-autosomal inheritance did not occur in the crosses studied.

In many studies it has been found that hybrids from crosses of inbred strains were less variable than their parents because they were both genetically uniform and heterozygous (more homeostatic). Variances of the S-22 strain (P_1) and R-13 strain (P_2), computed as the square of the reciprocal of the slope of the dose–response regression line (Goldstein 1964), were combined to provide an estimate of the variance of inbred strains, $V = 0.3022$. Variances of the F_{1a} and F_{1b} crosses, computed similarly, were combined to provide an estimate of the variance of hybrids from crosses of inbred strains, $V = 0.1540$. The variance ratio (0.3022/0.1540 = 1.96) was not statistically significant ($F = 1.96$, df = 4,4, $P > 0.05$). On this basis it was concluded that hybrids from crosses of inbred strains were not less variable than their parents in this study. This result is similar to that obtained in comprising the variability of laboratory strains and inbred strains (see above).

An independent estimate of the heritability of deet-tolerances (H^2) was obtained by comparison of data from Tables 2 and 5 as suggested by

Table 6. Data for joint scaling test of parent, cross, and backcross generations of *Aedes aegypti* in tests against deet.

Generation	Model[1]			log ED_{50}[2]		
	m	[d]	[h]	Observed	Expected	(Obs. − Exp.)^2
P_1	1.0	1.0	0.0	−2.0969	−2.0155	0.0066
P_2	1.0	−1.0	0.0	−1.6021	−1.8201	0.0475
F_{1a}	1.0	0.0	1.0	−1.9586	−1.8575	0.0102
B_{1a}	1.0	0.5	0.5	−1.8861	−1.9365	0.0025
B_{2a}	1.0	−0.5	0.5	−1.9568	−1.8388	0.0144
F_{1b}	1.0	0.0	1.0	−1.6198	−1.8575	0.0565
B_{1b}	1.0	0.5	0.5	−1.8239	−1.9365	0.0127
B_{2b}	1.0	−0.5	0.5	−2.1549	−1.8388	0.0999
					$\chi_{[5]}^2 = 0.25$[3]	

[1] Symbols used are defined as: m = midparent value; [d] = departure of parental line from midparent value; [h] = departure of heterozygote from midparent value (dominance deviation). See Mather and Jincks (1977) for discussion.

[2] Observed value is logarithm of ED_{50} as given in Table 5. Expected value is value obtained by substituting the estimates of m, [d], and [h] given in Fig. 1 into the model shown in columns 2 to 4 (see text).

[3] Value is not significant at the 5% level.

Suzuki et al. (1986). Variances of the MOYO INDOOR and MASAKA strains, computed as the square of the reciprocal of the slope of the dose–response regression line (Goldstein 1964), were combined to provide an estimate of phenotypic variance, $V_P = 0.1668$. Variances of the F_{1a} and F_{1b} crosses, computed similarly, were combined to provide an estimate of environmental variance, $V_E = 0.1540$. The genotypic variance was then obtained as $V_G = V_P - V_E = 0.0128$, and H^2 was obtained as $H^2 = V_G/V_P = 0.0767$.

This estimate agrees closely with that obtained earlier using independent data (Table 4). However, heritability in the broad sense (H^2), which is the proportion of phenotypic variance contributed by genetic variance, differs by population and environment and cannot be extrapolated from one population or environment to another (Suzuki et al. 1986). Heritability in the narrow sense (h^2), which is the proportion of phenotypic variance contributed by additive genetic variance only, could not be obtained in the present study because the F_2 generation was not reared.

The joint scaling test provides a test of the quantitative model and provides best possible estimates of the parameters required to account for differences among means when the model is adequate (Mather and Jincks 1977). Table 6 gives the data for the joint scaling test of the ED_{50} values of Table 5. An unweighted analysis was performed in lieu of the weighted analysis of Mather and Jincks (1977) because the numbers of mosquitoes, replications, and doses were equal in all tests.

Estimates obtained for the midparent value (m), the departure of the parental lines from the midparent value ([d]), and the departure of heterozygotes from the midparent value (dominance deviation, [h]) are shown in Fig. 1. These values were used in conjunction with the model shown in columns 2 to 4 of Table 6 to compute the expected values shown in column 6. For example, (1)(−1.9178) + (1)(−0.0977) + (0)(+0.0603) = −2.0155, as shown in column 6 for generation P_1. Observed values are shown in column 5. The χ^2 test of the quantitative model (Mather and Jincks 1977) is given by columns 5, 6, and 7. The value of χ^2 obtained was not

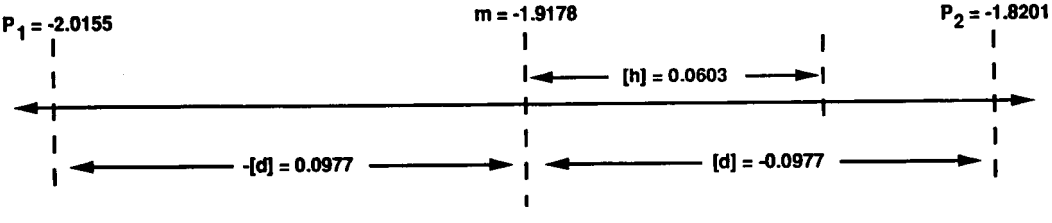

Fig. 1. Departures of parent strains ([d]) and heterozygotes ([h]) from the mid-parent value (m) as estimated by the joint scaling test. All values are in log mg/cm^2.

statistically significant, indicating that the observed data did not differ significantly from the quantitative model ($\chi^2 = 0.25$, df = 5, $P > 0.05$).

Figure 1 shows that the observed dominance deviation, [h], was positive and less than [d]. This result agrees with the prior finding of incomplete dominance for deet-tolerance in *D. melanogaster* by Becker (1970). The degree of dominance, calculated as [h]/[d] (Strickberger 1968), was 0.62.

Figure 1 shows that heterosis did not occur in the study, because overdominance ([h] > [d]) was not observed. This result was also in accord with expectation. To our knowledge there has been only one report of heterosis in mosquitoes to date. Asman et al. (1963) reported increased resistance to radiation and reduced time to pupation and adult emergence in progeny of certain crosses of laboratory and inbred strains of *Ae. aegypti*. In contrast, Rutledge et al. (1970) did not observe significantly increased ovogenesis, oviposition, or eclosion in progeny of crosses of laboratory strains of *Anopheles stephensi* Liston, and Shahid and Reisen (1981) did not observe heterosis in life table characteristics of progeny of crosses of laboratory and inbred strains of *Culex tritaeniorhynchus* Giles.

CONCLUSIONS

Several lines of evidence in the study indicated that the variation observed in the study was quantitative. Results of the joint scaling test were compatible with a quantitative model in which the effects of the genetic factors involved were multiplicative.

Heritability of repellent tolerance (heritability in the broad sense, H^2) was 0.05 for deet, 0.22 for ethyl hexanediol, 0.48 for dimethyl phthalate, and 0.51 for Indalone, based on a within- and between-strain analysis of variance. Deet-tolerance was incompletely dominant.

The conclusions of this study apply specifically to the populations studied. Studies utilizing other strains of *Ae. aegypti* would not necessarily involve the same loci or the same alleles and would not necessarily give the same results. The low value of H^2 obtained for deet indicates that environmental variance was the significant component of phenotypic variance of deet-tolerance in this study. Studies utilizing the same strains of *Ae. aegypti* under other environmental conditions would not necessarily give the same results.

ACKNOWLEDGMENTS

This paper is dedicated to George B. Craig, Jr. Moufied A. Moussa and Ruth Lynn Hooper provided executive support for the study at critical points. Numerous personnel of the Letterman Army Institute of Research provided technical assistance.

REFERENCES CITED

Asman, M. M., G. B. Craig and K. S. Rai. 1963. Heterosis in *Aedes aegypti*. Bull. Entomol. Soc. Am. 9:173.

Becker, H. J. 1970. The genetics of chemotaxis in *Drosophila melanogaster*: selection for repellent insensitivity. Mol. Gen. Genet. 107:194–200.

Brewbaker, J. L. 1964. Agricultural genetics. Prentice-Hall Inc., Englewood Cliffs, NJ.

Ehrman, L. and P. A. Parsons. 1976. The genetics of behavior. Sinauer Associates Inc., Sunderland, MA.

Falk, R. and J. Atidia. 1975. Mutation affecting taste perception in *Drosophila melanogaster*. Nature 254: 325–326.

Fay, R. W., J. W. Kilpatrick and G. C. Morris. 1958. Malathion resistance studies on the house fly. J. Econ. Entomol. 51:452–453.

Finney, D. J. 1947. Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge Univ. Press, Cambridge.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge Univ. Press, Cambridge.

Fuyama, Y. 1976. Behavior genetics of olfactory responses in *Drosophila*: I. Olfactometry and strain differences in *Drosophila melanogaster*. Behav. Genet. 6:407–420.

Fuyama, Y. 1978. Behavior genetics of olfactory responses in *Drosophila*: II. An odorant-specific variant in a natural population of *Drosophila melanogaster*. Behav. Genet. 8:399.

Gaaboub, I. A. and M. R. Dawood. 1975. Irritability status of adults of *Culex pipiens* L. under selection pressure with lethal concentrations of DDT and malathion. Z. Angew. Entomol. 77:126–132.

Goldstein, A. 1964. Biostatistics: an introductory text. Macmillan Co., New York.

Kikuchi, T. 1973. Genetic alteration of olfactory functions in *Drosophila melanogaster*. Jpn. J. Genet. 48:105–118.

Mather, K. and J. L. Jincks. 1977. Introduction to biometrical genetics. Cornell Univ. Press, Ithaca, NY.

Mehr, Z. A., L. C. Rutledge, M. D. Buescher, R. K. Gupta and M. M. Zakaria. 1990. Attraction of mosquitoes to diethyl methylbenzamide and ethyl hexanediol. J. Am. Mosq. Control Assoc. 6:469–476.

Pluthero, F. G. and R. S. Singh. 1984. Insect behavioural responses to toxins: practical and evolutionary considerations. Can. Entomol. 116:57–68.

Rutledge, L. C. and G. N. Piper. 1984. Inbreeding of *Aedes aegypti* and *Anopheles stephensi*. Mosq. News 44:43–50.

Rutledge, L. C., R. A. Ward and W. E. Bickley. 1970. Experimental hybridization of geographic strains of *Anopheles stephensi* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 63:1024–1030.

Rutledge, L. C., D. M. Collister, V. E. Meixsell and G. H. G. Eisenberg. 1983. Comparative sensitivity of representative mosquitoes (Diptera: Culicidae) to repellents. J. Med. Entomol. 20:506–510.

Rutledge, L. C., M. A. Moussa, C. A. Lowe and R. K. Sofield. 1978. Comparative sensitivity of mosquito species and strains to the repellent diethyl toluamide. J. Med. Entomol. 14:536–541.

Scirocchi, A. and A. Milita. 1986. Repellency of some insecticides towards *Musca domestica*. Ann. Ist. Super. Sanita 22:311–313. [In Italian.]

Shahid, A. A. and W. K. Reisen. 1981. *Culex tritaeniorhynchus*: failure to detect heterosis in the life table characteristics of outcrossed laboratory colonies. Entomol. Exp. Appl. 30:58–62.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill, New York.

Steiner, W. W. M., W. J. Tabachnick, K. S. Rai and S. Narang (editors). 1982. Recent developments in the genetics of insect vectors of disease. Stipes Publishing Co., Champaign, IL.

Strickberger, M. H. 1968. Genetics. Macmillan Co., New York.

Suzuki, D. T., A. J. F. Griffiths, J. H. Miller and R. C. Lewontin. 1986. An introduction to genetic analysis, 3rd ed. W. H. Freeman & Co., New York.

Threlkeld, S. F. H. 1986. Some effects of permethrin on the behavior of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Can. Entomol. 118:481–486.

Tompkins, L., M. J. Cardosa, F. V. White and T. G. Sanders. 1979. Isolation and analysis of chemosensory behavior mutants in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 76:884–887.

Wright, J. W. and R. Pal (editors). 1967. Genetics of insect vectors of disease. Elsevier, Amsterdam.