LINKAGE GROUPS IN *ANOPHELES QUADRIMACULATUS*[1]

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INTRODUCTION. Interest in mosquito genetics has grown considerably in the last few years. This growth, at least in part, is the result of ever increasing numbers of investigations which have demonstrated that the resistance of insects to insecticides has a genetic basis (Davidson and Mason, 1963). The realization that resistance to insecticides is the most important single problem facing the world-wide malaria eradication program has stimulated many workers to seek information on the genetics of *Anopheles* mosquitoes.

Basic genetic information on *Anopheles* mosquitoes is extremely limited. For a summary of the early work in this area, see the review by Kitzmiller (1953), and for later developments, see Rozeboom and Kitzmiller (1958), and Davidson and Mason (1963). Some progress has been made on the elucidation of certain fundamental aspects of anopheline genetics (French, 1963).

The present paper reports the linkage group relationships of the dieldrin-resistance locus and the morphological gene "nonstripe." The results provide an additional tool for further work on the inheritance of resistance phenomena and for the further study of the fundamental genetics of *Anopheles*.

MATERIALS AND METHODS. The genetic techniques utilized to determine the phenotypes for dieldrin resistance have been explained elsewhere (French and Kitzmiller, 1963a, 1963b) and will not be considered in detail here. The technique, in brief, consists of subjecting late fourth instar larvae to a given concentration of dieldrin (8 p.p.m.) and observing, at intervals, the mortality produced by this insecticide over a 24-hour period. It has been determined that when appropriate experimental standards are maintained, the various phenotypes (with respect to dieldrin resistance) will survive for differential lengths of time in the given concentration of insecticide; the phenotype of each larva tested can thus be clearly and precisely determined. The homozygous dieldrin-resistant larvae survive a 24-hour exposure to 8 p.p.m. dieldrin. The homozygous susceptible larvae die within the first 3 to 4 hours. The heterozygotes begin to die after the last susceptible is dead; all the heterozygotes are dead well within the 24-hour test period.

Typical mortality curves are shown in Figure 1. The probits of cumulative mortality are plotted against the log of time.

Line A shows typical results obtained from testing homozygous susceptibles. Line B depicts the results obtained from testing populations of known heterozygotes. F_2 results are shown by Line C, with the characteristic plateaus. The lower plateau distinguishes the susceptibles from the heterozygotes, and the upper plateau distinguishes the heterozygotes from the homozygous-resistant larvae. The results of backcrosses of heterozygotes to homozygous susceptibles are shown by Line D, and to homozygous resistants by Line E. The characteristic plateaus clearly separate the appropriate genotypes.

The results of crosses involving the nonstripe characteristic have been published (French and Kitzmiller, 1963c).

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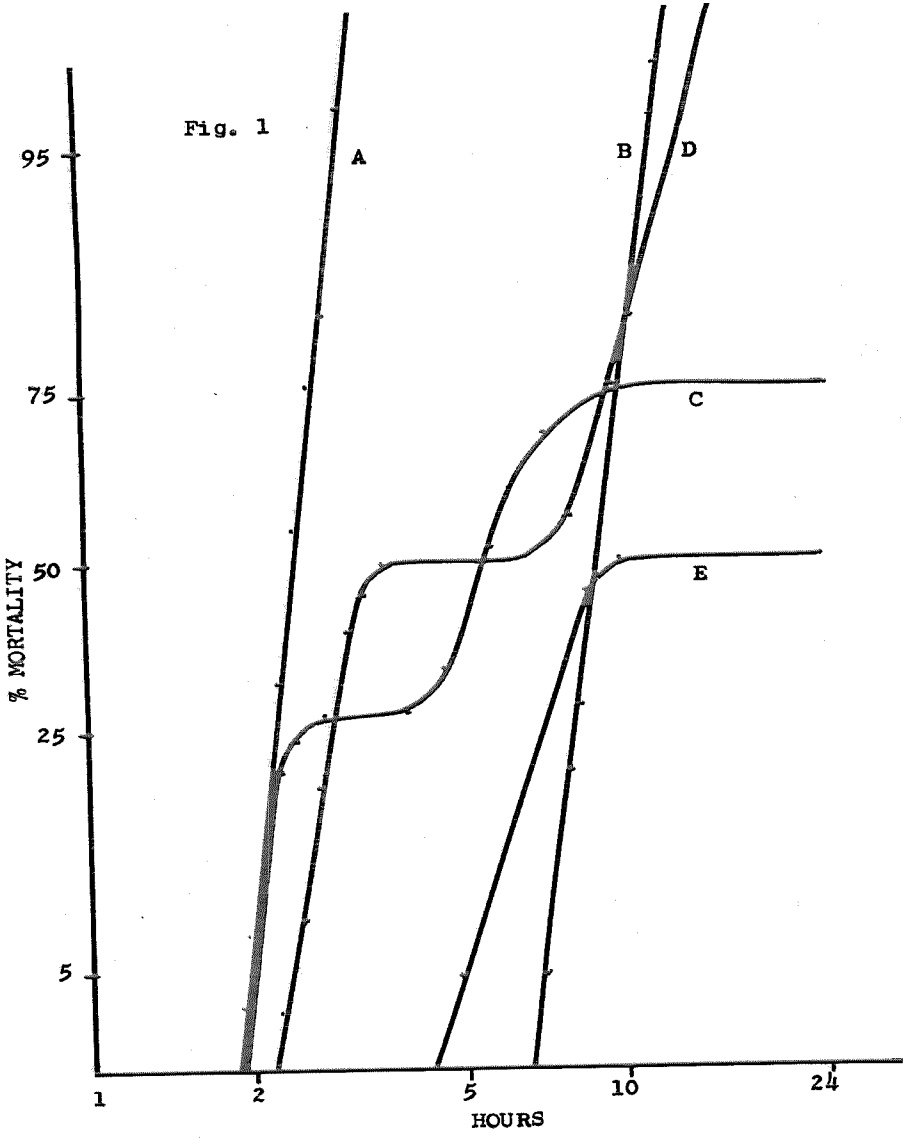
Fig. 1
A
B
D
C
E
95
75
50
25
5
% MORTALITY
1
2
5
10
24
HOURS

Nonstripe is an autosomal gene recessive to its allele, stripe. The stripe phenotype is characterized by the presence of a pronounced white pigmentation on the middorsal surface of the abdomen and thorax of larvae, pupae and adults. The nonstripe phenotype lacks such pigmentation.

The strains of mosquitoes utilized for these studies were developed from two populations of *A. quadrimaculatus* maintained in the laboratory at the University of Illinois. One of these populations contained individuals all of which were homozygous for stripe. The other population was pure for the nonstripe gene. It was determined that both of the above populations contained a relatively high frequency of the gene for dieldrin resistance.

Blood-fed females from both the stripe and nonstripe populations were isolated individually into shell vials and allowed to deposit eggs. The larvae were allowed to hatch in the vials and were reared in the usual manner. The progeny from each female were thus reared as a separate "family." Groups of 25–30 larvae were selected at random from each "family" and were screened for their dieldrin-resistant phenotypes by the method described above.

The remaining untreated mosquitoes from those families which had been demonstrated by the screening procedure to be composed of individuals homozygous either for dieldrin-susceptibility or for dieldrin-resistance, were utilized to establish the four strains used in the present study. These strains were: stripe susceptible, stripe resistant, nonstripe susceptible and nonstripe resistant.

Two factors were of particular importance in the establishment of pure dieldrin-resistant and pure dieldrin-susceptible strains in a single generation without selection. The first was the extremely low frequency of multiple inseminations of *Anopheles quadrimaculatus* females (French and Kitzmiller, 1963c). The second factor was the utilization of a screening technique which permitted the accurate determination of the phenotype of each larva tested.

RESULTS. Previous limited evidence (French and Kitzmiller, 1963a) indicated that the nonstripe gene assorted independently from the gene for dieldrin-resistance. The theoretical expectations from crosses involving these two genes are presented in Tables 1 and 2. In these tables classical genetic notations are used: *ns* denotes the recessive gene nonstripe; $+ns$ represents the normal allele, stripe, of nonstripe. Phenotypic designations are also given in Tables 1 and 2, where ST denotes the stripe phenotype, and NS the nonstripe phenotype. The symbol *Dl* is used to denote the mutant gene dominant for resistance to dieldrin; the symbol $+Dl$ represents the normal recessive allele for dieldrin susceptibility. The phenotypic designation used for dieldrin resistance is RES. The phenotype of the heterozygote is designated as INT to denote an intermediate susceptibility. SUS is the phenotypic symbol used to specify the susceptible individuals.

The following crosses were made to determine whether "nonstripe" and "dieldrin-resistance" come upon the same or upon different linkage groups to determine their possible association with the mechanism of sex determination:

Cross series I:
Dieldrin-susceptible stripe females were mated to dieldrin-resistant nonstripe males.

Cross series II:
Dieldrin-resistant nonstripe females were mated to dieldrin-susceptible stripe males.

Cross series III:
Dieldrin-susceptible nonstripe females were mated to dieldrin-resistant stripe males.

Cross series IV:
Dieldrin-resistant stripe females were mated to dieldrin-susceptible nonstripe males.

In each of these series of crosses an F_1 was obtained, and its phenotype determined for both characters. The stripe-

TABLE 1.—Theoretical expectations of crosses involving the genes for nonstripe and dieldrin resistance in *Anopheles quadrimaculatus*

CROSS SERIES I:

P_1 $+^{Dl}/+^{Dl}$ $+^{ns}/+^{ns}$ ♀ ♀ × Dl/Dl ns/ns ♂ ♂
(SUS, ST) (RES, NS)

F_1 $+^{Dl}/Dl+^{ns}/ns$
(INT, ST)

BC-1A $+^{Dl}/Dl$ $+^{ns}/ns$ (F_1) ♀ ♀ × $+^{Dl}/+^{Dl}$ ns/ns ♂ ♂
(INT, ST) (SUS, NS)

BC-1B $+^{Dl}/Dl$ $+^{ns}/ns$ (F_1) ♂ ♂ × $+^{Dl}/+^{Dl}$ ns/ns ♀ ♀
(INT, ST) (SUS, NS)

Results expected from BC-1A and BC-1B;

¼ $+^{Dl}/+^{Dl}$ $+^{ns}/ns$, ¼ $+^{Dl}/+^{Dl}$ ns/ns, ¼ $+^{Dl}/Dl$ $+^{ns}/ns$, ¼ $+^{Dl}/Dl$ ns/ns
1(SUS, ST): 1(SUS, NS): 1(INT, ST): 1(INT, NS)

BC-2A $+^{Dl}/Dl$ $+^{ns}/ns$ (F_1) ♀ ♀ × Dl/Dl ns/ns ♂ ♂
(INT, ST) (RES, NS)

BC-2B $+^{Dl}/Dl$ $+^{ns}/ns$ (F_1) ♂ ♂ × Dl/Dl ns/ns ♀ ♀
(INT, ST) (RES, NS)

Results expected from BC-2A and BC-2B;

¼ $+^{Dl}/Dl$ $+^{ns}/ns$, ¼ $+^{Dl}/Dl$ ns/ns, ¼ Dl/Dl $+^{ns}/ns$, ¼ Dl/Dl ns/ns
1(INT, ST): 1(INT, NS): 1(RES, ST): 1(RES, NS):

F_2 $+^{Dl}/Dl$ $+^{ns}/ns$ (F_1) ♀ ♀ × $+^{Dl}/Dl$ $+^{ns}/ns$ (F_1) ♂ ♂
(INT, ST) (INT, ST)

Expected F_2 results;

2/16 Dl/Dl $+^{ns}/ns$, 1/16 Dl/Dl ns/ns, 4/16 $Dl/+^{Dl}+^{ns}/ns$,
1/16 Dl/Dl $+^{ns}/+^{ns}$ 2/16 $Dl/+^{Dl}+^{ns}/+^{ns}$
3(RES, ST): 1(RES, NS): 6(INT, ST):
2/16 $Dl/+^{Dl}$ ns/ns, 2/16 $+^{Dl}/+^{Dl}$ $+^{ns}/ns$, 1/16 $+^{Dl}/+^{Dl}$ ns/ns
1/16 $+^{Dl}/+^{Dl}$ $+^{ns}/+^{ns}$
2(INT, NS): 3(SUS, ST): 1(SUS, NS)

TABLE 2.—Theoretical expectations of crosses involving the genes for nonstripe and dieldrin resistance in *Anopheles quadrimaculatus*

CROSS SERIES II:

P_1 Dl/Dl ns/ns ♀ ♀ × $+^{Dl}/+^{Dl}$ $+^{ns}/+^{ns}$ ♂ ♂
(RES, NS) (SUS, ST)

CROSS SERIES III:

$+^{Dl}/+^{Dl}$ ns/ns ♀ ♀ × Dl/Dl $+^{ns}/+^{ns}$ ♂ ♂
(SUS, NS) (RES, ST)

CROSS SERIES IV:

Dl/Dl $+^{ns}/+^{ns}$ ♀ ♀ × $+^{Dl}/+^{Dl}$ ns/ns ♂ ♂
(RES, ST) (SUS, NS)

Combined Expected Results from Cross Series II, III and IV

Combined results expected from BC-1A and BC-1B:
1 (SUS, ST): 1(SUS, NS): 1(INT, ST): 1(INT, NS)

Combined results expected from BC-2A and BC-2B:
1(INT, ST): 1(INT, NS): 1(RES, ST): 1(RES, NS)

Combined results expected from F_2
3(RES, ST): 1(RES, NS): 6(INT, ST): 2(INT, NS): 3(SUS, ST): 1(SUS, NS)

TABLE 3.—Data from Cross Series I

	Stripe Susceptible	Nonstripe Susceptible	Stripe Intermediate	Nonstripe Intermediate	Stripe Resistant	Nonstripe Resistant	Total	X^2	P
F_1									
Expected	...	...	810	...	...	...	...	...	...
Observed	...	...	810	...	...	...	810	...	...
Backcross of F_1 Females x Nonstripe, Susceptible Males									
Expected	298	298	298	298	...	...	...	...	...
Observed	308	312	286	286	...	...	...	...	...
Difference	10	14	12	12	...	...	...	...	...
D^2/E	.336	.658	.483	.483	...	...	1192	1.960	.50–.70
Backcross of F_1 Males x Nonstripe, Susceptible Females									
Expected	70.7	70.7	70.7	70.7	...	...	...	...	...
Observed	77	79	69	58	...	...	...	...	...
Difference	6.3	8.3	1.7	12.7	...	...	...	...	...
D^2/E	.561	.975	.048	2.28	...	...	283	3.864	.20–.30
Backcross of F_1 Females x Nonstripe, Resistant Males									
Expected	...	...	243	243	243	243	...	...	...
Observed	...	...	248	247	233	244	...	...	...
Difference	...	...	5	4	10	1	...	...	...
D^2/E	...	...	.102	.065	.411	.004	972	.582	.90–1.00
Backcross of F_1 Males x Nonstripe, Resistant Females									
Expected	...	...	89	89	89	89	...	...	...
Observed	...	...	84	96	77	99	...	...	...
Difference	...	...	5	7	12	10	...	...	...
D^2/E	...	...	.280	.550	1.617	1.123	356	3.570	.30–.50
F_2									
Expected	215	72	429	143	215	72	...	...	...
Observed	190	82	429	161	202	82	...	...	...
Difference	25	10	0	18	13	10	...	...	...
D^2/E	2.906	1.388	0	2.265	.786	1.388	1146	8.733	.10–.30

TABLE 4.—Combined Data from Cross Series II, III, and IV

	Stripe Susceptible	Nonstripe Susceptible	Stripe Intermediate	Nonstripe Intermediate	Stripe Resistant	Nonstripe Resistant	Total	X^2	P
				F_1					
Expected	...	...	1022	...	...	...	...	...	...
Observed	...	...	1022	...	...	...	1622	...	...
				Backcrosses to Nonstripe, Susceptibles					
Expected	49.5	49.5	49.5	49.5	...	...	...	...	...
Observed	43	57	47	51	...	...	...	...	...
Difference	6.5	7.5	2.5	1.5	...	...	...	...	...
D^2/E	.813	1.156	.126	.045	...	...	198	2.140	.50–.70
				Backcrosses to Nonstripe, Resistants					
Expected	...	...	338.75	338.75	338.75	338.75	...	...	...
Observed	...	...	306	338	353	358	...	...	...
Difference	...	...	32.75	.75	14.25	19.25	...	...	...
D^2/E	...	...	3.166	.001	.599	1.092	1355	4.818	.10–.30
				F_2					
Expected	293	98	587	195	293	98	...	...	...
Observed	264	109	624	204	279	84	...	...	...
Difference	29	11	37	9	14	14	...	...	...
D^2/E	2.870	1.234	2.332	.415	.688	2.0	1564	9.519	.05–.10

nonstripe phenotype was immediately recognizable; the dieldrin-resistance phenotype was determined by subjecting a portion of each F_1 to 8 p.p.m. dieldrin. Untreated individuals from each F_1 were used as parents for the F_2 and for the various backcross generations. BC-1A represents the backcross of F_1 females to dieldrin-susceptible nonstripe males. BC-1B denotes the cross of F_1 males to dieldrin-susceptible nonstripe females. BC-2A is the designation used for the backcross of F_1 females to dieldrin-resistant nonstripe males, while BC-2B is the reciprocal cross of F_1 males backcrossed to dieldrin-resistant nonstripe females. Finally, F_1 females were mated to F_1 males to produce an F_2.

All of the above matings were made from each F_1 in cross series I, II, III and IV, with the exceptions that backcrosses were not made in cross series II and that BC-1A was not made in cross series IV.

There were no significant differences in the F_1 results from cross series II, III and IV; the data are therefore combined in Table 4; similarly, the F_2 and equivalent backcross results are also combined in Table 4. The relevant theoretical information for cross series II, III and IV was combined in Table 2. The results obtained from cross series I are presented in Table 3. It may be seen from the data in Tables 3 and 4 that in all of the crosses, the experimental results did not differ significantly from the expected results. These results fit the hypothesis that 1) the nonstripe and dieldrin-resistance genes are on different autosomes and 2) an XY sex-determining mechanism is involved. If an XY mechanism were present, and if either of the above genes were sex-linked, the observed results could not be obtained.

The accepted m/m, M/m mechanism of sex determination in *Culex* and *Aedes* has not yet been established for *Anopheles*. The data obtained would also fit the M/m scheme if neither were sex-linked. However, exactly the same ratios would be obtained (assuming the M/m mechanism) if one or the other gene *were* sex-linked. The present data do not allow discrimination of these two possibilities.

Although no genetic information is available on the mechanism of sex determination in *Anopheles quadrimaculatus,* cytological investigations (Kitzmiller and French, 1961; French, 1963) show that an XY chromosomal mechanism is involved. Cytological preparations from pupal ovaries show the presence of two subtelocentric X-chromosomes about 8.25 microns long, and two pairs of mediocentric autosomes measuring 5.5 and 8.0 microns. Preparations from the cells of the pupal testis have autosomal chromosomes of approximately the same length as those in the ovaries. Testis cells, however, have only one X-chromosome measuring approximately 8.25 microns. The homologous Y-chromosome often found in synaptic association with the X is much smaller, measuring only about 2.5 microns. The same pronounced dimorphic sex chromosome pattern is found in the larval brain and nerve cord. The cytological evidence, therefore, strongly suggests a *Drosophila* type sex determination mechanism in *Anopheles quadrimaculatus*.

CONCLUSION. Three genetic characteristics have been studied in a series of crosses. These characteristics were shown to assort independently and are consequently considered to be members of different linkage groups. Some reservations, however, were made concerning the segregation of the mechanism for sex determination in *A. quadrimaculatus*. The present work should provide a skeletal framework for the linkage groups in *A. quadrimaculatus*. It can be reasonably expected that any additional genes such as those for DDT-resistance or malathion-resistance should show linkage with one of the characteristics herein studied. This work should therefore provide a tool useful for the further elucidation of the relationship existing between resistance genes, as well as for further study of the fundamental problems involved in anopheline inheritance.

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LARVAL HABITATS OF *CULEX TARSALIS* (COQ.) (DIPTERA: CULICIDAE) IN MINNESOTA[1]

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INTRODUCTION. Most studies on *Culex tarsalis* Coquillett have been carried out in the irrigated sections of the western United States. In the north central area of the country, work on the biology of this medically important species has been much less extensive. The present study of larval habitats in representative sections of Minnesota during 1958 through 1960 was one aspect of a broader investigation on the bionomics of *C. tarsalis* and western encephalitis viral activity (Price, *et al.*, 1960; Olson, *et al.*, 1961).

Minnesota, with its continental climate, differs from the irrigated regions and represents a transitional zone between these western areas and the eastern boundary limits of *C. tarsalis*. The extremes in temperature are great, ranging from a minimum of −59° F. to maximum of 112° F. The mean precipitation varies from 21.04 inches per year in the northwest to 29.23 in the southeast (Strub, 1960), with May and June being the wettest months. Prairie covers the southwestern portion of the lower half of the state and extends upward to Canada through the extreme western tier of counties (Fig. 1). Deciduous forest borders the prairie on the east, forming a diagonal belt narrow at the northern border of the state and widening in the lower eastern half of the state. The eastern two-thirds of the northern half of the state is predominantly coniferous forest. Many of the surface features of the state can be traced to the periods of intense glacial activity. The undulating and hilly surfaces of central and southern Minnesota are directly traceable to successive ice sheets which overspread the state, and the flat northwest corner was once the bed of the glacial Lake Agassiz. *Culex tarsalis* has been found in all parts of the state

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