# GENETICS OF RESISTANCE IN MOSQUITOES

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In the 10 years since the first genetic investigation of resistance in mosquitoes was reported, such studies have yielded weighty benefits and scientific insights. They have indicated the extent to which resistance could develop and have provided the means of detecting resistant genotypes long before their frequency increased sufficiently to cause failures in control. Genetical methods have figured importantly in delimiting cross-resistance spectra, identifying physiological mechanisms of resistance, and in monitoring the response of the genotype to measures designed to counter resistance. Also they have uncovered the gene-protein relationships that are among those best suited for investigation of the physiology of gene action in multicellular organisms.

DIELDRIN RESISTANCE. We are indebted to Davidson (1956) who just 10 years ago made the first study of resistance to insecticides in mosquitoes and determined the doses for discrimination between susceptible and resistant strains and their $F_1$ hybrids. With these diagnostic doses, Davidson and his co-workers (Davidson and Mason, 1963); Davidson, 1965) proved that resistance to dieldrin segregates as a single partially dominant autosomal factor in six anopheline species: *Anopheles albimanus* Wiedemann, *A. gambiae* Giles, *A. quadrimaculatus* Say, *A. stephensi* Liston, *A. pharoensis* Theobald, and *A. sundiacus* (Rodenwaldt). Davidson's studies suggested that the gene alleles for resistance to dieldrin are homologous in all six anophelines, since

the discriminating doses were identical and since the degree of resistance always decreased in the order aldrin>dieldrin>endrin, with the position of chlordane in this series varying among *A. gambiae, A. albimanus,* and *A. quadrimaculatus.* French and Kitzmiller (1964) showed that the allele for resistance to dieldrin (Figure 1) in these 3-chromosomed mosquitoes segregated independently of the sex chromosome and of the autosomal marker *Stripe.* In *A. albimanus,* Rozeboom (1963) found that the allele for dieldrin resistance was penetrant in all individuals but that its expression could be increased by selection for modifiers.

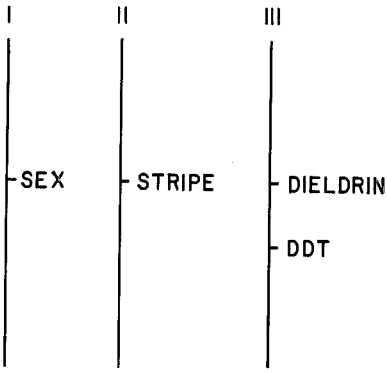


FIG. 1.—Linkage groups of *Anopheles quadrimaculatus.* Derived from French and Kitzmiller (1964) and Davidson (1965).

Davidson and Hamon (1962) discovered a gene allele for dieldrin resistance that they concluded may be fully dominant in *A. gambiae* from the Ivory Coast. Their conclusion was based on studies with diagnostic doses only and requires substantiation by studies made with a geometric series of concentrations. Further, a test of allelism is necessary to determine whether the same locus is involved as in classical dieldrin resistance.

Evidently the factor for dieldrin resistance does not handicap all genotypes. As many as 74 percent of *A. gambiae* carry the gene allele in untreated areas of Northern Nigeria (Service and Davidson, 1964). Also, in certain strains, resistance is stable: Gilotra (1965) showed that a dieldrin-resistant strain of *A. albimanus* from El Salvador possessed a higher reproductive potential than a susceptible Panama strain. On the other hand, Georghiou and Metcalf (1963) induced reversion of resistance by selection with a carbamate, and Keppler *et al.* (1964) observed spontaneous reversion.

Reversion may be a manifestation of ancillary factors required to make the allele for resistance to dieldrin compatible with the remainder of the genotype, and these ancillary factors may handicap the genotype when it is subjected to certain types of stress. Their existence and location might be evaluated through factorial analysis by using *Stripe, sex,* and *Dieldrin* itself as markers of the three chromosomes. The importance of ancillary factors could also be determined by studying the biotic potential and stability of resistance after the allele for resistance is first transferred into the susceptible genotype.

In *Aedes aegypti* (L.), Khan and Brown (1961) located the partially dominant allele for resistance to dieldrin on chromosome II; and Klassen and Brown (1964) showed that it was located in the same position on the linkage map in populations from the Grenadines, Jamaica, Curaçao, and Puerto Rico (Figure 2). The linkage measurements and toxicological data suggest that the factors in the various strains are allelic and identical.

Although this allele for resistance to dieldrin remained fully penetrant in crossing experiments, its penetrance and ex-

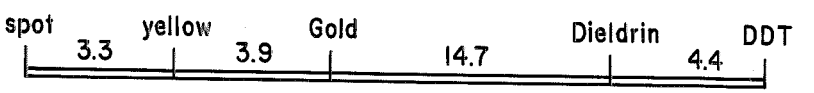


FIG. 2.—Location of the gene alleles for resistances to DDT and dieldrin in linkage group II of *Aedes aegypti* according to Klassen and Brown (1964).

pressivity were found to be influenced by modifiers. When Klassen and Brown (1964) selected a mixed population and used a dose poised to discriminate between the heterozygotes and the homozygous resistant individuals, five cycles of selection were required to achieve full expression of the allele in all individuals. Whether the separation of the modifiers from the allele for resistance is involved in the rapid reversion of resistance remains unknown; in preliminary studies Khan (1964) did not find differences in the reproductive potential between reverted and re-selected strains.

Another set of modifiers causing an increase in the cross-resistance determined by the allele for dieldrin resistance to other cyclodienes could be assembled by selection with isobenzan (Telodrin®) (Klassen, unpublished data). However, in spite of the influence of modifiers, dieldrin resistance is the best genetic marker in mosquitoes.

In *Culex pipiens quinquefasciatus* Say, Pennell and Hoskins (1964) found that resistance to dieldrin was inherited as a single partially dominant factor. A similar factor was found by Davidson (1964) in *Culex pipiens fatigans* Wiedemann. It was located by Tadano and Brown (see Tadano, 1966) on chromosome III (Figure 3) at 35 units from the marker *kps*

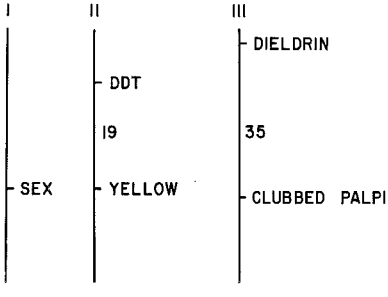


FIG. 3.—Location of the gene alleles for resistances to DDT and dieldrin in the linkage groups of *Culex pipiens fatigans* according to Tadano and Brown (see Tadano, 1966).

(clubbed palpi).

RESISTANCE TO DDT. Although resistance to DDT does not show clear-cut segregation in *Anopheles,* Davidson (1965) showed that it was largely determined by an autosomal factor which is recessive in *A. albimanus, A. sundiacus,* and *A. quadrimaculatus;* partially dominant in *A. pharoensis*; and nearly dominant, depending on ancillary factors, in *A. stephensi* (Davidson and Jackson, 1961). In larvae of the latter species a different set of minor factors modifies the degree of resistance to DDT than in adults (Mohan and Singh, 1965). In *A. quadrimaculatus,* Davidson (1965) found that resistance to DDT was linked with resistance to dieldrin. All these resistant strains studied by Davidson showed high cross resistance to the dehydrochlorinatable analogs of DDT, methoxychlor, DDD now called TDE, and diethyldiphenyldichloroethane. However, dehydrochlorination has not been established as the mechanism of resistance though it was demonstrated in *A. sundiacus* by Perry (1960).

Chromosomal aberrations and rearrangements in *Anopheles,* according to Hobbs (1962) and to Mason and Brown (1963), do not determine resistance or greatly modify its intensity; however, D'Alessandro *et al.* (1962), Mariani *et al.* (1964), and Bruno-Smiraglia *et al.* (1965) showed that selection with DDT or malathion and other types of stress induced chromosomal rearrangements.

Resistance to DDT in *Aedes aegypti* is neither dominant nor recessive: the hybrids between resistant and susceptible mosquitoes are intermediate, and the dosage-mortality lines of the three genotypes usually overlap broadly which prevents their complete classification. However, Qutubuddin (1958), working with an extremely resistant strain from Trinidad, was able to determine discriminating doses and to establish monofactorial inheritance.

Coker (1958) attacked the same problem by adopting a method originated by Wright (1952) in which the resistant strain is repeatedly backcrossed to a susceptible strain (Figure 4). Backcross

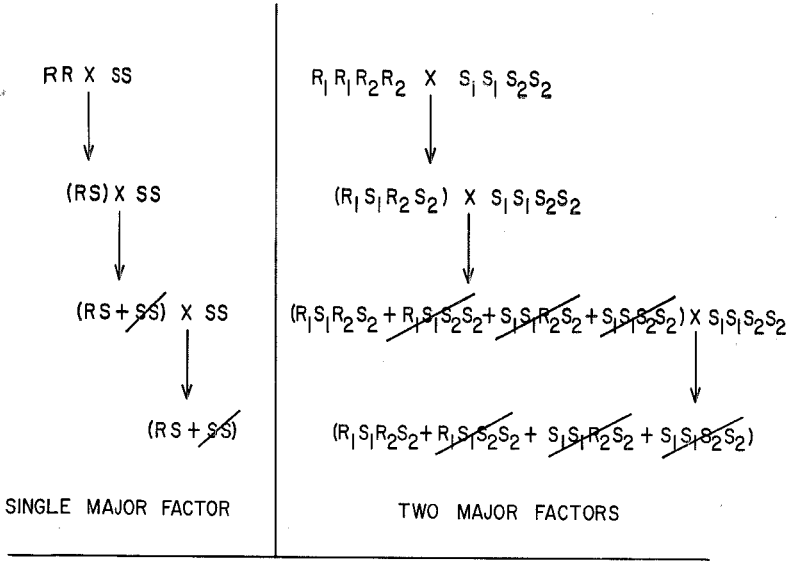


FIG. 4.—Method of determining the number of major factors that determine resistance according to Coker (1958). The oblique lines indicate the genotypes in each backcross generation which are selected away by a fixed dose. In these examples the maximum dose which is sublethal to $F_1$ hybrids is used for selection.

progeny are selected at the midpoint of their distribution to remove the susceptible individuals. If resistance is determined by one major factor that is not entirely recessive, the progeny of each successive backcross will fall into two categories; if several major factors determine the resistance, the level of resistance will decrease with each successive backcross, because the proportion of intermediate genotypes with low resistance increases with each successive backcross. Coker (1958) applied this procedure through two successive backcross generations to material from Trinidad, Haiti, and Malaya that was moderately resistant to DDT. Since the progeny of the second backcrosses of the Trinidad and Malaya strains to a susceptible strain segregated into two categories without any decline in resistance, monofactorial inheritance was indicated.

In the Haiti strain the procedure indicated one major factor with additional factors in males (cf. Wood, 1965); however, these results require confirmation because of the questionable purity of the strains. Coker then crossed the strains to test whether the factors were allelic in the various stranis. Greater variability in the $F_2$ than in the $F_1$ progeny might indicate the recovery of very susceptible and very resistant genotypes because of crossing over between the factors. By this method he obtained evidence of allelism between the Trinidad and Haiti factors but not between the Trinidad and Malaya factors. Possibly the failure was caused by impurity in the strains or by recombination between different systems of modifiers introduced by the two strains. Modifiers could also obscure results of a more sensitive test (Figure 5) devised by Coker (1964) in which the $F_1$ progeny of the interstrain crosses were test-crossed to a fully susceptible strain. If fully susceptible and extremely resistant individuals appeared in the backcross progeny, the hypothesis of allelism would be disproved.

Khan and Brown (1961) found that

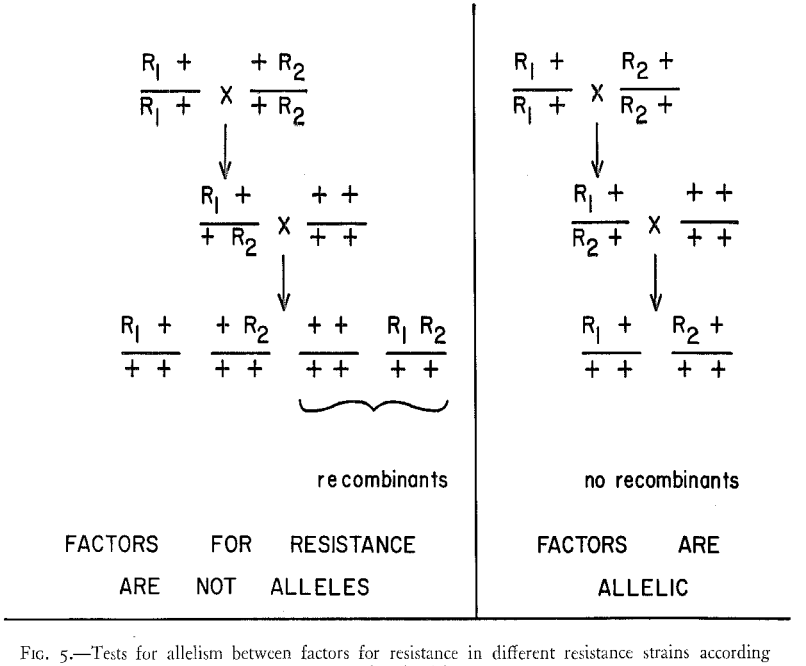


FIG. 5.—Tests for allelism between factors for resistance in different resistance strains according to Coker (1964).

resistance to DDT in a strain from Puerto Rico was determined exclusively by chromosome II and showed a 25 percent cross over with the mutant marker *yellow*. This result was confirmed by Brown and Abedi (1962) who found that it also applied to the Trinidad and Penang strains. In this study the strains were purified by sibling selection of single broods and then crossed. Since the $F_2$ progeny of these interstrain crosses showed no greater variance than the $F_1$ progeny, the resistance factors in the three strains may be considered to be alleles or closely linked.

Klassen and Brown (1964) found that the factor for resistance to DDT was similarly located on chromosome II in populations from Puerto Rico, Jamaica, Grenadines, and Curaçao (Figure 2). This factor determines the detoxifying enzyme DDT-dehydrochlorinase, whose substrate specificity is identical with Caribbean strains but differs in the Malayan strain, as shown by Kimura and Brown (1964) and Abedi *et al.* (1963). Kimura and Brown (1964) found that the amount of DDT-dehydrochlorinase that was produced could be increased by accumulating modifiers through protracted selection. However, Oppenoorth (personal communication) cautioned that some interstrain differences might be caused by non-identical alleles, as in the house fly (Oppenoorth, 1965).

Several factors for DDT resistance may be anticipated since mechanisms of resistance other than dehydrochlorination have been identified. These are excretion of the peritrophic membrane encrusted with DDT (Abedi and Brown, 1961) and reduced absorption (Fast and Brown, 1962).

A large measure of the heterogeneity in linkage values between DDT and visible markers may be caused by the presence of inversions. In an American strain, Klassen and Brown (1964) determined

the distance between *spot* and *yellow* to be 3.3 cross-over units compared with 9.7 in an African strain. By using the American marker strain, Klassen and Brown found the order of the genes in test crosses involving three points to be *spot—yellow—DDT*; Coker (1964), by using the African marker strain, produced $F_2$ data indicating the order *yellow—spot—DDT*.

By using the product method of analysis of badly disturbed $F_2$ data, Coker (1964) found closer linkage of DDT to *bpd* and *spot* in a moderately resistant strain than in a highly resistant Trinidad strain. Even though the highly resistant strain was derived from the moderately resistant strain by several generations of selection, Coker entertained the hypothesis that different loci determined resistance in these strains. To test allelism, the two strains were crossed, and the $F_1$ progeny were test-crossed to a fully susceptible strain. Of the $F_1$ progeny, 22 percent were susceptible; of the test-cross progeny, 20–22 percent were susceptible. Coker suggested that susceptible individuals in the test-cross progeny indicated recombination between the factors; however, the susceptible individuals of the $F_1$ progeny indicated contamination, at least, of the highly resistant strain so the evidence for crossing over between two separate factors is questionable.

Another measure of heterogeneity in linkage values is caused by modifiers of penetrance and expressivity of *DDT* (Coker, 1963; 1964) which prevent the reliable classification of genotypes by diagnostic doses in segregating generations. The greater the extent of the overlap between regression lines for various genotypes, the more parental type offspring will be misclassified as recombinants. Brown and Abedi (1962) overcame this error by a method suggested by Oppenoorth in which dosage-mortality regression lines for various percentages of crossing over are drawn up on the basis of regression lines for known genotypes. The theoretical plot which fits the observed data at the point of equal distance from the 50 percent mortality point, i.e. at the point of least variation, is then selected.

The opposite type of error in which recombinants are misclassified as parental type offspring also occurs. Wood (1965) reported a modifier of dominance near *yellow* which masks cross-over events in a moderately resistant strain. This effect was observed by Brown and Abedi (1962) who found that *yellow* itself does not pleiotropically produce a more susceptible phenotype even though, on the average, *yellow* larvae weigh less than wild larvae. Subsequently, Klassen and Brown (see Klassen, 1963) performed a test cross in which *yellow* (*y*) was coupled to susceptibility. The offspring were then classified by a diagnostic dose as

| | | |
|---|---|---|
| + | DDT | 221 |
| *y* | + | 308 |
| + | + | 113 |
| *y* | DDT | 40 |

The deficiency in the *y DDT* class and the inflation of the *y* + class suggests that a modifier of dominance on chromosome II had become separated from *DDT* by crossing over with the homologue from the susceptible parent. If a single modifier, *M*, is postulated, then—ignoring double cross overs—the following genotypic frequencies may be deduced:

| | | | |
|---|---|---|---|
| + | *M* | *DDT* | 221 |
| *y* | + | + | 221 |
| *y* | *M* | *DDT* | 40 |
| + | + | + | 40 |
| + | *M* | + | 80 |
| *y* | + | *DDT* | 80 |

The data yields the following map: *y*—11.7—*M*—23.4—*DDT*. (The values are slightly large since the discriminating dose killed 5 percent of the resistant heterozygotes). It seems likely that the modifier of dominance lies very near to *Gold*.

Wood (1965) detected modifiers of expressivity by differences in levels of resistance of strains derived by sibling selection, as did Pillai and Brown (1965) who crossed various resistant strains to produce enhanced resistance in the hybrid generations. By factorial analysis, Pillai and

Brown found these modifiers to be located on chromosome II. However, Pillai and Brown were also able to assemble factors for resistance to DDT on chromosome III for an unknown mechanism of resistance by selecting with a mixture of DDT and WARF anti-resistant (*N,N*-dibutyl-p-chlorobenzensulfonamide) to produce a high resistance to the mixture and to DDT alone. During initial generations before these modifiers have been assembled, mixture selection reverses DDT resistance (Pillai *et al.*, 1963).

Abedi and Brown (1960) found that modifiers are required to stabilize resistance to DDT by making the *DDT* factor compatible with the genotype as a whole: in Malayan *Aedes aegypti,* high resistance to DDT induced in the initial generations under selection rapidly reverted when pressure was relaxed, and these first plus variants were handicapped by poor egg production and hatch. By selection through nine generations, modifiers of normal biotic potential were accumulated, and a stable resistance was produced.

Differences in the systems of modifiers assembled in various strains necessitate a special diagnostic for genotypes in each strain. Separation of genotypes may be accomplished in some strains by time-to-knockdown and in others by time-of-recovery-from-knockdown; moreover, discrimination is most readily achieved during the last larval stadium (Klassen, 1963).

The chromosome II factor for DDT-dehydrochlorinase may influence other resistances pleiotropically: Pillai and Brown (1965) found it was selected with the non-dehydrochlorinatable analog Prolan (1, 1-bis- (*p*-chloro phenyl)-2-nitropropane) and by the scarcely dehydrochlorinatable analog, deutero-DDT (2,2-bis (*p-chlorophenyl*)-1,1,1-trichloroethane-2-d).

In *Culex pipiens fatigans* from the State of Delhi, Pal and Singh (1958) found that an 11-fold resistance to DDT was determined by a single autosomal recessive; Rozeboom and Hobbs (1960) found the 13-fold resistance of a Philippine strain was determined by a dominant factor. A nearly dominant factor was found by Davidson (1964) that determined a 200-fold resistance in a strain from south India. Similarly in a Rangoon strain, a partially dominant factor showing clear-cut segregation was found by Brown and Tadano (1965) on chromosome II about 19 units from *yellow* (Figure 3). It would be helpful if this chromosome II factor could be shown to be genetically inseparable from the enhanced dehydrochlorination of this strain.

In *Culex tarsalis* Coquillett, Plapp *et al.* (1961) tentatively stated that resistance to DDT was derived from a recessive factor. However, close examination of the data reveals that this resistant strain contained a mixture of genotypes, that one of the $F_1$ crosses was intermediate in resistance, and that one of the $F_2$ crosses segregated as though a partially dominant factor was involved. Since recent work showed that resistance does not derive from dehydrochlorination (Plapp *et al.*, 1965), genetic analysis is urgently required to determine the mode of inheritance and to identify the mechanism of resistance.

RESISTANCE TO ORGANOPHOSPHORUS AND CARBAMATE COMPOUNDS. Brown and colleagues found that resistance to malathion in *Aedes aegypti* did not exceed 13 times that of the susceptible strain and derived from many factors on chromosome II and III (Brown and Abedi, 1960; Matsumura and Brown, 1963b; Pillai and Brown, 1965). In all instances, cross resistance to DDT that was determined by the chromosome II factor for DDT-dehydrochlorinase had supervened. In the Penang strain, resistance to malathion extended to carbaryl but not to 3-isopropylphenyl-N-methylcarbamate.

Resistance to parathion in laboratory strains of *Aedes aegypti* was shown by Matsumura and Brown (1963a) not to exceed 3 times that of the susceptible strain and to derive from multiple factors. In *Aedes nigromaculis* (Ludlow), Brown *et al.* (1963) found a 4,000-fold resistance to parathion that extended to other organophosphorus compounds. The grad-

ual flattening of the regression line with increasing resistance suggested the emergence of a principal factor. Possibly this mosquito could be colonized by forced copulation to permit determination of the precise nature of its resistance.

A 90-fold resistance to malathion extending to diazinon was observed in populations of *C. pipiens fatigans* in the Cameroons, but it could not be studied because of its rapid reversion to susceptibility (Mouchet, 1964).

The specific 45-fold resistance to malathion of *C. tarsalis* was found by Matsumura and Brown (1961; 1963a) to derive from a partially dominant autosomal factor. This factor proved to be inseparable from a 13-fold increase in a slightly altered carboxyesterase. It would be interesting to know whether the heterozygote produces both malathion-degrading enzymes.

The 5-fold cross resistance in *A. aegypti* to the carbamate, carbaryl, induced by selection for resistance to malathion was shown by Brown and Abedi (1960) to be polygenic in origin. Georghiou and Metcalf (1963) failed to induce carbamate resistance in *Anopheles albimanus,* but in *Culex pipiens quinquefasciatus,* Georghiou (1965a, 1965b) induced a 7-fold increase in polygenic tolerance to Bayer 39007 (o-isopropoxyphenyl methylcarbamate) which continues to increase with selection (Georghiou, personal communication).

In house flies, the allele for the enzyme that hydrolyses methyl butyrate mutated to produce breakdown enzymes of organophosphates and carbamates. Plapp *et al.* (1965) found that *Aedes* hydrolized methyl butyrate but *Anopheles* and *Culex* did not. These authors suggested that *Culex* and *Anopheles* lack the allele which could mutate to form breakdown enzymes; thus, no resistance to organophosphorus and carbamate compounds has appeared in these genera (other than resistance to malathion in *Culex tarsalis*). However, in *Aedes aegypti,* organophosphorus breakdown enzymes have not arisen, and in *Culex tarsalis,* which is resistant to malathion, increased phosphatase activity proved to be genetically inseparable from cross resistance to malaoxon (Matsumura and Brown, 1961).

Studies by Brown and Tadano (1965), by Georghiou (1965b), and by Yu and Lu (1964) showed that principal factors for high resistance to organophosphorus and carbamate insecticides will not develop until ancillary factors have been accumulated through intensive selection.

In *Aedes aegypti,* resistance has developed to apholate (Hazard *et al.,* 1964) and to a lesser degree to metepa (Klassen and Matsumura, 1966). This species responded to metepa selection by increased egg hatchability and by degrading metepa duced in the larva in which spermatogonia predominate in the gonad extend to the at an accelerated rate. The important questions that remain unanswered deal with cross-resistance spectra of chemosterilants—will resistance to sterility in adult in which the sperm are fully formed and will resistance to kill and sterility derive from the same physiological mechanism?

Selection for behavioral changes that would result in less contact with an insecticide have yielded strains of *Anopheles atroparvus* that show increased spontaneous activity as well as hyperirritability when they are exposed to DDT and to Risella oil, according to Gerold and Laarman (1964). However, strains selected for such changes should be monitored for changes in physiological resistance, because behavior is thought to play a role in the development of physiological resistance in field populations (Davidson, 1965; Muirhead-Thomson and Bruce-Chwatt, 1964).

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