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Malathion Resistance Alleles and Their Fitness in the Red Flour Beetle (Coleoptera: Tenebrionidae)[1]

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J. Econ. Entomol. 79: 580–587 (1986)

ABSTRACT Variation in level of resistance to malathion was measured in six field-collected strains of the red flour beetle, *Tribolium castaneum* (Herbst), of diverse geographic origins. Levels of resistance ranged from 3.6- to 111-fold. In all cases, resistance was controlled by closely linked alleles in the sixth linkage group at or near the *Rmal* locus. Two incompletely dominant alleles at this locus, *Rmal-1* and *Rmal-2*, conferring high and low resistance, respectively, were resolved and purified. Expression of both alleles was found to be relatively independent of genetic background. The *Rmal-1* allele was intrinsically stable (fit) in laboratory populations when placed in competition with the wild-type (susceptible) allele over six generations.

VARIATION IN levels of insecticide resistance may stem from many causes. Such variation in field strains that are homozygous for single resistance factors (*R*) may reflect the existence of several *R* loci, several allelic forms at a single locus, single modifying genes, or the polygenic effects of genetic background on the expression of the same *R* allele in unrelated strains.

The presence of different *R* loci can be demonstrated by classical linkage studies. However, to differentiate between the other possibilities, isogenic strains must be developed by repeated backcrossing of the test strain to a reference strain. Ideally, the result of this process is a pair of strains that are genetically identical except for a small region of one chromosome that includes the *R* locus of interest. Without this precaution, any genetic analysis will be inconclusive. For example, Arnold & Whitten (1976) claimed to have resolved three *R* alleles at a locus on chromosome 4 of *Lucilia cuprina* (Wiedemann). However, since they did not use isogenic strains, their results are also consistent with differential expression of a single *R* allele in three different genetic backgrounds.

The major *R* alleles having been resolved, the problem of allele fitness can be addressed. This problem is important because it is directly related to the stability (persistence) of resistance. Once again, the use of isogenic strains is required to determine the intrinsic fitness of a particular allele or the relative fitnesses of several alleles in competition with each other, and to assess the extent of coadaptation of *R* and fitness (see also Whitehead et al. [1985]).

Some of these principles are illustrated by our analysis of malathion resistance in the red flour beetle, *Tribolium castaneum* (Herbst). For many years, malathion has been the primary insecticide for control of stored-product insect pests, including *T. castaneum*. As malathion-specific resistance in this species has become widespread in the field, the resistant type has largely or completely replaced the susceptible type in some cases (Zettler 1982, Haliscak & Beeman 1983). Such resistance is under the control of a single, major gene designated *Rmal*, located in the sixth linkage group (Beeman 1983).

Here we describe the resolution of alleles at the *Rmal* locus and their fitness in populations in the constant genetic background of a susceptible reference strain.

Materials and Methods

Rearing and Bioassay. Beetles were reared in wheat flour + brewer's yeast (95:5, wt/wt) at 30 ± 1°C. Bioassays were conducted with adult beetles 2–4 weeks old at a room temperature of 25 ± 2°C. The bioassay method was described by Haliscak & Beeman (1983). Time/mortality measurements were made at a single malathion dose of 10 mg per 7-cm filter paper. Because of day-to-day variation that occurred in the responses of beetles to bioassays, bioassays were done simultaneously when critical comparisons were made. Control mortality was zero in every case at exposure times of 24 h or less.

Probit analysis was calculated according to Finney (1978). Dose/response lines were compared by calculating the likelihood ratio statistic for comparing the equality of two models (Finney 1978).

Beetle Strains and *Rmal* Alleles. The GA-1,

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KS-1, and IA-7 strains are described by Haliscak & Beeman (1983) and Beeman (1983). The NC strain is the "field" strain described by Bansode & Campbell (1979). The TX strain was collected on stored rice in Beaumont, Tex. in 1981. The Kano strain is the resistant strain described by Dyte & Rowlands (1968) and by Wool et al. (1982). *Sooty* (*s*) is a susceptible strain homozygous for a recessive body color mutation (*s*) in the fourth linkage group. *Black* (*b*) is a susceptible strain homozygous for a semidominant body color mutation (*b*) in the third linkage group. The *s* and *b* strains were obtained from the *Tribolium* Stock Center, Department of Biology, California State University, San Bernardino.

Rmal-1 and *Rmal-2* are two semidominant alleles at the *Rmal* locus that confer high- and low-level resistance, respectively. These two, plus the wild-type, susceptible allele (*S*), constitute the polymorphic system described in this report[2]. *Rmal-1* was found in strain GA-1, while both *Rmal-1* and *Rmal-2* were extracted from strain KS-1. The IA-7, NC, TX, and Kano strains all contained pure alleles that were similar to either *Rmal-1* or *Rmal-2*.

As defined in this work, *Rmal-1* and *Rmal-2* are functional units (i.e., they are defined only in terms of R level). There may be several alleles that result in low R (the *Rmal-2* phenotype) and several others that result in high R (the *Rmal-1* phenotype). These distinctions must await successful resolution and identification of the specific allozymes that underlie resistance, either by electrophoresis or by DNA sequencing. Here, *Rmal* alleles that confer the same level of resistance are assumed to be identical. For each experiment the source of the allele is indicated, as in "*Rmal-2* ex TX."

Extraction and Purification of Alleles. The resolution and separation of *Rmal-1* and *Rmal-2* were based on good discrimination between their times of death during bioassay. Strain GA-1 was already homozygous for *Rmal-1* when collected. *Rmal-1* and *Rmal-2* were isolated from strain KS-1 as follows: KS-1 males were mass-crossed with *sooty* (*s*) susceptible (*S*) females. The virgin F_1 progeny were selected with malathion and genotypically *S/S* and *Rmal-2/S* beetles were selectively killed at a discriminating time of 8 h. A pure *Rmal-1* strain was derived from the survivors. To isolate *Rmal-2*, more of the same F_1 progeny were harvested and *S/S* beetles were killed at a discriminating time of 2 h. Genotypes of the survivors had to be determined in individual beetles without further selection, since *Rmal-2/S*, the desired genotype, was less resistant than *Rmal-1/S*, the contaminating genotype. This was accomplished by progeny testing. Surviving F_1 males were mated with virgin *s* females in single pairs. These matings were of two types, depending upon the male (sire) genotype: 1) *Rmal-1/S* × *S/S* → *Rmal-1/S* + *S/S*; 2) *Rmal-2/S* × *S/S* → *Rmal-2/S* + *S/S*.

The progeny of each mating should consist of a 1:1 ratio of susceptible and heterozygous resistant insects, as shown. The sires were saved, and ca. 50 progeny from each single pair were selected at two consecutive discriminating times, the first to kill *S/S* genotypes and the second to kill *Rmal-2/S* genotypes. At the end of the first discriminating time, all susceptible beetles were removed from the bioassay papers. At the end of the second discriminating time, sire genotypes could be inferred, since the mortality of the remaining beetles in each family was either 0% (*Rmal-1/S* sires) or 100% (*Rmal-2/S* sires). Identified *Rmal-2/S* sires were mass-crossed to *S/S* females and a pure *Rmal-2* strain was derived from their progeny.

Construction of Isogenic Strains. To eliminate genetic background as a variable in comparing allele phenotypes, we introduced the *Rmal* alleles into a uniform, susceptible genetic background. *Rmal-1* or *Rmal-2* beetles (ex GA-1 or KS-1) were crossed and backcrossed to the *s* strain for 5–10 consecutive generations. For the first cross and subsequent backcross, we used *Rmal* males and *s* females to replace all field-derived X chromosomes with those from the *s* strain. This method was arbitrarily continued through the penultimate backcross. For the last backcross we crossed *Rmal* females with *s* males to replace all field-derived Y chromosomes with those from the *s* strain. Before each backcross to *s*, resistant progeny from the previous backcross were selected at a discriminating time of 2 h.

The original field strains were all homozygous for the wild-type allele at the *sooty* (*s*) locus, whereas the susceptible strain was homozygous for the mutant allele *s*. Thus, for any field strain the progress of the backcrossing scheme could be monitored by the increase in frequency of the *s* allele with each successive backcross. The *s* allele represented a randomly chosen locus not linked to *Rmal* and not subject to intentional selection. After *Rmal* strains isogenic with *s* were constructed, each *Rmal* allele was repurified by selection for four consecutive generations at an exposure time sufficient to kill all *S/S* individuals and also to partially discriminate between *Rmal/S* and *Rmal/Rmal* genotypes.

Linkage Analysis and Test of Allelism. *Microphthalmic* (*Mo*) is a visible mutation linked to *Rmal* in the sixth linkage group. Analysis of linkage between *Mo* and suspected *Rmal* alleles was conducted as previously described (Beeman 1983).

For the test of allelism we used the GA-1 and TX strains as sources of *Rmal-1* and *Rmal-2*, respectively, since these strains were already pure

[2] The upper-case letter *S*, representing the semidominant, susceptible allele at the *Rmal* locus in the sixth linkage group, should not be confused with the lowercase symbol *s*, representing the recessive body color mutation *sooty* in the fourth linkage group. The *sooty* strain is homozygous for both *s* and *S*. The italicized letter *R* represents a gene allele that confers resistance. The roman letter R is used as an abbreviation for the term "resistance" in its more general sense.

when collected. We first crossed *Rmal-1/Rmal-1* females with *Rmal-2/Rmal-2* males as 10 single pairs. The F_1 progeny (genotypically *Rmal-1/Rmal-2*) were selected with malathion at a discriminating time of 2 h as a precaution against contamination by *S*. All 20 parents were recovered and individually testcrossed to virgin, susceptible *b* beetles to confirm that they were homozygous for resistance. The semidominant mutation *b* was used to determine paternity in groups of progeny whose mothers had mated with two males. Approximately 50 F_1 progeny from each *Rmal* × *b* testcross were selected at a discriminating time of 2 h. Lack of any mortality in the F_1 proved that the *Rmal* parent was homozygous. In the case of the *Rmal-1* female × *b* male testcrosses, only the bronze-colored progeny (genotypically *b*/+) were picked, since these must have been sired by the *b* male, not the *Rmal-2* male with which the female had previously mated (see Beeman & Nanis [1985] for a further description of the *b* locus; see Wool & Bergerson [1979] for a discussion of sperm precedence after multiple mating in *Tribolium*). The confirmed *Rmal-1/Rmal-2* F_1 progeny from the original 10 single-pair crosses were then outcrossed to *s* en masse, and the outcross progeny were selected to detect susceptible recombinants. The criterion for death during bioassay was paralysis, because the penetrance of *Rmal-2* was 100% under this definition of death, as compared with only 80–90% when uncoordination was the criterion.

Persistence of *Rmal-1* in a Population. To assess the relative fitness of resistance in a population under stress, we placed the *Rmal-1* allele in competition with the susceptible (*S*) allele in the isogenic *sooty* (*s*) background in the absence of insecticide. The frequency of the *S/S* genotype was monitored for six consecutive, discrete (nonoverlapping) generations in laboratory populations supplied with food that was not ideal, so that individuals would be forced to compete for survival. The medium used was coarsely ground corn with a particle size distribution as follows: >2 mm diameter, 3.3% of total weight; 0.85–2 mm, 69.6%; 0.595–0.85 mm, 8.8%; 0.354–0.595 mm, 10.6%; 0.15–0.354 mm, 7.6%; <0.15 mm, <0.4%.

Population cages consisted of ca. 1-liter (1-qt) mason jars containing 250 g of ground corn. Populations were initiated by adding 100 virgin adult beetles 2–3 weeks old to each jar. The initial frequency (f) of the *Rmal-1* allele was set at 0.1, 0.5, or 0.9 at Hardy–Weinberg (HW) equilibrium by adding *Rmal/Rmal*, *Rmal/S*, and *S/S* beetles in the proper ratios (1:18:81 for $f = 0.1$, 25:50:25 for $f = 0.5$, and 81:18:1 for $f = 0.9$). The *Rmal-1* allele (ex GA-1) had been backcrossed into *s* for 10 consecutive generations, then repurified before the experiment. The sex ratio was set at 1.0 for each genotypic class, except for an excess of one male in groups with odd numbers.

To ensure discrete generations, parents were removed and discarded after 2 weeks. After 8–10 weeks, all F_1 adult progeny were harvested from each jar. One hundred were immediately used to initiate the subsequent generation. The remainder (ca. 100–300) were held for 1 week in wheat flour + brewer's yeast (95:5 wt/wt), then selected for 2 h to determine $f_{S/S}$. After oviposition, the 100 subcultured adult progeny were removed and these too were selected. Thus, the entire F_1 adult population of each generation was tested. *Rmal/Rmal* and *Rmal/S* beetles were not differentiated because only *S/S* beetles were killed at the discriminating time used. The bioassay method was modified from that described previously. Beetles were confined in PVC rings (12 cm) on filter paper disks (15 cm) treated with 3.3-ml aliquots of a 20 mg/ml solution of malathion. All beetles harvested from one population jar were bioassayed simultaneously on one filter paper. The study was replicated six times.

Results

Resolution of *Rmal* Alleles. Levels of resistance (R) of the strains tested varied widely. Initial time/response analysis was performed on six unrelated, field-collected strains after they had first been selected (as virgins) for at least three consecutive generations at a discriminating time of 2 h. This was just sufficient to kill all homozygous susceptible (*S/S*) beetles. Data for three representative strains and their F_1 hybrids are plotted in Fig. 1. Results of probit analysis are shown in Table 1. Resistance in the field strains ranged from 3.6- to 111-fold at LT_{50} (Table 2), and degree of dominance ranged from incomplete in the GA-1 strain to overdominant in the TX strain.

From the data in Fig. 1 we suspected that several *Rmal* alleles might exist in different populations, each conferring a different level of R. In the case of KS-1, the shallow, biphasic slope for the *Rmal/S* hybrid might reveal a dimorphism at the *Rmal* locus within this strain. To confirm this we selected KS-1 for several more generations, then mass-crossed KS-1 males to *s* females. The F_1 sexes were segregated as pupae and bioassayed separately. The distinct inflection in the time/response curves of both sexes (Fig. 2) confirmed the presence of a dimorphism in the KS-1 strain. The frequency of the low-R form, indicated by the position of the inflection point, was only 0.12 in Fig. 2 compared with 0.4 in the original bioassay (Fig. 1). The decrease might have stemmed from partial kill of the low-R form during the selection that occurred between the two experiments. The two R morphs, designated *Rmal-1* and *Rmal-2*, were separated by selection and progeny testing as described previously. Each was then introduced into the *s* genetic background by five consecutive generations of backcrossing, and repurified by four consecutive generations of selection at LT_{50} to LT_{70}. After backcrossing, the frequency of the mutant "indicator" phenotype *s* was 0.95 for *Rmal-1* and

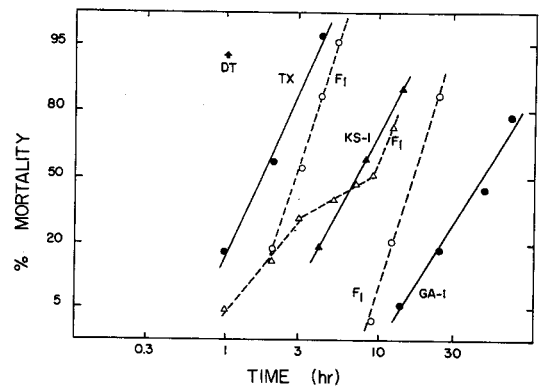

Fig. 1. Time/mortality data for malathion against three field-collected strains of *T. castaneum* and their F_1 hybrids with the susceptible strain, *sooty*. Data are for mixed sexes. The arrow labeled DT indicates the discriminating time (i.e., the minimum LT_{100} for the susceptible strain). This value was estimated by the time of death of the last surviving beetle in the sample (n = 100). Each datum point is the mean of four independent replicates of 25 beetles each.

0.87 for *Rmal-2* ($n > 300$). The expression and dominance of each backcrossed R morph were then determined by bioassay. The results (Fig. 3) revealed the segregation of two distinct *Rmal* alleles, even after backcrossing, indicating that the dimorphism was probably not caused by a modifying gene acting on the expression of a single *Rmal* allele.

The dominance of R was distinctly less in the purified, backcrossed alleles than in the original KS-1 strain (Fig. 1 and 3). We suspect that this reflects the elimination of heterotic effects in Fig. 3. In Fig. 1 the two parent strains were unrelated, and hybrid vigor (heterosis) may have enhanced the expression of R in the F_1. In Fig. 3 the two parent strains were isogenic, and the dominance observed should be a truer measure of allele interactions. The heterotic effect was especially noticeable in the TX strain, in which the F_1 hybrid was clearly more resistant than the R parent (i.e., R was overdominant) (Fig. 1). This difference was significant according to the likelihood ratio statistic for comparing the equality of two models ($P < 0.001$) (Finney 1978). Overdominance of resistance was also recorded in the Kano strain by Wool et al. (1982). Another possible basis for such overdominance could be a modifying gene in the susceptible strain that by itself does not confer resistance, but that enhances resistance conferred by *Rmal*.

Strain GA-1 appeared to be homozygous for an *Rmal* allele similar or identical to *Rmal-1* from the KS-1 strain. To confirm this, we made GA-1 isogenic with *s* by 10 consecutive generations of backcrossing, after which the strain was again made homozygous for *Rmal* by 3 consecutive generations of selection at ca. LD_{80}. By the ninth backcross generation, the R strain had become homozygous for the "indicator" mutation *s*, in spite of the absence of intentional selection for this trait. The phenotype of this *Rmal* allele (ex GA-1) was compared with that of the isogenic *Rmal-1* strain (ex KS-1) by combining the two alleles in heterozygotes, then testing for segregation of R morphs after outcrossing to *s*. Backcrossed, repurified males derived from the GA-1 strain were mated to isogenic *Rmal-1* females derived from the KS-1 strain. The F_1 males were outcrossed to *s* females. The male progeny from this outcross were bioassayed. The results (Fig. 4) show that no segregation occurred, and that *Rmal-1* ex GA-1 is indistinguishable from *Rmal-1* ex KS-1. In contrast, when the same experiment was done using GA-1 males and isogenic *Rmal-2* ex KS-1 females, the expected 1:1 segregation of two alleles occurred among the outcross progeny (Fig. 4).

Table 1. Toxicity of malathion to strains of *T. castaneum*[a]

Strain	n	Slope ± SE	LT_{50} (95% CL)
sooty	100	9.49 ± 0.74	0.47 (0.46–0.49)
TX	100	4.39 ± 0.42	1.71 (1.56–1.87)
F_1 (TX × *s*)	100	6.48 ± 0.56	2.80 (2.64–2.96)
KS-1	100	3.74 ± 0.39	6.86 (6.17–7.57)
GA-1	100	2.78 ± 0.42	52.0 (43.5–68.7)

[a] Linear regressions for all strains except *sooty* are plotted in Fig. 1. See also Fig. 1 legend.

Effect of Genetic Background on the Expression of Resistance. Strain GA-1 and its F_1 hybrid with *s* were bioassayed both before and after 10 generations of backcrossing to *s* to determine the dependence of *Rmal-1* expression on genetic background. The results (Fig. 5) indicate that the expression of *Rmal-1* ex GA-1 was only slightly reduced by transferring it from the original field background to the unrelated *s* background. The dominance was unchanged.

These results, in conjunction with the analysis of the KS-1 strain described previously, suggested that the expression of *Rmal* alleles is not strongly dependent upon genetic background.

Linkage Analysis and Test of Allelism. Malathion resistance factors in nonbackcrossed field strains GA-1, NC, KS-1, IA-7, TX, and Kano were all tested for linkage to *Mo* to determine whether

Table 2. Levels of malathion resistance in three field-collected strains of *T. castaneum* with reference to the susceptible strain, *sooty*[a]

Strain	Resistance factor
sooty	1.0
TX	3.6
KS-1	14.6
GA-1	111.0

[a] Resistance factor = LT_{50}(field strain) ÷ LT_{50}(*sooty* strain). See Table 1 for LT_{50} values.

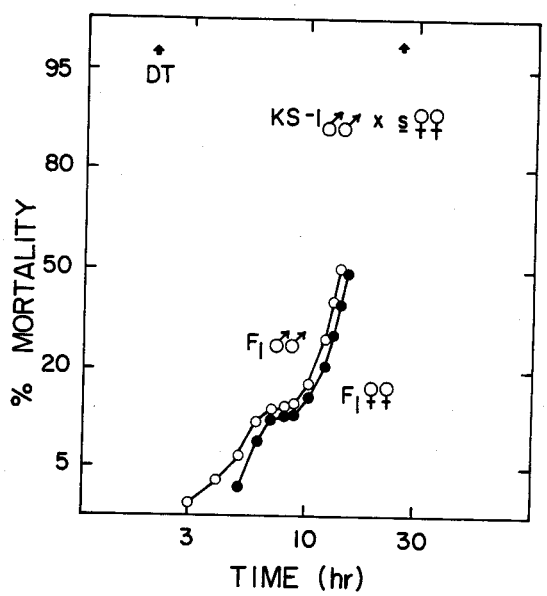

Fig. 2. Segregation of low- and high-resistance alleles in the dimorphic strain KS-1. Data for virgin males and virgin females are plotted separately. See Fig. 1 legend for an explanation of DT. Each datum point is the mean of eight independent replicates of 25 beetles each. The vertical arrow at time = 24 h indicates LT_{100} for the entire sample of 400.

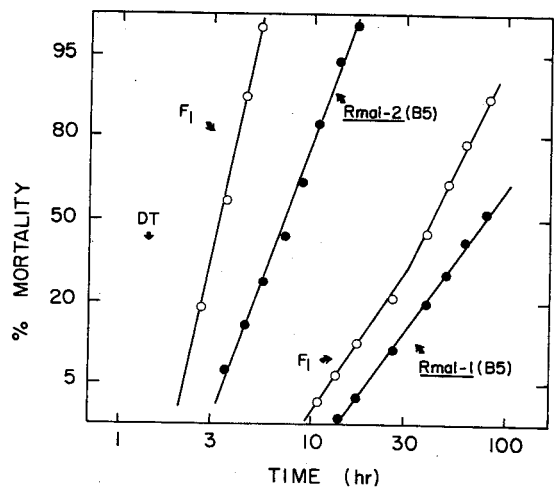

Fig. 3. Time/mortality data for malathion against the two alleles resolved in Fig. 2. Each allele was first made isogenic with the susceptible strain by five consecutive generations of backcrossing (B5), then repurified by four consecutive generations of virgin selection with malathion. Data are presented for each homozygous allele and its F_1 hybrid with the susceptible strain. See Fig. 1 legend for an explanation of DT. Each datum point is the mean of eight independent replicates of 25 beetles each.

a single chromosome controlled malathion resistance in a wide range of geographic races. In every strain tested, R was controlled by factors located in the sixth linkage group ca. 17–25 map units from *Mo* (Table 3). These results suggested that a single locus or closely linked group of loci in the sixth linkage group is of primary importance in the development of field resistance to malathion in *T. castaneum*. Another possibility is that two *Rmal* loci are located equidistant from *Mo* but on opposite sides of it. Three-point crosses to clarify this point were not performed, although such crosses are now possible because of the recent isolation of chromosome rearrangements with breakpoints on the *Rmal* chromosome (Beeman et al. 1986).

To determine whether low-resistance and high-resistance alleles exist at a single *Rmal* locus, we performed a test of allelism using *Rmal-1* ex GA-1 and *Rmal-2* ex TX. Both alleles were used in the original field background, but their expression was subsequently shown to be relatively unchanged after 5 and 10 consecutive generations of backcrossing into *s* for *Rmal-2* ex TX and *Rmal-1* ex GA-1, respectively (Fig. 5; unpublished observations). No susceptible recombinants were found among 2,700 chromosomes tested, indicating a nominal recombination value of <0.04%. Thus, *Rmal-1* and *Rmal-2* are either allelic or very closely linked.

Persistence of *Rmal-1* in a Population. The *Rmal-1* and *S* alleles were allowed to compete against each other in laboratory populations at three different initial frequencies of *Rmal-1* (f = 0.1, 0.5, and 0.9). There was no evidence of directed selection for either allele, since the mean frequency of the *S/S* genotype remained at or near its original value in all three tests, even after six generations (Fig. 6). Fluctuations in $f_{(S/S)}$ in indi-

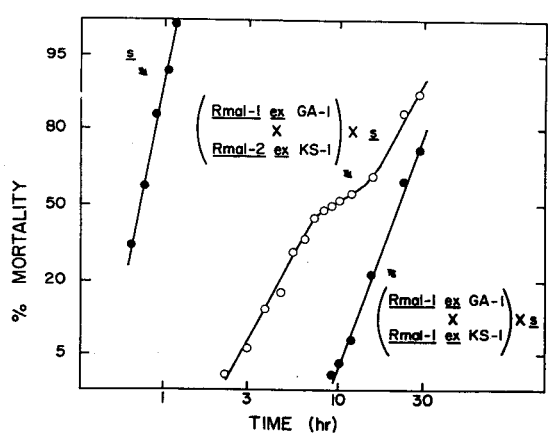

Fig. 4. Phenotypic comparison of *Rmal* alleles from the two unrelated strains, GA-1 and KS-1. Each allele was first made isogenic with the susceptible strain by 5–10 consecutive generations of backcrossing, then repurified by 3–4 consecutive generations of virgin selection with malathion. Each datum point is the mean of eight independent replicates of 25 beetles each.

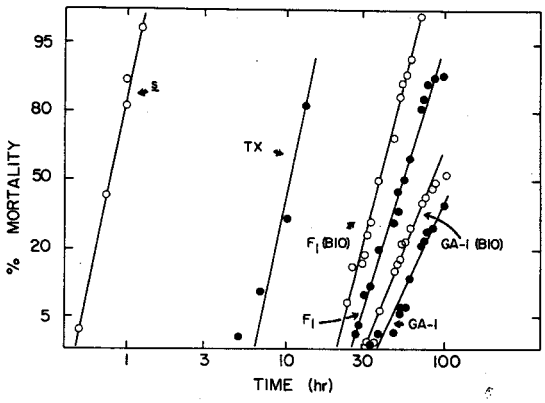

Fig. 5. Expression and dominance of *Rmal-1* in two different (unrelated) genetic backgrounds. Time/mortality data are shown for *Rmal-1* and its F_1 hybrid with *s*, both in the original GA-1 field background and in the *s* background. *Rmal-1* was transferred to the *s* background by 10 consecutive generations of backcrossing, then repurified by 3 consecutive generations of selection of virgin adults with malathion. Data for homozygous *Rmal-2* ex TX beetles are shown for comparison. All six bioassays were run simultaneously. Each datum point is the mean of eight independent replicates of 25 beetles each.

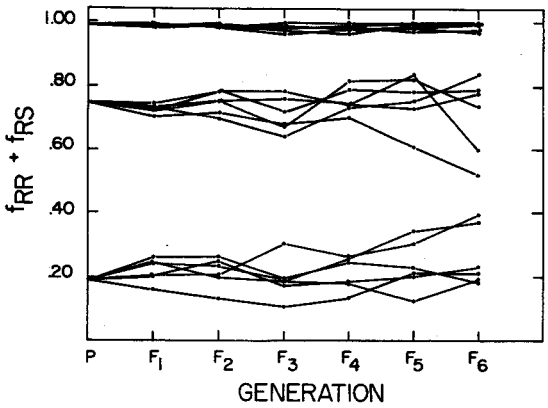

Fig. 6. Stability of *Rmal-1* in laboratory populations during six consecutive, nonoverlapping generations. Six independent lines were monitored for each of three initial *Rmal-1* frequencies. Each datum point represents the $f_{S/S}$ in the total population, not including the sample of 100 that was used to initiate the subsequent generation.

vidual replicates could be explained by random genetic drift. It is conceivable, although unlikely, that a shift away from HW equilibrium may have been exactly balanced by directional selection in favor of one allele, such that $f_{(S/S)}$ remained constant while $f_{(S/R)}$ changed. Within-line fluctuations in $f_{(S/S)}$ that occurred in the progeny each generation (Fig. 6) were not closely related to fluctuations in $f_{(S/S)}$ in the samples of 100 parent beetles used to initiate each generation (i.e., sets of parents with the highest $f_{(S/S)}$ did not as a rule give rise to sets of progeny with the highest $f_{(S/S)}$ [data not shown]).

Discussion

The *Rmal* locus controls malathion-specific resistance that is suppressed by carboxylesterase inhibitors (Haliscak & Beeman 1983). Thus, it is likely that *Rmal* is a carboxylesterase structural or regulatory locus. Plapp has promoted the novel idea that position effects, probably associated with chromosome inversions near a major regulatory locus, may be responsible for variations in level of insecticide resistance in several strains of the house fly (e.g., Plapp & Wang 1983). Because *Rmal-1/Rmal-1*, *Rmal-1/S*, and *S/S* genotypes are all fully fertile in *T. castaneum* (Beeman et al. 1986; unpublished observations) and because chromosome inversions are usually associated with partial sterility (Robinson 1975), position effects (at least those associated with large inversions) do not appear to contribute to *Rmal* expression in *T. castaneum*.

Our survey of field resistance to malathion (Haliscak & Beeman 1983) suggested that resistant red flour beetles throughout the United States grain belt were almost as numerous as susceptible ones. The mean percent mortality was 53% for all 36 field strains bioassayed at a discriminating dose. Evidence in the present work suggests that alleles at the *Rmal* locus may control most or all field resistance to malathion in this species. From our 1983 data, we calculated $f_{(S/S)}$ for each field strain both in the native condition and after one generation of selection at a dose that killed all *S/S* individuals, under the assumption of a two-allele locus at HW equilibrium, with the alleles of equal fitness in the absence of insecticide. Under these assumptions, and letting p and q = the frequencies of the *S* and *R* alleles, respectively, the frequencies of the *S/S* genotype before and after one generation of premating selection are p^2 and $\left(\frac{p}{2p+q}\right)^2$, respectively. The latter expression is derived algebraically as follows: Before selection, the assumption of HW equilibrium gives the frequencies of the *S/S*, *S/R*, and *R/R* genotypes as

Table 3. Tests of linkage of malathion resistance with *Mo* in six unrelated strains of *T. castaneum*[a]

Strain	Total n	% R	No. of progeny of given phenotype		% recom
			R, *Mo*	+, *Mo*	
GA-1	6,307	50.0	443	1,346	24.8 ± 1.0
NC	3,507	53.3	75	293	20.4 ± 2.1
KS-1	4,380	49.2	202	773	20.7 ± 1.3
IA-7	2,967	48.9	57	169	25.2 ± 2.9
TX	1,904	51.4	52	220	19.1 ± 2.4
Kano	3,272	47.5	63	300	17.4 ± 2.0

[a] Data for strain GA-1 are from Beeman (1983).

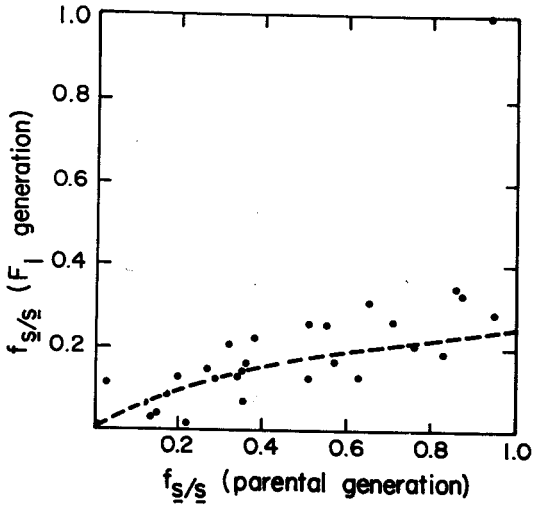

Fig. 7. Responses of 29 unrelated, partially resistant field strains of *T. castaneum* to one generation of selection with a discriminating dose of malathion. The data are replotted from table 2 of Haliscak & Beeman (1983). The dotted line is the theoretical response that would occur in an ideal population after one generation of premating selection at a discriminating dose for a dominant resistance allele (i.e., a dose that kills all S/S individuals) in HW equilibrium. If p equals the frequency of the S allele in the ideal population, then the frequency of genotypically S/S beetles is p^2 (abscissa) at HW equilibrium. After one generation of selection the theoretical frequency of S/S beetles can be shown to be $\left(\frac{p}{p+1}\right)^2$ (ordinate). The exceptional datum point in the upper right corner of the figure represents strain IA-10 (see text for explanation).

p^2, $2pq$, and q^2, respectively. After selection (elimination of S/S beetles), the relative frequencies of the two surviving genotypes, S/R and R/R, are $2pq$ and q^2, respectively. Among these survivors there is one S and one R allele for each S/R beetle and two R alleles for each R/R beetle. Thus, there are $2pq$ S alleles and $2pq$ R alleles for each $2pq$ S/R beetles, and there are $q^2 + q^2$ R for each q^2 R/R beetles. From this, the absolute frequency of the S allele after selection is calculated by dividing the relative frequency of the S allele by the sum of the relative frequencies of the S and R alleles, or

$$2pq \div (2pq + 2pq + q^2 + q^2) = \frac{p}{2p + q}.$$

Again applying the condition of HW equilibrium to the F_1 progeny of the survivors of the selection, the frequency of the S/S genotype in these progeny equals the square of the frequency of the S allele, or $\left(\frac{p}{2p+q}\right)^2$. Given that $p + q = 1$, the latter is equivalent to $\left(\frac{p}{p+1}\right)^2$.

Calculations based on the data for 29 field strains in Haliscak & Beeman (1983) indicate excellent overall agreement with the hypothesis of premating selection at a single diallelic locus in HW equilibrium (Fig. 7). The single exception was strain IA-10. This strain was probably homozygous for S, even though 6% of the beetles sampled survived the first exposure to a discriminating dose (see table and discussion on p. 719 of Haliscak & Beeman 1983).

Our population study provides a clear example of resistance that is intrinsically fit and that does not require coadaptation with fitness modifiers of the type described by McKenzie & Purvis (1984). After such resistance has become established in the field, the only strategies for re-establishing the susceptible type may be negatively correlated cross-resistance, or sophisticated and as yet experimental genetic methods such as the use of chromosome rearrangements as gene-transporting devices to reintroduce susceptibility.

Acknowledgment

This article is Contribution No. 86-103-J, Dep. of Entomology, Kans. Agric. Exp. Stn., Manhattan, and a cooperative investigation between the Kans. Agric. Exp. Stn. and USDA.

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Received for publication 28 October 1985; accepted 6 March 1986.