Genetics of Endosulfan Resistance in *Hypothenemus hampei* (Coleoptera: Scolytidae): Implications for Mode of Sex Determination

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ABSTRACT The genetics of endosulfan resistance in *Hypothenemus hampei* (Ferrari) was studied through classical crosses to determine the degree of dominance and number of genes involved. After adult beetles were sprayed, mortality was recorded at 6 h and 7 d. Tests of the males from reciprocal F_1 crosses indicated that resistance is sex-linked or that paternal chromosomes are inactive in the sons. Responses of F_1 females after 6 h indicated degrees of dominance of -0.38 ± 0.03 and -0.25 ± 0.03 for RR × S and SS × R crosses, respectively. In contrast, dominance after 7 d was -0.17 ± 0.02 and -0.02 ± 0.02, apparently indicating a trend toward codominance over time. Responses of backcrosses of the F_1 generation to both parental lines and of F_2 progeny were inconsistent with results predicted when assuming simple Mendelian inheritance. These results illustrate the practical weaknesses of backcrosses in estimating the number of genes that control resistance in situations where bioassays are inconsistent or where discrimination between genotypes is poor.

KEY WORDS *Hypothenemus hampei*, insecticide resistance, sex ratio bias

Hypothenemus hampei (FERRARI) is a major pest of coffee worldwide and is highly resistant to endosulfan in New Caledonia (Brun et al. 1989, 1992). Resistance has been confined to five regions on the East Coast, where the existence of resistance is significantly more frequent in the newer sunny plantations that had been treated with endosulfan during the preceding 12 mo than in fields that had not been treated recently or in older fields under native forest canopy (Brun et al. 1990). Parkin et al. (1992) related variation in resistance levels within fields reported in Brun & Suckling (1992) to operational factors such as application from truck-mounted sprayers and the type of field. Cessation of endosulfan use led to a reduction in resistance frequency, whereas continued use increased the resistance frequency (Brun & Suckling 1992).

Although much has been learned about the evolution of resistance in *H. hampei* through monitoring and without any understanding of the underlying genetics, several other important factors, such as the effective dominance of resistance (Roush & McKenzie 1987, Roush & Daly 1990), cannot be studied without determining the mode of inheritance of the trait. Thus, we sought to determine the degree of dominance and mode of inheritance of endosulfan resistance in *H. hampei* by using backcross methods.

Materials and Methods

Insect Strains. Susceptible (S) and resistant (R) strains of *H. hampei* were collected in July 1990 from coffee berries at the roadsides of fields near La Foa (LA 2, an S strain identified in Brun et al. 1989) and Ponérihouen (designated PN106). Females of the R strain were mated, selected with 10,000 ppm of endosulfan (as described below) for four generations, with 200–1,000 females selected per generation. This concentration was used because it exceeded the $LC_{99.9}$ (2,500 ppm) of the offspring of beetles collected directly from the field. Survivors were reared, mated and selected, and used as the parent R strain. Larvae were reared on the artificial diet described by Brun et al. (1993).

Direct-Spray Technique. Concentration–mortality responses of R and S *H. hampei* with the direct spray technique after 6 h have been reported previously (Brun et al. 1989, 1990). For examination of the genetics of resistance, we recorded

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the survivors after 6 h and 7 d. A glass ring was used to confine adults on a piece of filter paper during spray application. Each test of females consisted of 2–8 replicates (generally 90–120 beetles, with a range of 50–480) per concentration and genotype of the females. Highly female-biased sex ratio prevented extensive testing of males, which was limited to samples of 22–82 (4–9 replicates of 5–10 individuals dependent on emergence). A Potter spray tower (Potter 1952), calibrated to deliver 1.6 mg/cm^2, was used to apply 2 ml of liquid onto each sample. Adults were held at 25 ± 1°C and 80–85% RH before mortality was assessed. Concentration–mortality responses were estimated where sufficient females were available, with up to 16 concentrations and a water control. Control mortality was consistently <5%, even after 7 d. The criterion for death was the inability to move more than its own body length when touched with a fine paintbrush.

Crosses. After female pupae from the R or S strain were removed from rearing media, they were allowed to emerge and mate with males from the opposite strain and to oviposit on diet (Brun et al. 1993). Resultant larvae were reared for mating or testing. For the backcrosses, female F_1 pupae were removed from original rearing media to new media and allowed to emerge and mate with males from either of the parental strains. Oviposition occurred within a few days. The F_2 generation was obtained in a similar fashion by allowing F_1 adults from a given cross to mate with one another.

Statistical Analysis. Abbott's (1925) formula was used to correct for natural mortality. Logit analysis was done with S-Plus (Statistical Sciences 1991). An *F* test was used to test for equality of the reciprocal F_1 responses. Responses in each of these crosses were overdispersed (larger than expected under the binomial model). This extra variability was estimated by the heterogeneity (Statistical Sciences 1991) and used in analysis of deviance. The degree of dominance of resistance was calculated from the formula of Stone (1968), with its standard errors calculated as described by Preisler et al. (1990).

Expected mortalities in the backcrosses were calculated assuming a single locus model, in which the mortality at any given dose was 0.5 × (F_1 mortality at that dose + backcross parent strain mortality at that dose) (Roush & Daly 1990, Tabashnik 1991). The mortalities used for the F_1 and parent strains at any given concentration were those predicted by the appropriate regressions for each genotype. Although responses of the F_1 generation were significantly different, the differences were small and seemed unlikely to have biological significance. Therefore, the results were pooled to produce the curves of expected values in the Fig. 2. Expected values for the F_2 were calculated similarly, as described later. To test whether the data fit a single locus model, a dispersion parameter was first estimated by fitting a generalized additive model to the backcross responses. This method allowed us to model the responses nonparametrically with a scatterplot smoothing procedure (Chambers & Hastie 1992).

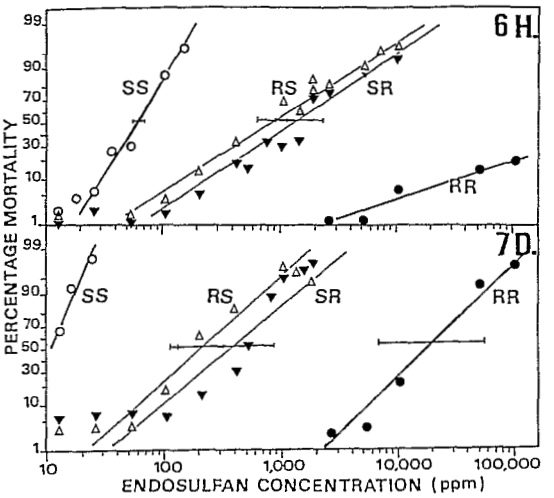

Fig. 1. Concentration–mortality responses of adult female *H. hampei* to endosulfan for susceptible and resistant parental strains and reciprocal F_1 hybrids after 6 h and 7 d. SR, the offspring of susceptible females crossed with resistant males. RS, the offspring of resistant females crossed with susceptible males.

Results and Discussion

Resistance Levels. During the initial selections, the mortality of females of the R parental strain after 7 d was 30–35% at 10,000 ppm of endosulfan and decreased slightly to 10% mortality during the course of selection. In contrast, females of the S strain were all killed at 400 ppm even after only 6 h (Fig. 1, Table 1). Susceptible males appear to be even more sensitive than S females. Whereas the S females had an LC_{50} of 25 ppm and 10% survived concentrations of 100 ppm at 6 h (Fig. 1), among the S males, 73% tested (n = 22) died at 25 ppm and none survived tests at 50 ppm. No mortality was observed in males of the selected resistant strain, scored at 6 h after treatment at 400 ppm (Table 1).

Dominance of Resistance. When the F_1 females were tested, resistance from both reciprocal crosses was incompletely recessive to semidominant in inheritance, depending on the length of time before mortality was tallied (Fig. 1). When mortality was recorded after 6 h, the degree of dominance was −0.38 ± 0.03 (S females × R males) and −0.25 ± 0.03 (R × S). After 7 d, D = −0.02 ± 0.02 (S × R) and D = −0.17 ± 0.02 (R × S), suggesting an increase in the relative expression (dominance) of the resistance during the bioassay. The LC_{50}s of the two F_1 crosses were not significantly different based on overlap of the 95%

Table 1. Mortality of *H. hampei* by genotype and time at 400 ppm endosulfan

Genotype	6-h mortality ± SEM	*n*	Genotype	7-d mortality ± SEM	*n*
S male	100 ± 3.2	22[b]	R male	3.7 ± 2.1	82
R male	0.0 ± 0.9	82	R(S)[a] male	3.3 ± 3.3	30
S(R)[a] male	100 ± 1.4	49			
SS female	100 ± 0.4	170	RR female	1.1 ± 11.0	90
SR[a] female	16.7 ± 3.4	120	RS[a] female	82.2 ± 4.0	74

[a] F_1s where the female parent is listed first.
[b] Tested at 50 ppm.

CL, but the hypothesis of equivalent responses was rejected for both 6 h and 7 d ($P \ll 0.01$).

Dominance expression is a phenotypic character liable to change with different types of bioassays (e.g., Roush & McKenzie 1987, Suckling et al. 1989, Roush & Daly 1990). Likewise, the length of time after treatment might also affect the expression of dominance. Nonetheless, resistance in this species shows the codominant inheritance that is typical of resistance to cyclodiene insecticides (Brown 1967).

Sex Linkage. In contrast with females, we detected clear differences in responses of the F_1 males that depended on which parent was R, implying that resistance is sex-linked. At 400 ppm, a concentration of endosulfan that killed all S males and females at 6 h, all 49 sons of S females crossed with R males (S × R) died within 6 h (four replicates) (Table 1). Thus, R fathers did not influence the phenotype of their sons. In contrast, only 1 of the 30 sons of R females and S males died (five replicates), even after 7 d (Table 1). This result (3% mortality) is similar to the mortality observed for R males and females at the same concentration, but much less than for RS females (Table 1). The difference between males from reciprocal crosses was highly significant ($P < 0.01$, Wilcoxon rank test of the percentage mortality of the replicates).

Hypothenemus hampei from Brazil were studied cytologically by Bergamin & Kerr (1953), who concluded that this species had an unusual sex-determination system. They reported that females had 12 autosomes (six pairs) and 2 X chromosomes, whereas males had 2 X chromosomes and 1 Y chromosome. Highly female-biased sex ratios, n = 10–12 females for every male (L.O.B. & V.G., unpublished data), were accounted for by loss of the Y chromosome through heteropycnosis in most of the sperm (i.e., the chromosome condenses into a densely staining mass, which is presumed to be inactive, and is eventually ejected from the cell). If Bergamin & Kerr's (1953) conclusions also apply to *H. hampei* populations from New Caledonia, our results suggest that the X chromosome contributed from the father is inactive in the sons, accounting for the phenotypic sex-linkage observed. However, recent observations suggest a more parsimonious hypothesis: a complete set of chromosomes may be inactive in the males (J. Stewart & L.O.B., unpublished data). A similar example is known from the predatory mite, *Metaseiulus occidentalis* (Nesbitt), where there is also a female-biased sex ratio (Roush & Hoy 1981) and where males are haploid because of the loss of the paternal set of chromosomes through heteropycnosis (Nelson-Rees et al. 1980). For this species, loss of the paternal set was also demonstrated by insecticide resistance, which served as a genetic marker (Roush & Plapp 1982). Thus, while the exact cytological system used by *H. hampei* requires further resolution, our data strongly suggest that gene or chromosome inactivation is occuring.

Number of Loci Controlling Resistance. The data from all backcrosses were signficantly different ($P \ll 0.01$ even at 6 h, data not shown) from the expected values for a single gene (Figs. 2A and B). If resistance were due to a male haploid (or sex-linked) single major gene, the results for the F_2 should be different than if resistence were autosomal, because male haploidy (or sex-linkage) would tend to show a pattern similar to backcrosses to either the R or S parents. Autosomal inheritance (0.25 SS + 0.5 RS + 0.25 RR) would give a more intermediate response. The results for the F_2 appear to be more consistent with sex-linked (or male haploidy) inheritance rather than autosomal inheritance, since the F_2 of S females by R males is much more suceptible than the F_2 of the reciprocal cross. However, neither F_2 shows a very close correspondence with the values expected (Fig. 2C).

Results from backcrossing experiments often give ambiguous results (Tabashnik 1991), and we suspect that these experiments provide a further example of ambiguity. The interpretation of backcrosses is especially difficult where consistent results are difficult to achieve in bioassays.

A single major mechanism has been described that could account for all of the resistance observed. DNA sequencing has shown that a mutation in the *Rdl* $GABA_A$ receptor gene that confers resistance to cyclodiene insecticides in *Drosophila* spp. (ffrench-Constant et al. 1993) and other insects also occurs in the *Rdl* homologue of *H. hampei* (ffrench-Constant et al. 1994). However, at present, we cannot discount the influence of other genes. The most direct way to test the influence of other genes would be by the use of genetic

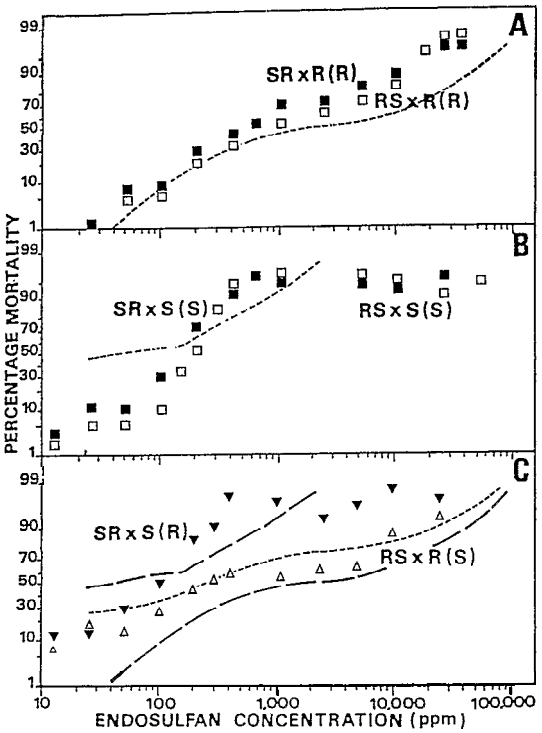

Fig. 2. Concentration–mortality responses of adult female *H. hampei* to endosulfan after 7 d for backcrosses of F_1 females to (A) resistant males and (B) susceptible males. Finely dashed curves show the expected responses if resistance resulted from a single gene. Allele indicated in parentheses, e.g., (R), indicates the allele that is apparently inactivated or lost in the males, as discussed in the text. Results for F_2 adult females are shown in C, where the middle, dotted curve shows the expected values assuming that the males were heterozygotes (i.e., the paternal allele could be passed via the F_1 males to their F_2 offspring) and the outer dashed curves show the expectations if males were hemizygotes (i.e., the F_1 males transmitted only the maternal allele).

markers so that the influence of all chromosomal regions (as done by McKenzie et al. 1980) could be assessed.

Two salient points emerge from these studies; first, the high levels of resistance found in the F_1s (Fig. 1), which are heterozygous for at least one major gene (*Rdl*), suggest that heterozygotes could survive field exposure (Roush & Daly 1990). This would help to account for the rapid changes in resistance frequency which were reported by Brun & Suckling (1992). Field bioassays (e.g., Parkin et al. 1992) of known genotypes would be required to investigate this further. Second, the strong apparent sex-linkage of resistance suggests, for the first time in this species, that either the paternal X chromosome or (more likely) the entire set of paternal chromosomes is inactivated in males.

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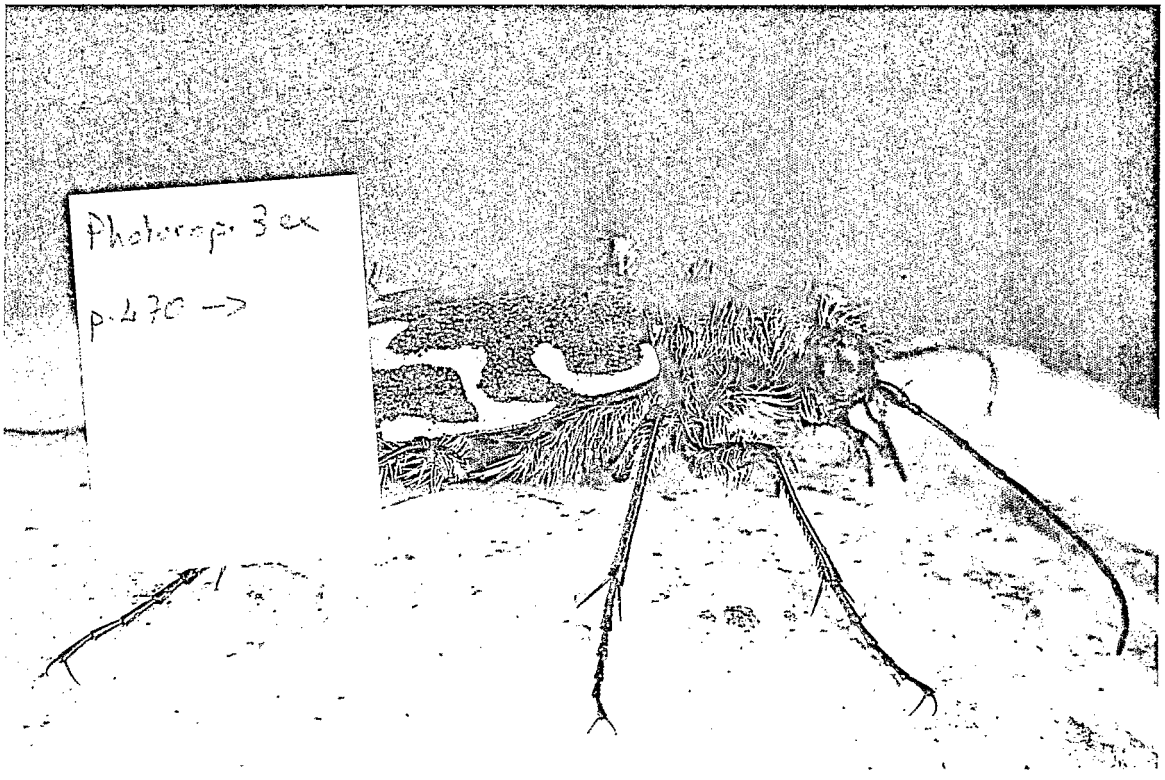

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