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Environmental and Genetic Effects on the Asymmetry Phenotype: Diazinon Resistance in the Australian Sheep Blowfly, *Lucilia cuprina*

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ABSTRACT

The asymmetry phenotype of diazinon-resistant flies lacking a fitness/asymmetry *Modifier* (+/+; *R*/−) was dominant and independent of developmental temperature, larval density and diazinon concentration. Asymmetry score, pooled over three bristle characters, was ~50% greater for these phenotypes than for those of modified genotypes (*M*/−; −/−) and unmodified susceptibles (+/+; *S*/*S*) reared under standard laboratory conditions. Modified and susceptible phenotypes showed increased asymmetry score for temperatures and larval densities above and below standard rearing conditions; a positive correlation was observed between diazinon concentration and asymmetry score. Single and multiple environmental stresses resulted in similar scores that approached, but never exceeded, those of unmodified resistant phenotypes. Irrespective of the developmental conditions anti-symmetry and fluctuating asymmetry were typically observed for each bristle character of unmodified resistant and the modified and susceptible phenotypes, respectively. Thus while similar asymmetry scores could arise from genetic or environmental effects, asymmetry pattern was genetically based. Population cage analyses at different temperatures and larval densities showed a negative association between mean asymmetry and relative fitness.

DEVELOPMENTAL stability has been used extensively to examine the effects of genetic and environmental stress (PALMER and STROBECK 1986; LEARY and ALLENDORF 1989; PARSONS 1992; GRAHAM *et al.* 1993a). Developmental stability refers to an individual's ability to withstand, or buffer, developmental disturbances that result in uncontrolled variation in its phenotype. The most common measure of this is departures from symmetry measured by comparing characters on the left and right sides of individuals that are bilaterally symmetrical (PALMER and STROBECK 1986, 1992).

The use of developmental stability as a measure of genetic and environmental perturbation has usually been restricted to the phenotypic level. Novel environmental and genetic changes may disrupt development, however, the ability to partition these influences and their possible interaction is often limited (GRAHAM 1992; LEARY *et al.* 1992; PARSONS 1992; MARKOW 1994, 1995 for reviews; MCKENZIE and YEN 1995). This is due to the difficulties in defining an organism's environment, its genotype, or both. Therefore, the individual components underlying the observed phenotype have frequently been based on conjecture. This may result in difficulties when interpreting the actual cause of changes in asymmetry.

Developmental stability is also often used as a measure of fitness. One of the assumptions of asymmetry is that there is an optimal developmental pathway (but see GRAHAM *et al.* 1993b). If this is true, deviations from this pathway would be expected to present a physiological cost to the organism (MAYNARD-SMITH *et al.* 1985; PARSONS 1993). Therefore, the ability of an organism to efficiently regulate its development may be considered a selective advantage. Although the relationship between asymmetry and fitness is repeatedly assumed, the evidence is ambiguous (GRAHAM 1992; PALMER and STROBECK 1992; MCKENZIE and O'FARRELL 1993). This may be due to the fact that many studies have been conducted on organisms where the evolutionary history or population biology is unknown or sample size is inadequate (GRAHAM 1992; PALMER 1994). Furthermore, many studies only look at single generations, or at only one fitness component (PACKER and PUSEY 1993). Therefore, these may not be representative of the organism's overall fitness. Further investigation is needed where the evolutionary history, population biology and the overall fitness of an organism are taken into consideration before the issue may be resolved.

There is also controversy over the patterns of asymmetry. Traditionally, fluctuating asymmetry has been the only accepted measure of developmental stability (VAN VALEN 1962; PALMER and STROBECK 1986, 1992). Anti-symmetry and directional asymmetry were believed to contain some unknown genetic component and were therefore considered inappropriate measures of environmental and developmental modification. However, recent studies have indicated that different patterns of asymmetry are interchangeable. This has led to growing support for the use of both anti-symmetry and direc-

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tional asymmetry as measures of developmental perturbation (McKenzie and Clarke 1988; Leary and Allendorf 1989; Graham *et al.* 1993b; McKenzie and O'Farrell 1993).

In this context there are three major issues to resolve in using asymmetry as a measure of developmental stress. First, what is the relative importance of genetic and environmental stresses (Markow 1994, 1995 for reviews; McKenzie and Yen 1995)? Second, can asymmetry be used as a measure of relative fitness (Palmer and Strobeck 1986; McKenzie and Clarke 1988; McKenzie and O'Farrell 1993)? Finally, can developmental instability only be measured by fluctuating asymmetry, or may other patterns be used (McKenzie and Clarke 1988; Leary and Allendorf 1989; Palmer and Strobeck 1992; Graham *et al.* 1993b; McKenzie and O'Farrell 1993; Markow 1995; McKenzie and Yen 1995)? Analysis of the asymmetry phenotypes of the diazinon-resistant genotypes in the Australian sheep blowfly, *Lucilia cuprina*, after development under different environmental conditions, allows an investigation of these questions in circumstances where the genotype is precisely defined.

Diazinon was used as the principle agent in the chemical control of *L. cuprina* between 1958 and 1980 (Hughes and McKenzie 1987). Resistance was first observed 10 years after the introduction of diazinon and is essentially controlled by allelic substitution at the *Rop-1* loci on chromosome *IV* (Arnold and Whitten 1976; Hughes and McKenzie 1987; McKenzie 1993). By the early 1970s, ~90% of the flies were resistant, and by 1980 many populations were close to fixation for the resistant allele (*R*). However, diazinon is still widely used as it continues to provide adequate protection against blowfly strike (McKenzie 1993).

The introduction of the resistant allele resulted in phenotypes of lowered relative fitness, in the absence of insecticide, and increased asymmetry (McKenzie *et al.* 1982; Clarke and McKenzie 1987; McKenzie and Game 1987; McKenzie and Clarke 1988). The effects of the resistant allele are dominant. Due to the continued use of diazinon once resistance occurred, a modifier was selected. This returned asymmetry and fitness levels of resistant flies to that of susceptibles in the absence of diazinon. Mapping experiments indicated that a single gene/gene complex, tightly linked to the *white* locus on chromosome *III*, was responsible for both fitness and asymmetry modification. This gene/gene complex was named *Modifier (M)*. The effects of *Modifier*, like *R*, are dominant for asymmetry and fitness (McKenzie and Purvis 1984; McKenzie and Game 1987; McKenzie and Clarke 1988; McKenzie 1993).

Three key variables in the ecology of the evolution of insecticide resistance in *L. cuprina* are developmental temperature (McKenzie 1990, 1994), larval density (Whitten *et al.* 1980) and insecticide concentration (McKenzie 1987, 1993). These have also been shown to effect developmental stability (Clarke and Ridsdill-Smith 1990; Clarke and McKenzie 1992; McKenzie and Yen 1995). Therefore, by altering these environmental conditions for known genotypes from the diazinon-resistance system, the relative contribution of environment, genotype, and their possible interaction may be examined. The effects of the changes on relative fitness and asymmetry patterns may also be considered. This paper reports such experiments and discusses the results in terms of the three questions that have been raised.

MATERIALS AND METHODS

Strains: Strains used were doubly homozygous for *Modifier/non-Modifier* and diazinon-resistant/susceptible alleles (*M/M;R/R, +/+;R/R, M/M;S/S* and *+/+;S/S*). The origin of these strains has been described in McKenzie and Game (1987). These strains were intercrossed to produce the nine possible *Modifier* and *Rop-1* genotypic combinations. Strains were maintained under standard laboratory conditions (27°, 100 larvae per 140 mL of meat meal medium) unless otherwise stated.

Asymmetry: One hundred flies for each of the nine possible genotypes of *Modifier* and *Rop-1* alleles were scored for asymmetry in each single environmental comparison (temperature, density and diazinon concentration). Fifty flies of each genotype were scored for asymmetry for the temperature/density environmental comparisons in which the genotypes examined were *M/M;R/R, +/+;R/R, M/M;S/S* and *+/+;S/S*. Fifty flies of each genotype were scored for asymmetry for the temperature/density/diazinon concentration environmental comparisons. The genotypes examined in these experiments were *M/M;R/R, +/+;R/R, M/M;R/S, +/+;R/S, M/M;S/S* and *+/+;S/S*. The genotypes for the double and triple environmental stress experiments were chosen based on the results of the single variable experiments, and the fact that the *Modifier* is dominant.

Calculation of asymmetry values followed those of Clarke and McKenzie (1987). The number of frontal head stripe, outer wing margin and R4+5 wing vein bristles were counted on the left and right sides of the fly. Asymmetry for each fly was calculated as the absolute difference between left and right side scores for each of the three characters. Asymmetry pattern was determined by the distribution of signed differences between left and right score for each character (McKenzie and Clarke 1988).

Environmental variables

Temperature: One hundred eggs were placed in cups containing 140 mL of standard laboratory medium. Flies emerged after development at 20°, 25°, 27° and 32°, ±1°.

Density: All experiments were conducted at 27 ±1°. Using first-instar larvae, densities of 25, 100, 250, 500 and 1000 larvae per 140 mL of standard medium were established.

Diazinon concentration: The experiments were conducted at 27 ±1° using 100 first-instar larvae per 140 mL of standard medium with concentrations of diazinon chosen to induce insecticide-dependent mortality in the range of 0–75%. For *S/S* genotypes the diazinon concentrations used were as follows: 0, 0.00008, 0.0001 and 0.00015% (w/v). *R/S* larvae developed on concentrations of 0, 0.0005, 0.0008 and 0.0009% (w/v), while *R/R* larvae were on concentrations of 0, 0.0007, 0.001 and 0.002% (w/v).

Temperature/density: Flies scored emerged after develop-

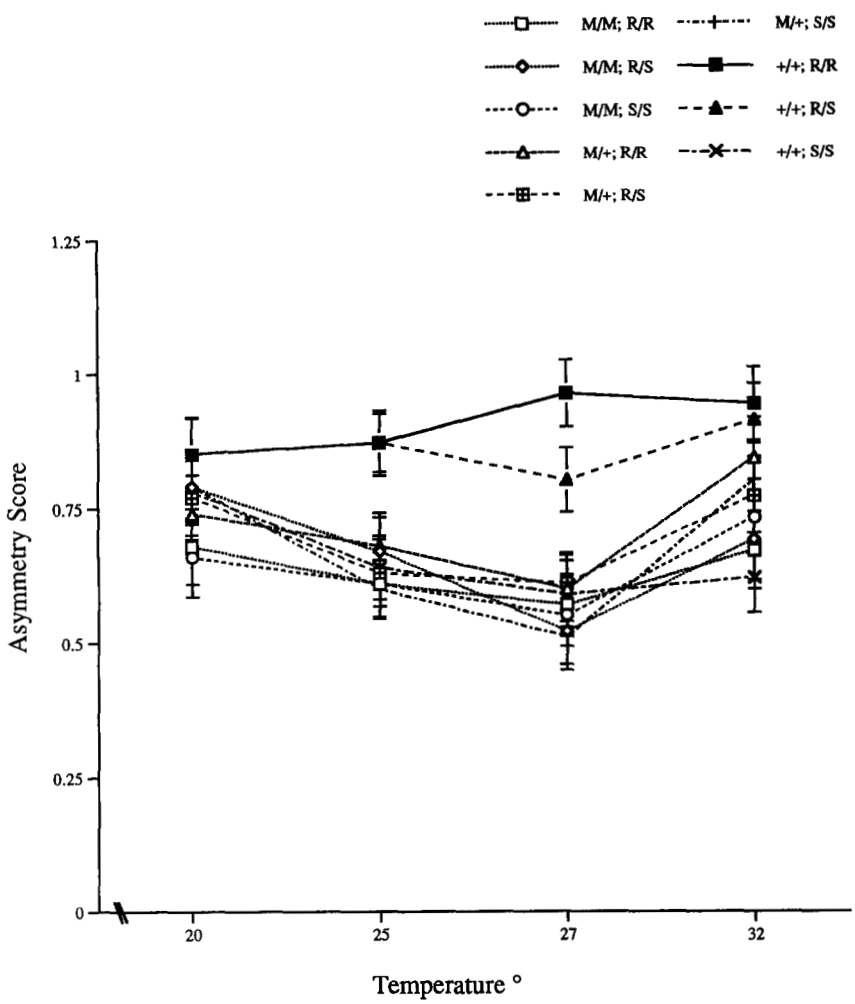

FIGURE 1.—Mean frontal stripe bristle asymmetry ± SE of the nine genotypic combinations of *Modifier* (*M*) and *Rop-1* (*R*) alleles after development at 20°, 25°, 27° and 32° at a density of 100 larvae per 140 mL of standard medium. One hundred individuals of each genotype were scored for asymmetry after development at each temperature.

ment at 20°, 25°, 27° and 32°, ±1°. Larval densities of 25, 100 and 500 larvae per 140 mL of standard medium were established at each temperature using first-instar larvae.

Temperature/density/diazinon concentration: Flies scored emerged after development at 27° and 32°, ±1°. Larval densities of 100 and 500 larvae per 140 mL of standard medium were established for each temperature using first-instar larvae. Diazinon concentrations that killed 0 and 75% of flies under standard laboratory conditions were established for each combination of temperature and density (see diazinon concentrations above).

Population cages: To generate *+/+R/S* flies, *+/+;R/R* females were crossed with *+/+;S/S* males. Similarly, *M/M;R/R* females were crossed to *M/M;S/S* males to generate *M/M;R/S* flies. Discrete generation population cages for *+/+* and *M/M* genotypic classes were initiated with *+/+;R/S* and *M/M;R/S* flies, respectively, and maintained from generation to generation by random egg samples of the adult population.

Population cages were reared at 20°, 25°, 27° and 32°, ±1°. Cages were maintained for eight generations at each temperature at a density of 100 larvae per 140 mL of standard medium. Population cages were also reared at 100 and 500 larvae per 140 mL of standard laboratory medium. These were maintained for 10 generations at 27 ± 1°. Two trials were conducted for each cage.

The proportion of *S/S* individuals in each cage at each generation was estimated by testing random samples of 20 flies with 0.5 μL of 0.01% (v/v) diazinon and scoring the number of dead flies 24 hr later (McKENZIE and GAME 1987).

RESULTS

In previous studies of asymmetry and insecticide resistance in *L. cuprina*, asymmetry scores have been calculated as the absolute difference between the left and right side values pooled over the three bristle characters (CLARKE and McKENZIE 1987; McKENZIE and CLARKE 1988; McKENZIE and O'FARRELL 1993; McKENZIE and YEN 1995). This practice is continued to allow comparison between present and previous data. It is important to note however that similar results are gained if each of the characters is analyzed separately (CLARKE 1987). A representative example is provided for the relationship between asymmetry values of frontal stripe bristles and developmental temperature (Figure 1).

Genotype ($F_{8,3564} = 8.91$, $P < 0.001$) and developmental temperature ($F_{3,3564} = 9.47$, $P < 0.001$) significantly influence asymmetry score, but the interaction between them ($F_{24,3564} = 0.88$, $P > 0.6$) does not. Separate analysis of the *+/+;R/−* phenotypes shows lack of significance for all comparisons (genotype, $F_{1,792} = 1.11$, $P > 0.2$; temperature, $F_{3,792} = 0.50$, $P > 0.6$; interaction, $F_{3,792} = 0.72$, $P > 0.5$), while only temperature significantly affects the asymmetry score of *M/−;−/−* and *+/+;S/S* phenotypes (genotype $F_{6,2272} = 0.81$, $P > 0.5$;

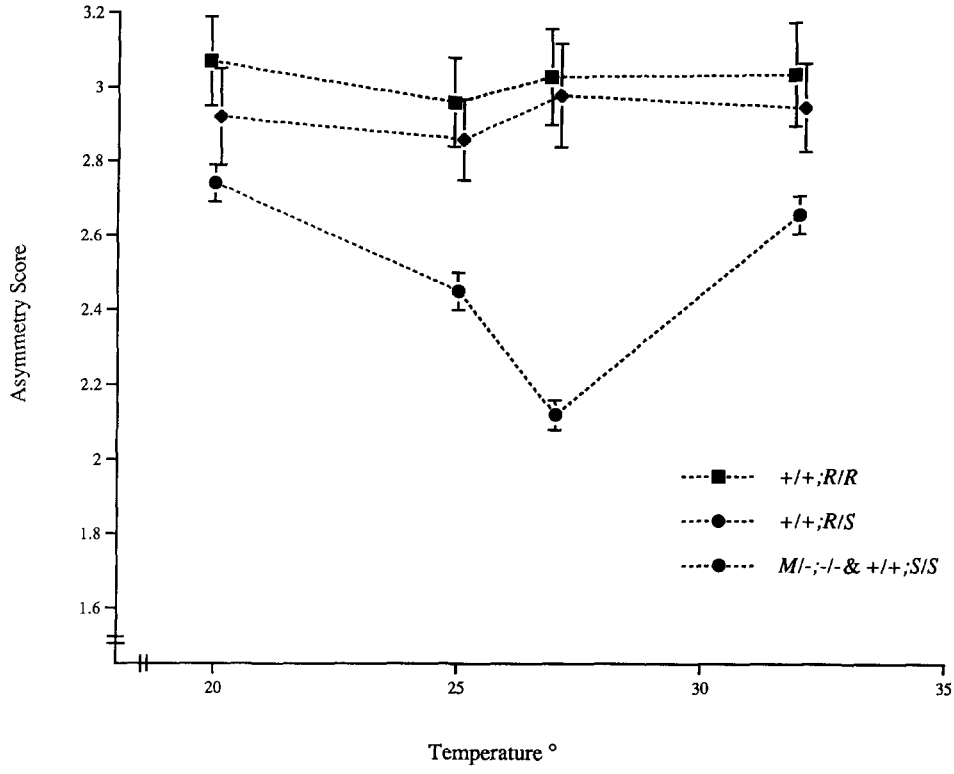

FIGURE 2.—Mean asymmetry ± SE of the nine genotypic combinations of *Modifier* (*M*) and *Rop-1* (*R*) alleles after development at 20°, 25°, 27° or 32° at a density of 100 larvae per 140 mL of standard medium. One hundred individuals of each genotype were scored for asymmetry after development at each temperature. The values of *M/M;R/R, M/M;R/S, M/M;S/S, M/+;R/R, M/+;R/S, M/+;S/S* and *+/+;S/S* individuals were pooled, as there was no significant difference between them.

temperature, $F_{3,2272} = 11.79$, $P < 0.001$; interaction, $F_{18,2272} = 0.60$, $P > 0.9$). Similar observations are made for the pooled data (Figure 2).

The signed differences between the left and right side scores of frontal stripe bristles showed fluctuating asymmetry for susceptible and modified phenotypes. The patterns for the phenotypes of *+/+;R/R* and *+/+;R/S* genotypes were anti-symmetric (data not shown), in accord with previous data (MCKENZIE and CLARKE 1988).

Temperature: When data, pooled over the three bristle characters, of all nine genotypes are analyzed, significant differences are observed between asymmetry levels for genotype ($F_{8,3564} = 11.763$, $P < 0.001$) and developmental temperature ($F_{3,3564} = 26.73$, $P < 0.001$), although there was no significant interaction ($F_{24,3564} = 1.15$, $P > 0.2$) (Figure 2). There is no significant difference between genotypes ($F_{1,792} = 1.19$, $P > 0.2$), temperatures ($F_{3,792} = 0.24$, $P > 0.8$) or interaction ($F_{3,792} = 0.06$, $P > 0.9$) for *+/+;R/−* phenotypes alone. Temperature significantly ($F_{3,2772} = 34.12$, $P < 0.001$) influences asymmetry score of *M/-;-/-* and *+/+;S/S* phenotypes but genotypic ($F_{6,2772} = 0.13$, $P > 0.9$) and interaction ($F_{18,2772} = 0.21$, $P > 0.9$) effects are not significant. For ease of comparison these genotypes were therefore pooled (Figure 2). The asymmetry scores of *+/+;R/R* and *+/+;R/S* are presented separately to demonstrate dominance of the resistant phenotype.

The asymmetry scores of unmodified resistant phenotypes remain relatively constant across the range of temperatures, however, the scores of susceptible and modified phenotypes increase above and below the standard rearing temperature of 27°. At more extreme temperatures the asymmetry scores approach those of unmodified resistant phenotypes (Figure 2).

Density: The results are similar to those observed for temperature. For analysis of the nine genotypes significant differences between asymmetry are observed for genotype ($F_{8,4455} = 9.92$, $P < 0.001$) and larval density ($F_{4,4455} = 10.24$, $P < 0.001$), but there is no significant interaction ($F_{32,4455} = 0.82$, $P > 0.7$). Resistant individuals that lack the *Modifier* have comparable mean asymmetry levels ($F_{1,990} = 0.24$, $P > 0.6$) that are independent of larval density ($F_{4,990} = 0.55$, $P > 0.6$) (Figure 3). However, the mean asymmetry of susceptible and modified phenotypes vary density dependently ($F_{4,3465} = 12.76$, $P > 0.001$) with all genotypes showing similar trends ($F_{6,3465} = 0.30$, $P > 0.9$). At densities above or below standard rearing densities of 100 larvae/140 mL of standard medium asymmetry scores increase with similar values being observed at each of these densities (Figure 3).

Diazinon concentration: Over the concentration range considered diazinon concentration had no significant influence on asymmetry for *+/+;R/R* and *+/+;R/S* individuals (Figure 4), but there is a significantly positive association between diazinon concentration and asymmetry for the susceptible and *Modifier* individuals, except for *M/M;S/S* and *M/M;R/S* genotypes (Figure 4). Although not significant, the latter genotypes also show a positive correlation between diazinon concentration and asymmetry score. That is, a similar trend was observed (Figure 4).

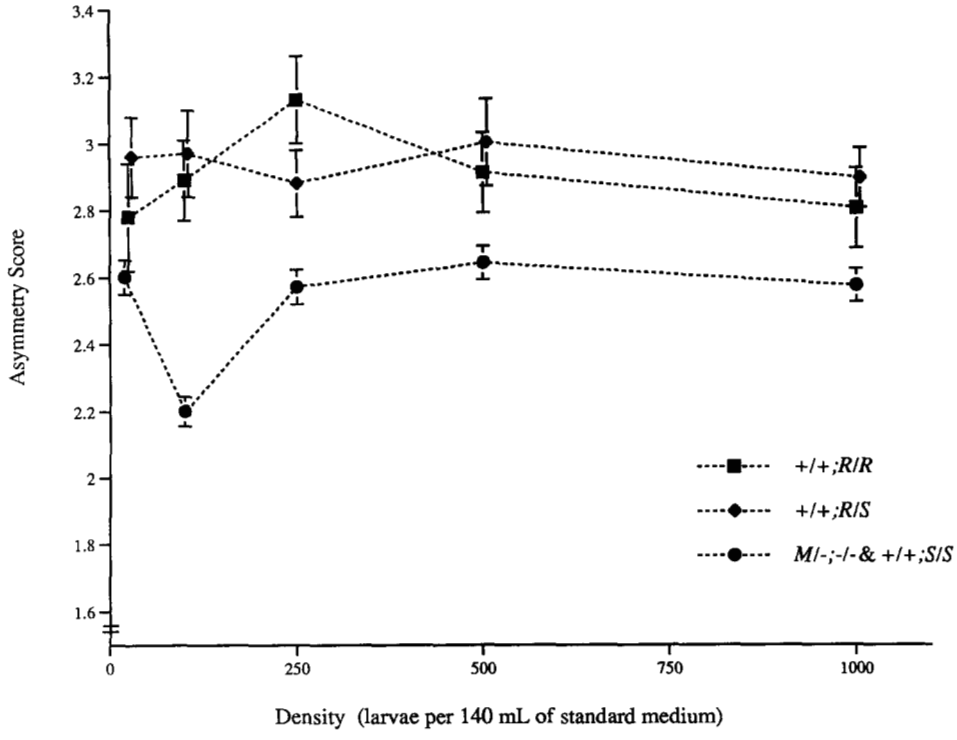

FIGURE 3.—Mean asymmetry ± SE of the nine genotypic combinations of *Modifier* (*M*) and *Rop-1* (*R*) alleles after development at 27° at densities of 25, 100, 250, 500 and 1000 larvae per 140 mL of standard medium. One hundred individuals of each genotype were scored for asymmetry after development at each larval density. The values of *M/M;R/R*, *M/M;R/S*, *M/M;S/S*, *M/+;R/R*, *M/+;R/S*, *M/+;S/S* and *+/+;S/S* individuals were pooled, as there was no significant difference between them.

Temperature/density: For ease of comparison the data are best considered within each of the genotypic classes (Figure 5). The asymmetry score of a genotype after development under normal laboratory conditions (100 larvae/140 mL medium at 27°) acts as a reference point. Within each genotypic class the responses observed when only temperature or larval density vary are similar to those observed previously (Figures 2 and 3). Asymmetry scores of *+/+;S/S*, *M/M;S/S* and *M/M;R/R* phenotypes increase as developmental conditions vary from the standard; scores of *+/+;R/R* flies are unaffected by either variable.

More importantly, in the context of this experiment, simultaneously varying both temperature and larval density does not significantly alter the developmental impact relative to that observed for a single variable alone. Thus, within each of the four genotypic classes, pairwise comparisons of the means of all phenotypes raised under nonstandard conditions give t_{98} values of <1.99 ($P > 0.05$). Therefore neither varying temperature nor larval density singly, or in combination, during development influences the asymmetry score of *+/+;R/R* flies. These variables, however, influence the asymmetry scores of *+/+;S/S*, *M/M;S/S* and *M/M;R/R* phenotypes in a nonadditive manner; single or combined changes of temperature and larval density produce similar asymmetry values within each of these genotypic classes (Figure 5).

Temperature/density/diazinon concentration: Statistical analysis followed that described for the temperature/density experiments. The mean asymmetry scores of *+/+;R/R* and *+/+;R/S* were similar for all developmental conditions ($t_{98} < 1.99$, $P > 0.05$). The remaining four genotypes showed similar increases in asymmetry score, relative to those observed under standard rearing conditions, whether developing phenotypes were subjected to one, two or three stresses (Figure 6). Differences between the asymmetry scores after development with single or multiple stresses were not significant ($t_{98} < 1.99$, $P > 0.05$).

Population cage studies: Population cages with individuals homozygous for the *Modifier* gave relatively constant *S/S* percentages across generations for all temperatures examined (Figure 7). However, the percentage of *S/S* increases with generation number for population cages homozygous for the *non-Modifier*. This occurred at all temperatures (Figure 7).

Similar results were observed for density population cages (Figure 8). That is, cages containing *Modifier* individuals had relatively constant *S/S* percentages, while population cages containing *non-Modifier* individuals had increasing *S/S* percentages with generation number (Figure 8).

A relationship between asymmetry and fitness was examined by comparing the observed and expected percentages of *S/S* individuals for the population cages. Expected values were calculated using mean asymmetry values from the temperature and density experiments as measures of relative fitness. For example, at 20° the mean asymmetry values were 2.70 for *+/+;S/S*, 2.92 for *+/+;R/S* and 3.07 for *+/+;R/R* individuals. Therefore, the relative fitness values are estimated as 1, 0.926, and 0.880, respectively. These fitness estimates were used to determine the expected percentage of *S/S* individuals

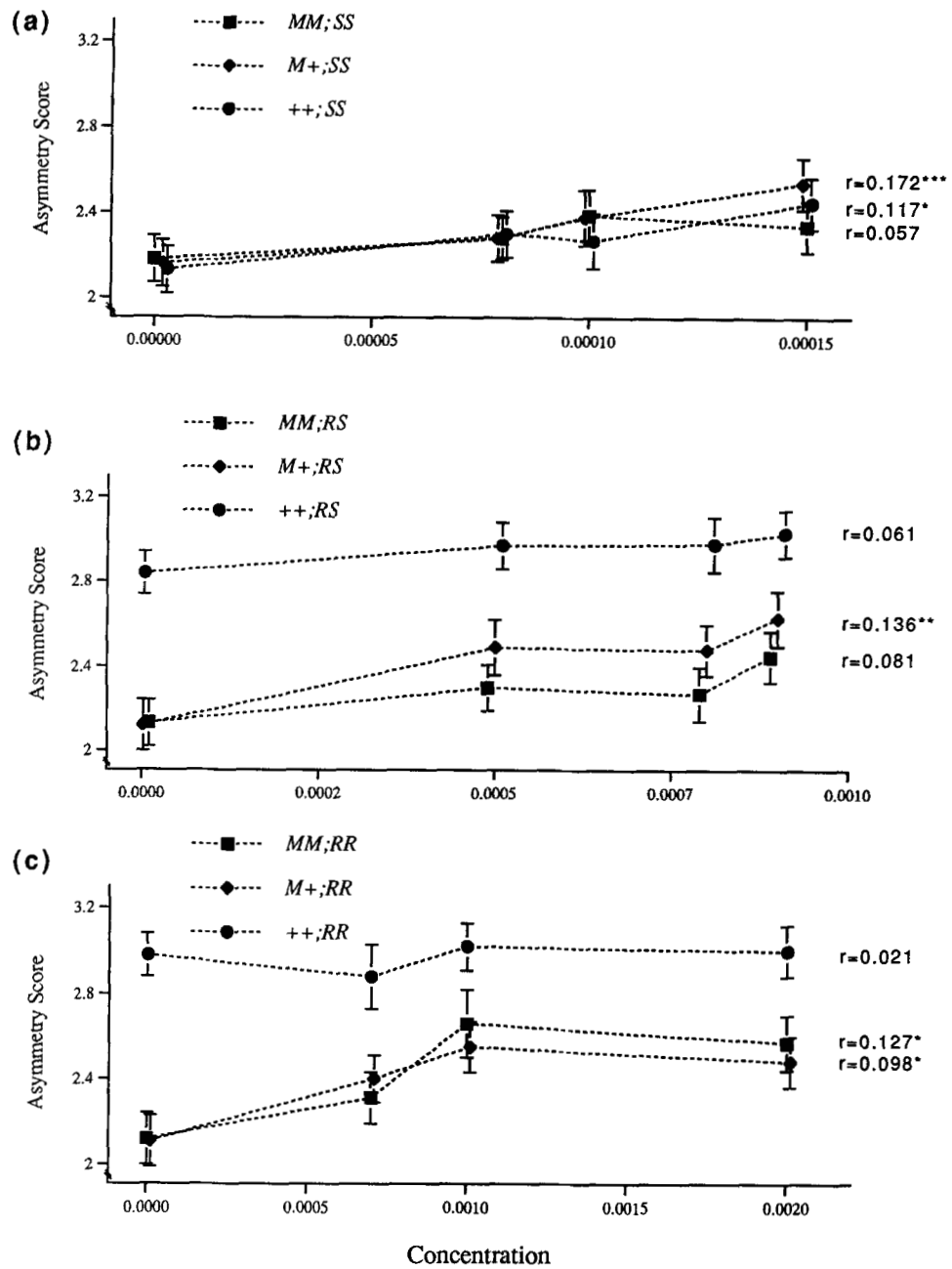

FIGURE 4.—Mean asymmetry ± SE of susceptible (a), heterozygous (b), and resistant homozygous (c) phenotypes after development at 100 larvae per 140 mL of standard medium supplemented with diazinon at the concentrations shown [percent (w/v)]. The rearing temperature was 27°. One hundred individuals of each genotype were scored for asymmetry after development at each diazinon concentration. *Pr < 0.05; **Pr < 0.01; ***Pr < 0.001.

using a discrete generation deterministic model (popsin program by JOHN SVED, personal communication). This was done for each generation, and each temperature and density examined for the population cage studies. A positive correlation between observed and expected was found for both temperature and density population cages (r = 0.712 and r = 0.837, respectively, $P < 0.001$).

DISCUSSION

The use of departures from bilateral symmetry as a measure of developmental stress poses many questions (MARKOW 1995). Three are considered here. What is the relative importance of genetic and environmental stresses? Does asymmetry score provide an estimate of relative fitness? Can patterns other than fluctuating asymmetry be used as indicators of developmental instability?

The answer to the first question implicates both genotype and environment in the determination of asymmetry scores. Furthermore, the influence of the environment is genotypically dependent, although statistically significant genotype × environment interactions are not observed for the characters and environmental vari-

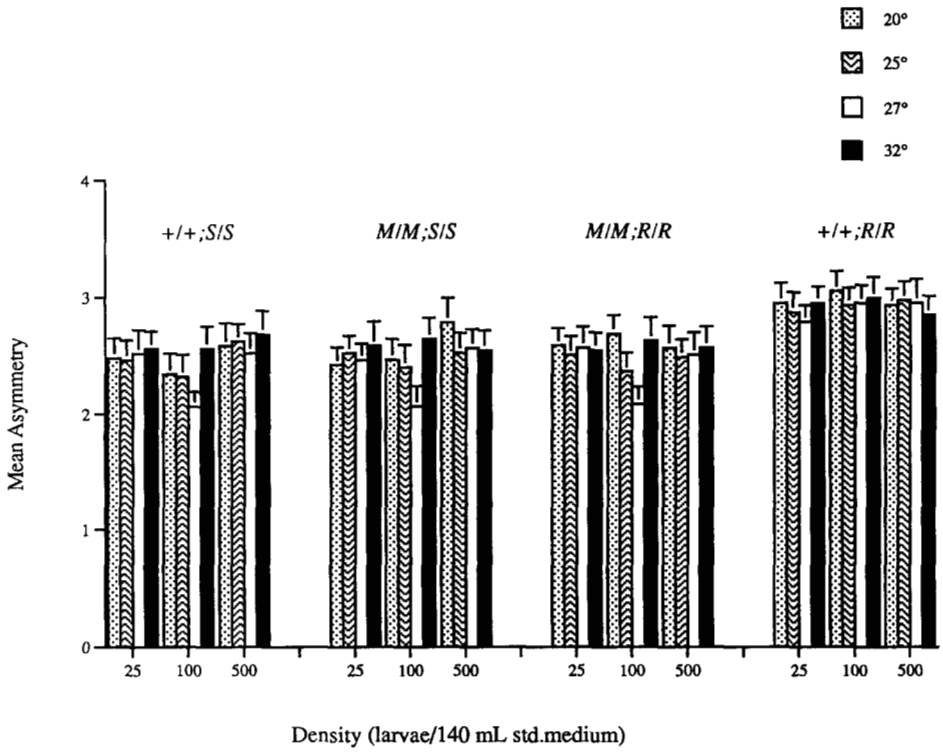

FIGURE 5.—Mean asymmetry ± SE of *M/M;R/R*, *+/+;R/R*, *M/M;S/S* and *+/+;S/S* flies after development at the different combinations of temperatures and densities shown. Fifty individuals of each genotype were scored for asymmetry after development at each condition.

ables considered. This lack of significance is a consequence of similar responses within two asymmetry classes. Unmodified resistant genotypes (*+/+;R/R* and *+/+;R/S*) show similar responses to temperature and larval density for all three characters (Figures 1–3). The responses of *M/−;−/−* and *+/+;S/S* differ from those of *+/+;R/−* but are similar to each other. Within each of the two classes genotype × environment influences are insignificant and this overides any effect within the data as a whole.

The asymmetry values of resistant individuals that lacked the *Modifier* were independent of their environment. That is, they had consistent asymmetry levels despite alterations in developmental temperature, larval density and diazinon concentration. Therefore, genotype appeared more important than environment in determining the asymmetry phenotype for these resistant genotypes. However, the environment played a very important role for susceptible and modified flies. Developmental temperature and larval density above and below standard rearing conditions (27°, 100 larvae per 140 mL of standard medium) resulted in an elevation in asymmetry for these genotypes (Figures 1–3). A general increase in asymmetry was also observed for these genotypes with increasing concentrations of diazinon. Comparisons of the influence of exposure to insecticide during development to asymmetry score are rather coarse because of the necessity to vary the range of concentrations relative to the resistance status of a genotype while generating comparable mortalities across resistance classes. However, the data are consistent with different responses for unmodified resistant and modified and susceptible phenotypes. Exposure to diazinon during development does not significantly influence the asymmetry score of *+/+;R/−* genotypes. This contrasts with the general response of *M/−;−/−* and *+/+;S/S* genotypes (Figure 4).

The results for developmental temperature, larval density and insecticide concentration demonstrate that genotype and environment are important to the asymmetry phenotype of the diazinon-resistance system of *L. cuprina*. Importantly, they emphasize that similar asymmetry scores may result from either genetic or environmental causes (Figures 1–4). Furthermore, a plateau effect was observed for all genotypes. That is, resistant individuals that lack the *Modifier* consistently had asymmetry values of ~3, whereas susceptible and modified flies had a maximum asymmetry of ~2.7. This was further confirmed by experiments where flies were exposed to different combinations of temperature and larval densities, and different combinations of temperature, larval density and concentrations of diazinon (Figure 5 and 6). Asymmetry values of susceptible and modified genotypes approach but never exceed the plateau observed for unmodified resistant phenotypes. The results indicate that in this system the impact of multiple developmental stresses is no more severe than the influence of a single stress. Therefore, the genotypes may place certain restrictions on the levels of asymmetry that may be induced by environmental stresses. This further emphasizes the importance of defining the relative importance of genotype and environment before measures are carried out in the field, particularly as conclusions may be system specific. Thus, while similar

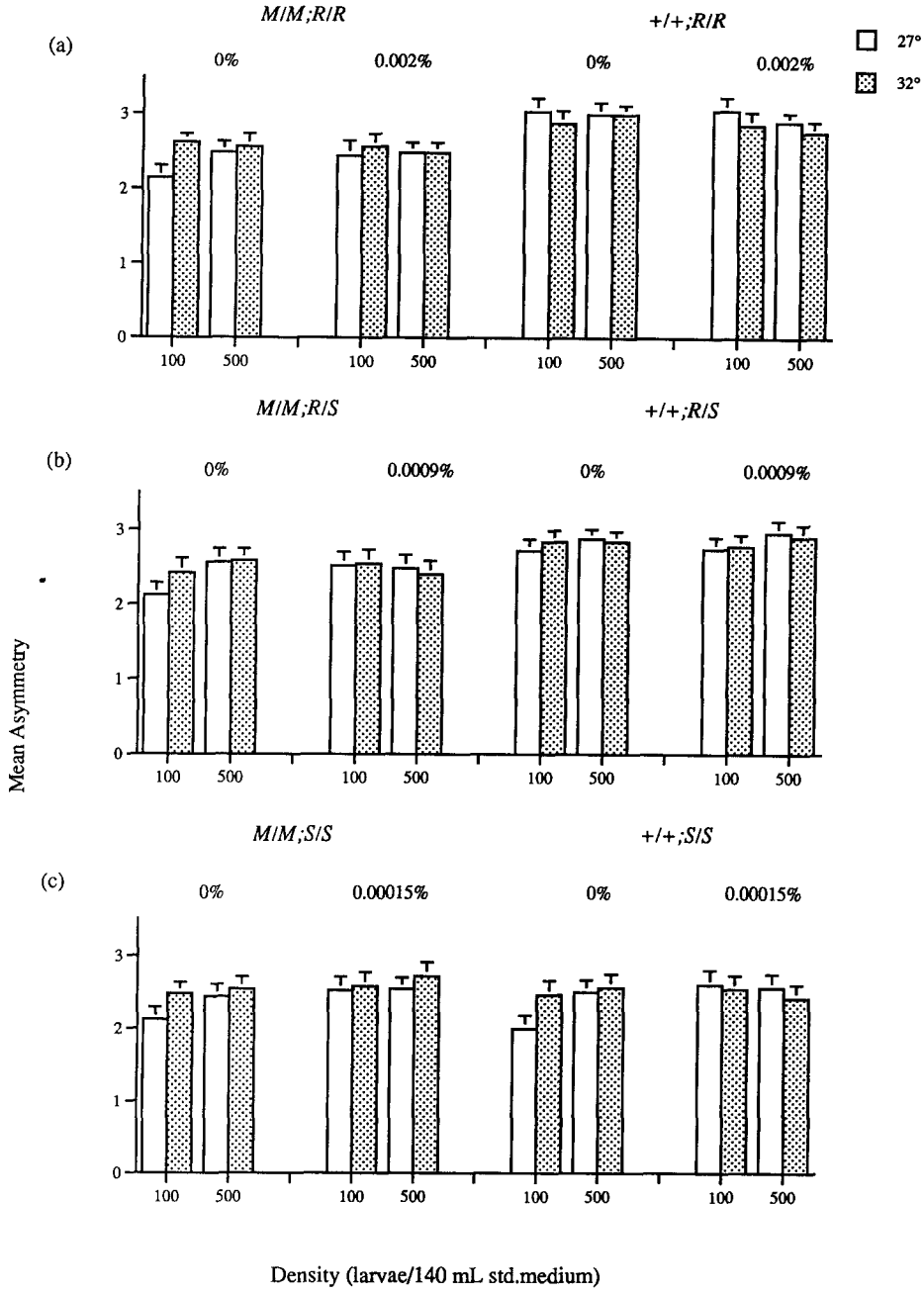

FIGURE 6.—Mean asymmetry ± SE of homozygous resistant (*M/M;R/R, +/+;R/R*) (a), heterozygous (*M/M;R/S, +/+;R/S*) (b), and susceptible (*M/M;S/S, +/+;S/S*) (c) flies. Development occurred at the various combinations of temperature, larval densities and concentrations of diazinon shown. Fifty individuals of each genotype were scored for asymmetry after development at each condition.

observations to those of diazinon-resistance phenotypes are made for the effect of developmental temperature and larval density on the asymmetry phenotype of dieldrin-resistant and susceptible genotypes of *L. cuprina*, asymmetry score is positively correlated with exposure to dieldrin concentration during the development of both resistant and susceptible phenotypes (McKenzie and Yen 1995). The impact of multiple stresses on asymmetry of dieldrin-resistance phenotypes has yet to be assessed.

The bristle characters used in this study were initially chosen for their ease and repeatibility of scoring and because each character displayed similar patterns of variation (Clarke 1987). These characters have formed the basis for several studies of asymmetry and resistance in the Australian sheep blowfly (Clarke and McKenzie 1987; McKenzie and Clarke 1988; McKenzie and O'Farrell 1993; McKenzie and Yen 1995). It is possible different characters may give different responses and any study must balance the choice between many and few characters (Zakharov 1992; Møller and Pomiankowski 1993; Palmer 1994). An assessment of the responses of the characters is also critical in comparative analyses (Palmer 1994; Markow 1995) as is the definition of stress (Parsons 1990, 1992, 1993).

In this study increasing diazinon concentration is clearly a stress as mortality increases as a function of concentration (Whitten *et al.* 1980). It is difficult to maintain cultures of *L. cuprina* at densities and temperatures above and below the high and low extremes of this

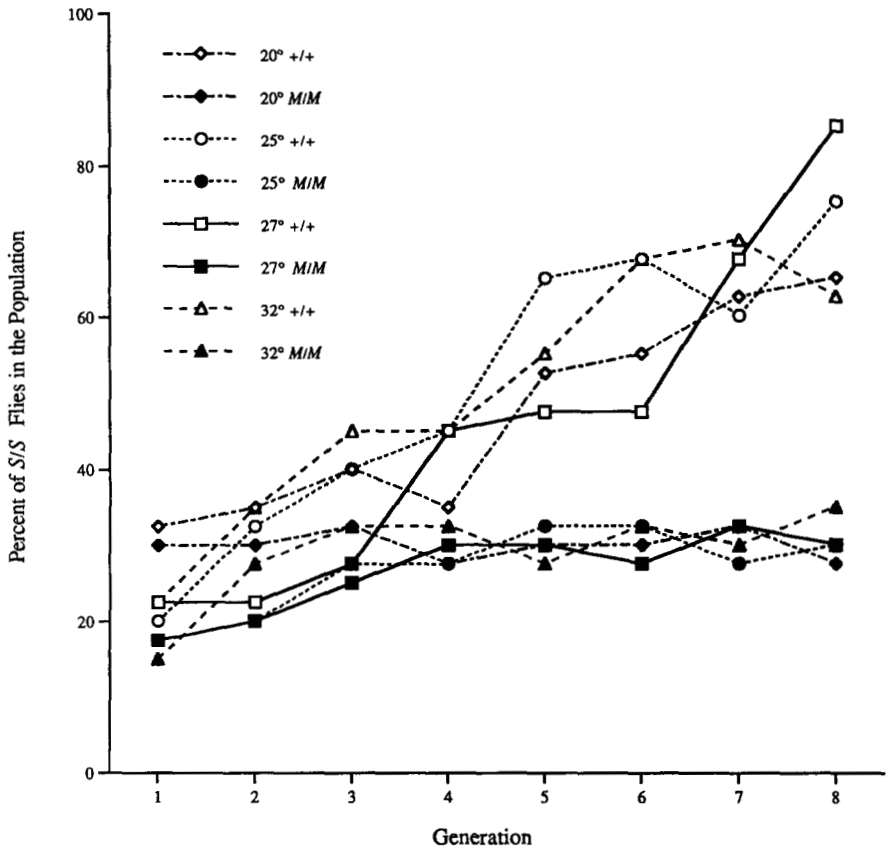

FIGURE 7.—Percentage of *S/S* individuals, averaged over two trials, at each generation for population cages reared at 20°, 25°, 27° or 32°. *M/M (Modifier)* population cages were initiated with *M/M;R/S* flies, while +/+ *(non-Modifier)* population cages were initiated with +/+;*R/S* flies. Each generation of each cage was reared at a density of 100 larvae per 140 mL of standard medium.

study (FOSTER *et al.* 1975; unpublished data), indicating that the extremes represent potential environmental stresses. However, while these three variables are important to the ecology of natural populations of the sheep blowfly (FOSTER *et al.* 1975; WHITTEN *et al.* 1980; MCKENZIE 1993, 1994), the direct relationship of laboratory and field stresses remains unknown. A relationship between asymmetry score and relative fitness in the laboratory is however indicated.

Population cages were examined for the influence of temperature or larval density on allele frequency changes at the *Rop-1* locus (Figures 7 and 8). The changes observed within a cage were correlated with those expected if the relative fitness of phenotypes was estimated by the reciprocal of relative asymmetry scores of phenotypes raised under the temperatures or larval densities experienced within the cage. In these circumstances an increase in relative asymmetry was associated with a decrease in relative fitness. These data are therefore supportive of using asymmetry as a measure of fitness.

Many studies and critical reviews of them have investigated the association between asymmetry and fitness by looking at more global comparisons such as changes following inbreeding, decreased heterozygosity, environmental stress or disruption of coadapted gene complexes (SOULÉ 1979; GRAHAM and FELLEY 1985; PALMER and STROBECK 1986; MARKOW 1995). Other studies have considered components of fitness (PACKER and PUSEY 1993). The current study is unusual in specifically relating asymmetry, fitness estimation and allele frequency changes. If the relationship between asymmetry and relative fitness is to be more generally defined, more studies where the influence of the genotype and the environment are known are required.

In the diazinon-resistance system of *L. cuprina,* both genotype and environment influence asymmetry score, and there is an association between asymmetry and fitness. The data presented contribute to, but do not resolve, the debate of the use of different patterns of asymmetry as indicators of developmental instability (MARKOW 1995).

The distributions of the signed differences of the left and right side scores of the three characters considered separately showed that susceptible and modified individuals generally exhibited fluctuating asymmetry, whereas resistant individuals that lack the *Modifier* usually displayed anti-symmetry. This was true for all environmental conditions tested and is in accord with previously published results (MCKENZIE and CLARKE 1988). As discussed, the levels of asymmetry of susceptible and modified flies were influenced by the environment. Asymmetry score of resistant flies that lacked the *Modifier* was independent of the environmental conditions. Thus the results of this study suggest that for the diazinon system anti-symmetrical distributions indicate genetic perturbation while fluctuating asymmetry may be used to monitor environmental stress. This is supportive

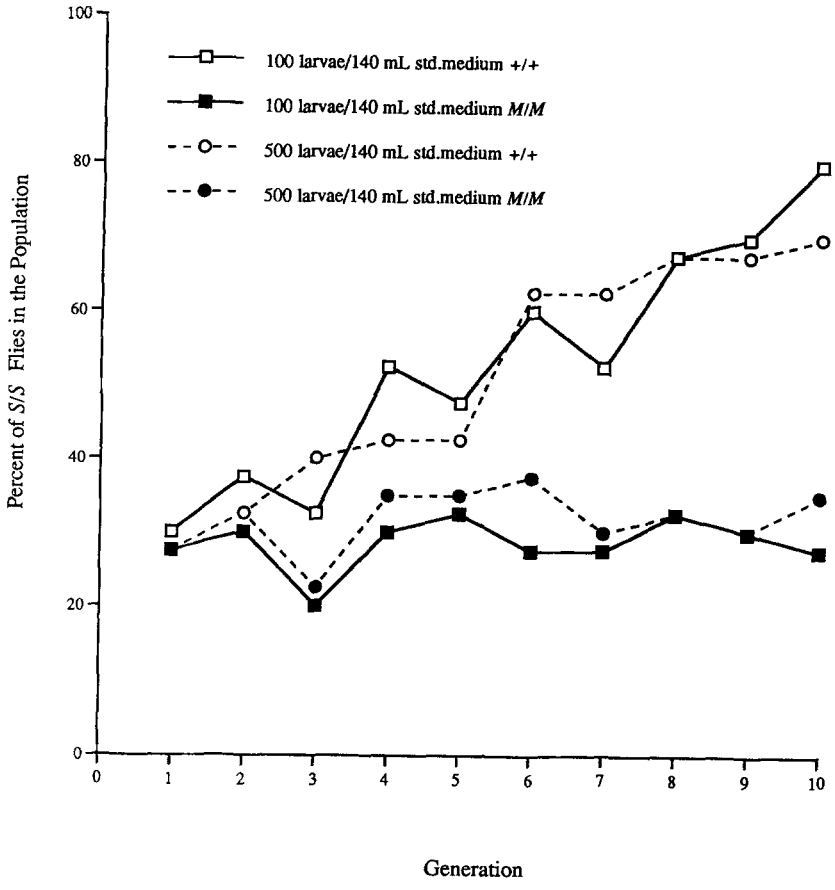

FIGURE 8.—Percentage of *S/S* individuals, averaged over two trials, at each generation for population cages reared at 100 or 500 larvae per 140 mL of standard medium. *M/M (Modifier)* population cages were initiated with *M/M;R/S* flies, while *+/+ (non-Modifier)* population cages were initiated with *+/+;R/S* flies. Population cages were maintained at 27°.

of PALMER and STROBECK'S (1992) view that only fluctuating asymmetry may be used as a measure of environmental perturbation during development. However, it may not be possible to generalize which patterns may be used as a measure of developmental instability. For instance, GRAHAM *et al.* (1993c) observed a transition from fluctuating asymmetry to directional asymmetry in sternopleural bristle patterns of *Drosophila melanogaster* in response to high levels of benzene. Furthermore, in *L. cuprina*, MCKENZIE and YEN (1995) found elevations in mean asymmetry in response to increasing dieldrin concentrations for genotypes that displayed anti-symmetry. A similar relationship was also observed for those genotypes with fluctuating asymmetry. In this case both fluctuating and anti-symmetric responses are indicative of environmental stress. This emphasizes that individual systems need to be considered on their own merits and that the impact of general and specific stresses on asymmetry values and patterns may be system specific (PARSONS 1961, 1990, 1992; MARKOW 1994; MCKENZIE and YEN 1995).

The diazinon-resistance system of *L. cuprina* is well defined. The genetic and environmental influences of general and specific stresses on asymmetry can be quantified. Ultimately, this system may be studied at a population, developmental, molecular and biochemical level, thereby enabling a more robust definition of developmental stability. This may contribute to the emerging field of evolutionary developmental biology, which integrates the study of development, fitness and adaptation (ARTHUR 1987; ATCHLEY and HALL 1991; HALL 1992; KIESER 1993).

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