Stress and asymmetry during arrested development of the Australian sheep blowfly

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SUMMARY

The dieldrin and diazinon resistance systems of the Australian sheep blowfly (*Lucilia cuprina*) have been used previously to relate stress, departures from bilateral symmetry, developmental stability and relative fitness. These systems are now used to consider stress and asymmetry in a developmental context. Larval to adult development is shown to be significantly impaired after arrested development at 8 °C, however the asymmetry score of adults of a given genotype is similar after arrested or continuous development. Selection against dieldrin-resistant and unmodified diazinon-resistant genotypes occurs during arrested development because greater proportions of these genotypes pupate at 8 °C than do susceptible or modified diazinon-resistant genotypes. Pre-pupae of all genotypes complete development equally successfully when transferred from 8 °C to 27 °C. Adults fail to emerge when pupae formed at 8 °C undergo this temperature transition. Temperature-shift experiments show the asymmetry score is determined between pre-pupal and pupal stages of the life cycle. This stage occurs at 27 °C in arrested and continuously developing cultures providing an explanation for the independence of stress, selective mortality during developmental arrest and asymmetry score. The results emphasize the need for genetic, environmental and developmental data before an asymmetry phenotype can be directly related to developmental stability and relative fitness.

1. INTRODUCTION

The effectiveness with which a genotype controls development provides a measure of relative fitness (Maynard Smith *et al.* 1985; Hall 1992; Palopoli & Patel 1996; Batterham *et al.* 1996). The more individuals maintain a particular course of development in the face of genetic or environmental pressures the relatively fitter they will be. Individuals that fail to maintain developmental fidelity incur a physiological cost and are selected against (Maynard Smith *et al.* 1985; Parsons 1992). In normally bilaterally symmetrical organisms, departures from symmetry are commonly used to estimate developmental instability (Palmer & Strobeck 1986; Markow 1995; Palmer 1996) although other methods are also used (Zakharov 1992).

Departures from symmetry are used to consider many different biological problems. For example, this approach can be used to assess the quality of the genotype (Leary *et al.* 1992; Clarke 1993), its capacity to withstand environmental stress (Parsons 1992; Freebairn *et al.* 1996), the impact of parasitism on host development (Polak 1993; Agnew & Koella 1997), and is used as a measure of sexual success (Møller 1992; Thornhill 1992). The rapidly expanding field is regularly reviewed (Palmer & Strobeck 1986; Watson & Thornhill 1994; Markow 1995; Palmer 1996; Møller 1996, 1997). There are, however, ongoing, sometimes acrimonious, debates.

There are several points of conjecture. Some workers (Palmer & Strobeck 1986, 1992; Palmer 1996) support the view that fluctuating asymmetry alone can be used to measure developmental stability, others believe directional and antisymmetrical patterns may also be appropriate (McKenzie & Clarke 1988; Leary & Allendorf 1989; Graham *et al.* 1993; Freebairn *et al.* 1996). There is also much debate about the genetic contribution to the asymmetry phenotype with heritability estimates ranging from negligible (Brakefield & Breuker 1996) to significant (Møller & Thornhill 1997*a*).

Møller & Thornhill's (1997*a*) meta-analysis of the heritability of developmental stability has excited much comment. While their approach and interpretations of the analysis have received some qualified support (Swaddle 1997), the majority of commentators are critical of the literature or traits selected for, or the methods of analyses used in their study (Houle 1997; Leamy 1997; Markow & Clarke 1997; Palmer & Strobeck 1997; Pomiankowski 1997; Whitlock & Fowler 1997). Even the reasons for the study and the conclusions drawn have been questioned (Markow & Clarke 1997; Palmer & Strobeck 1997).

Almost equal controversy surrounds any general relationship between levels of inbreeding and asymmetry (Leary & Allendorf 1989; Parsons 1992; Clarke *et al.* 1992; Fowler & Whitlock 1994), the choice of characters used to estimate developmental instability (Palmer & Strobeck 1986; Dufour & Weatherhead

1996), and the statistical methods of analysis (Palmer 1994). Indeed, the relationship between stress, asymmetry and relative fitness is not always perceived as unambiguous (Graham 1992; Clarke 1995; Palmer 1996; Møller 1997).

A major confounding factor in the debate is that it is rarely possible to directly partition the asymmetry phenotype into genetic and environmental components and to relate changes in asymmetry phenotype to differences in components of fitness. The dieldrin and diazinon resistance systems of *Lucilia cuprina* overcome these difficulties as estimates of asymmetry are repeatable in particular environments and are influenced by allelic substitution at single genetic loci (McKenzie & Yen 1995; Freebairn *et al.* 1996). The following summary demonstrates these systems are ideal to understand how changes in asymmetry occur and to caution against over-interpretation of data based only on phenotypic analysis.

Resistance to dieldrin, a cyclodiene, is controlled by a gene (*Rdl*) on chromosome V; resistance to diazinon, an organophosphorus insecticide, by a gene (*Rop1*) on chromosome IV (McKenzie 1987). Resistant phenotypes are significantly more asymmetric than susceptibles (Clarke & McKenzie 1987; McKenzie & Clarke 1988). The asymmetry phenotype of dieldrin-resistant flies is dominant and independent of larval density and developmental temperature, environmental variables that influence the asymmetry of susceptibles. Asymmetry score is positively correlated with the dieldrin-concentration of the larval growth medium of all *Rdl* genotypes (McKenzie & Yen 1995).

The dominant asymmetry score of diazinon-resistant phenotypes is reduced to that of susceptibles by a dominant fitness/asymmetry-modifier gene on chromosome III (McKenzie & Game 1987; McKenzie & Clarke 1988). The score is environmentally independent for unmodified resistant phenotypes, but modified genotypes and unmodified susceptibles (at the *Rop1* locus) are influenced by developmental temperature, larval density or concentration of diazinon in the larval medium (Freebairn *et al.* 1996).

Dieldrin-resistant and unmodified diazinon resistant phenotypes consistently display anti-symmetrical patterns, other genotypes fluctuating asymmetry (Palmer & Strobeck 1986; Palmer 1996), in all environments (McKenzie & Clarke 1988; McKenzie & Yen 1995; Freebairn *et al.* 1996). The modifier of the diazinon-resistance system does not influence genotypes of the dieldrin resistance system (Clarke & McKenzie 1987).

These resistance systems are thus unusual in the analysis of asymmetry in that genetic and environmental influences on the phenotype can be related to differences in fitness during continuous development. Furthermore, the results indicate similar asymmetry scores may result from genetic or environmental causes (McKenzie & Yen 1995; Freebairn *et al.* 1996). The present study uses this foundation and information about the population biology of *L. cuprina* to consider these relationships during arrested development.

L. cuprina overwinters as pre-pupae following temperature-dependent arrested development of wandering larvae. Significant mortality occurs during this stage of the life cycle (Foster *et al.* 1975; Dallwitz & Wardaugh 1984) with dieldrin-resistant and unmodified diazinon-resistant phenotypes selected against at low temperature (McKenzie 1990, 1994). The relationship between low temperature stress and asymmetry was therefore investigated. The developmental period during which temperature influences the bristle characters used to score asymmetry was also considered. The latter information proves necessary to interpret the genotypically dependent and environmentally independent asymmetry scores observed following arrested development.

2. MATERIALS AND METHODS

(*a*) *Strains*

For the dieldrin resistance system the strains used were homozygous dieldrin-resistant (*Rdl/Rdl*) and homozygous dieldrin-susceptible (+/+). These strains share a common genetic background (McKenzie & Yen 1995). The strains of the diazinon resistance system were doubly homozygous for modifier (*M*)/non-modifier and diazinon-resistant/susceptible alleles (*M/M*; *Rop1/Rop1*, +/+; *Rop1/Rop1*, *M/M*; +/+, +/+; +/+) (McKenzie & Game 1987). Appropriate crosses were conducted within a resistance system to produce larvae of the three dieldrin-resistance and the nine *M* and Rop_1 genotypes.

(*b*) *Development of wandering larvae to adults*

Larvae were raised at 27 ± 1 °C under standard laboratory conditions at a density of 100 per 140 ml of insecticide-free meat meal medium (standard density). In each trial, two samples of 100, 4-day-old, wandering larvae of each genotype were collected and each sample was placed in plastic containers containing vermiculite. One sample (control) was held at 27 ± 1 °C, the other (experimental) at 8 ± 1 °C. These are the respective conditions for continuous and arrested development (McKenzie 1990, 1994). After four weeks the experimental sample was transferred to the control temperature. The number of adults emerging in control and experimental samples was recorded. Five trials were conducted.

In these experiments it was noted that under experimental conditions wandering larvae either die, arrest as pre-pupae or pupate. The experiments were therefore repeated. The percentage of each genotype pupating was recorded. The percentage of pre-pupae and pupae which subsequently develop to flies after transfer to 27 °C was also recorded. For comparison, the percentage of wandering larvae that pupate in control conditions was recorded. Five trials were conducted.

(*c*) *Asymmetry*

For experimental and control samples, 50 flies of each genotype were chosen from those emerging in the developmental trials and scored for asymmetry. Calculation of asymmetry values followed that previously described (McKenzie & Clarke 1988; McKenzie & Yen 1995; Freebairn *et al.* 1996). The numbers of frontal head stripe, outer wing margin and R4 + 5 wing vein bristles were counted on the left and right sides of a fly. Each count was repeatable. Asymmetry score was 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 the left and right score for each character

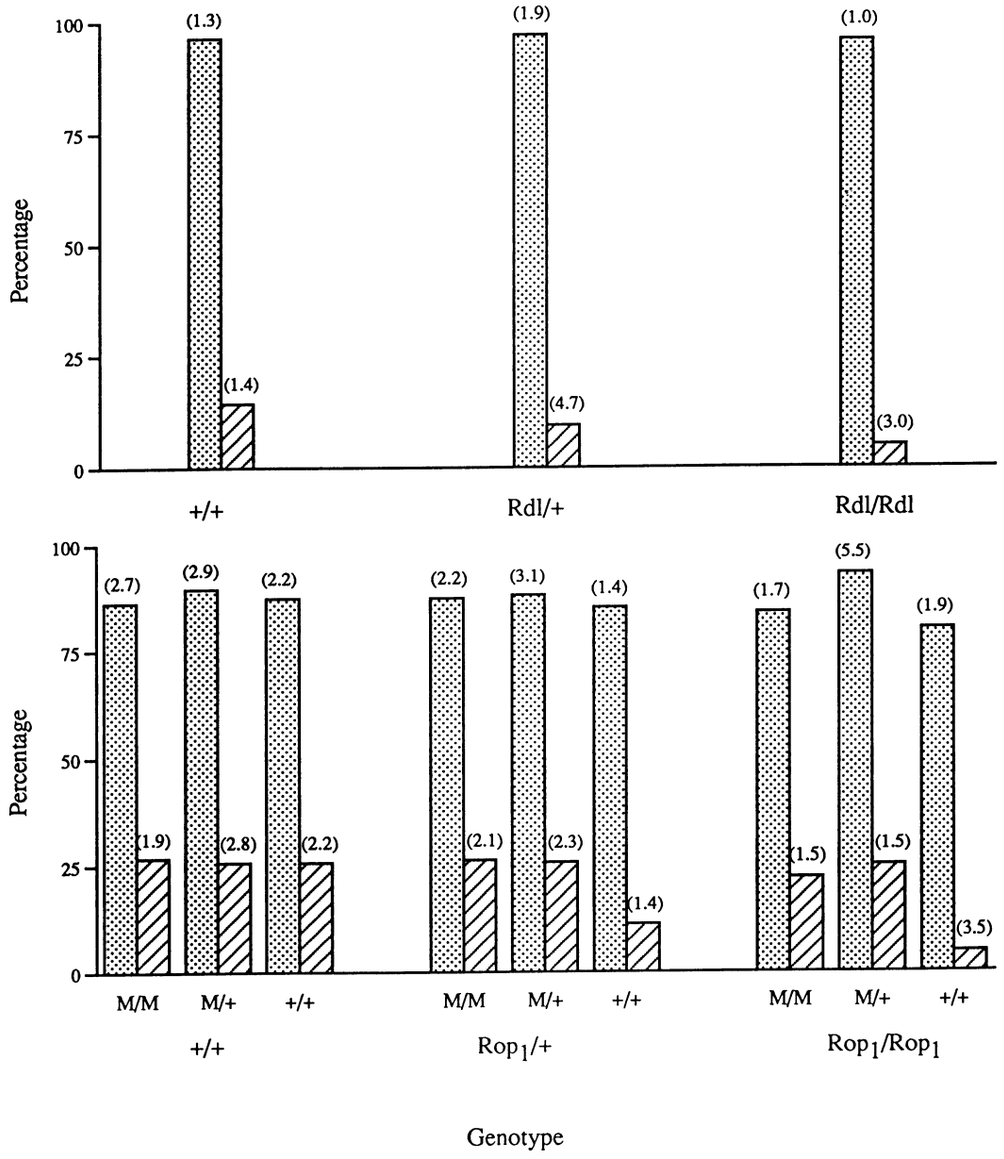

Figure 1. Mean percentage (s.e. in angular scale) of wandering larvae that develop to emerge as adults after continuous development at 27 °C (dotted) or after arrested development for four weeks at 8 °C (diagonal lines) followed by transfer to 27 °C. Genotypes are for the dieldrin-resistance system (*Rdl*, +) and for the diazinon-resistance system (*Rop1*, +) and its modifier gene (*M*, +).

(Palmer & Strobeck 1986; McKenzie & Clarke 1988; McKenzie & Yen 1995).

(*d*) *Temperature-shift experiments*

Larval cultures of an unmodified strain susceptible to dieldrin and diazinon (+/+; +/+; +/+) were raised at standard density at 27 ± 1 °C or 32 ± 1 °C.

Cultures were allowed to complete development at these temperatures or reciprocal transfers (27 °C to 32 °C, 32 °C to 27 °C) were made at the wandering larvae and pupal stages of the life cycle. Samples of 25 adults for each comparison were scored for asymmetry. Two trials were conducted.

(*e*) *Statistical analysis*

Standard analyses of variance were used. Percentage data were analysed after angular transformation. By convention, standard errors for such data are presented in the transformed scale (Sokal & Rohlf 1969). In accord with previous studies with *L. cuprina* (McKenzie & Clarke 1988; McKenzie & Yen 1995; Freebairn *et al.* 1996), asymmetry pattern analysis followed the methods of Palmer & Strobeck (1986).

3. RESULTS

(*a*) *Development of wandering larvae to adults*

The percentage of wandering larvae that develop to the adult stage of the life cycle is significantly ($p < 0.001$) greater during continuous development at 27 °C, than after arrested development at 8 °C, for both resistance systems (dieldrin, $F_{1,24} = 931.9$; diazinon, $F_{1,72} = 958.7$) (figure 1). Within each resistance system the genotypes perform differently (dieldrin, $F_{2,24} = 7.3$, $p < 0.01$; diazinon, $F_{8,72} = 5.0$, $p < 0.001$) but

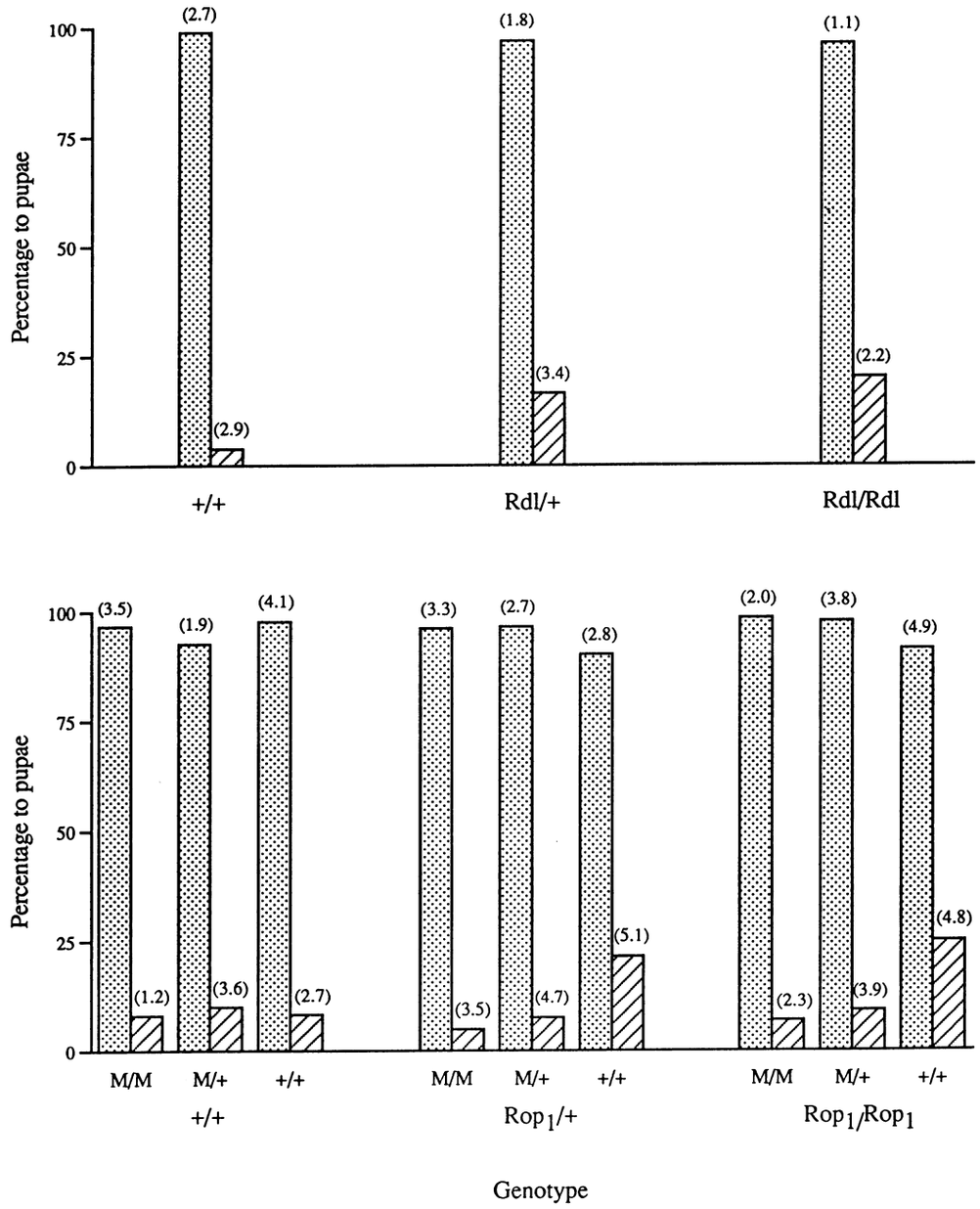

Figure 2. Mean percentage (s.e. in angular scale) of wandering larvae, of the three genotypes of the dieldrin-resistance system and the nine genotypes of the diazinon-resistance system and its modifier, that pupate when held at 27 °C (dotted) or 8 °C (diagonal lines).

genotype × environment interaction is observed only for the dieldrin resistance system ($F_{2,24}=5.4$, $p<0.025$). The difference between genotypes occurs during arrested development (dieldrin, $F_{2,12}=7.5$, $p<0.01$; diazinon, $F_{8,36}=8.6$, $p<0.001$) as there are no significant differences between genotypes within a resistance system during continuous development. Thus dieldrin-resistant and unmodified diazinon-resistant phenotypes are selected against during arrested development (figure 1).

The differences between the proportion of wandering larvae pupating during continuous or arrested development is clear (figure 2) and highly significant ($p<0.001$, dieldrin, $F_{1,24}=898.4$; diazinon, $F_{1,72}=1241.3$). Significant differences between genotypes are observed only during arrested development (dieldrin, $F_{2,12}=8.34$, $p<0.01$; diazinon, $F_{8,36}=2.42$, $p<0.05$). Dieldrin-resistant and unmodified diazinon-resistant phenotypes pupate more commonly than other phenotypes of their respective resistance systems in this developmental regime (figure 2).

In accord with the data of figure 1, the percentage of pupae from which flies emerge after continuous development is similar for all genotypes. It is in excess of 90% in each case. Likewise, after pupation during arrested development, the percentage of pupae from

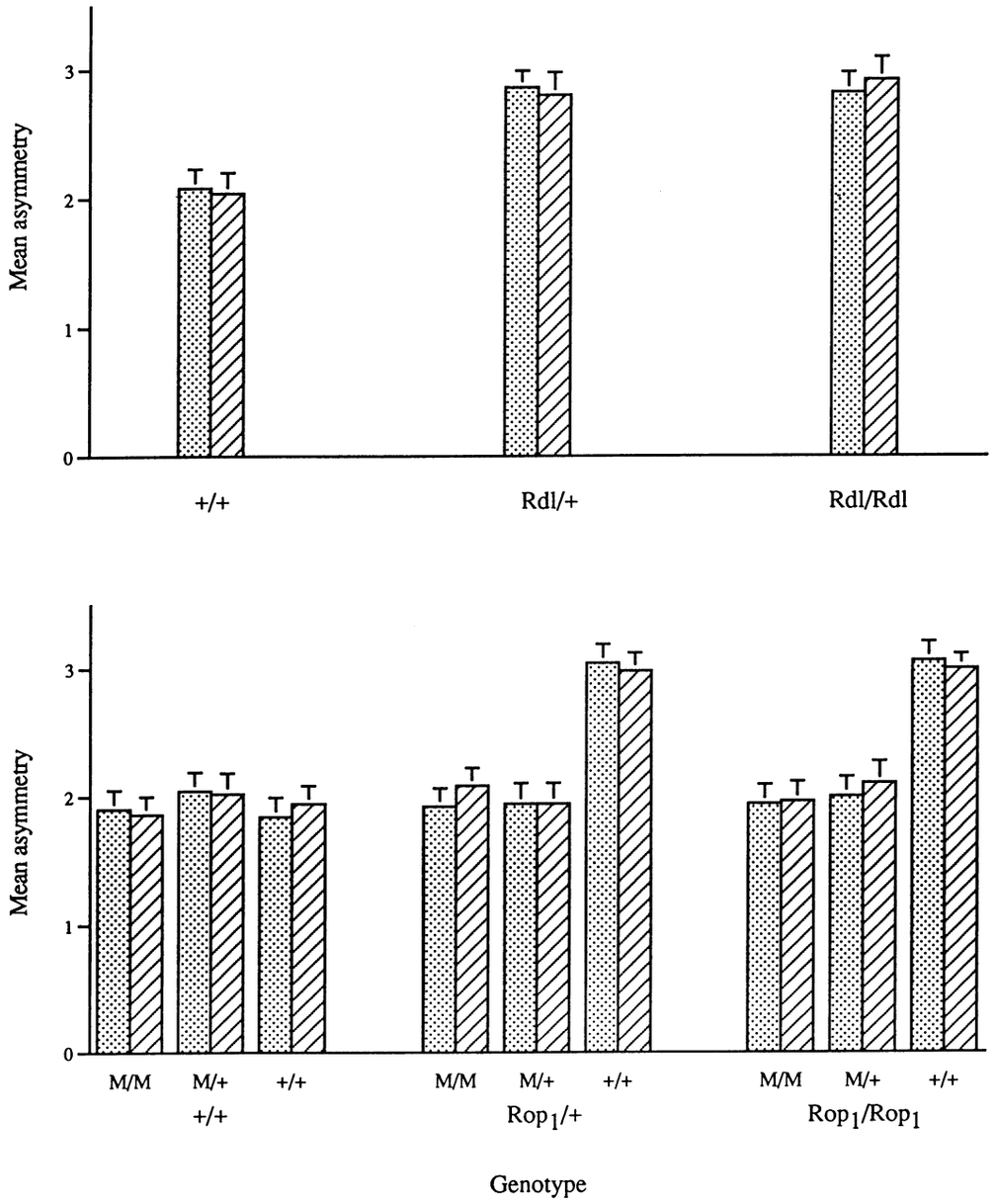

Figure 3. Mean asymmetry score (+ s.e.) of flies of the three genotypes of the dieldrin-resistance system and the nine genotypes of the diazinon-resistance system and its modifier after continuous (dotted), or arrested (diagonal lines), development.

which flies emerge after transfer to 27 °C is similar for all genotypes. However, in this case, it is essentially zero (range 0–0.7%).

(*b*) *Asymmetry*

For comparison with previous studies the asymmetry is calculated as the absolute difference over the three bristle characters (Clarke & McKenzie 1987; McKenzie & Clarke 1988; McKenzie & Yen 1995; Freebairn *et al.* 1996). Similar results are observed if the characters are analysed separately (Freebairn *et al.* 1996).

Within each resistance system mean asymmetry scores differ significantly ($p < 0.001$) between genotypes (dieldrin, $F_{2,294} = 16.1$; diazinon, $F_{8,882} = 20.2$). Within a resistance system, dieldrin-resistant and unmodified-diazinon resistant genotypes have greater scores than other genotypes (figure 3). The influence of the resistant alleles is dominant. The effect of the modifier in ameliorating this influence in the diazinon resistance system is also dominant.

The differences between genotypes are similar for continuous and arrested development and interactive effects are also insignificant (figure 3). There is also a consistency of asymmetry pattern for both developmental regimes. Dieldrin-resistant and unmodified diazinon-resistant genotypes are anti-symmetric for each bristle character. Fluctuating asymmetry is observed in every other case (data not shown). The results for asymmetry pattern agree with previous results (McKenzie & Clarke 1988; McKenzie & Yen 1995; Freebairn *et al.* 1996).

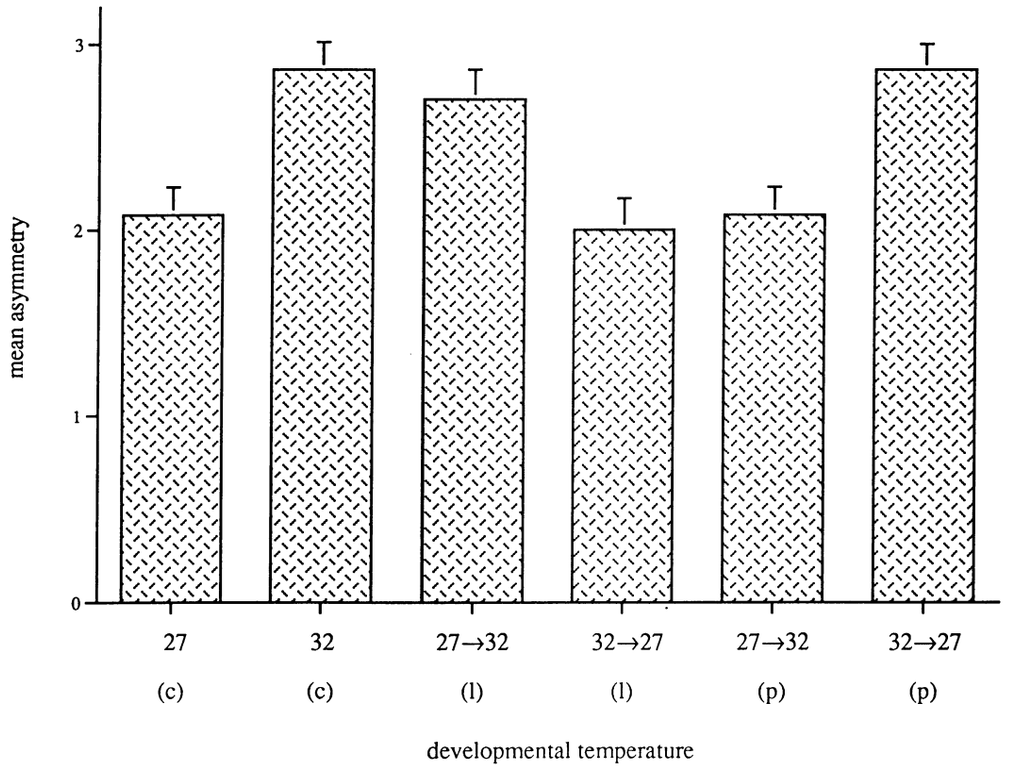

Figure 4. Mean asymmetry score (+ s.e.) of unmodified flies susceptible to dieldrin and diazinon experiencing different developmental temperature conditions. Flies complete development from the larval stage at constant temperatures (c) of 27 °C or 32 °C or after reciprocal transfer of larvae (l) or pupae (p) between 27 °C and 32 °C.

(*c*) *Temperature-shift experiments*

Mean asymmetry score is significantly influenced ($F_{5,294}=7.6$, $p<0.001$) by the temperature experienced at specific developmental stages (figure 4). Asymmetry scores cluster in two groups separated by least significant difference analysis (Sokal & Rohlf 1969). Complete development at 27 °C, transfer of larvae from 32 °C to 27 °C, or transfer of pupae from 27 °C to 32 °C yield flies of similar asymmetry score. The remaining developmental conditions also give flies of similar score, elevated above those of the previous grouping (figure 4). The temperature-shift experiments thus indicate the critical time for temperature to influence the asymmetry of the bristle characters used in this study lies between the wandering larvae and the pupal stages of the life cycle.

4. DISCUSSION

The observation that dieldrin-resistant and unmodified diazinon-resistant phenotypes are selected against during arrested development (figure 1) is in accord with previous results (McKenzie 1990, 1994). The present results provide at least a partial explanation for this selective effect. The physiological cost of resistance manifests itself as a relative failure to arrest development at the pre-pupal stage. Failure to arrest development at the pre-pupal stage is functionally lethal as adults fail to emerge from pupae collected after arrest at 8 °C. Conversely, 75–95% of pre-pupae transferred to permissive developmental temperatures eventually give rise to adults. The results are similar for all genotypes. Thus, differential survivorship does not occur for pre-pupae. This stage alone survives arrested development.

Temperature-shift experiments of life cycle stages show that during continuous development the critical period for temperature-dependent bristle asymmetry (McKenzie & Yen 1995; Freebairn *et al.* 1996) is between the wandering larvae and pupal stages (figure 4). Therefore genotypes that successfully arrest development make the pre-pupal to pupal transition at the normal developmental temperature of 27 °C. The similar asymmetries of a given genotype after continuous or arrested development reflect this (figure 3).

The data suggest that individuals that successfully arrest development at low temperature are unstressed by the environment. Those who are stressed fail to arrest development, fail to reach the adult stage and therefore cannot be scored for asymmetry. Environmental stress, in this instance, is lethal. Its influence cannot be measured by changes in asymmetry.

The present study is unusual in being able to so closely associate genotype, developmental conditions and asymmetry score. The potential limitations of more broad-scale studies to relate asymmetry and fitness have been recognized (May 1995; Clarke 1995; Palmer 1996; Møller 1997). When taken in conjunction with previous studies of the insecticide resistance systems of *L. cuprina* (McKenzie & Yen 1995; Freebairn

et al. 1996) the current results support this concern and help to focus other arguments. For example, there is considerable debate about the use of departures from symmetry other than fluctuating asymmetry for estimates of developmental perturbation (Graham *et al.* 1993; Palmer 1996; Møller & Thornhill 1997*b*). Previous studies of the dieldrin and diazinon resistance systems of *L. cuprina* have shown that asymmetry pattern is genetically determined while asymmetry score is influenced by genotype and environment during continuous development. Both pattern and score provide estimates of developmental perturbation (McKenzie & Clarke 1988; McKenzie & Yen 1995; Freebairn *et al.* 1996). Some environmental stresses influence both fluctuating and antisymmetrical distributions (McKenzie & Yen 1995). It is therefore concluded that in the absence of data on the genetic and environmental components of the asymmetry phenotype and information of the population and developmental biology of the organism, any generalizations concerning asymmetry, developmental stability and relative fitness should be interpreted with extreme caution. Debates about which patterns of asymmetry are appropriate indicators of developmental stability should be viewed in this context as should advocacy of departures from symmetry, of whatever variety, as general monitors of adaptation (Sommer 1996).

When these conclusions are coupled with the concern of others for the choice of characters (Dufour & Weatherhead 1996; Brakefield & Breuker 1996), and the methods of analysis (Palmer 1994), used to assess developmental stability it is clear that much work is still necessary before departures from symmetry will be universally regarded as appropriate to address questions of ecological, evolutionary or developmental interest (Markow 1995; May 1995; Palmer 1996).

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