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Wings and bristles: character specificity of the asymmetry phenotype in insecticide-resistant strains of *Lucilia cuprina*

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We investigated the hypothesis that observed higher levels of asymmetry displayed by insecticide-resistance genotypes of *Lucilia cuprina* are restricted to bristle characters, due to the action of resistance genes in bristle cell development, rather than through the disruption of genomic coadaptation. We compared the level of asymmetry of three bristle characters and three wing characters in non-modified- and modified-resistance genotypes. Consistent with previous studies, resistance genotypes displayed greater levels of bristle asymmetry than either susceptible or modified genotypes. However, there were no differences among genotypes for any of the wing characters. To confirm that this result is attributable to the action of the resistance and modifier genes themselves, we also examined the responses of both bristle and wing characters to the more general developmental stress of extreme temperature. Sub-optimal temperature was shown to increase both bristle and wing asymmetry, suggesting that there are no underlying differences between the two character types which could, of themselves, explain the differential response observed in the resistance genotypes.

Keywords: developmental stability; fluctuating asymmetry; insecticide resistance; sheep blowfly

1. INTRODUCTION

Selection for genes conferring insecticide resistance within the genome of the Australian sheep blowfly, *Lucilia cuprina*, has been shown to lead to an increase in the level of asymmetry for three bristle characters, presumably due to disruption of the underlying developmental stability of these characters (Clarke & McKenzie 1987; McKenzie & Clarke 1988; McKenzie & O'Farrell 1993; McKenzie & Yen 1995). In the cases of diazinon and malathion resistance, subsequent selection for background modifier genes has ameliorated the negative impacts of these resistance genes, reducing asymmetry scores to levels similar to those present in susceptible genotypes (McKenzie & O'Farrell 1993). Bristles act as sensory organs within the fly and their development is controlled by a series of proneural and neurogenic genes. It has been hypothesized that the gene products of both the resistance and modifier genes have cell-adhesion properties and are involved in cell decision-making processes within the nervous system. Thus, the interaction between these gene products has been suggested to influence bristle-cell development directly (Batterham *et al.* 1996; Clarke 1997). Under this hypothesis, it would be predicted that the influence of resistance and modifier genes on changes in asymmetry would be restricted to bristle characters.

Alternatively, it has been argued that the introduction of resistance genes into the genome has resulted in a disruption of coadapted gene complexes (Clarke & McKenzie 1987; McKenzie & Clarke 1988). Such genomic coadaptation has been generally seen to represent a possible genetic basis for developmental stability in a number of systems (reviewed by Clarke 1993). Within such a framework, selection of the modifier gene in *L. cuprina* is considered to act to restore the genic balance, resulting in a concomitant reduction in levels of asymmetry. Under this hypothesis it might be expected that the influence of resistance genes would be more general and impact upon a number of different characters. To date, it has not been possible to discriminate between these hypotheses, as only bristle characters have been examined.

In this study we examined levels of asymmetry in six characters within resistance and modified genotypes of *L. cuprina*. The characters include the three meristic bristle characters previously examined within this system and an additional three metric wing characters (wing vein lengths). Under the first hypothesis, we predict an increase in bristle asymmetry, relative to susceptibles, in non-modified-resistance genotypes, but no increase in wing asymmetry when blowflies develop under standard laboratory conditions. Under the second hypothesis, we predict increased levels of both bristle and wing asymmetry in non-modified-resistance genotypes. Modified-resistance genotypes should display background levels of asymmetry in both cases.

Differential response between bristle and wing characters in resistance genotypes could, however, be attributable to factors other than the hypothesized action of resistance and modifier gene products in bristle formation. For example, it might be argued that wing characters may be better buffered against disturbance than bristle characters, given that they might be expected to be more closely associated with fitness (through their role in locomotion). It has been argued that the greater the functional importance of a character, the more developmentally stable it is (Stearns & Kawecki 1994; Clarke 1995; Gummer & Brigham 1995; Swaddle & Witter 1997; Woods *et al.* 1999). Thus, the identification of any asymmetry–stress relationship may be character specific and

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reflect the underlying buffering capacity of the character (Leung & Forbes 1997). To test for this possibility we conducted a second series of experiments that compared the responses of bristle and wing characters of a single susceptible genotype to another known developmental stress in *L. cuprina*: extreme temperature. Sub-optimal rearing temperatures have previously been shown to result in increased bristle asymmetry in this system (Clarke & McKenzie 1992).

2. METHODS

(a) *Strains and experimental conditions*

The strains used in the experiments, and their derivation, have been described in detail elsewhere (McKenzie & Yen 1995; Freebairn *et al.* 1996). Resistances to diazinon (an organophosphorus insecticide) and to dieldrin (a cyclodiene) are controlled by separate single genes (*Rop*$_1$ and *Rdl*) located on chromosomes IV and V, respectively. There is no cross resistance. A modifier gene, which influences the fitness and asymmetry of diazinon- but not dieldrin-resistant phenotypes, maps to chromosome III (Clarke & McKenzie 1987; McKenzie & Clarke 1988). The reference susceptible strain used in these experiments, which carries the wild-type allele at the modifier locus and the susceptible allele at the diazinon-resistance locus, is designated as genotype $+/+;S/S$ (Freebairn *et al.* 1996). This strain is also susceptible to dieldrin. The strain $M/M;S/S$ is also susceptible to both insecticides but homozygous for the modifier allele. Strains $+/+;R/R$ and $M/M;R/R$ are diazinon resistant; Rdl/Rdl and $Rdl/+$ are dieldrin resistant.

In experiment 1, all strains were raised under standard laboratory conditions (100 larvae per 140 ml of standard medium at 27 °C). Ten flies were collected for each strain and asymmetry scored for each of six characters for each individual. Five independent trials were conducted.

In experiment 2, the reference susceptible strain was raised under standard conditions and also at 32 °C on standard medium at standard larval density. Five independent trials were conducted with ten individuals scored for the six characters in each trial.

(b) *Character scoring*

The three meristic bristle characters (FSB, WMB, R4+5) examined are described elsewhere (Clarke & McKenzie 1987). Left- and right-side scores were made for each individual for each character under a dissecting microscope at magnification ×50. Wings were removed from each individual using forceps, mounted on a microscope slide and three wing-vein measurements made under a dissecting microscope, also at magnification ×50. The wing-vein measurements were the distances between the intersection points of wing veins R_1 and R_{2+3} on the outer wing margin, between the r–m junction of the R_{4+5} wing vein and the intersection point of the distal cross vein and the length of this wing vein from the point of intersection to the junction with wing vein M_{3+4} (see Colless & McAlpine 1991, fig. 39.7C). No formal estimate of measurement error was undertaken. However, the $+/+;S/S$ sample of experiment 1 and the 27 °C sample of experiment 2 represent identical treatments. Each of these samples were scored by two different observers (J.L.Y. and J.A.M., respectively). The congruence of mean and variance values between these two data sets suggests that any measurement error is negligible, compared with asymmetry differences observed among samples. All measurements were performed blind without reference to the genotype or temperature.

(c) *Statistical analysis*

The asymmetry distributions and analyses for the bristle characters have been described previously (McKenzie & Clarke 1988; McKenzie & Yen 1995). For the wing-vein measurements, there was no evidence of directional asymmetry or antisymmetry, and no association between asymmetry values and character size, following standard statistical procedures (Palmer 1994). Thus, these characters display fluctuating asymmetry and provide an estimate of developmental stability. For each character and sample, the index of asymmetry calculated was the sum of the absolute differences between left and right sides of an individual divided by the sample size, $\Sigma(|L_i - R_i|)/N$. In addition to examining each character independently, two combined character indexes were calculated: BRISTLES and WINGS were simply the sum of the individual bristle- and wing-character scores, respectively, for each individual divided by the sample size. Multivariate and univariate analyses of variance were conducted to examine differences among genotypes and temperature treatments. In cases of significant differences among treatments, post-hoc pairwise comparison tests were undertaken. Bonferroni corrections for multiple tests were applied in all analyses. All analyses were performed using the statistical software package SYSTAT[1] v. 9 (SPSS 1999).

3. RESULTS

(a) *Resistance experiment*

Mean asymmetry values and their standard errors for the six individual and two combined characters for each genotype are given in table 1. Multivariate analysis of variance (MANOVA) involving the six individual characters revealed significant differences among genotypes (Wilk's $\lambda = 0.7392$, $F_{30,1158} = 3.0288$, $p < 0.001$). Univariate analysis revealed significant differences among genotypes for only two of the bristle characters (FSB, $F_{5,294} = 4.3564$, $p < 0.001$; and WMB, $F_{5,294} = 10.7477$, $p < 0.001$). In the case of FSB only the Rdl/Rdl genotype displayed significantly greater asymmetry than the susceptible ($+/+;S/S$) and the two modified genotypes ($M/M;+/+$ and $M/M;R/R$), although both the other resistance genotypes ($Rdl/+$ and $+/+;R/R$) showed higher (although non-significant) asymmetry levels. For character WMB both Rdl/Rdl and $+/+;R/R$ genotypes were significantly more asymmetrical than $+/+;S/S$, $M/M;+/+$ and $M/M;R/R$ genotypes while the $Rdl/+$ genotype was significantly more asymmetrical than the $+/+;S/S$ genotype. There were no significant differences among genotypes for either R_{4+5} or any of the three wing-vein characters.

For the combined BRISTLES character there were significant differences among genotypes ($F_{5,294} = 6.5259$, $p < 0.001$) with all non-modified-resistance genotypes displaying significantly higher asymmetry values than either the susceptible or the modified genotypes. The combined WINGS character failed to reveal any significant differences among genotypes ($F_{5,294} = 0.3907$, $p = 0.855$).

(b) *Temperature experiment*

Mean asymmetry values and their standard errors for the six individual and the two combined characters for each

Table 1. *Mean asymmetry values ± s.e.m. for the six individual and the two combined characters for each genotype*

	BRISTLES				WINGS			
genotype	FSB	WMB	R_{4+5}	combined	wing 1	wing 2	wing 3	combined
+/+;*S*/*S*	0.52 ± 0.08	0.50 ± 0.07	1.14 ± 0.12	2.16 ± 0.14	1.02 ± 0.12	0.80 ± 0.11	0.66 ± 0.08	2.48 ± 0.20
M/*M*;*S*/*S*	0.54 ± 0.09	0.54 ± 0.09	0.96 ± 0.11	2.04 ± 0.17	0.88 ± 0.14	0.80 ± 0.09	0.60 ± 0.10	2.28 ± 0.22
+/+;*R*/*R*	0.68 ± 0.08	1.12 ± 0.10	0.90 ± 0.10	2.70 ± 0.15	0.96 ± 0.13	0.70 ± 0.11	0.64 ± 0.10	2.30 ± 0.22
M/*M*;*R*/*R*	0.58 ± 0.08	0.54 ± 0.08	0.90 ± 0.13	2.02 ± 0.18	0.86 ± 0.10	1.00 ± 0.12	0.62 ± 0.10	2.48 ± 0.17
Rdl/+	0.80 ± 0.09	0.88 ± 0.07	1.06 ± 0.11	2.74 ± 0.15	0.92 ± 0.08	0.88 ± 0.09	0.70 ± 0.10	2.50 ± 0.16
Rdl/*Rdl*	0.96 ± 0.08	1.06 ± 0.10	0.94 ± 0.14	2.96 ± 0.18	0.80 ± 0.09	0.96 ± 0.11	0.82 ± 0.10	2.58 ± 0.16

Table 2. *Mean asymmetry values ± s.e.m. for the six individual and the two combined characters for each temperature*

	BRISTLES				WINGS			
temperature	FSB	WMB	R_{4+5}	combined	wing 1	wing 2	wing 3	combined
27 °C	0.60 ± 0.09	0.60 ± 0.08	0.82 ± 0.11	2.02 ± 0.15	0.92 ± 0.10	0.76 ± 0.11	0.94 ± 0.10	2.62 ± 0.18
32 °C	0.90 ± 0.09	0.84 ± 0.10	1.20 ± 0.14	2.94 ± 0.17	1.18 ± 0.13	1.24 ± 0.10	1.06 ± 0.11	3.48 ± 0.19

temperature are given in table 2. MANOVA involving the six individual characters revealed significant differences between temperatures (Wilk's $\lambda = 0.7646$, $F_{6,93} = 4.7718$, $p < 0.001$). For all characters the 32 °C sample displayed higher asymmetry levels than the 27 °C sample; however, this result was only significant for FSB ($F_{1,98} = 5.7273$, $p < 0.05$), R_{4+5} ($F_{1,98} = 4.4568$, $p < 0.05$) and Wing2 ($F_{1,98} = 10.4071$, $p < 0.01$). These differences were also significant for both the combined characters (BRISTLES, $F_{1,98} = 17.0253$, $p < 0.001$; WINGS, $F_{1,98} = 11.0314$, $p < 0.01$).

4. DISCUSSION

Results of the insecticide-resistance experiment are consistent with those of previous studies in this system (McKenzie & Clarke 1988; McKenzie & Yen 1995). In general, non-modified-resistance genotypes display significantly greater levels of asymmetry than susceptibles or modified genotypes for bristle characters. The lack of response for wing characters is consistent with the hypothesis that the action of both the resistance and the modifier genes on developmental stability is restricted to bristle characters. This differential response of bristle and wing characters does not appear to be attributable to any inherent differences in susceptibility to disturbance between the two character types, as both bristle and wing characters responded in a similar and predicted manner to the more general temperature stress. The result for the bristles is in accordance with previous studies (McKenzie & Yen 1995; Freebairn *et al.* 1996; McKenzie 1997). Similar responses to temperature stress have been shown in two *Drosophila* species in which both bristle and wing characters displayed elevated asymmetry following exposure to extreme temperature (Imasheva *et al.* 1997).

The effects of single genes on developmental stability have been little studied. Most genetic studies of developmental stability have been concerned with whole-genome processes such as heterozygosity or genomic coadaptation. However, two studies of rainbow trout have found that individuals containing certain alleles at two loci (phosphoglucomutase and lactate dehydrogenase) showed reduced developmental stability for five meristic characters (Allendorf *et al.* 1983; Leary *et al.* 1993). It should be noted, however, that due to the nature of the asymmetry index used in these studies it is not possible to ascertain whether the responses were character specific.

The literature on the impacts of genetic perturbation on developmental stability is characterized by considerable heterogeneity of results. No matter whether the studies involved genomic or specific-locus heterozygosity, inbreeding or outbreeding regimes or selection, the results are spread evenly between negative and positive effects (Palmer & Strobeck 1986; Markow 1995). Given that evidence from this study indicates that specific genes may directly influence developmental stability in a character-specific manner, such heterogeneity might be expected. Any particular result may depend on which genes are being affected by the genetic perturbation and which characters are being examined. The separation of the genetic and the environmental components has proved possible in the resistance systems of *L. cuprina*, informing the debate of the importance of general and specific stresses, which may educe different evolutionary and development responses (Hoffmann & Parsons 1991). In the absence of such separation the high degree of genetic and character specificity may mean that the delineation of a generalized underlying genetic mechanism for developmental stability will remain elusive.

Relationships between asymmetry, stress and fitness have recently been reviewed (Leung & Forbes 1996; Møller 1997, 1998; Clarke 1998). Empirical data have revealed such relationships to be generally weak and again characterized by considerable heterogeneity. Modelling these relationships has suggested that such heterogeneity may reflect underlying differences in the capacity of different characters to buffer developmental perturbation (Leung & Forbes 1997). To date, few empirical data exist that specifically examine the possibility of stress by character interactions. Specific questions such as whether

different stressors elicit different responses in a given character and whether different characters show different responses to a given stress have been little studied. Such experiments will need to be conducted within a constant genetic background. Until such data exist for a suite of characters, stressors and taxa, any generalized relationships between character asymmetry, stress and fitness will remain speculative.

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