The relationship between genotype, developmental stability and mating performance: disentangling the epigenetic causes

Michal Polak^{*} and Elizabeth M. Stillabower

Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-0006, USA

Developmental stability (DS) may confer an advantage in competition for mates. The present study tests this hypothesis using *Drosophila immigrans*, and proposes a novel approach to help broadly define the epigenetic factors causing such an effect. We first estimated the magnitude of isofemale heritability in sternopleural bristle fluctuating asymmetry (FA), using replicate genetic lines extracted from nature. Positional FA (PFA) exhibited significant among-line variation, and the heritability estimate of 0.10 (0.046 s.e.m.) was statistically significant. Among individual males, there was a significant positive relationship between PFA and copulation latency (time elapsed between introduction of females and copulation) and duration, but not copulation frequency. Moreover, high-DS lines exhibited significantly shorter copulation latency and duration compared with low-DS lines. When these components of sexual performance were again contrasted between lines with among-individual differences in bristle asymmetry controlled statistically, significant line effects on copulation latency and duration disappeared. The results suggest that deficits in the developmental apparatus underlying one particular trait can compromise individual sexual performance, and weaken the hypothesis that FA is a cue of overall 'genetic quality'.

Keywords: developmental stability; *Drosophila immigrans*; epigenetic control; genetic quality; positional fluctuating asymmetry; sexual selection

1. INTRODUCTION

Developmental stability (DS) is the outcome of corrective mechanisms that buffer the effects of the minor, intrinsic perturbations to developmental processes (Waddington 1957). Under the assumption that lack of developmental fidelity incurs physiological costs, reduced DS has been predicted to compromise individual fitness components (Palmer & Strobeck 1986; Møller & Swaddle 1997; Gangestad & Thornhill 1999). One area of particularly intensive research has been the relationship between mating success and fluctuating asymmetry (FA) (reviewed in Tomkins & Simmons 2003), the subtle deviations from perfect bilateral symmetry in morphological structures that reflect underlying DS (Van Valen 1962). Although the overall effect of FA on sexual selection is weak and highly variable, FA does appear to explain a significant portion of the variation in mating success in some species (Tomkins & Simmons 2003).

FA and male mating success may come to be associated by way of at least two general mechanisms. First, asymmetry itself may functionally generate male mating success variance in courtship or contest competition (Møller & Pomiankowski 1993; Watson & Thornhill 1994; Uetz & Taylor 2003). Alternatively, asymmetry may become negatively correlated with mating success incidentally because symmetrical individuals are healthier or more vigorous in competition for mates (Møller & Pomiankowski 1993; Polak 1997a).

When FA and mating success come to covary by either of these mechanisms, an important next step will be to decipher the source(s) of DS. One popular hypothesis states that FA in a particular trait reflects genome-wide DS, and hence taps an organism's overall genetic quality. According to this 'generalized effects' hypothesis, the degree of phenotypic symmetry in a particular trait indicates generalized (organism-wide) buffering capacity, influenced by segregating variation at loci throughout the genome, including loci that also influence viability, reproductive success, physiological vigour and so forth. This hypothesis is the foundation of 'good genes' arguments for female mate choice (Andersson 1994) in favour of symmetry *per se* in male secondary sexual traits (Ridley 1992; Møller & Pomiankowski 1993).

Alternatively, the 'local effects' hypothesis states that FA in a particular trait reflects DS arising from trait-specific (local) developmental processes. Disharmony among developmental gene products building a particular trait is itself the cause of that trait's DS and resultant FA. Covariation between FA and fitness attributes (e.g. mating success) arises because deficits in the developmental apparatus specific to the trait, disrupt physiological processes elsewhere in the body. Such linkage would be a form of epigenetic control, defined by Hall (1983) as the influence genes regulating one cell or group of cells have on different cells in other parts of the body (see Cowley & Atchley 1992). Discriminating between the generalized versus local effects mechanisms is important because each hypothesis defines a different class of loci expected to be influenced by selection. Which of these two mechanisms contributes to sexual selection in natural populations is unknown.

^{*} Author for correspondence (polakm@email.uc.edu).

The present study discriminates the local and generalized effects hypotheses using a drosophilid fly as test organism. We introduce a novel approach that involves extracting genetic isolates (full-sib families) from natural populations, and identifying replicate low-DS and high-DS lines on the basis of mean absolute FA in a particular trait. Mating performance of individuals sampled from within each line is then measured, and tests for differences in performance between high-DS and low-DS lines are conducted before and after statistically correcting performance values for *individual* asymmetry (DS) differences. The local effects hypothesis predicts that differences in performance detected before the FA correction will be lost subsequent to the correction. By contrast, if differences in performance are a result of genome-wide differences in quality (the generalized effects hypothesis), high-DS genetic isolates should continue to exhibit relatively greater fitness despite the correction.

The role of positional FA (PFA), which reflects asymmetry in the placement (as opposed to mere number) of components of meristic traits, is of particular interest, because both a developmental model (Starmer *et al.* 2002) and empirical data (e.g., Polak 1997b; Polak *et al.* 2004) suggest that it may be a sensitive indicator of DS.

The goals of the present study are: (i) test for differences in sternopleural bristle FA between genetic isolates (isofemale lines) of *Drosophila immigrans* derived from nature; (ii) estimate isofemale heritability (h^2_{iso}) for trait FA; (iii) test for a relationship between male FA and components of mating success at the level of the individual and genetic line; and (iv) discriminate between the local effects versus generalized effects hypotheses to help unravel the underlying 'epigenetic cause' of any relationship between sexual performance and morphological symmetry.

2. METHODS

(a) Isofemale lines

Isofemale lines were derived from wild-caught female *D. immigrans* Sturtevant collected by baiting in June 2000 from two sites in the USA: Independence, KY (38.929° N, 84.538° W), and Fayetteville, NY (43.030° N, 75.998° W). In the laboratory, females were allowed to oviposit into individual eight fluid-dram shell vials containing powdered Betty Crocker instant mashed potato flakes, instant *Drosophila* medium (Carolina Biological Supply Co.), deionized H₂O, and 4–5 mg of Fleischmann's active dry yeast. Virgin offspring were harvested and allowed to reach sexual maturity in single-sex food vials. A randomly selected virgin brother–sister pair was used to propagate each isofemale line. Lines were not propagated directly from wild-caught females to restrict segregating genetic variation within lines; wild-caught females were presumed to have been multiply inseminated. Lines were cultured in half-pint bottles in a Percival incubator with a 12 L (25 °C): 12 D (23 °C) photoperiod. Adults were transferred to fresh bottles after 2 days to retain moderate larval densities across the lines; there were two bottles per line. This method of controlling larval densities served to avoid handling the larvae, which could cause stress and influence FAs. Bottles were placed at random in the incubator to avoid systematic environmental effects. During the first 4 days of eclosion, approximately 20 adult progeny (10 flies of each sex) were randomly selected from each bottle and bristle number and thorax length were determined.

Isolines were maintained at least three generations in the laboratory before characterization (e.g. Polak & Starmer 2001).

(b) Bristle number and fluctuating asymmetry

FA was estimated in sternopleural bristle number. Each fly was killed with ether and its anterior and transverse sternopleural bristles counted (fig. 1 in Polak 1997b). Overall FA is the absolute value of the difference in the total number of sternopleural bristles (i.e. anterior plus transverse bristles) between right and left body sides. FA1 and FA2 refer to absolute asymmetry in the number of anterior and transverse sternopleural bristles, respectively. Positional asymmetry, which reflects the difference between the two sides of the body in the placement of bristles on the sternopleuron, was calculated as $PFA = |\ln(\text{number of right anterior bristles} - \text{number of right transverse bristles}) + 0.5| - |\ln(\text{number of left anterior bristles} - \text{number of left transverse bristles}) + 0.5|$ (Polak 1997b).

Trait 'size' is the total number of a given bristle type. Tot1 and tot2 are the total number of anterior and transverse sternopleural bristles, respectively. Tot1,2 is the sum of tot1 and tot2, and is the associated measure of trait size for overall FA and PFA. Thorax length, measured from the anterior end of the thorax to the posterior end of the scutellum using an ocular micrometer of a stereo-microscope, estimates adult body size. Distributions of signed PFA values were examined for the presence of directional asymmetry, skewness and kurtosis (Sokal & Rohlf 1995). Tests are reported for PFA only because it is the focus of subsequent analyses.

(c) Measurement error

Two replicate counts were conducted on a sample of 28 flies. After the first count, flies were stored frozen for 3 days, and a second set of counts was made in an order different from the first and which was unknown to the observer. Flies were physically repositioned before the second count. Counts were analysed using a two-factor ANOVA (Palmer 1994), in which individual and side were entered as factors. Variation in total bristle number asymmetry owing to measurement error was 0.0926 (mean square error), representing 2.2% of the individual $\times$ side mean square (4.30). In addition, the individual $\times$ side interaction was strongly significant ($F_{26,54} = 46.44$, $p < 0.0001$). Thus, measurement error was small compared with the between-sides variance representing morphological differences.

(d) Components of variation

A total of 22 lines (11 per site) were characterized in respect to FA, trait size and thorax length. For each isofemale line, the use of replicate bottles permitted environmental effects to be partitioned (Polak & Starmer 2001). A nested ANOVA was used to test for the presence of genetic variation underlying FA; 'bottle' was nested within 'line', which in turn was nested within 'site'. ANOVAs were performed on absolute ($|R-L|$) FA values. 'Bottle' and 'line' were entered as random factors, and *F*-statistics were computed using appropriate mean squares (Sokal & Rohlf 1995). Trait size was entered as a covariate in all ANOVAs on FA.

Lines were ranked in ascending order of PFA and FA2 means, and the two lines with lowest and highest combined rank were identified as 'high-DS' and 'low-DS' lines, respectively (PFA and FA2 means were strongly correlated across lines: $r = 0.65$, $p = 0.001$). These four chosen lines were used in subsequent mating tests, as well as to evaluate whether asymmetry differences would persist across generations of laboratory rearing. For this latter analysis, data from six laboratory generations were available for the two lines exhibiting the most extreme FA differences;

ANOVAs were performed with 'line' and 'generation' entered as factors, and 'trait size' as the covariate.

(e) Heritability estimates

Variation as a result of trait size was removed from all FA traits by regressing absolute FA ($|R - L|$) on trait size. $\log_{10}(y + 3)$ -transformed residuals from these analyses were then entered into model 3 of the MIXED-MODEL LEAST-SQUARES AND MAXIMUM LIKELIHOOD program, v. PC-2 (Harvey 1990) to estimate variance components between and within lines. Site and line within site were factors. Isofemale heritability (h^2_{iso}) and its standard error (s.e.m.) were calculated following Hoffmann & Parsons (1988), but corrected by deleting the square of the denominator in the equation of s.e.m. for h^2 (Loeschcke *et al.* 1999).

(f) Male reproductive performance

Reproductive performance was assayed in males from high-DS and low-DS lines only. All flies used in mating experiments were between 11 and 14 days old, and males and females used on the same day differed in age by no more than 1 day. All females were collected from general stocks as virgins and held at densities of 10 flies in single-sex vials containing agar medium and 10–12 mg live yeast. Females were moved to fresh vials every 2 days. Males were collected within 24 h of eclosion and held in densities of 10 flies per agar vial without live yeast. Males were moved to fresh vials every 2–3 days.

Lines were characterized in terms of reproductive performance by way of mating assays. An assay consisted of testing an approximately equal number of males from both a high-DS and a low-DS line. A total of 38–81 (mean 48) males were tested in any given assay. Four replicate assays, each conducted on a separate day, were used to characterize each line. All assays began 30 min after the incubator lights were switched on. Males were placed individually into clean and sterile shell vials without food; males from the high and low line were randomly dispersed across vials lined up along a desktop. After 15 min of acclimatization, two virgin females were gently introduced into each vial by using an aspirator. Two females were used as a precaution in case one female was not receptive, for example, because of subtle injury not apparent to the observers, or because of unknown causes. Vials were each observed by two to three observers for exactly 2 h after the first set of virgin females was introduced. During this period, the beginning and end times of all copulations were recorded. Immediately after any copulation, both females were removed from the vial and replaced with two fresh, virgin females. The following variables for each male were quantified: (i) the number of copulations in the 2 h observation period; (ii) the copulation latency (time elapsed from introduction of females to first copulation); and (iii) the copulation duration (time elapsed between onset and termination of copulation). Because the frequency at which males copulated did not differ across lines (see § 3), mean copulation duration was calculated for each male that mated more than once. The thorax length of all males was measured.

(g) Data analysis

Because PFA was the only FA trait to show both significantly between-line differences and isofemale heritability (see § 3), the relationship between PFA and the three components of mating success were examined for this FA trait only. In a first evaluation, we tested for the relationship between PFA and sexual performance across individual males using data pooled across the four experimental lines (i.e. two low-DS and two high-DS lines). Multiple regression analysis was used, with thorax length as the

covariate. Initial analyses, with total bristle number entered as an additional covariate, revealed that in no case was the effect of bristle number significant. Bristle number was therefore excluded from these analyses. Analysis of covariance (ANCOVA), with thorax length as the covariate, was performed to test for PFA differences between males that either did or did not copulate. Copulation status (yes or no) and line (high or low) were entered as factors.

Multiple analysis of variance (MANOVA) was conducted to discriminate between the generalized versus local effects hypotheses. We first tested for effects of 'rep' (replicate day) and 'line' (high versus low) on the three dependent variables: copulation number, latency and duration. Both the above hypotheses predict significant effects of line (see § 1) on performance. In a second evaluation, MANOVA was conducted on the same three dependent variables but that were first corrected for PFA variation using a procedure based on regression. Each performance trait was regressed on PFA using data pooled across lines. Residuals from each regression were obtained, and each summed with mean trait value ($e_i + \bar{Y}$) before analysis. In an alternative analysis, multiple analysis of covariance (MANCOVA) was conducted on the three dependent variables that were uncorrected but in which PFA was the covariate, and rep, line and their interaction were factors. The generalized effects hypothesis predicts that the significant effect of line will persist despite correcting for PFA, whereas the local effects hypothesis predicts that the line effect observed in the first analysis will be lost. All analyses were performed using SAS (SAS Institute Inc. 1990).

3. RESULTS

(a) Descriptive statistics

For the pooled dataset across lines and sites, the mean signed PFA value of 0.0157 was not significantly different from zero ($p > 0.05$, $n = 886$), and no significant skewness was detected (skewness = 0.057, $p > 0.05$). Significant leptokurtosis was, however, detected (kurtosis = 1.48, $p < 0.001$) (see Polak 1997b; Polak & Starmer 2001). The PFA distribution for the four experimental lines also exhibited a mean that was not significantly different from zero (mean = 0.0049, $n = 387$, $p = 0.86$). This distribution did not exhibit significant skewness (skewness = 0.043, $p > 0.05$), but significant leptokurtosis was again detected (kurtosis = 5.43, $p < 0.01$).

(b) Among-line differences and isofemale heritability

Nested ANOVA detected no effect of site or bottle on any of the three FAs (all p -values > 0.1). No line effects were detected for FA1 ($F_{20,22.7} = 1.63$, $p = 0.13$). Significant line effects were, however, detected for both FA2 ($F_{20,24} = 2.82$, $p = 0.008$) and PFA ($F_{20,23} = 3.70$, $p = 0.0016$). Out of these two traits, only PFA exhibited a significant isofemale heritability (PFA: $h^2_{\text{iso}} = 0.102 \pm 0.046$, $p = 0.02$; FA2: $h^2_{\text{iso}} = 0.011 \pm 0.019$, $p = 0.3$). ANOVA revealed that PFA did not differ significantly across the six generations of laboratory rearing ($p = 0.24$), indicating stability of line-specific PFA values.

(c) Mating performance

Out of the 386 males tested, 290 (75%) mated with at least one female during the 2 h observation period, whereas 96 males failed to mate. Out of the males that mated, 179 (62%) mated once, 106 (37%) mated twice and five (2%)

mated three times. In a first overall evaluation, ANCOVA revealed that, whereas there was a strong effect of line on PFA (high: 0.506 ± 0.032 ; low: 0.114 ± 0.031 ; $F_{1,381} = 76.6$, $p < 0.0001$), there was no difference in mean PFA between males that either did or did not copulate ($F_{1,381} = 0.12$, $p = 0.73$). The interaction between line and copulation status was likewise non-significant ($F_{1,381} = 0.84$, $p = 0.36$).

Relationships between PFA and components of sexual performance on data pooled across lines were also sought using multiple regression. A significant positive linear relationship was found between PFA and both copulation latency and duration (figure 1); PFA explained 2.0% and 6.4% of the variation in these variables, respectively. By contrast, there was no relationship between PFA and number of copulations, consistent with the above ANCOVA. Finally, latency and duration were not themselves correlated, irrespective of whether this relationship was examined in the pooled data ($r = 0.019$, $p = 0.76$, $n = 283$), or separately in the high-DS ($r = 0.025$, $p = 0.76$, $n = 141$) or low-DS ($r = -0.10$, $p = 0.23$, $n = 142$) experimental lines.

In a second evaluation, performance traits were contrasted *between* high- and low-DS lines. Probability of copulation did not differ between lines: 24.1% and 25.8% of males from high-DS and low-DS lines, respectively, failed to copulate ($\chi^2 = 0.14$, d.f. = 1, $p > 0.5$). Moreover, contingency table analysis revealed that among males that copulated at least once, the probability of copulating one, two or three times did not differ among lines ($\chi^2 = 2.9$, d.f. = 2, $p > 0.1$).

MANOVA on the three PFA-*uncorrected* mating success components revealed a strong overall effect of line (table 1, model 1). Standardized canonical coefficients were greatest for duration and latency, identifying these traits as most important in explaining between-line variation (figure 2). Table 2 presents tests of significance for the effect of line on each performance trait separately; the effects of line were significant for latency and duration, but as expected not for copulation number.

A second MANOVA on PFA-*corrected* performance traits revealed no significant overall effect of line (table 1, model 2). Indeed, the correction reduced the overall explanatory power of model by a full order of magnitude (model r^2 fell from 11% to less than 1%). In an alternative evaluation, in which uncorrected performance values were analysed using MANCOVA with PFA as the covariate, the loss of the overall effect of line on the response variables was confirmed ($F_{3,363} = 1.30$, $p = 0.3$). Tests of significance using univariate ANOVAs (table 2) on corrected values indicated non-significant line effects for each mating success component considered separately (figure 2). This loss of detectable differences in sexual performance between high-DS and low-DS lines resulting from the PFA correction supports the local effects hypothesis.

4. DISCUSSION

Significant sternopleural PFA variation among isofemale lines (full-sib families) was detected, reflected in a significant isofemale heritability ($h^2_{iso} \pm \text{s.e.m.}$) of 0.10 ± 0.046 . Thus, a significant genetic component to PFA variation exists in natural populations of *D. immigrans*. This

Figure 1. Relationships between components of sexual performance and PFA in *Drosophila immigrans* pooled across lines. Slopes ($\pm$ s.e.m.) for (a) number of copulations, (b) copulation latency and (c) copulation duration are: -0.043 ± 0.093 ($p = 0.64$); 0.17 ± 0.070 ($p = 0.013$); and 0.12 ± 0.028 ($p < 0.0001$), respectively.

Table 1. Results of MANOVA on uncorrected (model 1) and PFA-corrected (model 2) sexual performance traits in *Drosophila immigrans*.

	standardized canonical coefficient			$F_{3,265}^a$	squared canonical coefficient
	latency	duration	number of copulations		
model 1	0.530	1.036	0.0568	10.37, $p < 0.0001$	0.11
model 2	0.709	0.595	-0.263	0.67, $p = 0.6$	0.0077

^a Evaluating H_0 : no overall effect of genetic line.

Figure 2. Mean copulation latency and duration in low-DS lines (white bars) and high-DS lines (grey bars), (a) before and (b) after correcting for individual PFA deviations. See table 2 for ANCOVA results. Significance values in the figure are assigned using a Tukey–Kramer procedure.

heritability estimate, however, should not be viewed as equivalent to the narrow-sense heritability, because the iso-female line approach does not separate additive genetic effects from other forms of genetic activity, such as dominance and epistasis (Falconer & Mackay 1996; Bublić *et al.* 2000). Only one study has examined the relative importance of these different forms of genetic activity affecting

PFA. Polak & Starmer (2001) found that in *D. falleni*, both additive and dominance effects contributed to sternopleural PFA, whereas epistatic, maternal and cytoplasmic effects were relatively unimportant.

Thus, if sternopleural PFA in *D. immigrans* is controlled at least in part by additive genetic activity, as in *D. falleni*, then combined with the data presented here, PFA and its underlying DS may possess significant evolutionary potential. Importantly, we found that PFA positively correlated with male copulation latency and duration at both the level of the individual and the genetic line, meaning that developmentally stable genotypes required significantly less time to initiate copulation with virgin females, and that they copulated for a significantly shorter duration of time. However, no relationship between PFA and copulation number was detected. Reasons for this heterogeneity are unknown, but perhaps a relationship with copulation number would have been detected had males been assayed across multiple reproductive episodes, or in the context of male–male competitive interactions.

Both a reduced copulation latency and duration could confer fitness advantages to males in natural mating arenas. For example, a reduced copulation latency, or ‘search time’ (Simmons 2001), could yield a greater number of lifetime mating partners, or to disproportionate access to high-quality females if male mate choice were to exist in this system (Thornhill & Alcock 1983). In a similar vein, shortened copulation duration may liberate time for additional mate searching (Thornhill & Alcock 1983; Alcock 1994; Simmons 2001); high-DS males could be predisposed to such gains because they may transfer sperm at greater rates (and see Simmons & Parker 1992; Parker & Simmons 1994; Simmons 2001).

We used virgin females that were well beyond the age at which they achieve sexual maturity to increase female receptivity to any first male they encountered. By minimizing the effects of female choice, this approach helped ensure that any observed differences in male copulation characters would more closely reflect physiological differences among the males. Thus, we interpret the reduced copulation latency and duration observed among high-DS genotypes to reflect their intrinsic superiority, such as in vigour and physiological competence. Moreover, by their very nature, sternopleural bristles in *Drosophila* are unlikely to be the target of female choice, nor otherwise involved in functionally mediating the mating process (e.g. Polak *et al.* 2004). These bristles are not a secondary sexual trait; they occur in both sexes, and are not exaggerated in either sex. Their small size and placement (most occur on the underside of the thorax, posterior to the foretarsi) also make it unlikely that they would be evident to a female or to a rival, and during no phase of courtship or copulation does the

Table 2. Results of ANCOVA on uncorrected and PFA-corrected components of male mating performance. Thorax length was entered as the covariate. Line effects are in bold.

copulation	source	d.f.	uncorrected		PFA-corrected	
			<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
number	thorax	1	1.03	0.31	1.02	0.31
	rep	7	0.99	0.44	0.83	0.56
	line	1	0.78	0.38	0.06	0.80
	rep*line	7	1.72	0.10	1.93	0.064
	error	368				
latency	thorax	1	2.46	0.12	2.90	0.090
	rep	7	0.51	0.83	0.73	0.64
	line	1	7.82	0.006	1.58	0.21
	rep*line	7	0.36	0.92	0.25	0.97
	error	272				
duration	thorax	1	1.89	0.17	1.10	0.29
	rep	7	10.28	< 0.0001	6.69	< 0.0001
	line	1	18.37	< 0.0001	0.28	0.59
	rep*line	7	0.64	0.72	1.58	0.078
	error	265				

female appear to come into physical contact with these bristles in the males.

Our results suggest that DS underlying *Drosophila* sternopleural bristles is trait-specific, i.e. that bristle DS emanates largely from the developmental architecture of the bristles themselves (the local effects hypothesis). This conclusion follows from two lines of reasoning. One is that bristle PFA is not itself the likely cause of differences in sexual performance, which implicates underlying physiological differences among males, as discussed above. The second is that the significant differences in performance between genetic lines could be greatly reduced by statistically correcting performance values for DS in this single bristle trait. Thus, to explain the significant differences in performance between genetic lines observed before the correction, we suggest the intriguing possibility that epigenetic effects (Hall 1983; Cowley & Atchley 1992; West-Eberhard 2003, p. 112), exerted by the products of bristle developmental genes themselves, alter physiological processes elsewhere in the body that affect behaviour. In other words, the subtle defects in developmental processes that give rise to bristle PFA may be acting globally to harm male sexual performance expressed later in life.

The data do not support the generalized effects hypothesis because they suggest that FA in one trait does not reflect properties of the genome. Our results therefore weaken the more general hypothesis, that female choice for low FA in one trait could yield indirect benefits that increase offspring fitness, owing to transmission of genetic factors (e.g. viability or 'good genes') segregating at loci outside those controlling the trait itself (e.g. Møller 1990; Ridley 1992; Møller & Pomiankowski 1993; Polak & Trivers 1994; Watson & Thornhill 1994; Møller & Swaddle 1997; Møller & Thornhill 1998; Tomkins & Simmons 2003).

Of course the possibility exists that our findings are specific to sternopleural bristles in *D. immigrans*, and that they are not relevant for understanding the relationship between DS, FA and performance more generally. Moreover, these bristles are not a secondary sexual characteristic, so perhaps

our conclusions regarding the signalling properties of a trait's FA would have been different had we focused on a sexual trait; however, the intent of the present study was to focus on a non-sexual trait for the reasons discussed above. Further research into the origins of DS and its relationship to fitness in other traits and species therefore may be warranted to assess the generality of the findings.

The laboratory where the research was conducted was supported by the National Science Foundation (NSF) USA, grant no. 0128999 (to M.P.) and the University of Cincinnati. We thank A. Cooperman, K. Petren and W. T. Starmer for discussing with us the ideas presented and/or comments on the manuscript, and W. T. Starmer for sending us flies from New York.

REFERENCES

- Alcock, J. 1994 Postinsemination associations between males and females in insects: the mate-guarding hypothesis. *A. Rev. Entomol.* **39**, 1–21.
- Andersson, M. 1994 *Sexual selection*. Princeton University Press.
- Bubli, O. A., Loeschcke, V. & Imasheva, A. G. 2000 Effect of stressful and nonstressful growth temperatures on variation of sternopleural bristle number in *Drosophila melanogaster*. *Evolution* **54**, 1444–1449.
- Cowley, D. E. & Atchley, W. R. 1992 Quantitative genetic models for development, epigenetic selection, and phenotypic evolution. *Evolution* **46**, 495–518.
- Falconer, D. S. & Mackay, T. F. 1996 *Introduction to quantitative genetics*, 4th edn. London: Longman.
- Gangestad, S. W. & Thornhill, R. 1999 Individual differences in developmental precision and fluctuating asymmetry: a model and its implications. *J. Evol. Biol.* **12**, 402–416.
- Hall, B. K. 1983 Epigenetic control in development and evolution. In *Development and evolution* (ed. C. Goodwin, N. J. Holder & C. C. Wylie), pp. 187–222. London: Cambridge University Press.
- Harvey, W. R. 1990 *User's guide for LSMLMN and MIXMDL v. PC-2*. Copyright W. R. Harvey.
- Hoffmann, A. A. & Parsons, P. A. 1988 The analysis of quantitative variation in natural populations with isofemale strains. *Genet. Selection Evol.* **20**, 87–98.

- Loeschcke, V., Brudgaard, J. & Barker, J. S. F. 1999 Reaction norms across and genetic parameters at different temperatures for thorax and wing size traits in *Drosophila aldrichi* and *D. buzzatii*. *J. Evol. Biol.* **12**, 605–623.
- Møller, A. P. 1990 Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. *Anim. Behav.* **40**, 1185–1187.
- Møller, A. P. & Pomiankowski, A. 1993 Fluctuating asymmetry and sexual selection. *Genetica* **89**, 267–279.
- Møller, A. P. & Swaddle, J. P. 1997 *Asymmetry, developmental stability, and evolution*. Oxford University Press.
- Møller, A. P. & Thornhill, R. 1998 Bilateral symmetry and sexual selection: a meta-analysis. *Am. Nat.* **151**, 174–192.
- Palmer, A. R. 1994 Fluctuating asymmetry analyses: a primer. In *Developmental instability: its origins and evolutionary implications* (ed. T. A. Markow), pp. 335–364. Dordrecht, The Netherlands: Kluwer.
- Palmer, A. R. & Strobeck, C. 1986 Fluctuating asymmetry: measurement, analysis, patterns. *A. Rev. Ecol. Syst.* **17**, 391–421.
- Parker, G. A. & Simmons, L. W. 1994 Evolution of phenotypic optima and copula duration in dung flies. *Nature* **370**, 53–56.
- Polak, M. 1997a Parasites, fluctuating asymmetry and sexual selection. In *Parasites and pathogens: effects on host hormones and behavior* (ed. N. E. Beckage), pp. 246–276. New York: Chapman & Hall.
- Polak, M. 1997b Ectoparasitism in mothers causes higher positional fluctuating asymmetry in their sons: implications for sexual selection. *Am. Nat.* **149**, 955–974.
- Polak, M. & Starmer, W. T. 2001 The quantitative genetics of fluctuating asymmetry. *Evolution* **55**, 498–511.
- Polak, M. & Trivers, R. 1994 The science of symmetry in biology. *Trends Ecol. Evol.* **9**, 122–124.
- Polak, M., Starmer, W. T. & Wolf, L. L. 2004 Sexual selection for size and symmetry in a diversifying secondary sexual trait in *Drosophila bipectinata* Duda (Diptera: Drosophilidae). *Evolution* **58**, 597–607.
- Ridley, M. 1992 Swallows and scorpionflies find symmetry is beautiful. *Nature* **257**, 327–328.
- SAS Institute Inc. 1990 *SAS user's guide*, v. 6. Cary, NC: SAS Institute.
- Simmons, L. W. 2001 *Sperm competition and its evolutionary consequences in the insects*. Princeton University Press.
- Simmons, L. W. & Parker, G. A. 1992 Individual variation in sperm competition success of yellow dung flies, *Scathophaga stercoraria*. *Evolution* **46**, 366–375.
- Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*. New York: Freeman.
- Starmer, W. T., Patten, M. & Polak, M. 2002 The statistics of detecting positional fluctuating asymmetry. *Biol. J. Linn. Soc.* **77**, 491–498.
- Thornhill, R. & Alcock, A. 1983 *The evolution of insect mating systems*. Cambridge, MA: Harvard University Press.
- Tomkins, J. L. & Simmons, L. W. 2003 Fluctuating asymmetry and sexual selection: paradigm shifts, publication bias, and observer expectation. In *Developmental instability: causes and consequences* (ed. M. Polak), pp. 231–261. New York: Oxford University Press.
- Uetz, G. W. & Taylor, P. W. 2003 Developmental instability and animal communication: fluctuating asymmetry as a signal and as an influence on the signaling process. In *Developmental instability: causes and consequences* (ed. M. Polak), pp. 213–230. New York: Oxford University Press.
- Van Valen, L. 1962 A study of fluctuating asymmetry. *Evolution* **16**, 125–142.
- Waddington, C. H. 1957 *The strategy of the genes*. London: Allen & Unwin.
- Watson, P. J. & Thornhill, R. 1994 Fluctuating asymmetry and sexual selection. *Trends Ecol. Evol.* **9**, 21–25.
- West-Eberhard, M. J. 2003 *Developmental plasticity and evolution*. New York: Oxford University Press.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.