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THE QUANTITATIVE GENETICS OF FLUCTUATING ASYMMETRY

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Abstract.—Fluctuating asymmetry (subtle departures from identical expression of a trait across an axis of symmetry) in many taxa is under stabilizing selection for reduced asymmetry. However, lack of reliable estimates of genetic parameters for asymmetry variation hampers our ability to predict the evolutionary outcome of this selection. Here we report on a study, based on analysis of variation within and between isofemale lines and of generation means (line-cross analysis), designed to dissect in detail the quantitative genetics of positional fluctuating asymmetry (PFA) in bristle number in natural populations of *Drosophila falleni.* PFA is defined as the difference between the two sides of the body in the placement or position of components of a meristic trait. Heritability (measured at 25°C) of two related measures of PFA were 13% and 21%, both of which differed significantly from zero. In contrast, heritability estimates for fluctuating asymmetry in the total number of anterior (0.7%) and transverse (2.4%) sternopleural bristles were smaller, not significant, and in quantitative agreement with previously published estimates. Heritabilities for bristle number (trait size) were considerably greater than that for any asymmetry measure. The experimental design controlled for the potentially confounding effects of common familial environment, and repeated testing revealed that PFA differences between lines were genetically stable for up to 16 generations in the laboratory at 25°C. We performed line cross analysis between strains at the extremes of the PFA distribution (highest and lowest values); parental strains, F_1, F_{1r} (reciprocal), F_2, backcross, and backcross reciprocal generations were represented. The inheritance of PFA was described best by additive and dominance effects localized to the X-chromosomes, whereas autosomal dominance effects were also detected. Epistatic, maternal, and cytoplasmic effects were not detected. The inheritance of trait size was notably more complex and involved significant autosomal additive, dominance, and epistatic effects; maternal dominance effects; and additive and dominance effects localized to the X-chromosomes. The additive genetic correlation between PFA and its associated measure of trait size was negative (−0.049), but not statistically significant, indicating that the loci contributing additive genetic effects to these traits are probably different. It is suggested that PFA may be a sensitive measure of developmental instability because PFA taps the ability of an organism to integrate interconnected developmental pathways.

Key words.—Coefficient of additive genetic variation, developmental instability, dominance, *Drosophila falleni*, epistasis, fluctuating asymmetry, genotypic variation, heritability, isofemale lines, line-cross analysis, quantitative genetics, X-chromosomes.

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Fluctuating asymmetry (FA) commonly refers to subtle deviations from perfect bilateral symmetry in morphological traits (Ludwig 1932; Van Valen 1962). The magnitude of this deviation reflects a point of balance between at least two opposing processes. On the one hand, random errors of development, referred to as developmental noise (sensu Waddington 1957), cause disturbances to patterns of cell division, differentiation, and growth, thereby resulting in observable asymmetry in trait expression across the midline. Developmental stability, on the other hand, dampens developmental noise and reduces asymmetry by helping to ensure that a trait develops along its genetically predetermined pathway, or creode (Waddington 1957). In other words, developmental stability limits the range of potential FA values an individual or genotype can develop in a given environment. An important consequence of this model, if correct, is that much of the FA variation in a population will reflect developmental noise and FA will correlate only loosely with developmental stability (Whitlock 1996; Van Dongen 1998).

The expected looseness of this association is at the heart of current controversy about the genetic basis of FA and about its value as a predictor of individual reproductive fitness (e.g., Leung and Forbes 1996; Palmer 1996; Houle 1997; Gangestad and Thornhill 1999). In addition, because the magnitude of deviations from symmetry will often be very small and thus relatively difficult to quantify, measurement error may even further erode the relationship between FA and developmental stability (Palmer and Strobeck 1986; Palmer 1994). Despite these potential pitfalls and although there are many exceptions, FA exhibits repeatable associations with the genetic characteristics of individuals as well as with certain estimates of individual phenotypic quality. Some of the best evidence for genetic involvement comes from studies of resistance against pesticide in Australian sheep blow flies (*Lucilia cuprina*; Clarke and McKenzie 1987). In this species, the presence of an unmodified resistance allele disrupts developmental stability and elevates FA through the breakdown of genic coadaptation. Evidence for a similar general mechanism elevating FA comes from hybridization studies of stickelbacks (*Pungitius*), sunfishes (*Enneacanthus*), fruit flies (*Drosophila*), and willows (*Salix*; Tanaka 1982; Graham and Felley 1985; Markow and Ricker 1991; Hochwender and Fritz 1999). But increasing genetic diversity within species may have a very different outcome; in rainbow trout (*Oncorhyncus mykiss*), cutthroat trout (*O. clarki*), and house mice (*Mus musculus*), the most heterozygous individuals express the least FA (Leary et al. 1983, 1984; Alibert et al. 1994). However, studies of honeybees (*Apis mellifera*) and *Drosophila melanogaster* do not support this association (Clarke et al. 1992; Fowler and Whitlock 1994). In relation to phenotypic quality, FA covaries negatively, for example, with survivorship in forest tent caterpillars (*Malacosoma disstria*; Naugler and Leech 1994) and house flies (*Musca domestica*; Møller 1996), although two recent studies of stalk-eyed flies

(*Cyrtodiopsis dalmanni*) found no association between symmetry and individual condition (David et al. 1998; Bjorksten et al. 2000).

Despite advances in our understanding of certain genetic correlates and the nature of selection for reduced FA, there is a dearth of studies on the genetic basis of FA variation in natural populations. This question, together with knowing the degree of genetic covariance between FA and traits in other fitness domains, is fundamental toward predicting the evolution of developmental stability. Heritability estimates of FA are especially needed to evaluate a popular good-genes model of the evolution of adaptive mate choice (Møller and Pomiankowski 1993). This model predicts that mate choice for symmetrical males will be rewarded by genetic factors (good genes) passed to offspring that confer superior developmental stability and thus elevate offspring survival (Møller and Pomiankoski 1993; Møller and Swaddle 1997). Mate choice in favor of symmetry has been documented in some taxa (reviewed by Møller and Thornhill 1998), such as in barn swallows (*Hirundo rustica*; Møller 1992) and wolf spiders (*Schizocosa ocreata*; Uetz and Smith 1999), but not in earwigs (*Forficula auricularia*; Radesäter and Halldórsdóttir 1993; Tomkins and Simmons 1998) and house flies (*Musca domestica*; Goulson et al. 1999).

Reliable estimates of FA heritability, unfortunately, are uncommon. A recent meta-analysis revealed a significant mean heritability estimate of 27% for FA encompassing 14 species of plants and animals (Møller and Thornhill 1997). This study, however, has been criticized for the inclusion of data from studies faulted for their lack of control of potential confounds such as maternal (and paternal) effects and effects of common familial environment. Whitlock and Fowler (1997), having excluded these questionable studies from Møller and Thornhill's analysis, came up with a considerably smaller mean heritability of 2.3%, but one that was also statistically significant (and see Gangestad and Thornhill 1999). The significance of this effect was attributable entirely to data derived from sternopleural bristles in *Drosophila melanogaster*. Indeed, Mather (1953) and Reeve (1960) used this species in their pioneering experiments in which they successfully selected for high and low asymmetry in sternopleural bristles in replicate populations; Reeve's experiment yielded heritability estimates of 2–3% at 25°C. However, both these studies have limited applicability to the question of FA heritability in natural populations: Mather's base population was derived from crosses between two ''long inbred stocks,'' and Reeve's study has been criticized for inadequate control of potentially confounding environmental effects (Woods et al. 1998). A recent study of heritability of bristle FA in *D. melanogaster* derived from nature yielded a relatively smaller mean heritability of 1.1% at 25°C (Woods et al. 1998).

The present study examines the quantitative genetics of sternopleural bristle FA in natural populations of *Drosophila falleni.* Our experiments were conducted at 25°C so that our heritability estimates could be compared to those from the growing number of studies done at this temperature. We first determined the magnitude and repeatability of genotype-specific differences in FA separately in each of two types of bristles on the sternopleuron of *D. falleni*. A unique feature of the present study is that it considers the genetic basis of positional fluctuating asymmetry (PFA) in sternopleural bristles (Polak 1997). PFA is a measure of the difference between the two sides of the body in the placement or position of the components of a meristic trait. Transverse bristles and anterior bristles are located in two distinct patches or rows on the sternopleuron, and their numbers are significantly positively correlated. In a previous study, PFA among offspring increased with experimentally applied maternal stress (parasitism by mites), whereas FA based simply on the difference in the total number of bristles between the two sides of the body was invariable in this regard. Consequently, it was suggested that PFA is a more sensitive measure of developmental stability than the more traditional FA in sternopleural bristles (Polak 1997).

The goals of the present study are as follows: (1) test for repeatable differences in trait FA and size between isofemale strains derived from two natural populations of *D. falleni*; (2) determine the degree of stability of any FA differences across multiple generations in the laboratory; (3) estimate at 25°C the heritability of FA in two bristle characters separately, as well as of PFA, and contrast heritability values to that for trait size; (4) using line-cross analysis, examine the relative importance of the following determinants of PFA variation: additivity, dominance, epistasis and cytoplasmic effects; and (5) estimate the magnitude of genetic covariance between PFA and trait size. No estimates of quantitative genetic parameters exist for positional FA. Moreover, there are no studies that have examined in detail the relative contribution of higher-order genetic effects, such as epistasis on FA variation in natural populations, predicted to be important determinants of long-term evolutionary change in any trait.

Methods

Isofemale Lines

Isofemale lines of *D. falleni* were established in the laboratory from progeny of one virgin pair and expanded to large population size in the laboratory prior to characterization. Flies were obtained from June to August 1998 from two sites in northeastern United States: North Hupper Island, Maine (43°55′N, 69°16′W; HUP site) and Green Lakes State Park, Syracuse, New York (43°03′N, 75° 59′W; GLS site). The distance between these sites is 550 km. *Drosophila falleni* is widespread throughout eastern North America and utilizes mushrooms as its primary breeding site (Jaenike 1978).

Flies were collected in the field by gently aspirating them from wild mushrooms and from bait buckets containing rotting commercial *Agaricus* mushrooms. On the day of capture, a single male-female pair was placed in an 8 fluid-dram shell vial containing instant *Drosophila* medium (Carolina Supply Co., Burlington, NC), distilled H_2O, 1 g commercial mushroom, and approximately four grains Fleischmann's active dry yeast. A single virgin female and male offspring resulting from this original field-caught pair were used to initiate a fresh vial. From this vial, five to 10 male-female pairs of progeny (full siblings) were collected and held until sexual maturity in shell vials containing cornmeal/agar substrate and 1–2 g commercial mushrooms. Groups of mature siblings were transferred together into 400 ml Mason jars containing

25 g instant *Drosophila* medium, 90 ml distilled H_2O, and 13 g commercial mushrooms. After 4 days of allowing flies to oviposit into this first jar, they were transferred to a new jar with fresh food supply. All jars were positioned at random within a single incubator with a 12:12 L:D photoperiod at 25°C and rotated daily to avoid any systematic microenvironmental effects. Thus, there were two replicate jars per isofemale line; progeny from both jars were collected and used for bristle number determination (see below). A total of 11 lines were derived from HUP and 14 lines were derived from GLS, for a total of 25 lines.

Fluctuating Asymmetry and Size Determination

Each fly was anesthetized with ether, and under an Olympus SZX12 stereomicroscope, its anterior sternopleural bristles and transverse sternopleural bristles were counted. For consistency with prior work (Polak 1993, 1997), sternal bristles were not counted. Thorax length, measured from the anterior end of the thorax to the posterior end of the scutellum, was used as an estimate of adult body size.

Trait size was calculated as the mean number of bristles across the two sides of the body ([R + L]/2) for a given trait. Size1 and Size2 correspond to trait size in anterior sternopleural bristles and transverse sternopleural bristles, respectively. Size1,2 is the sum of Size1 and Size2.

Asymmetry was calculated as the value of a given trait on the right side of the body minus its value on the left (R − L). FA1 and FA2 refer to FA in the anterior sternopleural bristles and transverse sternopleural bristles, respectively. Each type of bristles (referred to as setulae in Polak 1997) are located in a distinct area on the sternopleuron (see fig. 1 in Polak 1997). Positional FA (Polak 1997) was calculated in two ways, each reflecting differences between the two sides of the body in the placement of bristles between these areas of the sternopleuron, and thus can be viewed as a measure of bristle position or ''shape'' differences between the two sides:

$$\begin{aligned}\text{PFA1} = {} & (\text{right number anterior bristles} \\ & \div \text{right number transverse bristles}) \\ & - (\text{left number anterior bristles} \\ & \div \text{left number transverse bristles}) \quad \text{and} \end{aligned} \tag{1}$$

$$\begin{aligned}\text{PFA2} = {} & \log_e(|\text{right number anterior bristles} \\ & - \text{right number transverse bristles}| + 0.5) \\ & - \log_e(|\text{left number anterior bristles} \\ & - \text{left number transverse bristles}| + 0.5). \end{aligned} \tag{2}$$

PFA1 and PFA2 are highly correlated (GLS: $r = 0.811$, $n = 652$; HUP: $r = 0.850$, $n = 480$; both $P < 0.001$). For traits exhibiting FA, the distribution of signed (R − L) values will have a mean zero and exhibit no apparent bimodality (Van Valen 1962; Palmer and Strobeck 1986, 1992). Departure in these moments can signal directional asymmetry or extreme antisymmetry (Palmer and Strobeck 1992). To test for FA, we calculated mean, skewness, and kurtosis for signed (R − L) values for each of FA1, FA2, PFA1, and PFA2 separately by site as well as for data pooled across sites.

Measurement error is potentially problematic because it can inflate the between-sides variance and obscure the variance due to actual FA. A previous study of *D. nigrospiracula* (Polak 1997) has demonstrated that measurement error associated with counts of anterior and transverse bristles performed by the present investigators is sufficiently small. This previous study showed using methods described by Palmer and Strobeck (1986) that the magnitude of the variance due to measurement error in both these traits, expressed as a percentage the variance representing FA, was negligible. Measurement error was assessed using two-way ANOVA in which sides and individuals were entered as factors (Palmer and Strobeck 1986; Palmer 1994) for 32 flies randomly sampled from laboratory stock bottles. Two replicate counts of each bristle type were made per fly, each on a different day. Two counting errors were made between replicate counts, each differing by one bristle. The resultant variance estimates due to measurement error for PFA was 0.00044 (MS_{error}). The magnitude of this variance as a percentage of the between-sides variance representing FA (sides × males interaction MS) was 0.72%. In the present study, counts were made when flies were young (1–4 days old) and the dark bristles were clearly visible against the pale background of the cuticle, so that we can assume that measurement error was probably even smaller than that reported in Polak (1997).

Data Analysis

A major goal was to estimate the magnitude of genotypic variance underlying bristle number asymmetry in natural populations of *D. falleni* and to contrast it to that for trait size (bristle number). We also evaluate the relative significance of the environmental variance for FA, which can confound interpretation of heritability estimates and which is at the heart of much current discussion regarding the heritable basis of FA.

We analyzed the effects of site, line, and jar on each measure of FA (unsigned values) and trait size. Line was nested within site and jar within line. *F*-statistics for site and line were computed using line(site) and jar(line site) mean squares, respectively, whereas *F*-values for jar were computed using error mean square. Because FA and trait size were correlated in the case of FA1 and FA2 (see Results), FA data were analyzed using nested ANOVA, using the GLM procedure of SAS (SAS Institute 1990), in which trait size was entered as a covariate. This analysis is equivalent to ANOVA (without the covariate) on residuals from regression of FA on trait size. The following size measures were used as covariates for the various FAs: Size1 for FA1; Size2 for FA2; and Size1,2 for PFA1 and PFA2. All FAs were corrected for size so that there was consistency between measures. Residuals in FA generated using the size covariate were desired because they more closely fit the normal distribution compared to raw unsigned FA values (see also Polak 1997). In the case of trait size, nested ANOVAs were performed with thorax length as a covariate; trait size and thorax length are significantly positively correlated (see Results).

To test for cross-generational stability of line FA and size

measures, we evaluated whether observed differences in FA and size would persist across one generation of laboratory rearing. We selected seven HUP and four GLS lines for characterization in a subsequent generation (fifth generation removed from the field). Flies were reared in Mason jars under similar incubator conditions as described above. ANOVA was used to test effects of site, line (nested within site), and generation (nested within site and line). Prior to analysis, FA and size data were corrected using regression for trait size and thorax length, respectively. *F*-statistics were computed as described above.

To test for longer-term stability of FAs, we retained lines GLS 2.3 and 3.5 in the laboratory at 25°C and characterized them again at generation 15 and 16 and 14 and 15, respectively. These lines were the most diverged in terms of magnitude of PFAs at GLS (see Results). Factorial ANOVA, in which generation and line were factors, tested for FA differences between isofemale lines and generations. PFA values were corrected for trait size for consistency with above analyses.

Heritability and Additive Genetic Coefficient of Variation

Analyses outlined in the previous section showed that jar effects (i.e., common familial environmental effects) were not significant for either FA or trait size (see Results). Nevertheless, we removed variability due to jar (common familial environment) from both FA and size prior to computing heritability estimates for these traits to avoid even subtle bias. FA measures were also corrected for trait size prior to computing heritability estimates; we used ANOVA in which jar was the class variable and trait size the continuous variable, or covariate. Trait size was corrected for both jar effects and thorax length (the covariate) in a similar manner. ANOVAs were run using the GLM procedure in SAS (SAS Institute 1990); we used residuals in FA and trait size from these ANOVAs as computational units for heritability estimates.

Heritability (h^2) and its standard error (SE) for FA and size were computed using the method of Hoffman and Parsons (1988, p. 95), but corrected by deleting the square of the denominator in their equation for the standard error of h^2. Estimation of variance components for between and within isofemale lines was done using model 3 of the PC-2 version of the mixed-model least-squares and maximum likelihood computer program (Harvey 1990). Heritability estimates from this approach have been shown to be similar to that derived from conventional sibling analysis, provided that strains are tested within five generations after establishment and maintained in the laboratory at large population sizes (Hoffman and Parsons 1988).

Additive genetic coefficients of variation were calculated for each measure of FA and trait size as:

$$CV_A = 100\sqrt{V_A}/\bar{X} \quad (3)$$

(Houle 1992), where $\bar{X}$ is the character mean and V_A is the additive genetic variance. V_A was taken as twice the value of the between-line variance component determined as described above (Hoffman and Parsons 1988; Falconer and Mackay 1996).

TABLE 1. Sources of genetic lines used in generation means analysis.

Line	Source	
	Female	Male
High	high	high
Backcross high (Bch)	high	F_1
Backcross high reciprocal (Bchr)	F_1	high
First filial generation (F_1)	high	low
Second filial generation a (F_{2a})	F_1	F_1
Second filial generation b (F_{2b})	F_{1r}	F_{1r}
First filial generation reciprocal (F_{1r})	low	high
Backcross low reciprocal (Bclr)	F_{1r}	low
Backcross low (Bcl)	low	F_{1r}
Low	low	low

Genetic Architecture of PFA2 and Trait Size

We chose isofemale lines 2.3 and 3.5, which exhibited the highest and lowest mean PFA2, respectively, at the GLS site (see Results) to dissect the quantitative genetics of PFA, and trait size (Size1,2) variation using analysis of generation means (or means of line crosses; Mather and Jinks 1982; Lynch and Walsh 1997). Ten lines were formed (Table 1), of which four were nonsegregating lines (two parental lines and F_1 and F_{1r}) and six were segregating lines (two F_{2s} and four backcrosses). All lines were reared at a 12:12 L:D photoperiod and at 25°C in the same incubator previously used to rear all isofemale lines.

Size1,2 was corrected for body size using ANCOVA, whereas PFA2 was not corrected prior to generation means analysis. This analysis estimates the net difference between additive effects, average dominance, and net directional epistasis. The parameters of a given model collectively summarize the overall effects across all loci. It is assumed that some alleles will contribute positive and others negative effects to the phenotype, but models are essentially arbitrary for the number and distribution of loci involved. Estimates of parameters are used to predict the line means as the algebraic sum of the contribution of each parameter associated with the expected genotype of that line (Mather and Jinks 1982).

The basic genetic model was based on the means of self, F_1, F_2, and backcross lines. This model was used to evaluate the importance of different forms of genetic activity with and without maternal effects. Because we distinguished between the sexes, we also were able to evaluate the importance of the effects of X-chromosomes. The full model when sexes were pooled included the following composite genetic effects: additive, [d]; dominance, [h]; digenic epistasis, [i], [j], and [l]; maternal additive, [dm]; maternal dominance, [hm]; and cytoplasmic effects, [cyto]. Because we observed 10 means, models with more than nine parameters could not be evaluated statistically.

Genetic models were also constructed for both PFA2 and trait size in which sexes were separate. In addition to examining the parameters used when sexes were pooled, we also evaluated X-chromosomal additive, [dx], and X-chromosomal dominance, [hx], effects.

For all models, least-squares procedures were used to estimate model parameters contained in vector *Y* and their variances from the diagonal of their variance covariance matrix

TABLE 2. **C** matrix for full generation means model, by sex. See Table 1 for description of lines; parameters are defined in the Methods.

Sex/ Line	Parameter										
	m	d	h	i	j	l	dm	hm	dx	hx	cyto
Female											
High	1	1	0	1	0	0	1	0	1	0	1
Bch	1	0.5	0.5	0.25	0.25	0.25	1	0	1	0	1
Bchr	1	0.5	0.5	0.25	0.25	0.25	0	1	0.5	0.5	1
F_1	1	0	1	0	0	1	1	0	0	1	1
F_{2a}	1	0	0.5	0	0	0.25	0	1	0.5	0.5	1
F_{2b}	1	0	0.5	0	0	0.25	0	1	−0.5	0.5	−1
F_{1r}	1	0	1	0	0	1	−1	0	0	1	−1
Bclr	1	−0.5	0.5	0.25	−0.25	0.25	0	1	−0.5	0.5	−1
Bcl	1	−0.5	0.5	0.25	−0.25	0.25	−1	0	−1	0	−1
Low	1	−1	0	1	0	0	−1	0	−1	0	−1
Male											
High	1	1	0	1	0	0	1	0	1	0	1
Bch	1	0.5	0.5	0.25	0.25	0.25	1	0	1	0	1
Bchr	1	0.5	0.5	0.25	0.25	0.25	0	1	0	0	1
F_1	1	0	1	0	0	1	1	0	1	0	1
F_{2a}	1	0	0.5	0	0	0.25	0	1	0	0	1
F_{2b}	1	0	0.5	0	0	0.25	0	1	0	0	−1
F_{1r}	1	0	1	0	0	1	−1	0	−1	0	−1
Bclr	1	−0.5	0.5	0.25	−0.25	0.25	0	1	0	0	−1
Bcl	1	−0.5	0.5	0.25	−0.25	0.25	−1	0	−1	0	−1
Low	1	−1	0	1	0	0	−1	0	−1	0	−1

S (Mather and Jinks 1982; Lynch and Walsh 1997). The estimates of Y and **S** are obtained as:

$$\hat{Y} = (\mathbf{C}^T\mathbf{V}^{-1}\mathbf{C})^{-1}\mathbf{C}^T\mathbf{V}^{-1}X \quad \text{and} \tag{4}$$

$$\hat{\mathbf{S}} = (\mathbf{C}^T\mathbf{V}^{-1}\mathbf{C})^{-1}, \tag{5}$$

where **C** is the coefficient matrix (Table 2) for the contribution of effects to each line mean, **V** is the diagonal matrix of the error variances of each line mean, and X is the vector of observed line means. All parameters of each model were estimated in a full model to provide a guide to which parameters might be important in reduced models (Mather and Jinks 1982). Exploratory analyses were perfomed to find models that provided a reasonable fit to the generation means by stepwise addition and deletion of parameters; models that offered a good fit to the data are presented along with full models. Goodness of fit of each model was tested using χ^2, where

$$\chi^2 = X^T\mathbf{V}^{-1}X - X^T\mathbf{V}^{-1}\,\mathbf{C}\hat{Y} \tag{6}$$

(Hayman 1958). The degrees of freedom for this χ^2 is the number of line means minus the number of parameters estimated in the model. F-statistics were used to evaluate the improvement in the goodness of fit after modifying number of model parameters (Graybill 1961). This F-statistic was calculated as:

$$F_{p_2-p_1,n-p_2} = \frac{(\chi^2_{n-p_1} - \chi^2_{n-p_2})/(p_2 - p_1)}{\chi^2_{n-p_2}/(n - p_2)}, \tag{7}$$

where n is the number of observations, p_1 is the number of parameters in a basic model, and p_2 is the number of parameters of an expanded model.

Genetic Correlation between PFA2 and Trait Size

A correlation matrix of genetic effects between PFA2 and Size1,2 was calculated using a technique of resampling lines used in the generation means. Resampling (1000 replicates) was done by sampling each line n_i times (n_i = sample size of that line) with replacement and calculating each line mean. The line means were then subjected to generation means analysis to estimate the parameters of a linear model:

$$\hat{Y} = (\mathbf{C}^T\mathbf{C})^{-1}\mathbf{C}^TX, \tag{8}$$

where **C** is the coefficient matrix, X is the vector of mean values from the resample, and Y is the vector of parameter estimates. The analysis was done without weights (i.e., the reciprocals of the line variances) because resampling according to sample size will result in appropriate weights. From these r estimates, the ordered values for each statistic provide the confidence interval based on number of resamples (the 95% confidence interval falls between the ordered resamples number 25 and 975 if r = 1000). The parameter estimates for means and additive effects for each trait were paired in each resample to calculate correlation coefficients (r), for example r for [m] for PFA2 versus [m] for Size1,2, and [d] for PFA2 versus [d] for Size1,2. The matrix of these correlation coefficients thus estimates the genetic effects correlation matrix between these traits.

RESULTS

Descriptive statistics for signed (R − L) asymmetry values for each trait within site are presented in Table 3. For all traits, mean signed (R − L) values did not differ significantly from zero, and there was little skewness or leptokurtosis detected for FA in any bristle trait considered separately (Table 3). However, distributions of signed PFA1 and PFA2 were significantly skewed to the left and to the right, respectively (Table 3). Significant leptokurtosis was also detected for both PFA1 and PFA2 (for similar findings for PFA1 in *D. nigrospiracula*, see Polak 1997). Leptokurtosis is consistent

TABLE 3. Mean signed fluctuating asymmetry (R − L), skewness, and kurtosis in sternopleural bristle traits of *Drosophila falleni*. FA, fluctuating asymmetry; PFA, positional fluctuating asymmetry.

		Signed asymmetry (R − L)			
Trait	Site (*n*)	Mean (VAR)	*P*[1]	Skewness	Kurtosis
FA1	GLS (656)	−0.0747 (1.107)	0.070	−0.000248	0.208
	HUP (482)	0.0187 (.966)	0.68	−0.103	0.226
	Pooled (1138)	−0.0352 (1.049)	0.25	−0.0477	0.214
FA2	GLS (655)	−0.0886 (1.900)	0.10	−0.0371	−0.0643
	HUP (481)	−0.0166 (2.096)	0.80	0.302**	0.360
	Pooled (1136)	−0.0581 (1.983)	0.17	0.122	0.155
PFA1	GLS (654)	−0.00483 (.0174)	0.35	−0.170*	1.085***
	HUP (481)	0.00355 (0.0168)	0.55	−0.442***	1.853***
	Pooled (1135)	−0.00128 (0.0171)	0.74	−0.282***	1.369***
PFA2	GLS (652)	0.00531 (0.134)	0.71	0.930***	6.019***
	HUP (480)	−0.0125 (0.118)	0.43	0.423***	1.954***
	Pooled (1132)	−0.00223 (0.128)	0.83	0.742***	4.575***

[1] *P* evaluating H_0: mean (R − L) = 0.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

with data presented below demonstrating significant genetic heterogeneity affecting PFA within populations (Palmer and Strobeck 1986; Gangestad and Thornhill 1999).

The phenotypic correlation between unsigned (|R − L|) FA1 and FA2 values and trait size ([R + L]/2) was significantly positive for each bristle trait taken separately (Table 4). Neither PFA1 or PFA2 was significantly correlated with trait size (Table 4).

Size1 and Size2 were significantly correlated (GLS: $r = 0.223$, $n = 654$; HUP: $r = 0.222$, $n = 481$; both $P < 0.0001$), but unsigned (|R − L|) FA1 and FA2 were not (GLS: $r = 0.00783$, $n = 654$; HUP: $r = -0.0369$, $n = 481$; both $P > 0.05$).

Genetic and Environmental Effects on Fluctuating Asymmetry

A total of 25 isofemale lines were characterized in respect to number of anterior and transverse bristles. Table 5 presents least-square means (corrected for trait size) for each isofemale line within sites calculated for each of the four FA measures.

Nested ANOVA revealed that the site and jar effects were not significant for any of these FAs (Table 6). Likewise, the effect of line was not significant for either FA1 or FA2. In contrast, the line effect was significant at $\alpha = 0.01$ for both PFA1 and PFA2 (Table 6).

We tested whether these significant PFA differences between lines would persist across one generation of laboratory rearing. We used 11 isofemale lines derived from both sites. Factorial ANOVA indicated that generation effects were not significant for either PFA1 ($F_{1,597} = 0.02$) or PFA2 ($F_{1,594} = 1.36$), although line effects remained significant for both PFAs ($F_{10,597} = 2.48$, $P = 0.0064$; $F_{10,594} = 3.73$, $P < 0.001$, respectively). Generation × line interactions were not significant.

We tested for longer-term stability of PFA differences by evaluating line effects between the high (2.3) and low (3.5) lines from the GLS site after generations 14 and 15 for line 3.5 and between generations 15 and 16 for line 2.3. Factorial ANOVA showed that line effects were significant for both PFA measures (PFA1: $F_{1,116} = 7.92$, $P = 0.0057$, PFA2: $F_{1,116} = 10.22$, $P = 0.0018$, respectively), but generation effects were not significant for either PFA1 ($F_{1,116} = 0.20$) or PFA2 ($F_{1,116} = 0.03$). The generation × line interaction was not significant for either PFA1 or PFA2. Least-square means (± SE, *n*) in PFA1 for lines 2.3 and 3.5 were 0.163 (± 0.016, 60) and 0.0991 (± 0.016, 60), respectively, whereas values for PFA2 were 0.533 (± 0.053, 60) and 0.287 (± 0.056, 60). Thus, line differences persisted across multiple generations, strengthening the conclusion that genetic effects underlie PFA variation in *D. falleni*.

Genetic and Environmental Effects on Trait Size

Table 7 presents least-square means and standard errors for number of each type of bristle. Means are presented for each line within site and for each site separately. Nested ANOVA was performed for each size measure, in which site, line (nested within site), and jar (nested within line) were entered as factors. Because trait size and thorax were positively correlated ($P < 0.001$), thorax length was entered as a covariate.

Effects of site were not significant at $\alpha = 0.05$ for any of

TABLE 4. Phenotypic correlation coefficients for unsigned fluctuating asymmetry (|R − L|) versus trait size at sites GLS and HUP. Sample sizes are given in parentheses. FA, fluctuating asymmetry; PFA, positional fluctuating asymmetry.

	FA measure			
Site	FA1	FA2	PFA1	PFA2
GLS	0.128*** (656)	0.0866* (654)	−0.0645 (654)	−0.0372 (652)
HUP	0.175*** (482)	0.0106 (481)	−0.0367 (481)	−0.0341 (480)

* $P < 0.05$; *** $P < 0.001$.

TABLE 5. Least-square means (corrected for trait size), standard errors (SE), and sample sizes (*n*) for unsigned fluctuating asymmetry (|R − L|) measures in *Drosophila falleni* by line within site and by site. FA, fluctuating asymmetry; PFA, positional fluctuating asymmetry.

Site/ Line	FA1			FA2			PFA1			PFA2		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
GLS												
1.2	0.590	0.075	88	0.982	0.097	88	0.0873	0.0089	88	0.229	0.026	88
1.13	0.862	0.087	65	0.937	0.12	66	0.0842	0.010	65	0.168	0.030	65
1.14	0.692	0.10	48	0.952	0.13	48	0.0933	0.012	48	0.27	0.035	48
2.1	0.707	0.090	60	1.027	0.12	59	0.0976	0.011	59	0.249	0.031	59
2.3	0.709	0.11	44	1.013	0.14	43	0.159	0.013	43	0.555	0.037	43
2.7	0.611	0.14	24	1.608	0.19	24	0.105	0.017	24	0.293	0.049	24
2.8	0.865	0.097	52	1.057	0.13	52	0.0962	0.012	52	0.221	0.033	52
3.2	0.739	0.11	41	1.308	0.15	41	0.109	0.014	41	0.281	0.039	41
3.4	0.916	0.12	35	1.203	0.15	35	0.102	0.014	35	0.260	0.041	35
3.5	0.566	0.13	29	0.766	0.17	29	0.0643	0.016	29	0.137	0.045	29
3.9	0.735	0.11	44	1.082	0.14	44	0.102	0.013	44	0.248	0.036	44
4.1	0.890	0.14	25	1.042	0.19	25	0.140	0.017	25	0.363	0.049	25
8.2	1.006	0.098	53	1.007	0.13	53	0.108	0.012	53	0.242	0.034	51
9.2	0.809	0.10	48	1.005	0.13	48	0.119	0.012	48	0.299	0.035	48
Total	0.764	0.029	656	1.071	0.038	655	0.105	0.0035	654	0.269	0.010	652
HUP												
4.2	0.694	0.11	44	1.201	0.14	44	0.0927	0.013	44	0.235	0.036	44
5.2	0.726	0.091	68	1.079	0.11	68	0.0779	0.010	68	0.173	0.030	67
7.2	0.641	0.092	58	1.402	0.13	58	0.109	0.011	58	0.326	0.033	58
9.2	0.669	0.17	17	0.682	0.22	17	0.0780	0.020	17	0.151	0.058	17
10.2	0.517	0.15	21	1.251	0.20	21	0.0798	0.018	21	0.226	0.053	21
13.2	0.313	0.40	3	1.354	0.53	3	0.108	0.048	3	0.328	0.138	3
14.2	0.541	0.10	48	0.915	0.13	48	0.0774	0.012	48	0.205	0.035	48
16.2	0.797	0.10	49	1.177	0.13	49	0.0105	0.012	49	0.240	0.034	49
18.2	0.888	0.086	73	0.911	0.11	72	0.128	0.0099	72	0.334	0.029	72
19.2	0.725	0.099	52	1.028	0.13	52	0.0996	0.012	52	0.256	0.035	52
20.2	0.659	0.10	49	1.247	0.13	49	0.0796	0.012	49	0.209	0.034	49
Total	0.652	0.049	482	1.113	0.064	481	0.0941	0.0058	481	0.244	0.017	480

the size measures (Size1: $F_{1,20} = 0.40$, Size2: $F_{1,20} = 1.74$, Size1,2: $F_{1,20} = 1.87$). Jar effects were also nonsignificant (Size1: $F_{20,505} = 1.47$, Size2: $F_{20,503} = 1.17$, Size1,2: $F_{20,502} = 1.51$). In contrast, there was a strong line effect for each size measure, demonstrating significant genetic effects on bristle number (Size1: $F_{20,20} = 13.14$, Size2: $F_{20,20} = 11.79$, Size1,2: $F_{20,20} = 9.62$, all $P < 0.001$).

TABLE 6. Nested analysis of variance for fluctuating asymmetry, testing for site, line and jar effects, and in which trait size was entered as a covariate.

Trait	Source	df	MS	*F*
FA1	Size1	1	7.25	14.40***
	Site	1	1.55	2.52
	Line(site)	21	0.615	1.13
	Jar (line (site))	23	0.544	1.08
	Error	777	0.504	
FA2	Size2	1	1.17	1.43
	Site	1	0.0484	0.04
	Line(site)	21	1.22	1.48
	Jar (line (site))	23	0.821	1.00
	Error	775	0.818	
PFA1	Size1,2	1	0.00276	0.40
	Site	1	0.0403	2.29
	Line(site)	21	0.0176	3.31**
	Jar (line (site))	23	0.00533	0.78
	Error	774	0.00684	
PFA2	Size1,2	1	0.0243	0.41
	Site	1	0.276	0.94
	Line(site)	21	0.293	3.56**
	Jar (line (site))	23	0.0823	1.40
	Error	772	0.179	

** $P < 0.01$; *** $P < 0.001$.

We also tested for cross-generational effects on bristle number. Nested ANOVA indicated that the generation effect was significant at $\alpha = 0.05$ in the case of anterior and transverse bristles (Size1: $F_{11,598} = 1.90$, Size2: $F_{11,598} = 1.91$, both $P < 0.05$), but not significant for Size1,2 ($F_{11,597} = 1.64$). These significant generation effects reflect a significant environmental component of size variation. Nevertheless, the analysis also revealed strong line effects for all size measures (Size1: $F_{9,11} = 22.18$, Size2: $F_{9,11} = 11.11$, Size1,2: $F_{9,11} = 15.44$, all $P < 0.001$), supporting the conclusion that there is a significant genetic component to trait size variation.

Heritability and CV_A of Fluctuating Asymmetry and Trait Size

Heritability (h^2) estimates for PFA and trait size, standard errors, and tests of significance (i.e., H_0: $h^2 = 0$) are presented in Table 8. Heritability of FA for each trait taken separately was not significantly different from zero. In contrast, significant heritability was found for both PFA1 and PFA2, with values being 13% and 21%, respectively.

In marked contrast to these estimates, heritability of size (i.e., total number of bristles) for each trait taken separately was highly significant, with values ranging from 73% to 100% (Table 8). The greatest heritability value was for the anterior sternopleural bristles.

TABLE 7. Least-square means and standard errors (SE) for trait size (Σ [R_i + L_i]/2) in *Drosophila falleni* by line within site and by site. Sample sizes for Size1, Size2, and Size1,2 are as given in Table 5 for FA1, FA2, and PFA1, respectively.

Site/ Line	Size1		Size2		Size1,2	
	Mean	SE	Mean	SE	Mean	SE
GLS						
1.2	8.40	0.18	19.41	0.26	27.81	0.35
1.13	7.71	0.21	22.46	0.31	30.17	0.41
1.14	8.92	0.24	20.85	0.36	29.77	0.48
2.1.2	8.15	0.22	18.39	0.32	26.54	0.43
2.3.2	12.02	0.25	17.95	0.38	30.00	0.51
2.7.2	9.67	0.34	20.38	0.51	30.04	0.68
2.8.2	7.87	0.23	20.50	0.34	28.37	0.46
3.2.2	10.34	0.26	23.34	0.39	33.68	0.52
3.4.2	9.11	0.28	19.49	0.42	28.60	0.56
3.5.2	9.66	0.31	22.41	0.46	32.07	0.62
3.9.2	9.32	0.25	21.07	0.37	30.39	0.50
4.1.2	7.64	0.34	15.92	0.50	23.56	0.66
8.2	7.04	0.23	18.45	0.34	25.49	0.46
9.2	9.48	0.24	20.35	0.36	29.83	0.48
Total	8.95	0.069	20.07	0.10	29.02	0.14
HUP						
10.2	6.90	0.37	19.05	0.54	25.95	0.73
13.2	9.00	0.97	18.67	1.43	27.67	1.92
14.2	9.02	0.24	20.65	0.36	29.67	0.48
16.2	9.37	0.24	19.92	0.35	29.29	0.47
18.2	10.75	0.20	19.24	0.29	29.97	0.39
19.2	6.87	0.23	16.62	0.34	23.48	0.46
20.2	8.49	0.24	18.61	0.35	27.10	0.47
4.2	8.36	0.25	20.82	0.37	29.18	0.50
5.2	5.94	0.20	19.37	0.30	25.31	0.40
7.2	8.21	0.22	16.26	0.32	24.47	0.44
9.2	8.18	0.41	20.71	0.60	28.88	0.81
Total	8.28	0.12	19.08	0.17	27.36	0.23

Phenotypic and additive genetic coefficients of variation for FA and trait size are presented in Table 9. The highest CV_A of 45 was for PFA2, which exhibited also greatest heritabilty. There was a close correspondence between the magnitude of heritability and CV_A across the four FAs. For trait size, CV_As ranged from 21 to 45.

Genetic Architecture of Positional Fluctuating Asymmetry

Table 10 presents means and standard errors used in generation means analysis of PFA (PFA2) and trait size (Size1,2). Means are presented for each sex separately and for pooled data. For PFA2, examination of the full genetic model that included additive effects, dominance, epistasis, and maternal effects indicated that only additive effects were significant. But the overall fit of this full model to the data was not good ($P < 0.05$ for χ^2, Table 11). In contrast, we found that the simple additive model (PFA2 = m + [d]) was the most appropriate (Table 11); addition of either epistatic or maternal and cytoplasmic effects to this simple model did not improve fit to the data, as indicated by *F*-tests. Thus, additive effects of genes are the most important factor determining the difference in PFA2 among diverged GLS lines. Figure 1 presents the least-squares regression line for this additive model.

The parameter estimates using least-squares procedure were nearly identical to that derived from bootstrapped values (Table 12). This result indicates that any violation of the normality assumption in the parametric analysis of PFA2 were inconsequential to the outcome of the analysis and that conclusions about the genetic underpinnings of FA derived from least-squares procedures are robust.

TABLE 8. Heritability (h^2) and standard error (SE) estimates for fluctuating asymmetry (FA) and trait size in bristle characters of *Drosophila falleni*. Fluctuating asymmetry data are corrected for trait size and jar effects, whereas trait size data are corrected for body size and jar effects. PFA, positional fluctuating asymmetry.

Trait	h^2	SE	P (H_0: $h^2 = 0$)
FA:			
FA1	0.007	0.0198	0.36
FA2	0.024	0.0248	0.17
PFA1	0.132	0.0548	0.01
PFA2	0.209	0.0740	0.004
Size:			
Size1	1.049	0.209	< 0.0001
Size2	0.814	0.188	0.0001
Size1,2	0.726	0.177	0.0002

Evaluation of a simple additive model in which sexes were analyzed separately indicated that although the additive effect proved to be significantly different from zero, this model did not adequately describe the generation means. Importantly, this significant additive effect was lost upon the addition of X-chromosomal additive and X-chromosomal dominance effects. Both these effects, together with the dominance effect from the autosomes, differed significantly from zero (Table 11); this mixed model provided an adequate fit to the generation means ($P > 0.05$). The model created with the addition of autosomal additive and maternal effects also adequately described the means, but these new terms did not differ significantly from zero, and an *F*-test showed no significant improvement in the fit to the data ($F_{3,13} = 1.18$, $P = 0.36$). Likewise, adding terms to create a full model did not improve fit ($F_{7,9} = 0.59$, $P = 0.75$).

Thus, inheritance of PFA is adequately described by additive and dominance contributions of genes, without epistasis and maternal effects. These additive genetic effects and some dominance effects localize to the X-chromosomes.

TABLE 9. Mean, standard error (SE), sample size (*n*), and coefficient of phenotypic variability (CV_P) and evolvability (CV_A) of size and fluctuating asymmetry (FA). PFA, positional fluctuating asymmetry.

Trait	Mean[1]	SE	*n*	CV_P	CV_A
FA					
FA1	0.738	0.0211	1138	96.26	8.14
FA2	1.070	0.0272	1136	85.50	13.16
PFA1	0.0997	1.523	1135	85.01	30.08
PFA2	0.255	2.380	1132	98.03	44.86
Size					
Size1	4.301	0.0319	1138	24.99	44.63
Size2	9.808	0.0448	1136	15.39	23.40
Size1,2	14.105	0.0610	1135	14.54	20.80

[1] Means for FA calculated as Σ ($|R_i - L_i|$)/*n*, whereas means for size calculated as Σ [($R_i + L_i$)/2]/*n*.

TABLE 10. Generation means (standard errors) and sample sizes for PFA2 and Size1,2 for each genetic line used in generation means analysis. See Table 1 for description of lines.

	Female		Male		Pooled	
Line	PFA2	Size1,2	PFA2	Size1,2	PFA2	Size1,2
High	0.592 (0.0775) 64	29.530 (0.413)	0.400 (0.0590) 59	27.908 (0.368)	0.500 (0.0355) 123	28.644 (0.311)
Bch	0.447 (0.0496) 70	31.382 (0.393)	0.531 (0.0614) 70	29.233 (0.434)	0.489 (0.0333) 140	30.282 (0.0289)
Bchr	0.390 (0.0495) 65	30.169 (0.433)	0.379 (0.0497) 65	28.468 (0.390)	0.385 (0.0345) 130	29.193 (0.303)
F_1	0.291 (0.0597) 45	31.415 (0.438)	0.517 (0.0734) 45	29.714 (0.403)	0.404 (0.0415) 90	30.570 (0.360)
F_{2a}	0.417 (0.0441) 70	31.622 (0.471)	0.422 (0.0532) 70	29.109 (0.424)	0.420 (0.0333) 140	30.430 (0.290)
F_{2b}	0.256 (0.0255) 70	30.637 (0.371)	0.347 (0.0434) 70	28.891 (0.375)	0.302 (0.0333) 140	29.789 (0.289)
F_{1r}	0.387 (0.0761) 45	32.226 (0.442)	0.352 (0.0561) 45	29.476 (0.503)	0.370 (0.0415) 90	30.777 (0.361)
Bclr	0.249 (0.0236) 70	32.192 (0.357)	0.357 (0.0474) 70	30.919 (0.412)	0.304 (0.0333) 140	31.587 (0.289)
Bcl	0.239 (0.0312) 60	32.483 (0.408)	0.313 (0.0415) 60	30.192 (0.494)	0.276 (0.0360) 120	31.365 (0.312)
Low	0.166 (0.0205) 65	33.544 (0.378)	0.213 (0.0350) 45	30.990 (0.422)	0.185 (0.0376) 110	32.665 (0.334)

Genetic Architecture of Trait Size

Investigation of models for SIZE1,2 in which sexes were pooled indicated that the simple additive model and the one containing significant additive and maternal dominance effects (no-epistasis model) were adequate to describe the generation means (Table 13). However, the addition of the terms from the simple additive model to form the no-epistasis model did not significantly improve the fit to the data ($F_{4,4} = 1.04$, $P = 0.49$). Figure 2 presents the least-squares regression line for the additive model.

In the case where sexes were analyzed separately, no model offered an adequate fit to the data (for all χ^2 statistics, $P < 0.0001$). Examination of these models nevertheless suggest a considerably more complex pattern of genetic determination for trait size than for PFA. In the full model for size, the following terms were significant: autosomal additive, dominance, and dominance × dominance epistatis (parameter [l]); maternal dominance; and X-chromosomal additive and dominance effects (Table 13).

Genetic Correlation between Fluctuating Asymmetry and Trait Size

Table 14 presents the genetic effects correlation coefficient matrix for bootstrapped values of population mean, m, and additive genetic effects, [d], between PFA2 and Size1,2. The correlation between additive genetic contributions was −0.049 and was not statistically significant. This result suggests that evolutionary change in PFA will be largely independent of additive genetic effects exerted on the total number of bristles on the sternopleuron and vice versa.

DISCUSSION

We analyzed variation in bristle trait FA and size within and between isofemale strains (full-sib families), the full range of values for which are presented in Tables 5 and 7. To gain a deeper understanding of the genetic basis of FA in natural populations, we combined our analysis of this variation with generation means analysis. The data indicate significant h^2 of PFA, whereas h^2 estimates of FA in two bristle traits taken separately were much smaller and did not differ significantly from zero. Generation means analysis indicated that the inheritance of PFA can best be described by additive [d] effects of genes on the X-chromosomes, as well as dominance [h] deviation from X-chromosomal and autosomal genetic factors.

In contrast, h^2 values for trait size (bristle number) in each trait taken separately and in linear combination were significant and high—higher than that for all FAs, including PFA. Trait size exhibited a notably more complex pattern of inheritance. A full-generation-means model with sexes separate

TABLE 11. Estimates of genetic parameters (SE) for generation means analysis on PFA2 when sexes were either pooled or kept separate. Bold numbers indicate significant ($P < 0.05$) contributions for that parameter. Parameters are defined in the Methods.

	Models				
	Sexes pooled		Sexes separate		
Parameter	Full	Additive	Full	Mixed	Additive
m	0.405 (0.137)	0.363 (0.0113)	0.370 (0.114)	0.308 (0.0182)	0.339 (0.0101)
d	**0.104 (0.0353)**	**0.153 (0.0206)**	0.0464 (0.0366)		**0.155 (0.0162)**
h	−0.0028 (0.298)		−0.0217 (0.256)	**0.137 (0.0377)**	
i	−0.0628 (0.134)		−0.0457 (0.111)		
j	−0.0944 (0.0957)		−0.0982 (0.0884)		
l	−0.0156 (0.184)		0.0862 (0.174)		
dm	0.0171 (0.0253)		−0.0464 (0.0320)		
hm	−0.0394 (0.0343)		0.0022 (0.0327)		
cyto	0.0360 (0.0209)		0.0158 (0.0211)		
dx			**0.130 (0.0411)**	**0.125 (0.0142)**	
hx			−0.0940 (0.0447)	**−0.114 (0.0364)**	
χ^2 (df) P	4.48 (1) 0.034	13.03 (8) 0.11	9.36 (9) 0.41	13.66 (16) 0.62	39.78 (18) 0.002

TABLE 12. Comparison of genetic parameters for PFA2 (sexes pooled) derived from bootstrapping (resampling) and weighted least-squares methods.

Parameter	Least-squares estimate (SE)	Resample mean (SE)
m	0.3629 (0.0113)	0.3626 (0.0112)
[d]	0.1533 (0.0206)	0.1546 (0.0207)

indicated significant contributions of additive [d], dominance [h], and dominance × dominance [l] epistatic effects as well as maternal dominance effects. For PFA, X-chromosome involvement was detected in the form of additive effects and dominance deviation. Because genetic covariance between PFA and trait size (i.e., total number of bristles on the sternopleuron) was not significant, different loci are probably involved in contributing genetic effects to these traits (cf. Klingenberg and Nijhout 1999).

Heritability estimates for FA were 0.7% for anterior and 2.4% for transverse bristles, whereas h^2 estimates for size were 100% for anterior and 81% for transverse bristles. These h^2 estimates for size are within the range of other published values for bristles in *Drosophila,* although those for anterior and transverse bristles fall toward the high end of the distribution for all morphological traits (fig. 5 in Roff and Mousseau 1987). The estimates of h^2 for FA in traits taken separately are in close quantitative agreement with those of others. Reeve (1960) estimated 2% heritability for FA in sternopleural bristles of *D. melanogaster.* Woods et al. (1998) examined FA in sternopleural bristles, orbital bristles, and in three wing measures of *D. melanogaster* and found h^2 for all these traits to be nonsignificant and with positive estimates ranging from 3% to 10% (an approximately equal number of estimates were negative). Scheiner et al. (1991) estimated heritability of FA to be 2.7% in sternopleural bristles in the same species. Leamy et al. (1998) found in a study of quantitative trait loci (QTLs) in house mice (*M. musculus*) that dominance effects played a significantly more important role than additive effects of genes on FA variation in skeletal traits. In a more recent study, Leamy (1999) reported an average FA heritability (derived from offspring-midparent regression) of 3% across 10 mandibular traits and concluded that ''there is no evidence for additive genetic variation of FA in the mandible characters of the random-bred mice.''

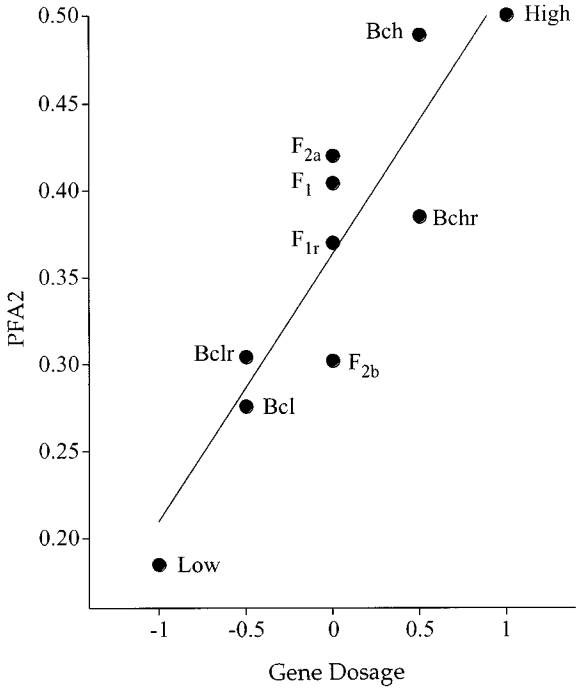

FIG. 1. Observed (filled circles) and expected (line) generation means for positional fluctuating asymmetry (PFA2) versus gene dosage (proportion genes) in a simple additive genetic model PFA2 = m + [d] in which sexes were pooled.

We have reached a different conclusion in regards to additive genetic variation for PFA. Heritability estimates were 13% for PFA1 and 21% for PFA2, both of which are statistically significant and up to 30 times greater than that for FA in individual bristle traits. These values for PFA are in close agreement with h^2 estimates for life-history traits in *Drosophila* reviewed by Roff and Mousseau (1987). Using the h^2 estimate for PFA2, we can also estimate h^2 of developmental stability (h^2_{DS}), governing the placement of bristles on the sternopleuron. The h^2_{DS} estimate was found to be 0.786

TABLE 13. Estimates of genetic parameters (SE) for generation means analysis on Size1,2 when sexes were either pooled or kept separate. Bold numbers indicate significant ($P < 0.05$) contributions for that parameter. Parameters are defined in the Methods.

	Models					
	Sexes pooled			Sexes separate		
Parameter	Full	No epistasis	Additive	Full	No epistasis	Additive
m	29.483 (1.192)	30.716 (0.207)	30.517 (0.0982)	28.782 (1.155)	30.799 (0.183)	30.517 (0.0920)
d	**−2.265 (0.309)**	**−2.234 (0.295)**	**−1.896 (0.0181)**	**−2.312 (0.299)**	**−2.293 (0.283)**	**−1.860 (0.163)**
h	3.079 (2.594)	0.0373 (0.341)		**6.626 (2.510)**	**−0.942 (0.338)**	
i	1.172 (1.170)			1.734 (1.138)		
j	0.350 (0.835)			0.0655 (0.801)		
l	−1.890 (1.599)			**−6.134 (1.573)**		
dm	0.165 (0.220)	0.152 (0.215)		−0.275 (0.267)	−0.282 (0.252)	
hm	−0.441 (0.298)	**−0.484 (0.198)**		**−1.237 (0.305)**	**−0.670 (0.189)**	
cyto	0.0901 (0.181)	0.122 (0.163)		−0.0433 (0.184)		
dx				**0.739 (0.325)**	**0.683 (0.311)**	
hx				**2.796 (0.351)**	**2.355 (0.332)**	
χ^2 (df) P	5.96 (1) 0.015	7.59 (4) 0.11	15.49 (8) 0.050	62.18 (9) 0.0001	80.69 (13) 0.0001	142.15 (18) 0.0001

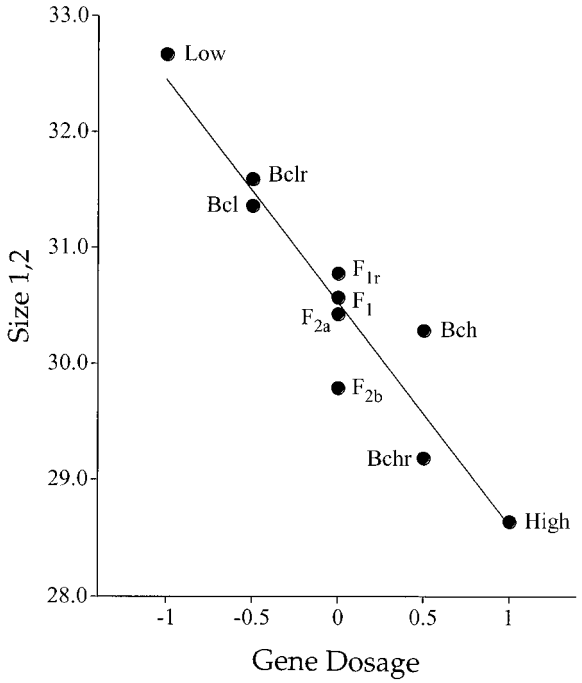

FIG. 2. Observed (filled circles) and expected (line) generation means for trait size (Size1,2) versus gene dosage (proportion genes) in a simple additive model Size1,2 = m + [d] in which sexes were pooled. Genetic line designations correspond to those for PFA2 (see Table 1).

by translating h^2 for PFA2 (Table 8) using a value of R, the hypothetical repeatability, of 0.266 (calculated following Whitlock 1996, 1998).

The feature of our experimental design that validates these estimates of genetic parameters is that we were able to control for or assess the magnitude of two potentially confounding factors, namely common familial environment and maternal effects, that can routinely bias heritability upward (Falconer and Mackay 1996). Isofemale lines were reared in replicate jars within a common incubator, which allowed assessment and removal of potentially confounding variance due to shared rearing environment. For example, if lines were each reared in a single jar, any variance due to jar effects would inflate the between lines variance component and bias heritability estimates upward. Jar effects, however, were not significant for either measure of PFA, indicating that environmental factors had little effects on heritability estimates. Jar effects were nevertheless statistically removed from PFA data prior to computing heritability. These results, together with the finding from generation means analysis showing an absence of maternal effects on PFA, strengthens the view that our heritability estimates are relatively free of bias arising from these potential confounds.

Heritability is a dimensionless parameter reflecting the ratio of additive genetic variance (V_A) to total phenotypic variability (V_P; Falconer and Mackay 1996). It has been used to make inferences about the relative magnitude of additive genetic variation underlying a variety of different traits of organisms. For example, one generalization has been that life-history traits have lower heritability and thus lower evolutionary potential than morphological traits (Mousseau and Roff 1987; Roff and Mousseau 1987). Houle (1992), however, suggested that because we are often more interested in evolutionary change in proportion to trait size, coefficients of additive genetic variation (CV_A, ratio of additive genetic variance to trait mean) will be a more appropriate measure of trait evolvability. In a review of such coefficients, Houle (1992) found that fitness traits have higher CV_A values, a conclusion opposite to that reached if h^2 values are considered (Mousseau and Roff 1987; Roff and Mousseau 1987). In our study, h^2 and CV_A values were highly positively correlated when considered among FA measures or among trait size measures. Thus, CV_A is a good substitute measure of trait evolvability, but only within categories of traits measured on the same scale. Interpretation of CV_A differences between categories of traits of very different dimension (e.g., trait size versus trait FA) must be done with care, because misleading conclusions may be reached. Consider, for example, the case of PFA2 and its associated measure of trait size. Heritability for PFA2 was found to be 21%, whereas h^2 for size was 73%. This relatively higher h^2 value indicates considerable greater evolutionary potential of trait size. However, a very different conclusion is reached when we consider CV_A as an index of evolvability, which is two fold *greater* for PFA2 than for size. Based on these CV_A values, one might conclude that FA possesses an equivalent, and indeed even greater, evolvability than trait size. The high CV_A for PFA relative to that for size results from the great disparity in their means; the reversal in their magnitude of evolvability is therefore an attribute of differences in dimensionality of scale. This problem was not addressed by Møller and Thornhill (1997) who concluded that FA has greater evolvability than trait size based on a higher mean CV_A for the former. If CV_A is to be used to contrast character evolvability, calculations of CV_A for trait FA should use trait size (i.e., $\{[\Sigma (R_i + L_i)]/2\}/n$) as the size standard, as opposed to mean unsigned asymmetry ($\Sigma |R_i - L_i|/n$). In this way, CV_As for both traits will be expressed in proportion to trait size and lead to estimates of evolvability that can be compared meaningfully.

Based on present h^2 estimates, we conclude that the additive genetic component of PFA variation is significant and considerably greater than that for FA in individual bristle characters and approximately 3.5-fold *less* than that for its respective measure of trait size. Moreover, X-chromosomal genes appear to be mainly responsible for these significant additive genetic effects. Thus, according to the standard equation $R = h^2S$, where R is the response to selection and S is the selection differential, PFA has the capacity to evolve under selection pressure, at least in the short term. Longer-term evolutionary dynamics, however, will depend on the

TABLE 14. Genetic correlation coefficient matrix for 1000 bootstrapped parameter estimates of population mean (m) and additive genetic effects (d) for PFA2 and trait size. For all coefficients $P < 0.1$.

	Trait size	
PFA2	m	d
m	−0.030	−0.024
d	−0.007	−0.049

influence of other genetic effects other than additivity (Barton and Turelli 1989). For example, epistasis can evolve toward limiting the range of phenotypes exposed to selection and thus mask additive genetic variation (Gimelfarb 1989). The generation means analysis, however, showed the absence of epistatic effects on PFA variation at the GLS site, suggesting that evolutionary dynamics of PFA variation may be generally unaffected by intergenic interactions. Likewise, Blows and Sokolowski (1995) found lack of epistatic variation for FA in sternopleural bristle number, using line cross analysis. However, we cannot completely exclude the possibility of epistatic effects on PFA because, whereas crosses between individuals within a population have often revealed additive effects on fitness, crosses between populations reveal epistasis (e.g., Hard et al. 1992; Armbruster et al. 1997; Starmer et al. 1998), consistent with the Sewall Wright's view of evolution (Wright 1968). Further experiments are therefore needed to survey for epistatic variance for FA between spatially and demographically separated population (e.g., Chakir et al. 1995).

The generation means analysis also showed lack of maternal effects on PFA, a result that facilitates making reasonable predictions about the course of evolutionary change in PFA under selection because, for example, with maternal effects, evolutionary response under selection may occur in a direction opposite of that expected (Barton and Turelli 1989). Another simplifying feature of the genetics of PFA is a lack of a genetic correlation between PFA and bristle number. This finding suggests that any evolutionary shift in PFA will be largely independent of additive genetic effects on trait size (i.e., total number of bristles on the sternopleuron), suggesting that changes in PFA may be unconstrained by selection acting on trait size and vice versa.

Thus, given additive genetic variance underlying PFA and assuming the presence of selection for some optimal value of PFA, we might expect that spatially isolated populations will exhibit differentiation in respect to PFA value. This predictions was not borne out by our data because we were unable to detect differences in PFA between the GLS and HUP sites, which are approximately 550 km apart. Similarly, trait size was invariant between sites. However, a recent study by Shoemaker and Jaenike (1997) shows that this lack of a difference may in fact not be anomolous. Using both nuclear and mitochondrial genetic markers, they found that *D. falleni* exhibited no detectable genetic differentiation between five populations sampled from Maine to Pennsylvania. They attributed this lack of differentiation to the homogenizing effect of gene flow among populations due to long-distance, windborne migrants. Thus, lack of a difference in PFA between sites may not be surprising because high levels of gene flow may continually constrain populations from reaching fitness peaks within their adaptive topography.

Origins of PFA and Its Connection with Sexual Selection

An intruiging and novel possibility is that PFA reflects the fidelity of buffering mechanisms operating at the interface of interconnected developmental pathways; thus, in a more general sense, PFA may reflect genotype-specific ability to integrate such pathways. To the extent that there are unusual biochemical and physiological challenges associated with ability to buffer a developmental interface, PFA will be a more sensitive measure of developmental instability compared to traditional FA that reflect buffering of a single, *individual* pathway; traditional measures are based on the difference merely in the total number of components of a meristic trait.

This scenario may be extended to fit current thinking about the role of FA in sexual selection. If trade-offs do indeed exist between attaining positional symmetry and other important physiological processes, then only individuals of good genetic quality should be able to afford to express low PFA (Zahavi 1975; Møller and Pomiankowski 1993). We might therefore predict that low PFA genotypes will have greater expression of fitness traits, such as male mating success, not necessarily because PFA in *Drosophila* bristles directly mediates sexual competition (indeed we deem this possibility to be very unlikely), but because of its covariance with underlying physiological traits (e.g., vigor) that themselves may mediate reproduction and survival. However, the available data, though meager, indicate that PFA variation is uncoupled from that in male reproductive success in at least one species. Polak (1997) contrasted mated versus unmated male *D. nigrospiracula* sampled in nature in terms of PFA1 and found no difference between these categories, suggesting a lack of a relationship between PFA variation and sexual selection. However, a conclusion based on a single species is clearly tenuous. Moreover, cross-sectional studies have serious limitations, for one, because they do not control for potentially confounding differences among males in terms, for example, of age or parasite load, which are known to interact in complex ways to influence male mating success (Forbes 1993; Polak and Starmer 1998). Detailed studies of the fitness correlates of PFA variation are needed to evaluate whether PFA is a honest indicator of phenotypic quality sensu Polak and Trivers (1994).

Any trade-offs between PFA and expression of fitness traits may also help explain the relatively large heritability of PFA compared to the typically nonsignificant heritability of FA in most other morphological traits (e.g., Woods et al. 1998; Tomkins and Simmons 1999; Leamy 1999; for a brief review of earlier studies, see Whitlock and Fowler 1997). If maximizing PFA incurs a physiological costs, then alleles that increase symmetry may compromise traits such as larval survival, developmental time, and/or body size at eclosion. Such antagonistic effects of alleles may contribute to the maintenance of genetic polymorphism (but see Curtsinger et al. 1994; Hedrick 1999) and should be detectable by observing correlated responses to artificial selection on PFA (e.g., Rose and Charlesworth 1981).

It is conceivable also that genetic variation for PFA results from pleiotropic effects of alleles segregating at loci with major direct effects on other traits. The discovery of strong X-chromosomal involvement in mediating PFA variation suggests that any of the number of loci known to occur on the X-chromosomes, such as those involved in the expression of sexually antagonistic traits (e.g., Rice 1984, 1996), secondary sexual traits (Reinhold 1998), and hybrid sterility (Coyne and Orr 1989) potentially are involved in such a mechanism.

We encourage the further study of the fitness correlates and the genetic and molecular bases of PFA variation because PFA appears to be a more sensitive indicator of underlying developmental instability than traditional measures of asymmetry. The method of PFA works when meristic units are located in two or more distinct but closely spaced areas (e.g., rows or patches) on each side of the body, and when the expression in number of units between areas are correlated or otherwise shown to be developmentally interconnected. For example, we are currently studying the genetic and molecular bases of PFA in the sex combs of *D. bipectinata*, which are located in two distinct, closely associated rows of prominent teeth on both front legs of males. Finally, analogues of PFA exist in metrical traits in the form of shape asymmetries, which also appear to be highly sensitive indicators of developmental instability and which are been effectively examined using geometric morphometrics and multivariate statistical methods (e.g., Klingenberg and McIntyre 1998; Auffray et al. 1999). A deeper understanding of the developmental and genetic bases and fitness consequences of PFA variation should lead to a better resolution of the properties and evolutionary consequences of developmental instability.

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