Variation in morphological traits and trait asymmetry in field *Drosophila serrata* from marginal populations

N. L. JENKINS* & A. A. HOFFMANN

Department of Genetics and Evolution, La Trobe University, Victoria 3083, Australia

Keywords:

asymmetry;
Drosophila;
field heritability;
marginal populations;
morphology;
species borders.

Abstract

Drosophila serrata occurs along the eastern coast of Australia with a southern range boundary near Sydney. To compare levels of phenotypic variation in marginal and central populations, we examined morphological variation in populations of this species from the southern range boundary and two more northerly populations. The populations differed for wing traits and there was an increase in wing size in the marginal locations which persisted under laboratory culture. The means of wing and bristle traits increased under laboratory culture, whereas wing trait coefficients of variation and variances decreased. Heritability estimates for wing size traits tended to be lower in the field compared with the laboratory, whereas bristle and crossvein length heritabilities were similar across environments. There was evidence for heritable variation in wing and bristle traits in both the marginal and more northern populations, suggesting that genetic variation was not limiting in marginal populations. Fluctuating asymmetry (FA) was also assessed as a measure of genomic and environmental stress. There were no consistent differences among populations for the FA of individual traits, or for a total FA score summed across traits. FA levels in field parents and laboratory-reared progeny were similar. Overall, the results do not support the conjecture that levels of phenotypic and genetic variability differ between central and marginal *D. serrata* populations.

Introduction

It is unclear why evolutionary changes in border populations do not allow continual expansion of a species range. Several evolutionary hypotheses exist to explain the limited distribution of species (Hoffmann & Blows, 1994; Hoffmann & Parsons, 1997), but there are almost no empirical data to evaluate them. Instead, much of the focus has been on describing and explaining patterns of variability in central and marginal populations. In plants, it has been proposed that marginal populations should show lower variances due to more intense stabilizing selection in marginal environments (Agnew, 1968). Under intense selection, levels of phenotypic variance may decline within a generation and influence variance levels across generations. However, plant evidence for this hypothesis is equivocal (Wilson *et al*., 1991). One problem is that selection pressures in marginal populations may be more heterogeneous and this could increase rather than decrease levels of variation in marginal populations (Safriel *et al*., 1994).

More recently, levels of variability measured within individuals (particularly fluctuating asymmetry) have also been related to the marginality of populations, particularly in birds. Fluctuating asymmetry (FA) refers to nondirectional differences between sides in a bilaterally symmetrical organism (Thoday, 1955). Increased FA of morphological traits is thought to occur under environmental and genomic stress. A range of different factors such as inbreeding (Leary & Allendorf, 1989), larval crowding (Clarke, 1992) and temperature (Beardmore, 1960; Parsons, 1962) affect FA. Møller

Correspondence: Ary A. Hoffmann, Department of Genetics and Evolution, La Trobe University, Victoria 3083, Australia.
Tel.: +61 394792769; fax: +61 39479 2480;
e-mail: Genaah@gen.latrobe.edu.au
**Present address:* Biological Gerontology, University of Manchester, Oxford Road, Manchester M13 9 PL, UK.

(1995) found that levels of FA in marginal populations of several bird species were elevated compared with central populations. Moreover, Carbonell & Telleria (1998) found that FA in tarsus length of blackcaps (*Sylvia atricapilla*) increased in a marginal dry environment. Fluctuating asymmetry has also been shown to increase in marginal plant populations (Siikamaki & Lammi, 1998). This raises the issue of whether FA generally increases in organisms towards the range boundary.

In *Drosophila*, data on genetic variability in marginal vs. central populations are largely restricted to allozyme variation and chromosome inversion polymorphisms. The former do not show differences between central and marginal populations, whereas inversions are generally less polymorphic in marginal populations (Soulé, 1973; Brussard, 1984). Data on quantitative traits are sparse; Carson (1959) found that lines of *D. robusta* from a marginal location responded to a lesser extent to selection for motility than lines from a central location, and Blows & Hoffmann (1993) obtained a similar result when *D. serrata* was selected for desiccation resistance.

In this paper, we consider morphological variability in populations of *D. serrata* that occur near or north of the southern range boundary of its distribution. This species is endemic to Australia and Papua New Guinea (Ayala, 1965) and occurs in a range of habitats as far south as Sydney. We are interested in several questions. Firstly, do populations differ in levels of morphological variability? A marginal population of *D. serrata* may have low morphological variability if it has been exposed to historical processes that have depleted genetic variance. Alternatively, stressful conditions in marginal populations may have increased the phenotypic variance of morphological traits (e.g. Imasheva *et al.*, 1997; Woods *et al.*, 1999), although this increased variance is not expected to persist away from the stress. To examine these possibilities, we consider variation in field-reared flies.

Secondly, is there evidence for heritable variation in marginal as well as more northern populations for morphological traits? The heritability of field populations can be assessed using comparisons of field-collected flies and their laboratory-reared offspring (Prout, 1958; Riska *et al.*, 1989). This method has been widely employed in a number of *Drosophila* species, particularly for morphological traits such as body size and bristle number (e.g. Coyne & Beecham, 1987; Prout & Barker, 1989; Ruiz *et al.*, 1991; Sgrò & Hoffmann, 1998). While we are also interested in differences in heritable variation between populations of *D. serrata* as previously suggested for desiccation resistance (Blows & Hoffmann, 1993), small sample sizes prevent direct comparisons of heritabilities.

Thirdly, are there differences between populations for morphological traits in *D. serrata*? These may arise because of latitudinal clines in morphological traits as found in species such as *D. melanogaster* (e.g. James & Partridge, 1995) and *D. kikkawai* (Karan *et al.*, 1998) where low-latitude populations tend to have a relatively smaller body size. Such variation can be indicative of selection pressures acting directly or indirectly on the morphological traits. By rearing flies under laboratory conditions, we can also test the heritable basis of any population differences in morphological traits.

Fourthly, do populations differ in levels of fluctuating asymmetry and can this be linked to environmental marginality? It is expected that in marginal environments FA will be higher than in more optimal environments (Parsons, 1992) and habitat fragmentation as well as marginality may increase levels of FA in marginal populations (Møller, 1995; Sarre, 1996). However, recent evidence suggests that FA does not always increase in suboptimal environments (e.g. Hurtado *et al.*, 1997; Woods *et al.*, 1999), although this may depend on the traits and species being investigated (Woods *et al.*, 1999). As there are few data on levels of FA in marginal populations, field *D. serrata* and their laboratory-reared offspring were measured for FA in four traits. Comparisons were made between central and marginal populations and between pre- and post-winter collections. Because combinations of FA from different traits may provide a better estimate of developmental stability than the FA of single traits (Leary & Allendorf, 1989; Palmer, 1994), total asymmetry values were examined, as well as individual trait FAs.

Materials and methods

Collections and rearing

Flies were collected from Forster, Taree, Coffs Harbour and Grafton using banana-baited traps. The former two sites are at the 'permanent' southern range boundary of *D. serrata* where this species can be collected all year round. They are 30 km apart and around 230 km north of Sydney. Coffs Harbour is 180 km north of these sites, while Grafton is 50 km north of Coffs Harbour. *D. serrata* occurs at a low frequency at the border sites and is normally vastly outnumbered by other *Drosophila* species, whereas *D. serrata* is often the predominant *Drosophila* species in collections at Coffs Harbour and Grafton (Jenkins, unpublished data). Two collections were made: in October – December (post-winter) 1995, and during March 1996. Field *D. serrata* were identified following ether treatment to anaesthetize the flies. The numbers of field flies collected from the four sites are shown in Table 1.

Up to 70 flies per sex per population from the March 1996 collection were set up in vials in the laboratory to obtain F_1 progeny. Single field females were placed in 40-mL vials containing 8 mL of culture medium (agar–sucrose–dead yeast–potato), with 0.02 mL antibiotics (streptomycin/penicillin) to reduce bacterial infection from field-caught flies. To obtain F_1 values from field males, the males were crossed to virgin females from

Table 1 Number of *D. serrata* flies obtained in field collections.

Site	Sex	October–December 1995	March 1996
Forster	Female	30	41
	Male	37	126
Taree	Female	57	100
	Male	79	212
Coffs Harbour	Female	38	55
	Male	63	109
Grafton	Female	38	64
	Male	66	79

isofemale lines originating from an earlier collection (April 1995). Males from each collection site were crossed to a single isofemale line from the same collection site in the case of Grafton, Coffs Harbour and Forster. The isofemale line from the Forster site was also used in crosses with the Taree males (i.e. three isofemale lines were used in total). These isofemale lines had been reared in 250-mL bottles containing 50 mL media, at a density of 100–120 adults per bottle. Four bottles were maintained for each isofemale line. Both field females and field males (crossed with laboratory females from the isofemale lines) were set up individually in vials and flies were tipped into fresh vials every 2 days to minimize larval crowding. F_1 flies were reared at 25 ± 1 °C in constant light.

Morphological measurements

Field-caught flies and their F_1 progeny were scored for three wing traits and one bristle trait. Wing length was determined from length of the third longitudinal vein from its intersection with the anterior crossvein to the wing tip, wing width was determined from the tip of the second longitudinal vein to the tip of the fifth longitudinal vein and crossvein was determined from the length of the posterior crossvein. The wing measurements were made using an image analysis system (Trace). The repeatability of the wing traits, based on repeat measures of 40 individuals and expressed as the correlation coefficient computed between these repeat measures, was 0.998 for length, 0.996 for width and 0.963 for crossvein. The bristle trait, measured under a dissecting microscope (80× magnification), was the number of chaetae on the sternopleural plate. This trait was scored without error.

The left and right sides were averaged to obtain trait scores for individuals. Wing length and width measurements provided an overall indication of wing size. These measures were generally positively correlated in the collections whereas bristle number was usually uncorrelated with them. Crossvein measurements are closely correlated to wing width because the crossvein runs parallel to the width measurement. To consider the size of the crossvein relative to the wing width, measurements were divided by the width and arcsine transformed prior to analysis. The resulting proportions were uncorrelated with both wing length and wing width. Principal component analyses were undertaken on the four field data sets (sexes separated) and two laboratory data sets to extract a general measure of wing size. This was always the first component on which both length and width loaded heavily, whereas the other variables tended to load onto different components.

Fluctuating asymmetry

Asymmetry has not previously been examined in *D. serrata*. As any measurement error can cause bias in studies of FA, repeat measures of at least 30 individuals is recommended in an initial analysis (Palmer, 1994; Swaddle *et al.*, 1994). To determine whether fluctuating asymmetry in wing traits was significant relative to measurement error, repeat measurements were made on 40 field females from the March 1996 collection. Ten females were chosen at random from each of the four populations. A mixed model, two-way analysis of variance (ANOVA) was used to determine if the 'between-sides' variation was significantly greater than the measurement error.

The ANOVA results for the three wing traits are given in Table 2. These indicate a significant level of FA relative to measurement error for all of the wing traits, as reflected by the significance of the 'side × individual' term. The significance of the 'individual' term and large proportion of the variance accounted for by this factor indicate a high degree of variation in overall trait size among individuals. The ratio of mean squares of individuals/(side × individuals) is also significant, indicating the need to use a relative measure of asymmetry, where relative FA is the absolute difference between sides divided by the mean of the two sides (Palmer, 1994).

Directional asymmetry is indicated by a significant 'sides' term in the mixed model ANOVA (Palmer & Strobeck, 1986; Palmer, 1994). As can be seen in Table 2, directional asymmetry was found for wing crossvein.

Table 2 ANOVA results for repeatability of fluctuating asymmetry in wing traits following the analysis in Palmer (1994). Entries are mean squares, while numbers in parentheses are the percentage of the total variance accounted for by each factor.

Source of variation	d.f.	Crossvein ($\times 10^4$)	Wing width ($\times 10^3$)	Wing length ($\times 10^3$)
Sides	1	0.64 (7.7)**	0.01 (0)	0.003 (0)
Individuals	39	7.83 (83.8)**	14.91 (98.1)**	30.25 (99.2)**
Side × Individuals	39	0.31 (6.0)**	0.13 (1.6)**	0.11 (0.7)**
Measurement error	80	0.05 (2.3)	0.01 (0.3)	0.01 (0.1)

**$P < 0.01$.

Single factor ANOVAS were used to test for directional asymmetry in all four traits measured for each population and each collection, but no further instances of directional asymmetry were detected. There was also no evidence for antisymmetry in the metric traits (as tested with Kolmogorov–Smirnov tests).

As there was no evidence of antisymmetry or consistent directional asymmetry, the differences between left and right sides were considered to be a measure of FA. Relative FA was used for all population comparisons and is henceforth referred to as asymmetry. As this measure is not normally distributed, nonparametric tests were used in all comparisons.

Because asymmetry measures for crossvein length were not significantly correlated with those of the other wing traits, as is also the case in *D. melanogaster* (Woods *et al*., 1998), the actual measurements of crossvein length were used in computing asymmetries rather than measurements expressed as proportions of wing width. Wing length and width asymmetries were also mostly uncorrelated, as previously found for *D. melanogaster* (Woods *et al*., 1998).

Analysis

For the traits, analyses of variance (ANOVAS) were used to determine if there were population differences within collections. Tukey HSD *post hoc* tests determined which populations were significantly different. Changes in trait means across field and laboratory environments within populations were assessed with *t*-tests. As a number of morphological traits were measured on each individual, the Dunn–Šidák correction for multiple comparisons (Sokal & Rohlf, 1995) was used throughout. Differences between CVs among populations and across environments were examined using the approach outlined in Zar (1996, pp. 144–6, 206–7) and variances were compared with Levene's test.

Heritabilities of the traits were estimated from parent–offspring regressions and regression coefficients were assessed with one-tailed *t*-tests to determine if they were significantly greater than zero. Heritability estimates for the comparisons of either the female or the male field parent and the average value of their laboratory offspring were obtained by doubling the regression coefficients. This assumes the absence of genotype–environment interactions and similar genetic variances in the field and laboratory (Riska *et al*., 1989) but appears to provide a satisfactory estimate of field heritability for morphological traits in *Drosophila* (Hoffmann, 1999). Comparisons of F_1's and their laboratory-reared female parents provided an estimate of laboratory heritability. Because field males from a population were mated to females from the same isofemale line, doubling the regression coefficients may underestimate the laboratory heritability to some extent. Nevertheless, since each isofemale line was maintained at a large population size (>400 individuals) directly after it was established, a line should contain around 75% of the additive genetic variance (Hoffmann & Parsons, 1988) and the degree of underestimation should therefore be relatively minor.

Mean asymmetries were calculated for each population in each environment/collection and confidence intervals were obtained by bootstrapping. The significance of any differences among populations and differences between the field and laboratory generations was assessed using Kruskal–Wallis tests. As four traits were measured on each individual, probabilities were corrected for multiple comparisons.

Asymmetry measures were combined to obtain a total asymmetry index for each individual. Such indices are expected to provide a better estimate of developmental imprecision than individual trait FAs that are subjected to a high degree of error. While trait FAs are only weakly correlated they are nevertheless assumed to measure the same underlying processes leading to developmental imprecision (see Gangestad & Thornhill, 1999). The total asymmetry index was used to compare each population within collections and to compare differences between the field and laboratory environments. To compute the index the asymmetry of individual traits was standardized to a mean of zero and a standard deviation of one by determining the mean and standard deviation across the populations (or environments) being compared using

$$X_{\text{std}} = (X_i - \bar{X})/\text{SD}.$$

This standardized asymmetry was then summed across all four traits to determine the total asymmetry of each individual, and hence the mean total asymmetry for each population (or environment). For comparisons across all four populations, the mean and standard deviation used was that of the four populations combined. When the two collections were compared, the mean and standard deviation were determined within populations for the combined collections. The 95% confidence intervals for total asymmetry values were determined by bootstrapping.

Results

Means, variances and CVs – population comparisons

Population differences were examined in both the field- and the laboratory-reared flies. For the March 1996 collection, comparisons on F_1s were only made for the male and female offspring from field females. Offspring of field males were not compared because these males had been mated to laboratory females from isofemale lines. As a different isofemale line was used for each population, any F_1 differences between the populations could reflect variation among isofemale lines rather than source populations.

Trait means for the field flies are summarized in Fig. 1. ANOVAS indicate population differences for the female

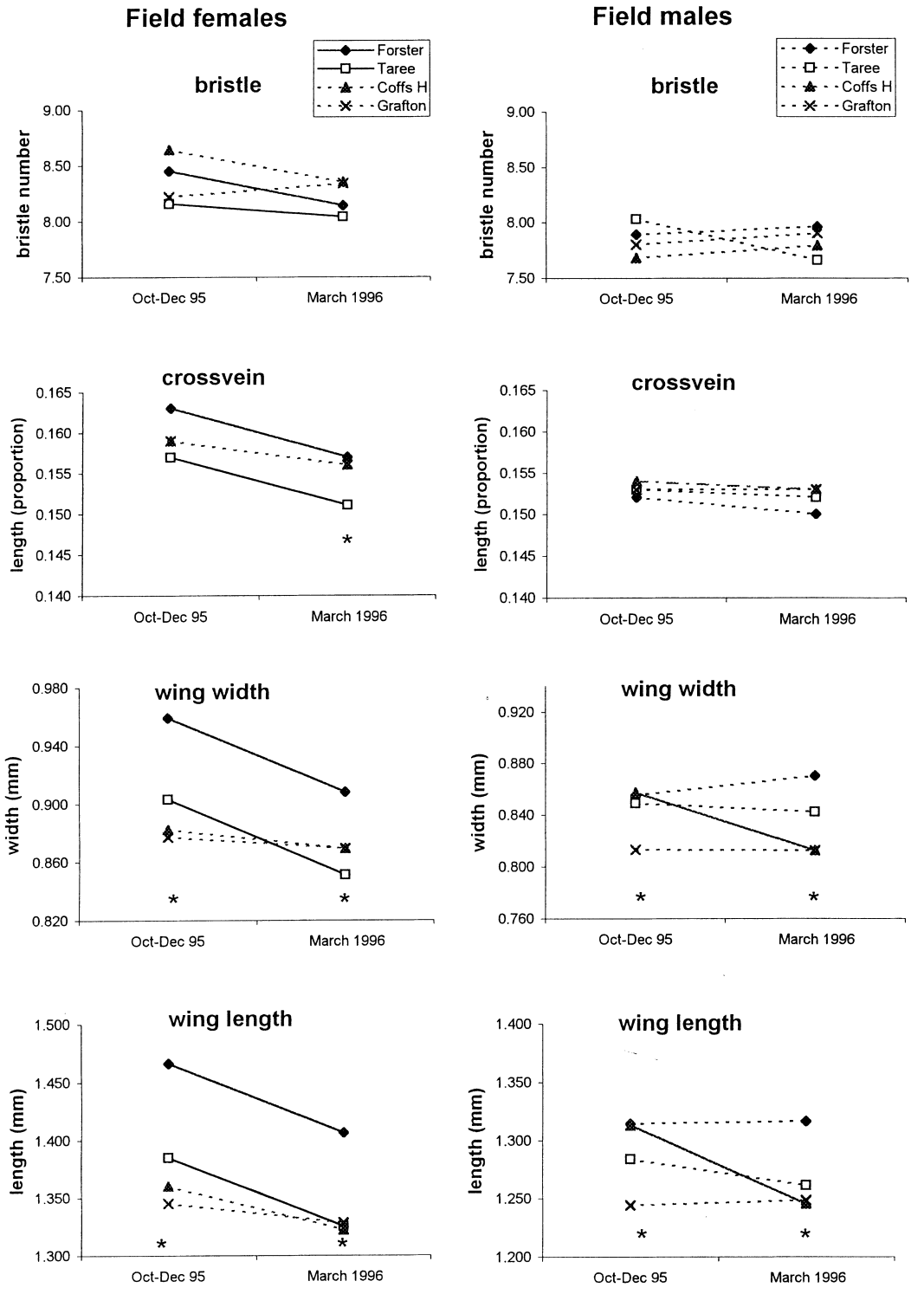

Fig. 1 Trait means for field-collected females and males. Solid lines represent significant within-population differences between October–December 1995 and March 1996. Broken lines are nonsignificant. Asterisks represent significant differences between populations within collections.

Table 3 Results of ANOVAS comparing populations for morphological traits in field flies from the October–December 1995 collections.

	Population		Error	
	d.f.	MS	d.f.	MS
Females				
Sternopleural bristles	3	1.98	144	0.73
Wing crossvein† (×1000)	3	0.37	132	0.21
Wing width (×100)	3	4.12*	132	0.60
Wing length (×100)	3	8.70*	143	1.31
First principal component	3	6.12*	130	0.88
Males				
Sternopleural bristles	3	1.41	221	0.73
Wing crossvein† (×1000)	3	0.04	198	0.13
Wing width (×100)	3	2.31*	219	0.44
Wing length (×100)	3	5.94*	215	0.93
First principal component	3	4.52*	198	0.95

†ANOVAS on arcsin-transformed proportions (crossvein length/wing width). *$P < 0.05$ after correcting for multiple comparisons.

data for two wing traits and the first principal component reflecting general size (Tables 3 and 4). For the October–December 1995 collection, Tukey *post hoc* tests indicate that this is largely due to females from the marginal Forster population having higher scores for wing width and wing length (as well as the principal component) than Coffs Harbour and Grafton females. Forster females also had significantly higher means than females from Taree. There were no significant differences between trait (or principal component) means of the two northerly populations. For the March 1996 collection Tukey *post hoc* tests indicate that Forster flies had wider wings than those from Taree and Grafton, and that Forster females had longer wings (and higher principal component scores) than those from all other populations (see Fig. 1). Forster females therefore generally had larger wings than those from the other populations.

For the male data, there were significant effects of population on two of the wing traits in both collections consistent with the female data (Tables 3 and 4). In the October–December 1995 collection, Tukey *post hoc* tests indicate that this is due to Forster males having larger wings (and higher principal component scores) than Grafton males. For the March 1996 collection, Tukey *post hoc* tests indicate that Forster males had larger wings than males from the other populations. Taree males also had wider wings than Grafton males. Overall, Forster males therefore had larger wings than those of the other populations, particularly the northern ones.

Population differences in levels of trait variability were also assessed. CV values were compared as well as variances. The comparisons (Table 5) indicate no significant differences in levels of variability for either the field females or field males, with the exception of the CVs for crossvein length for the March 1996 collection when Forster females had a higher CV than the other populations (Fig. 2). Trait variability was therefore similar in the four populations. In general, CV values for bristles were higher than for the wing traits (Fig. 2).

Population comparisons for the F_1 flies from the March 1996 collection suggest that the differences in wing size were heritable. For the female data, there were significant population differences for all wing traits and the principal component (Table 4). F_1 females from both Forster and Taree had significantly wider and longer wings than those from Coffs Harbour and Grafton (Fig. 3). In addition, there was a difference in crossveins; Taree F_1's had higher crossvein scores than those from Coffs Harbour and Grafton. These population differences were also evident in the F_1 male data (Table 4). Tukey

Table 4 Results of ANOVAS comparing populations for morphological traits in the March 1996 field flies.

	Females				Males			
	Population		Error		Population		Error	
	d.f.	MS	d.f.	MS	d.f.	MS	d.f.	MS
Field flies								
Sternopleural bristles	3	1.34	214	0.53	3	1.12	266	0.56
Wing crossvein† (×1000)	3	0.50	158	0.69	3	0.43	197	0.13
Wing width (×100)	3	1.99*	158	0.37	3	4.53*	217	0.33
Wing length (×100)	3	5.90*	176	0.74	3	7.36*	261	0.70
First principal component	3	7.61*	158	0.87	3	7.35*	182	0.86
Field female progeny								
Sternopleural bristles	3	2.55	247	0.75	3	0.98	242	0.58
Wing crossvein† (×1000)	3	1.57*	145	0.08	3	1.25*	192	0.35
Wing width (×100)	3	0.30*	179	0.06	3	1.21*	192	0.07
Wing length (×100)	3	1.62*	242	0.01	3	3.48*	237	0.14
First principal component	3	27.98*	143	0.43	3	15.82*	191	0.77

†ANOVAS on arcsin-transformed proportions (crossvein length/wing width). *$P < 0.05$ after correction for multiple comparisons.

Table 5 Tests for homogeneity of coefficients of variation and variances among the population samples. For CVs, numbers are χ^2 values with three degrees of freedom. For variances, *F* ratios are for Levene's test with three degrees of freedom in the numerator and the degrees of freedom for the denominator indicated in parentheses.

		CV comparison		Variance comparison	
		Females	Males	Females	Males
Field flies (Oct–Dec 1995)	Sternopleurals	1.42	1.08	0.52 (144)	0.46 (221)
	Wing crossvein	6.19	2.46	2.42 (132)	0.35 (198)
	Wing width	4.70	7.76	1.05 (132)	1.70 (199)
	Wing length	1.67	7.20	1.13 (143)	1.98 (215)
Field flies (Mar 1996)	Sternopleurals	1.63	0.93	0.98 (214)	0.97 (273)
	Wing crossvein	16.89*	1.38	1.99 (158)	1.24 (197)
	Wing width	6.97	5.04	1.03 (128)	1.43 (201)
	Wing length	5.62	3.56	0.95 (176)	1.31 (235)
F1s (Mar 1996)	Sternopleurals	3.35	2.14	3.41 (259)	0.94 (242)
	Wing crossvein	0.23	9.22	2.82 (145)	2.39 (191)
	Wing width	10.98*	2.31	4.05 (146)*	0.54 (192)
	Wing length	16.10*	3.21	3.87 (250)*	0.54 (237)

*$P < 0.05$ after correcting for multiple comparisons due to the number of traits scored.

post hoc tests indicated that wing size differences among populations were due to F_1 males from Forster and Taree having higher mean values than those derived from Coffs Harbour and Grafton (Fig. 3).

For both the trait CVs and variances in the F_1 values, differences among populations were not significant for the males but there were significant differences for the females (Table 5). For both wing length and wing width, Grafton F_1 females had higher CVs than those from the other populations (Fig. 4) suggesting differences in trait variability not evident at the phenotypic level. Variances showed the same patterns (data not shown). For all populations the CVs were higher for bristle number than for the wing traits, in agreement with trait differences for the field flies.

Means, variances and CVs – comparisons across collections and environments

For the females, trait means tended to decline in the March 1996 collection compared with the October–December 1995 collection (Fig. 1) and this probably reflects the cooler conditions under which the 1995 flies were raised (in winter/spring). This trend was not significant in the two northern subtropical populations. For the males, a decrease in the wing traits was evident only in the Coffs Harbour population. CVs were similar across the collections except for a significant decrease in variability in males from the Grafton population (Fig. 2).

There were a number of significant changes in trait means between the field and laboratory generations in both sexes (Fig. 3). In all cases, trait means were higher in the laboratory compared with the field, presumably because flies reared under more favourable and cooler laboratory conditions had a larger body size. There was also a marked change in the CVs of the wing size traits but not for bristle number or crossveins (Fig. 4). In all populations, wing trait CVs declined under laboratory rearing. The same patterns were evident in the variances (not shown).

Trait heritability

Regression coefficients from parent–offspring comparisons and probability values from one-tailed *t*-tests are given in Tables 6 and 7. There were 20 estimates for each trait. Family sample sizes were small in some cases, particularly for field female to F_1 comparisons.

For the 1995 collection, many of the coefficients were negative (Table 6). Only one of the coefficients was significant after correction for multiple comparisons, involving the comparison of field males from Coffs Harbour to F_1 females for sternopleural bristles, which had a coefficient of 0.432 (95% confidence intervals 0.183–0.681). The combined probability across both populations for this sternopleural bristle comparison (computed following Sokal & Rohlf (1995, p. 195)) was less than 0.001. This suggests heritable variation for this trait in the field flies with a heritability value estimated at around 70% (mean regression coefficient of 0.345). However, regressions for sternopleurals involving laboratory-reared males were not significant, and neither were combined probabilities for the other comparisons.

For the 1996 collection, most of the regression coefficients were positive for all four traits (Table 7). Two of the coefficients were significant after correction for multiple comparisons. In addition, several comparisons were significant after combining probabilities across populations: the field female – F_1 male comparison of sternopleural bristles, both comparisons involving field females for crossveins, the field male – F_1 female comparison for wing width, and the field female – F_1 male comparison for wing length. Mean coefficients for the sternopleural bristles (0.14), crossvein (0.26), wing width (0.004) and wing length (0.05) suggest low to

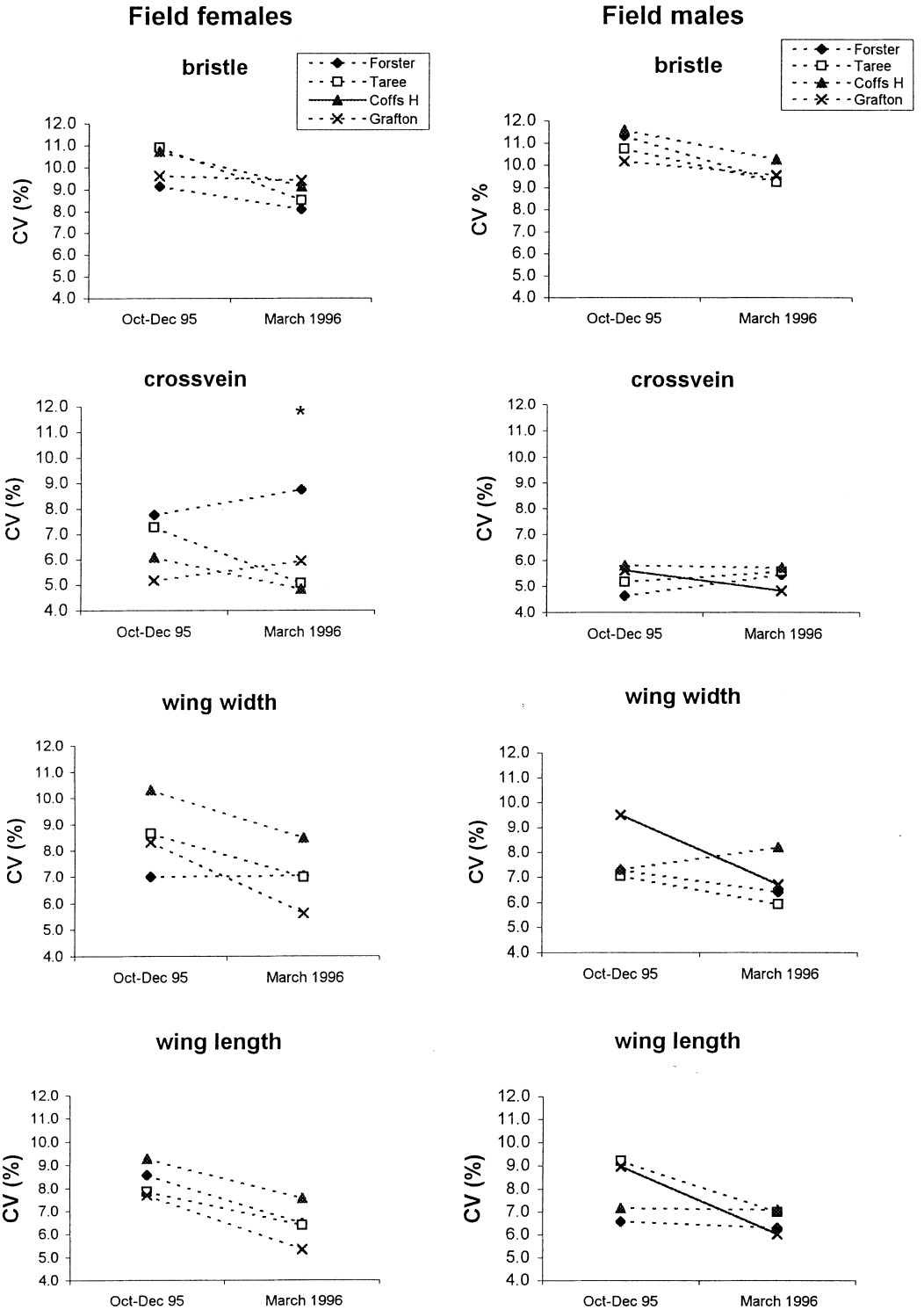

Fig. 2 Trait CVs for field-collected females and males. Solid lines represent significant within-population differences between October–December 1995 and March 1996. Dashed lines are nonsignificant. Asterisks represent significant differences between populations within collections.

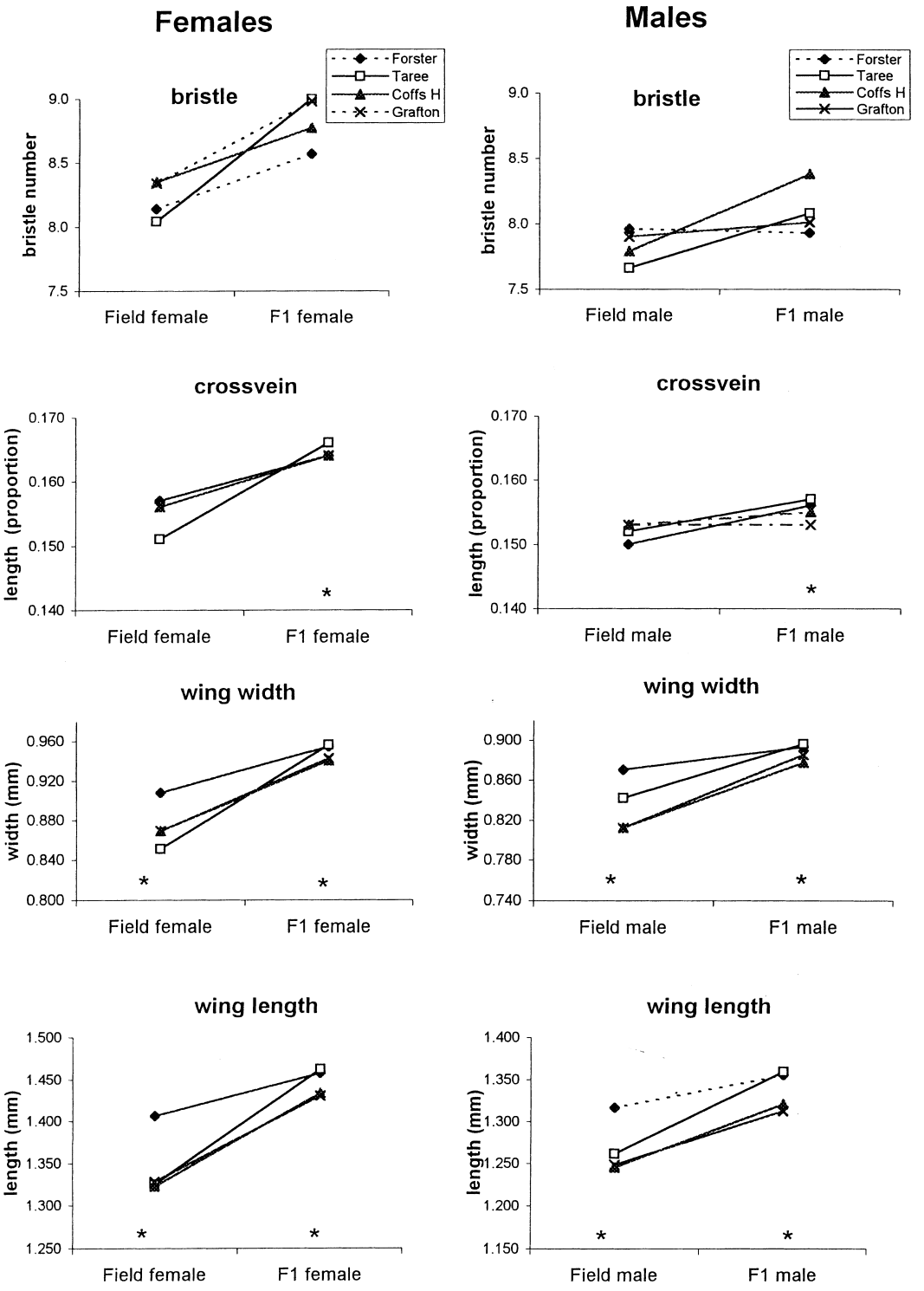

Fig. 3 Trait means for March 1996 field-collected females and males and their laboratory-reared progeny. Solid lines represent significant within-population differences between field- and laboratory-reared flies. Dashed lines are nonsignificant. Asterisks represent significant differences between populations within environments.

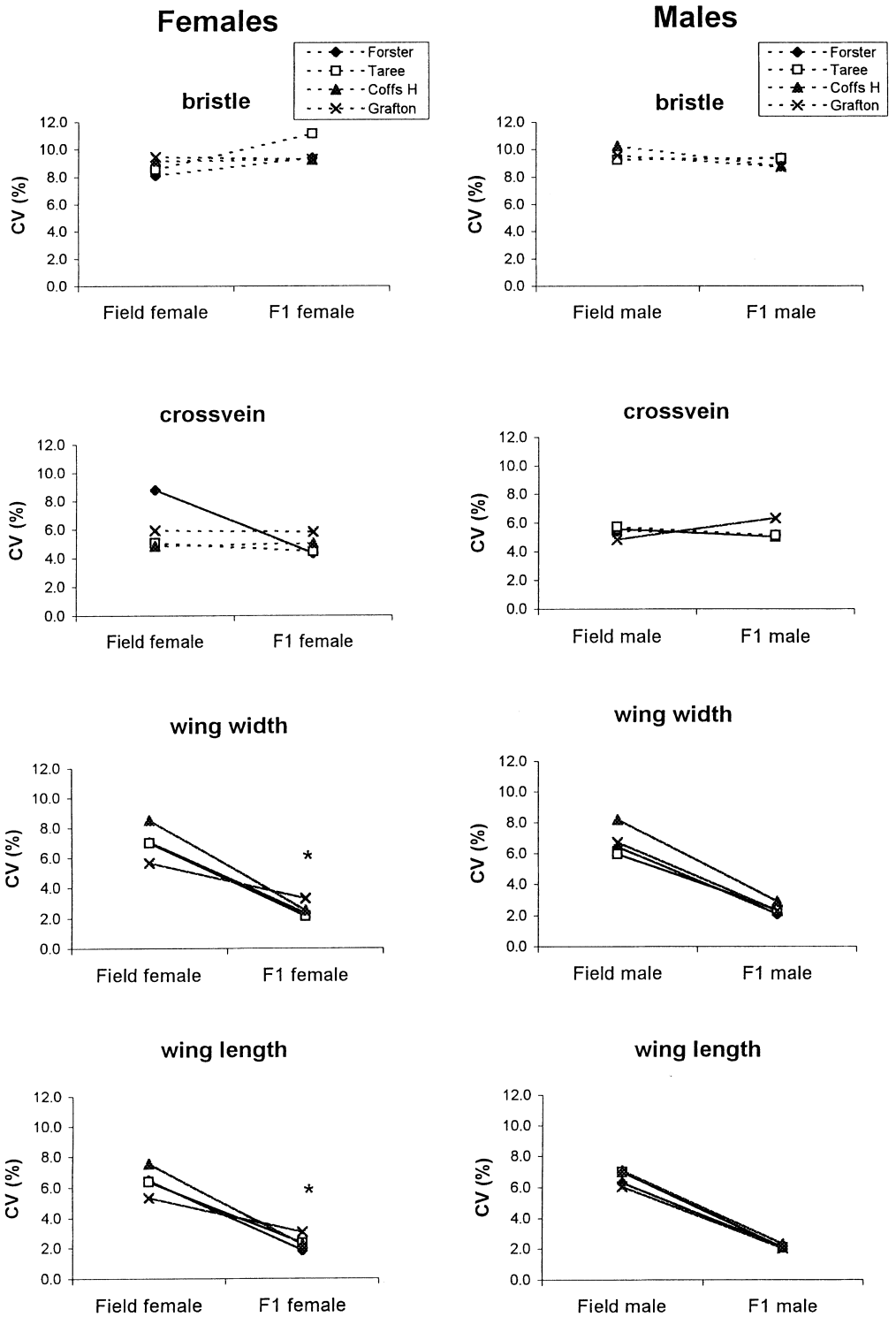

Fig. 4 Trait CVs for March 1996 field-collected females and males and their laboratory-reared progeny. Solid lines represent significant within-population differences between field- and laboratory-reared flies. Dashed lines are nonsignificant. Asterisks represent significant differences between populations within environments.

Table 6 Regression coefficients (*b*) from parent–offspring comparison of traits in the 1995 collection. Probability values (*P*) are for one-tailed *t*-tests to determine if the regression coefficients are significantly different from zero. Sample sizes are also shown.

	Forster			Coffs Harbour		
	N	*b* (SE)	*P*	*N*	*b* (SE)	*P*
Sternopleural						
Field male – F1 female	29	0.258 (0.153)	0.103	48	0.432 (0.123)	0.001*
Field male – F1 male	29	–0.028 (0.091)	0.760	49	0.047 (0.105)	0.658
Crossveins						
Field male – F1 female	24	0.391 (0.334)	0.262	38	0.088 (0.131)	0.505
Field male – F1 male	24	0.041 (0.174)	0.816	38	0.116 (0.142)	0.420
Wing width						
Field male – F1 female	24	0.018 (0.189)	0.925	43	0.007 (0.046)	0.880
Field male – F1 male	24	0.020 (0.063)	0.755	40	–0.068 (0.114)	0.555
Wing length						
Field male – F1 female	28	–0.051 (0.072)	0.486	48	–0.077 (0.052)	0.148
Field male – F1 male	28	–0.017 (0.048)	0.727	48	–0.068 (0.043)	0.121

*$P < 0.05$ after correction for multiple comparisons.

intermediate heritabilities (estimates of 1–52%). Although the small family sizes preclude a direct comparison of heritability estimates across populations, the data provide no evidence that levels of genetic variation are lower in Forster and Taree compared with the more northern populations. A Friedman test was undertaken to compare overall heritability levels by computing the mean heritability of each trait across comparisons and then testing for differences among the populations. This test indicated no significant differences among the populations when all the traits were considered ($\chi^2 = 5.71$, d.f. $= 3$, $P = 0.13$).

Finally, the laboratory heritability estimates (Table 8) are mostly positive (29 out of 32 cases), suggesting heritable variation for these traits under laboratory conditions. Combined probabilities were significant in both of the sternopleural comparisons, one of the crossvein comparisons (female – F_2 male) and both wing length comparisons. Mean heritabilities for the traits were 28% for sternopleural bristles, 34% for crossveins, 26% for wing width and 36% for wing length. There was no evidence that the marginal populations had lower heritabilities for the traits under laboratory conditions. A Friedman test conducted as above indicated no significant differences among the populations when all traits were considered ($\chi^2 = 3.60$, d.f. $= 3$, $P = 0.31$).

Asymmetry – population comparisons

Overall, the mean relative FA values of the populations were highest for bristle asymmetry, ranging from 0.066 to 0.134, and lowest for wing length asymmetry, ranging from 0.006 to 0.009. Mean wing crossvein asymmetries ranged from 0.032 to 0.055 and those for wing width ranged from 0.008 to 0.016.

Kruskal–Wallis tests (not presented) were used to compare the populations. For the field flies, only one of the 16 comparisons was significant after correction for multiple comparisons. This involved sternopleural bristle asymmetry in the October–December 1995 males ($z = 48.56$, $P < 0.001$) when Forster males had a relatively higher asymmetry than those from other populations. For the F_1 flies, there were no significant differences among populations in the eight comparisons. Thus, there is no evidence that environmental or genomic stresses in marginal populations caused an increase in FA.

Total FA scores for field flies and 95% confidence intervals are summarized in Fig. 5. As the total asymmetry of each population is expressed relative to that of the other populations, positive values reflect populations that have a relatively higher asymmetry and negative values reflect populations that are relatively more asymmetric. In the October–December 1995 collection, Forster flies had the highest total asymmetry and differences were significant by a Kruskal Wallis test for the male data ($\chi^2 = 9.84$, d.f. $= 3$, $P = 0.02$) but not the female data ($\chi^2 = 4.94$, d.f. $= 3$, $P = 0.18$). For the March 1996 collection, differences among the populations were not significant for either the male ($\chi^2 = 0.64$, d.f. $= 3$, $P = 0.89$) or female ($\chi^2 = 2.00$, d.f. $= 3$, $P = 0.57$) data.

Table 7 Regression coefficients (*b*) from parent–offspring comparison of traits in 1996 collection. Probability values (*P*) are for one-tailed *t*-tests to determine if the regression coefficients are significantly different from zero. Sample sizes are also shown (*N*).

	Forster			Taree			Coffs Harbour			Grafton		
	N	*b* (SE)	*P*	*N*	*b* (SE)	*P*	*N*	*b* (SE)	*P*	*N*	*b* (SE)	*P*
Sternopleurals												
Field female – F1 female	28	0.214 (0.161)	0.197	35	0.071 (0.240)	0.769	30	0.077 (0.109)	0.485	34	0.174 (0.161)	0.195
Field female – F1 male	29	0.333 (0.171)	0.062	36	0.467 (0.174)	0.011*	28	0.070 (0.160)	0.665	34	0.120 (0.107)	0.269
Field male – F1 female	51	0.052 (0.103)	0.617	54	0.196 (0.113)	0.090	57	0.128 (0.083)	0.128	65	0.148 (0.115)	0.204
Field male – F1 male	52	–0.001 (0.158)	0.995	52	0.068 (0.123)	0.584	58	0.061 (0.117)	0.603	66	0.035 (0.108)	0.748
Crossveins												
Field female – F1 female	20	0.435 (0.181)	0.027	19	0.306 (0.130)	0.031	14	0.586 (0.423)	0.191	25	0.505 (0.134)	<0.001*
Field female – F1 male	20	0.400 (0.150)	0.016	20	0.303 (0.166)	0.084	17	0.202 (0.268)	0.462	24	0.179 (0.162)	0.282
Field male – F1 female	37	–0.107 (0.181)	0.558	27	–0.173 (0.195)	0.384	40	0.033 (0.134)	0.807	39	0.126 (0.586)	0.831
Field male – F1 male	36	0.837 (0.689)	0.233	30	0.128 (0.220)	0.566	38	0.184 (0.120)	0.133	40	0.225 (0.147)	0.135
Wing width												
Field female – F1 female	20	0.013 (0.053)	0.811	20	0.050 (0.078)	0.529	16	0.009 (0.056)	0.873	26	–0.009 (0.042)	0.832
Field female – F1 male	20	0.106 (0.087)	0.241	21	0.068 (0.072)	0.354	19	0.034 (0.059)	0.576	25	0.002 (0.054)	0.971
Field male – F1 female	37	0.096 (0.048)	0.053	37	–0.083 (0.080)	0.307	40	0.048 (0.031)	0.126	40	–0.410 (0.254)	0.115
Field male – F1 male	36	0.023 (0.308)	0.941	40	0.027 (0.068)	0.692	43	0.064 (0.045)	0.164	42	0.019 (0.050)	0.704
Wing length												
Field female – F1 female	34	0.030 (0.044)	0.499	21	0.012 (0.078)	0.879	21	0.004 (0.040)	0.923	30	0.130 (0.093)	0.173
Field female – F1 male	25	0.152 (0.058)	0.015	25	0.048 (0.055)	0.394	20	0.065 (0.041)	0.130	30	0.191 (0.082)	0.028
Field male – F1 female	45	0.021 (0.038)	0.580	45	–0.021 (0.045)	0.645	53	0.078 (0.036)	0.033	52	0.014 (0.047)	0.767
Field male – F1 male	45	0.071 (0.038)	0.066	43	0.035 (0.036)	0.332	54	0.031 (0.050)	0.541	54	0.035 (0.037)	0.356

*$P < 0.05$ after correction for multiple comparisons.

For the F_1 data, there were significant differences between populations for the males ($\chi^2 = 9.56$, d.f. = 3, $P = 0.02$) but not the females ($\chi^2 = 1.75$, d.f. = 3, $P = 0.63$). For the F_1 males, the Forster population had a relatively lower level of asymmetry than one of the more northern populations (Fig. 5). Overall there was no consistent evidence that FAs in marginal populations are relatively higher than in the northern populations.

Asymmetry – comparisons between environments

Within populations, comparisons were made between the field and laboratory environments in the March 1996 collection using Kruskal–Wallis tests. For the individual traits, there were no significant changes between the environments (results not shown). Total asymmetries were also compared across the environments (Fig. 6). For the female data, differences were not significant for comparisons (all with one degree of freedom) involving the Forster ($\chi^2 = 0.69$, $P = 0.41$), Taree ($\chi^2 = 0.65$, $P = 0.42$), Coffs Harbour ($\chi^2 = 0.19$, $P = 0.66$) and Grafton ($\chi^2 = 0.05$, $P = 0.82$) populations. For the male data, comparisons for the Forster ($\chi^2 = 3.73$, $P = 0.05$), Taree ($\chi^2 = 0.07$, $P = 0.79$), Coffs Harbour ($\chi^2 = 1.48$, $P = 0.22$) and Grafton ($\chi^2 = 0.86$, $P = 0.35$) populations were also nonsignificant. Thus there is no evidence that laboratory flies were exposed to lower stress levels than field flies as assessed by changes in FA.

Table 8 Regression coefficients (*b*) from parent–offspring comparison of traits in the laboratory. Probability values (*P*) are for one-tailed *t*-tests to determine if the regression coefficients are significantly different from zero. Sample sizes are also shown (*N*).

	Forster			Taree			Coffs Harbour			Grafton		
	N	*b* (SE)	*P*	*N*	*b* (SE)	*P*	*N*	*b* (SE)	*P*	*N*	*b* (SE)	*P*
Sternopleurals												
F1 female – F2 female	66	0.295 (0.092)	0.002*	55	0.132 (0.141)	0.353	60	0.087 (0.102)	0.398	66	−0.061 (0.118)	0.607
F1 female – F2 male	51	0.178 (0.116)	0.131	53	0.194 (0.153)	0.211	59	0.179 (0.073)	0.017	67	0.116 (0.111)	0.302
Crossveins												
F1 female – F2 female	26	0.211 (0.443)	0.638	19	−0.031 (0.219)	0.889	35	0.094 (0.154)	0.547	32	0.344 (0.188)	0.077
F1 female – F2 male	23	−0.021 (0.271)	0.939	23	0.121 (0.132)	0.371	34	0.021 (0.148)	0.888	32	0.636 (0.174)	<0.001*
Wing width												
F1 female – F2 female	26	0.122 (0.159)	0.450	19	0.093 (0.114)	0.428	36	0.151 (0.169)	0.379	32	0.163 (0.162)	0.322
F1 female – F2 male	24	0.024 (0.111)	0.831	24	0.180 (0.101)	0.089	36	0.230 (0.222)	0.308	33	0.088 (0.178)	0.625
Wing length												
F1 female – F2 female	45	0.300 (0.101)	0.005	53	0.023 (0.086)	0.79	58	0.317 (0.125)	0.014	64	0.139 (0.108)	0.202
F1 female – F2 male	47	0.136 (0.082)	0.105	53	−0.005 (0.029)	0.866	57	0.225 (0.098)	0.025	66	0.295 (0.092)	0.002

*$P < 0.05$ after correction for multiple comparisons.

Discussion

Population comparisons

We tested whether marginal populations of *D. serrata* showed relatively higher levels of morphological variability, and whether this could be related to environmental effects and/or historical factors. The CVs and variances provide no evidence for an association between variability and marginality. Where population differences were evident, these involved only one of the marginal or northern populations and patterns were not consistent across environments or sexes. For instance, for wing size traits Grafton females tended to have the highest CVs and variances under laboratory conditions but not field conditions. Across populations, CVs were higher for sternopleural bristles than for the wing traits, consistent with data from *D. melanogaster* (Imasheva *et al.*, 1997; Woods *et al.*, 1998).

The asymmetry data did not provide convincing evidence for increased variability associated with marginal conditions even when a composite index of FA was used. Forster flies had a higher total FA in field males only in the October–December 1995 collection, but in this collection Taree had a relatively low total FA. In the F_1 flies there was a decrease in FA in the Forster flies rather than an increase. In general the FA data suggest that environmental and genomic stress are absent in the marginal populations, or else that FA is a poor indicator of these stresses in *D. serrata*.

Failure to find differences between populations in trait asymmetry may be related to several factors. For accurate comparisons between populations, sample sizes of more than 30 individuals are recommended (Palmer, 1994). All field populations from the March 1996 collection and all F_1 populations exceeded this number. It is also possible that the level of stress encountered by the populations was insufficient to cause an elevation in FA (Parsons, 1992). FA may only increase in marginal environments under some conditions and it would be worthwhile testing additional large samples of *D. serrata* after winter when stressful developmental conditions are likely. Finally, FA may have been examined in the wrong traits because it is known that FA changes with stress can be highly trait-specific in *Drosophila* (Woods *et al.*, 1999).

While there were no consistent population differences for trait variability, there were patterns for trait means. Field flies from Forster and to a lesser extent Taree tended to be larger than those from the other collections. Size differences under field conditions have been described in several *D. melanogaster* studies (e.g. Coyne & Beecham, 1987) and are commonly interpreted in terms of clinal selection mediated by climatic factors, although adult size is also influenced by larval density at breeding sites (Atkinson, 1979).

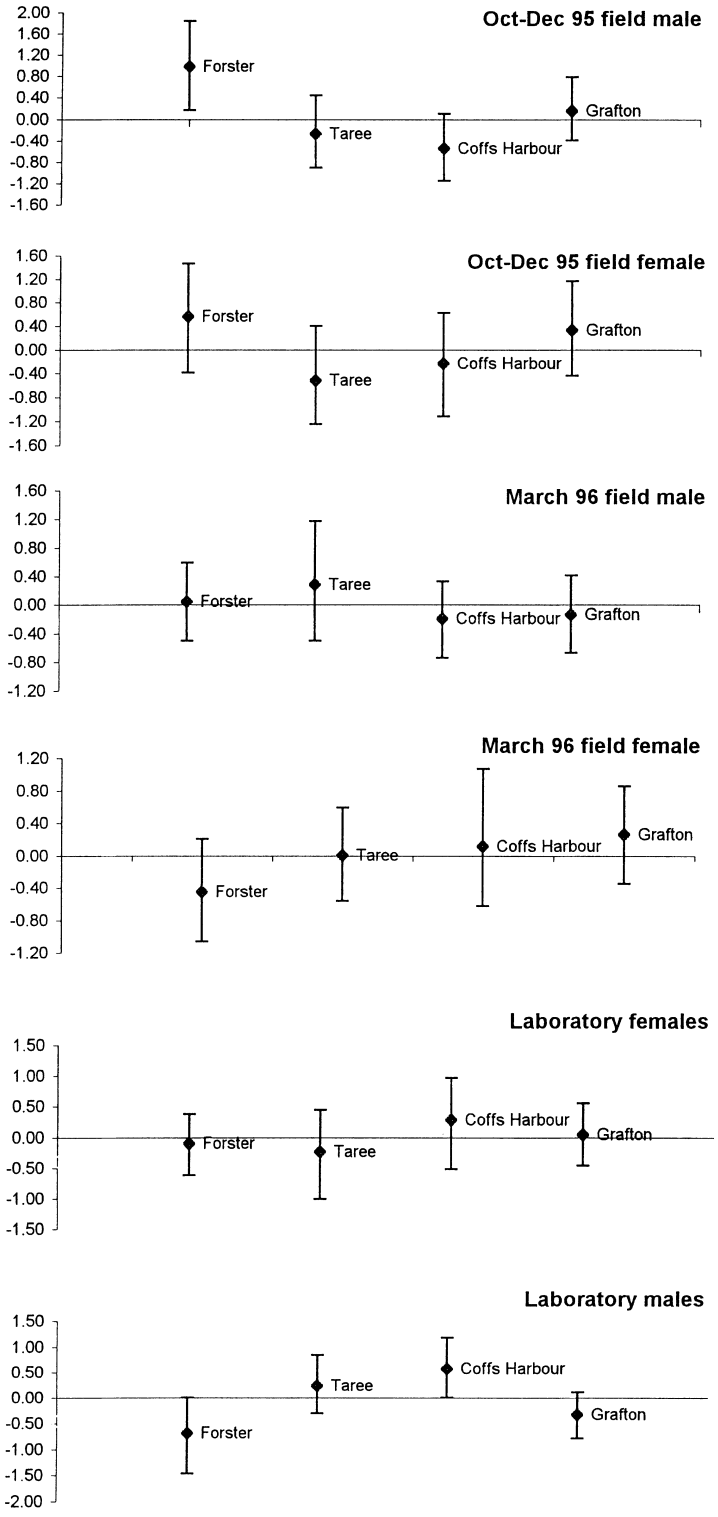

Fig. 5 Mean total asymmetry values for population comparisons of field-collected females and males and laboratory-reared offspring from field females. Error bars are 95% confidence intervals.

The F_1 data indicated population differences for wing width and wing length, in that Taree and Forster flies were larger than those from the more northern populations. Heritable differences among populations for size-related traits that follow latitude are well known for *D. melanogaster* and other species (Tantawy & Mallah,

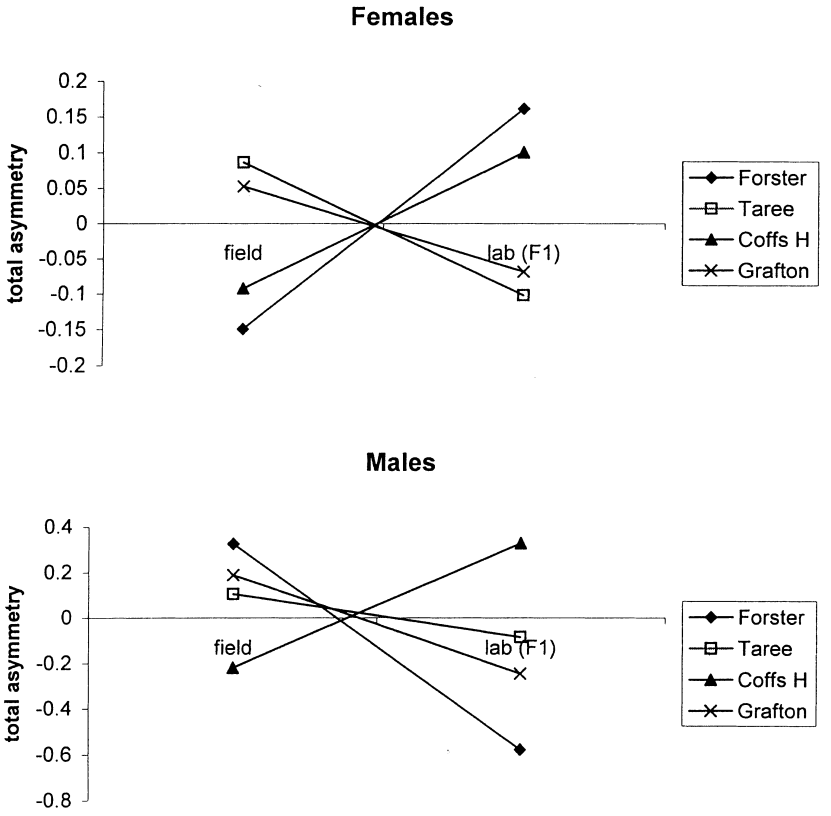

Fig. 6 Mean total asymmetry values for within-population comparisons of field-collected and laboratory-reared females and males from the March 1996 collection.

1961; Coyne & Beecham, 1987; Capy *et al.*, 1993; Karan *et al.*, 1998) and thought to be related in some way to temperature (e.g. David *et al.*, 1983; Partridge *et al.*, 1994). However, additional populations over a wider latitudinal range need to be considered before a size cline can be firmly established in *D. serrata*.

Overall, the data are inconsistent with comparisons of variability in marginal and central populations in other organisms. In particular, it appears that FA may not increase in marginal *Drosophila* populations, unlike in birds where even studies of FA in one trait have shown an association with marginality (cf. Møller, 1995). In future collections, it may be worthwhile focusing on flies from areas south of Forster where *D. serrata* can occasionally be collected in late summer to autumn. However, *D. serrata* has never been collected south of Forster after winter when developmental conditions are most likely to be stressful.

Comparisons across environments and collections

Wing size was greater when flies were reared in laboratory conditions, a pattern evident in all populations. Changes in bristle number were less clear cut, although bristle number did increase under laboratory rearing in some populations. The increase in size in the laboratory may reflect the effects of temperature and/or nutrition. Flies were reared in the laboratory at 25 °C which is higher than culture temperatures likely to have been experienced during development in the 1995 collection, though not the 1996 collection.

Changes in mean size were accompanied by a decrease in variability. This decrease probably reflects a lower level of environmental heterogeneity under laboratory conditions although it may also reflect the fact that environmental conditions were more favourable in the laboratory. In previous comparisons of CVs in *D. melanogaster* (e.g. Gibert *et al.*, 1998; Woods *et al.*, 1998), trait variability also tended to decrease under laboratory rearing. No patterns were evident for bristle CVs in *D. serrata*, consistent with *D. melanogaster* data (Woods *et al.*, 1998).

In contrast to these changes, there was no evidence that levels of FA were higher in the field compared with laboratory conditions, regardless of whether individual trait FA or total FA were considered. In *D. melanogaster*, Woods *et al.* (1998) found that total asymmetry was higher in the field than in the laboratory in two collections, but the opposite result was obtained in a third collection. This may reflect the fact that FA is a poor measure of environmental quality in *D. serrata* or that a similar stress level is experienced in different populations and in the laboratory and field environments.

Genetic variation

Overall, regression coefficients involving comparisons of field and laboratory-reared flies tended to be positive, reflecting heritable variation in these traits in natural populations. Estimates of heritable variation tended to be low to intermediate. However, these may be inaccurate because regression of field parents onto laboratory offspring can be a poor estimate of the true field heritability (Riska *et al*., 1989). The regression of laboratory offspring onto the midparent from nature is given by

$$\beta_{(OL,PN)} = \frac{\gamma\sigma_{AL}\sigma_{AN}}{\sigma^2_{PN}} \quad (1)$$

where γ is the additive genetic correlation between the trait in nature and the same trait in the laboratory, σ^2_{AL} and σ^2_{AN} are the additive genetic variances in the laboratory and nature, respectively, and σ^2_{PN} is the phenotypic variance in nature. Heritability estimates obtained from this equation will only equal the true field heritability ($\sigma^2_{AN}/\sigma^2_{PN}$) if γ is one (no genotype–environment interactions) and if the additive genetic variance is the same in the field and the laboratory. However, a lower-bound estimate can be obtained (Riska *et al*., 1989) from

$$\beta^2_{(OL,PN)}\left(\frac{\sigma^2_{PN}}{\sigma^2_{AL}}\right) = \gamma^2 h^2_N \leq h^2_N. \quad (2)$$

This equation describes the way the regression coefficient, the additive genetic variance in the laboratory and phenotypic variance in nature (σ^2_{AL},σ^2_{PN}) and the genetic correlation between environments (γ) relate to heritability in nature when measurements on both field parents are available. Whether this is an accurate reflection of field heritability depends on how close the squared genetic correlation between laboratory and field environments (γ^2) is to one and whether $\sigma_{AL} = \sigma_{AN}$.

Hoffmann (1999) reviewed all *Drosophila* cases where field and laboratory estimates were available, allowing estimates obtained with eqns 1 and 2 to be compared. He found that the association between estimates closely fitted a straight line with a slope of one, indicating very similar estimates from these approaches. This suggests that $\gamma = 1$ and that $\sigma_{AL} = \sigma_{AN}$. Therefore, either the minimum estimate or the regression slope may provide an estimate of field heritability, at least for morphological traits. The limited data available from this study support this conjecture. Using the above formula, average estimates of heritabilities from the 1996 collection and phenotypic variabilities from the field, minimum field estimates were 23% for bristle number, 69% for crossveins, 0.1% for wing width and 27% for wing length. These are similar to the average estimates of 28%, 52%, 1% and 10%, respectively, for these traits from regressions of laboratory offspring onto field parents.

For wing width and wing length, heritabilities in the field appear to be relatively low regardless of the population being considered. This is consistent with estimates for size-related traits in other *Drosophila* species. In *D. melanogaster*, heritability estimates for size-related traits in field flies are around 20% (Coyne & Beecham, 1987; Gibert *et al*., 1998; Sgrò & Hoffmann, 1998; Woods *et al*., 1998), while in *D. buzzatii*, estimates tend to be lower (Prout & Barker, 1989; Ruiz *et al*., 1991; Liebowitz *et al*., 1995). This may reflect a large impact of environmental conditions on variation in body size in *Drosophila*. For crossvein length, the mean regression estimate across all collections and comparisons was fairly high and suggests a heritability of around 50%. Because crossvein length was measured relative to wing length, heritability estimates cannot be compared with those of Woods *et al*. (1998) where an uncorrected measure of crossvein length was considered. Finally, heritability estimates for sternopleural bristle numbers are comparable with those obtained for *D. melanogaster* in the field where values of 20–50% have been obtained (Coyne & Beecham, 1987; Woods *et al*., 1998).

The tests comparing the heritability of traits across populations were weak because of the small number of families tested for any of the populations. Nevertheless, there was no indication that there were low levels of genetic variation for quantitative traits in the marginal population, and some of the regression coefficients for this population were significant. This contrasts with Blows & Hoffmann (1993) who found that marginal *D. serrata* responded less to selection than more centrally located populations (although these populations covered a broader geographical gradient than in the present study). The present data suggest that overall levels of genetic variance at Forster are unlikely to be responsible for the southern limit of *D. serrata*'s distribution.

Conclusions

The findings provide no evidence that flies from the permanent border of *D. serrata* are particularly stressed or particularly variable for the morphological traits measured. Whilst these findings will need to be extended by including flies from more southerly locations where low numbers of *D. serrata* can be collected in autumn, the similar variability across populations at both the genetic and phenotypic level suggests that overall levels of genetic variation are not limiting. We are presently testing if variability in traits likely to be under selection at the border show a similar pattern.

Acknowledgments

This research was supported by a grant from the Australian Research Council. We thank G. McColl, M. J. Hercus, A. Magiafoglou and two anonymous reviewers for comments on the manuscript.

References

Agnew, A.D.Q. 1968. Variation and selection in an isolated series of populations of *Lysimachia volkensii*. *Evolution* **22**: 228–236.

Atkinson, W.D. 1979. A field investigation of larval competition in domestic *Drosophila*. *J. Anim. Ecol.* **48**: 91–102.

Ayala, F.J. 1965. Sibling species of the *Drosophila serrata* group. *Evolution* **19**: 538–545.

Beardmore, J.A. 1960. Developmental stability in constant and fluctuating environments. *Heredity* **14**: 411–422.

Blows, M.W. & Hoffmann, A.A. 1993. The genetics of central and marginal populations of *Drosophila serrata*. I. Genetic variation for stress resistance and species borders. *Evolution* **47**: 1255–1270.

Brussard, P.F. 1984. Geographic patterns and environmental gradients: the central-marginal model in *Drosophila* revisited. *Ann. Rev. Ecol. Syst.* **15**: 25–64.

Capy, P., Pla, E. & David, J.R. 1993. Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. *Gen. Sel. Evol.* **25**: 517–536.

Carbonell, R. & Telleria, J.L. 1998. Increased asymmetry of tarsus-length in three populations of Blackcaps *Sylvia atricapilla* as related to proximity to range boundary. *Ibis* **140**: 331–333.

Carson, H.L. 1959. Genetic conditions which promote or retard the formation of species. *Cold Spring Harb. Symp. Quant. Biol.* **24**: 87–103.

Clarke, G.M. 1992. Fluctuating asymmetry: a technique for measuring developmental stress of genetic and environmental origin. *Acta Zool. Fenn.* **191**: 31–35.

Coyne, J.A. & Beecham, E. 1987. Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. *Genetics* **117**: 727–737.

David, J.R.R., Allemand, J., Van Herrewege & Cohet, Y. 1983. Ecophysiology: abiotic factors. In: *The Genetics and Biology of Drosophila* (M. Ashburner, H. L. Carson & J. N. Thompson, eds), pp. 105–170. Academic Press, London.

Gangestad, S.W. & Thornhill, R. 1999. Individual differences in development precision and fluctuating asymmetry: a model and its implications. *J. Evol. Biol.* **12**: 402–416.

Gibert, P., Moreteau, B., Moreteau, J.-C. & David, J.R. 1998. Genetic variability of quantitative traits in *Drosophila melanogaster* (fruit fly) natural populations: analysis of wild-living flies and of several laboratory generations. *Heredity* **80**: 326–335.

Hoffmann, A.A. 1999. Laboratory and field heritabilities: some lessons from *Drosophila*. In: *Adaptive Genetic Variation in the Wild* (T. A. Mousseau, B. Sinervo & J. A. Endler, eds), pp. 200–218. Oxford University Press, New York.

Hoffmann, A.A. & Blows, M.W. 1994. Species borders: ecological and evolutionary perspectives. *Trends Ecol. Evol.* **9**: 223–227.

Hoffmann, A.A. & Parsons, P.A. 1988. The analysis of quantitative variation in natural populations with isofemale strains. *Gen. Sel. Evol.* **20**: 87–98.

Hoffmann, A.A. & Parsons, P.A. 1997. *Extreme Environmental Change and Evolution*. Cambridge University Press, Cambridge.

Hurtado, L., Castrezana, S., Mateos, M., McLaurin, D., Tello, M.K., *et al*. 1997. Developmental stability and environmental stress in natural populations of *Drosophila pachea*. *Ecotoxicology* **6**: 233–238.

Imasheva, A.G., Loeschcke, V., Zhivotovsky, L.A. & Lazenby, O.E. 1997. Effects of extreme temperatures on phenotypic variation and developmental stability in *Drosophila melanogaster* and *Drosophila buzzatii*. *Biol. J. Linn. Soc.* **61**: 117–126.

James, A.C. & Partridge, L. 1995. Thermal evolution of rate of larval development in *Drosophila melanogaster* in laboratory and field populations. *J. Evol. Biol.* **8**: 315–330.

Karan, D., Munjal, A.K., Gibert, P., Moreteau, B., Parkash, R., *et al*. 1998. Latitudinal clines for morphometrical traits in *Drosophila kikkawai*: a study of natural populations from the Indian subcontinent. *Genet. Res.* **71**: 31–38.

Leary, R.F. & Allendorf, F.W. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends Ecol. Evol.* **4**: 214–217.

Liebowitz, A., Santos, M. & Fontdevila, A. 1995. Heritability and selection on body size in a natural population of *Drosophila buzzatii*. *Genetics* **141**: 181–189.

Møller, A.P. 1995. Patterns of fluctuating asymmetry in sexual ornaments of birds from marginal and central populations. *Amer. Nat.* **145**: 316–327.

Palmer, A.R. 1994. Fluctuating asymmetry analyses: a primer. In: *Developmental Instability: its Origins and Evolutionary Implications* (T. A. Markow, ed.), pp. 335–364. Kluwer Academic Publishers.

Palmer, A.R. & Strobeck, C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Ann. Rev. Ecol. Syst.* **17**: 391–421.

Parsons, P.A. 1962. Maternal age and developmental variability. *J. Evol. Biol.* **39**: 251–260.

Parsons, P.A. 1992. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity* **68**: 361–364.

Partridge, L., Barrie, B., Fowler, K. & French, V. 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution* **48**: 1269–1276.

Prout, T. 1958. A possible difference in genetic variance between wild and laboratory populations. *Dros. Inf. Ser.* **32**: 138–139.

Prout, T. & Barker, J.S.F. 1989. Ecological aspects of the heritability of body size in *Drosophila buzzatii*. *Genetics* **123**: 803–813.

Riska, B., Prout, T. & Turelli, M. 1989. Laboratory estimates of heritability and genetic correlations in nature. *Genetics* **123**: 865–871.

Ruiz, A., Santos, M., Barbadilla, A., Quezada-Diaz, E., Hasson, E., *et al*. 1991. Genetic variation for body size in a natural population of *D. buzzatii*. *Genetics* **128**: 739–750.

Safriel, U.N., Volis, S. & Kark, S. 1994. Core and peripheral populations and global climate change. *Israel J. Plant Sci.* **42**: 331–345.

Sarre, S. 1996. Habitat fragmentation promotes fluctuating asymmetry but not morphological divergence in two geckos. *Res. Pop. Ecol.* **38**: 57–64.

Sgrò, C.M. & Hoffmann, A.A. 1998. Heritable variation for fecundity in field-collected *D. melanogaster* and their offspring reared under different environmental temperatures. *Evolution* **52**: 134–143.

Siikamaki, P. & Lammi, A. 1998. Fluctuating asymmetry in central and marginal populations of *Lychnis viscaria* in relation to genetic and environmental factors. *Evolution* **52**: 1285–1292.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*, 3rd edn. W. H. Freeman, New York.

Soulé, M. 1973. The epistasis cycle: a theory of marginal populations. *Ann. Rev. Ecol. Syst.* **4**: 165–187.

Swaddle, J.P., Witter, M.S. & Cuthill, I.C. 1994. The analysis of fluctuating asymmetry. *Anim. Behav.* **48**: 986–989.

Tantawy, A.O. & Mallah, G.S. 1961. Studies on natural populations of *Drosophila*. 1. Heat resistance and geographical variation in *Drosophila melanogaster* and *D. simulans*. *Evolution* **15**: 1–14.

Thoday, J.M. 1955. Balance, heterozygosity and developmental stability. *Cold Spring Harb. Symp. Quant. Biol.* **20**: 318–326.

Wilson, J.B., Ronghua, Y., Mark, A.F. & Agnew, A.D.Q. 1991. A test of the low marginal variance (LMV) theory in *Leptospermum scoparium* (Myrtaceae). *Evolution* **45**: 780–784.

Woods, R.E., Hercus, M.J. & Hoffmann, A.A. 1998. Estimating the heritability of fluctuating asymmetry in field *Drosophila*. *Evolution* **52**: 816–824.

Woods, R.E., Sgrò, C.M., Hercus, M.J. & Hoffmann, A.A. 1999. The association between fluctuating asymmetry, trait variability and stress: a multiply-replicated experiment on combined stresses in *Drosophila melanogaster*. *Evolution* **53**: 493–505.

Zar, J.H. 1996. *Biostatistical Analysis*. Prentice Hall International, Inc., Upper Saddle River, New Jersey.

Received 6 July 1999; accepted 3 August 1999