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Bilateral variation and the evolutionary origin of macroscopic asymmetries

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Abstract

Given that characters exhibiting macroscopic asymmetry have evolved in a wide variety of taxa, heritable variation for bilateral asymmetry must have arisen at some point in their history. The recognition that heritable variation may underlie some statistical asymmetries not only raises concerns about the incautious use of statistical estimates of FA in studies of developmental stability, but it suggests some intriguing questions about the possible evolutionary origins of macroscopic asymmetries. First, we developed an additive model of bilateral variation based on some simple assumptions about the developmental control of bilateral variation. Second, using a new approach for studying statistical asymmetries, we conducted an analysis of bilateral variation in eight metrical traits of a harpacticoid copepod (*Tigriopus californicus*) to search for novel forms of statistical asymmetries. The model we developed revealed three independent statistical asymmetries of potential evolutionary significance: *a*) a previously unrecognized form of asymmetry (referred to here as *normal covariant asymmetry*), *b*) antisymmetry, and *c*) directional asymmetry. Because each pattern of variation would seem to require different amounts and kinds of developmental-genetic information [*a*- only negative feedback between sides (*bilateral inhibition*), *b*- both *bilateral inhibition* and average departure from symmetry (*bilateral offset*), *c*- *bilateral inhibition*, *bilateral offset*, and a consistent overdevelopment of one side or the other (*side-bias control*)], those requiring less information would seem more likely to represent earlier stages in the evolution of macroscopic asymmetries. Our analysis of bilateral variation in *Tigriopus* revealed no evidence for any form of statistical asymmetry other than fluctuating asymmetry. However, a significant positive covariation between sides, even after correction for body size variation, suggested that factors influencing relative limb length (whether genetic or environmental) affected both sides equally rather than one side at the expense of the other. Finally, we note that certain statistical asymmetries (directional asymmetry, any form of covariant asymmetry) may render characters unreliable for estimating developmental stability because, unlike pure fluctuating asymmetry, they may signal a genetic component to asymmetry variation.

Introduction

Symmetry and departures from symmetry

Although the external body form of most animals is bilaterally symmetrical, departures from symmetry may take a variety of forms (Ludwig, 1932). Sometimes these departures are quite conspicuous, as in the case of gastropods, male fiddler crabs, American lobsters, and flatfishes (Neville, 1976). More commonly, departures from symmetry are quite subtle and require careful measurement to detect (Palmer & Strobeck, 1986). No character, strictly speaking, will exhibit perfect bilateral symmetry, except perhaps by chance, since the mechanisms guiding development simply do not have that kind of precision. Even for characters that are bilaterally symmetrical on avarage, the right and left sides of

any given individual will depart subtly from symmetry at some level of measurement. To what extent might such subtle variation provide the raw material from which macroscopic asymmetries evolve?

The biological significance of subtle, statistical departures from bilateral symmetry has stimulated much interest (Palmer & Strobeck, 1986; Zakharov, 1992). Underlying this interest is a belief that one form - fluctuating asymmetry (FA), or small, random departures from bilateral symmetry - can be used to describe 'developmental stability'. In its simplest expression, perfect symmetry provides a convenient reference against which to measure developmental precision. The less able an organism is to buffer itself against disturbances during development, the more likely one or more of its characters will depart from symmetry. In some organisms, FA may increase in response to extrinsic environmental stresses (Leary & Allendorf, 1989; Zakharov, 1992) or to intrinsic genetic stresses (Parsons, 1990). In others, FA appears to decrease with increasing heterozygosity, suggesting a connection between heterosis and developmental stability (Mitton & Grant, 1984; Allendorf & Leary, 1986; Palmer & Strobeck, 1986).

In the extensive literature on subtle, statistical departures from symmetry, however, an important observation is often overlooked: macroscopically asymmetrical structures have, in fact, evolved in a wide variety of taxa (Ludwig, 1932; Neville, 1976). Presumably, such macroscopic asymmetries arose initially as subtle departures from symmetry that had a heritable basis and were subsequently amplified by natural selection. This view of asymmetry raises three questions to which we address this paper. 1) What forms might evolutionarily significant variation in asymmetry take? 2) Of alternative forms, which is the most likely starting point for the evolution of macroscopic asymmetries? 3) What are the implications of such an evolutionary perspective for studies of developmental stability based upon variation in FA?

Components of bilateral variation

An additive model of bilateral variation

The following model attempts to incorporate some simple developmental processes that influence bilateral variation and describe how they contribute to the patterns of variation observed between sides. Building upon the notation of Palmer and Strobeck (1986), let the right and left members of a bilateral trait-pair for the ith individual of a particular sample be

$$R_i = \mu + s_i + (D + \delta_i)A_i/2 + r_i = \mu + s_i + DA_i/2 + \delta_i/2 + r_i, \text{ and} \quad (1)$$

$$L_i = \mu + s_i - (D + \delta_i)A_i/2 + l_i = \mu + s_i - DA_i/2 - \delta_i/2 + l_i. \quad (2)$$

where the terms are defined as in Table 1. One half of $D + \delta_i$ is added to the right side and subtracted from the left so that variation in these terms has no affect on average trait size. Note also that because δ_i has a mean of zero and is normally distributed, its contribution to the variation in R or L is not influenced by *side bias*, A_i, which may only take values of $\pm$ 1. Hence both equations simplify to those on the right. Given these components of bilateral variation, the expected means and variances for each side will be:

$$E(R_i) = \mu + D\,E(A_i)/2 = \mu + D\,[p - q]/2, \quad (3)$$

$$E(L_i) = \mu - D\,E(A_i)/2 = \mu - D\,[p - q]/2, \text{ and} \quad 4)$$

$$\mathrm{Var}(R_i) = \mathrm{Var}(L_i) = \sigma_s^2 + (D/2)^2\,\mathrm{Var}(A_i) + \sigma_d^2/4 + \sigma_e^2 = \sigma_s^2 + D^2pq + \sigma_d^2/4 + \sigma_e^2. \quad (5)$$

The covariance between sides will be

$$\mathrm{Cov}(R_i,L_i) = E\{[R_i - (\mu + D[p - q]/2)]\,[L_i - (\mu - D[p - q]/2)]\}.$$

Substituting for R_i and L_i from equations (1) and (2), and rearranging yields

$$\begin{aligned}\mathrm{Cov}(R_i,L_i) &= E\{(s_i + DA_i/2 - D[p - q]/2 + \delta_i/2 + r_i)\,(s_i - DA_i/2 + D[p - q]/2 - \delta_i/2 + l_i)\} \\ &= E\{\sigma_i^2 - (DA_i/2 - D[p - q]/2)^2 - (\delta_i/2)^2\} \\ &= \sigma_s^2 - D^2\,pq - \sigma_d^2/4. \quad (6)\end{aligned}$$

Table 1. Description of variables for an additive model of bilateral variation where there is no variation in body size.

Symbol*	Description	Effect on distribution of bilateral variation	Interpretation of developmental origin
μ	A positive, arbitrary constant defining the mean size of a trait *for a given body size*	n.r.	n.r.
s_i	A random normal deviate (mean 0, variance σ_s^2) added to both sides of individual i	Results in positive covariation between sides	Reflects environmental or genetic factors that increase or decrease development of both sides equally (*positive covariation*)
D	A positive, arbitrary constant representing the fixed difference between sides	Results in directional asymmetry or antisymmetry depending on A_i	Reflects presumably genetic factors that determine average magnitude of departure from symmetry (*bilateral offset*)
δ_i	A random normal deviate (mean 0, variance σ_d^2) added to the fixed difference between sides (D) in individual i	Results in continuous negative covariation between sides	Reflects environmental or genetic factors that influence the extent of bilateral offset in a given individual (*offset variation*)
A_i	A binomial random variable that takes the values +1 or -1 with probability p and $q = 1 - p$ respectively, and indicates whether the fixed difference between sides (D) is added to the right (+1) or subtracted from the right (-1) of individual i	Influences the direction of directional asymmetry if $p = 1$ or $p = 0$, or the shape of the frequency distribution of $(R - L)$ if $0 < p < 1$	Reflects environmental or genetic factors that influence the direction of departure from symmetry (*side bias*)
r_i and l_i	Random normal deviates (mean 0, variance σ_e^2) added to the right and left side respectively of individual i	Results in residual variation between sides	Reflects non-genetic factors that cause random, independent variation in final trait size (*developmental noise*)

* as in equations 1 & 2; n.r. - not relevant.

The difference between sides, obtained from equations 1 and 2 is

$$R_i - L_i = DA_i + \delta_i + r_i - l_i, \quad (7)$$

and the expected mean and variance of this difference will be

$$E(R_i - L_i) = DE(A_i) = D\,[p - q] \text{ and} \quad (8)$$

$$\begin{aligned} \mathrm{Var}(R_i - L_i) &= D^2\,\mathrm{Var}(A_i) + \sigma_d^2 + 2\sigma_e^2 \\ &= 4D^2\,pq + \sigma_d^2 + 2\sigma_e^2. \end{aligned} \quad (9)$$

Equation 9 could also have been derived by recognizing that

$$\mathrm{Var}(R_i - L_i) = \mathrm{Var}(R_i) + \mathrm{Var}(L_i) - 2\mathrm{Cov}(R_i, L_i). \quad (10)$$

Note that according to this formulation, variation in the difference between sides (equation 9) includes a contribution due to *bilateral offset* (D), variation in *side bias* (pq), and *offset variation* (σ_d^2). Hence, only if these terms are zero will the variation in $(R_i - L_i)$ reflect pure *developmental noise* (σ_e^2).

A graphical view of bilateral variation

Conventional forms of statistical asymmetries. Frequency distributions of $R - L$ are traditionally used to view different forms of statistical asym-

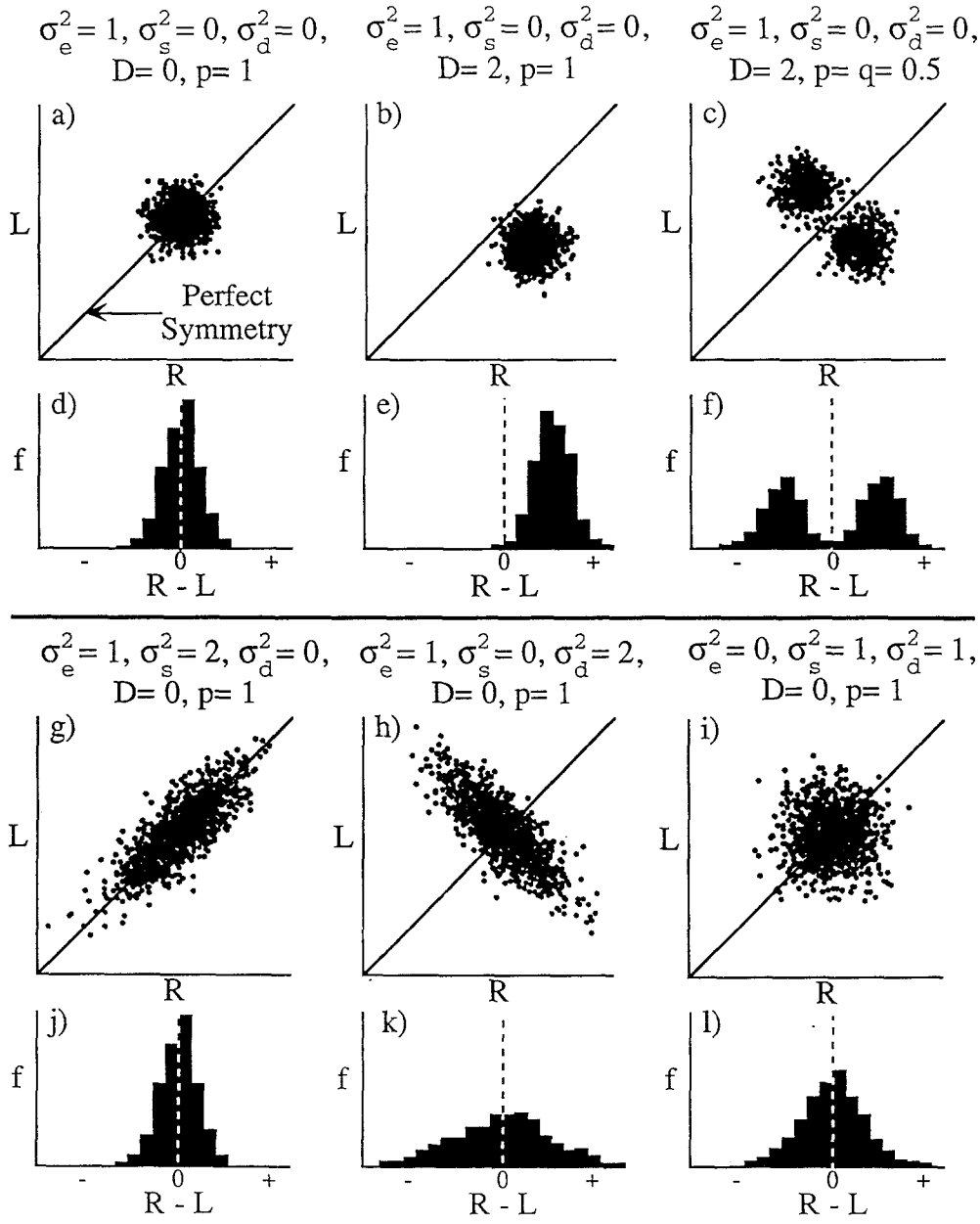

Fig. 1. Computer simulations of bilateral variation using the developmental model described in equations 1 and 2. Variables above each figure are defined in Table 1. Each form of asymmetry is presented as a pair of figures: *scatterplots* of *R* versus *L* (upper figure of each vertical pair), and *frequency distributions* of *R - L* (lower figure of each vertical pair, dashed vertical line corresponds to perfect symmetry), (a,d) fluctuating asymmetry. (b,e) directional asymmetry, (c,f) antisymmetry. (*g,j*) positive covariation between sides at a given body size. (h,k) negative covariation between sides at a given body size (normal covariant asymmetry). (i,l) apparent FA in the absence of *developmental noise* (σ_e^2), but where *positive covariation* sides (σ_s^2) and *negative covariation* between sides (σ_d^2) are nonzero and equal.

metries. However, once general size effects have been factored out (see *Scaling variation to a common body size* below), plots of R versus L reveal more information about bilateral covariation. No such covariation is present in either pure fluctuating asymmetry or pure directional asymmetry (Fig. 1a,b). Of the three currently named forms of statistical asymmetries (Fig. 1a-c), only antisymmetry signals covariation unambiguously (Fig. 1c). All three of these asymmetries may be distinguished by knowing the mean and shape of the frequency distribution of $R - L$ (Fig. 1d-f; Van Valen, 1962).

A novel form of statistical asymmetry. An important point to which we wish to draw attention is that negative covariation between sides need not be expressed only as a bimodal or platykurtic distribution of $R - L$ (Fig. 1c,f). Negative covariation between sides may also be continuous (Fig. 1h). Such covariation would correspond to *offset variation* in our formulation (σ_d^2 in equation 9).

Unlike *positive covariation* between sides (σ_s^2), negative covariation *does* contribute to the variance of $R - L$ (equation 9), even though the frequency distribution of $R - L$ may still exhibit what appears to be pure FA (mean zero, normal; Fig. 1k,l). This may be visualized most easily by considering the shape of the frequency distribution that would result from bilateral variation about the line of perfect symmetry when viewed from the origin. Increasing the *positive covariation* between sides (e.g. σ_s^2 in Fig. 1g) will have no effect of the frequency distribution of $R - L$ (Fig. 1j), whereas increasing the negative covariation between sides (e.g. σ_d^2 in Fig. 1h) will increase the variance of the frequency distribution of $R - L$ (Fig. 1k). Hypothetically, a normal frequency distribution with mean zero could arise even in the absence of any *developmental noise* ($\sigma_e^2 = 0$), so long as σ_d^2 was not zero (Fig. 1i,l).

Terminology for different forms of negative covariation between sides. In keeping with the tradition of naming asymmetries based on their pattern of bilateral variation, as opposed to the presumed mechanism giving rise to asymmetry, we suggest that all forms of negative covariation between sides be referred to as *covariant asymmetry*. However, because the term *antisymmetry* is so deeply entrenched in the literature, we suggest retaining it to describe the case illustrated in Figure 1c,f, even though this is just a special case of covariant asymmetry. We suggest *normal covariant asymmetry* to describe the case illustrated in Figure 1h,i. Skewed frequency distributions of $R - L$ would correspond to another form of antisymmetry (*skewed covariant asymmetry*) where $p \neq q$, and where neither p nor q nor $D = 0$ (see equation 9 and Palmer & Strobeck, 1992).

Distinguishing among different forms of covariant asymmetry

Although directional asymmetry may be detected easily by a variety of analytical procedures (Palmer & Strobeck, 1986), forms of covariant asymmetry are more difficult to detect. As a first step, any form of covariant asymmetry will be revealed as a statistically significant sides X individuals interaction in the ANOVA procedure outlined by Palmer and Strobeck (1986), so long as an estimate of measurement error is available. However, this ANOVA procedure cannot distinguish among forms of covariant asymmetry, hence some additional analysis is required.

Skewed covariant asymmetry and antisymmetry. Both skewed covariant asymmetry and antisymmetry will appear as departures from zero of the third or fourth statistical moments of frequency distributions of $R - L$ (skew and kurtosis). Both are thus readily detected by conventional tests for departures from normality [see Shapiro, Wilk & Chen (1968) and Palmer & Strobeck (1992) for discussion of such tests].

Normal covariant asymmetry: Positive covariation *between sides absent.* Normal covariant asymmetry is more difficult to detect than skewed covariant asymmetry or antisymmetry because the frequency distribution of $R - L$ in this case is indistinguishable from that of pure FA (compare Fig. 1k or 1l with 1d). Where *positive covariation* between sides is absent ($\sigma_s^2 = 0$) and where *developmental noise* is small ($\sigma_e^2 < \sigma_d^2$), *offset variation* (σ_d^2 can be detected as a statistically significant negative correlation between sides (Fig. 1h). Note, though, that if the *offset variation* is small relative to *developmental noise* ($\sigma_d^2 < \sigma_e^2$), then the negative correlation may be difficult to detect statistically for routine sample sizes.

Normal covariant asymmetry: Confounding effects of positive covariation *between sides.* Unfortunately, average character size $[(R_i + L_i)/2]$ may vary even where overall body size does not (e.g. relative arm length may still vary among humans of the same body size). When character size varies in this manner (i.e. where *positive covariation* between sides (σ^2_s) is greater than 0), the detection of *offset variation* becomes problematical. An extreme example should make this clear. Consider a single sample where factors contributing to *positive covariation* between sides (σ^2_s) equal those contributing to *offset variation* (σ^2_d), as in Figure 1g and 1h respectively, but where *developmental noise* is absent ($\sigma^2_e = 0$). If each of these patterns of covariation is bivariate normal, then the joint distribution will appear to be pure FA (Fig. 1i and 1l)!

Regrettably, the confounding effect of *positive covariation* between sides is not simply an empirical problem. If all three variances (σ^2_s, σ^2_d, and σ^2_e) are non-zero, it is not possible to partition them out precisely. For example, the distribution illustrated in Figure 1h could result either from a) a combination of positive and negative covariation ($\sigma^2_d > \sigma^2_s > 0$, $\sigma^2_e = 0$), b) a combination of negative covariation and *developmental noise* in the absence of *positive covariation* ($\sigma^2_d > \sigma^2_e > 0$, $\sigma^2_s = 0$), or c) some combination of all three variance components (e.g. $\sigma^2_d >> \sigma^2_e = \sigma^2_s > 0$).

Normal covariant asymmetry: A pragmatic approach. The major practical concern for studies of the evolution of bilateral asymmetry is whether the magnitude of normal covariant asymmetry, and its presumed genetic underpinnings, is large enough to respond to selection. For studies of FA, the concern is whether normal covariant asymmetry is large enough to confound inferences about developmental stability. Thus, although it may never be detectable when slight, a pragmatic approach to this problem would be to ask, as illustrated below with *Tigriopus*, whether the *offset variation* (σ^2_d) is large relative to *positive covariation* between sides (σ^2_s) or to *developmental noise* (σ^2_e), among individuals of comparable body size. Continuous negative covariation between R and L will only be apparent statistically where the *offset variation* (σ^2_d) *is greater than positive covariation* between sides (σ^2_s).

A case study of statistical asymmetries in a harpaticoid copepod

To test for the presence of various alternative forms of statistical asymmetries, we analyzed data collected as part of a larger study of geographic differentiation in the common, high-tidepool harpacticoid copepod *Tigriopus californicus* (Chippindale & Palmer unpublished). Burton (Burton & Feldman, 1981; Burton, 1987) has reported extensive divergence at allozyme loci among local populations and we wished to determine if developmental stability was reduced in hybrids between geographically distant populations. Except for its rather small size, *T. californicus* is an ideal animal for developmental-genetic studies because of the ease with which it may be bred and raised in the lab.

Methods

Collection, culture, and preparation. Individuals of *T. californicus* were collected from three sites over a two year period. Laboratory populations were initiated with 125-150 mature females in the early stages of ovigery. Both parents and offspring were reared on a mixed diet of the cultured unicellular green alga *Isochrysis* sp. (Provasoli, Shiraishi & Lance, 1959) and powdered commercial fish food (TetraMin) (Burton, pers. comm.). The cultures were maintained in two liters of sterilized, normal-strength seawater within four liter glass containers left open to the air and held at room temperature. Before initiating the cultures, females were rinsed four times with sterilized, aerated seawater.

When a sufficient number of individuals in a culture had reached their adult moult [copepodid VI (C-VI); approximately 2-3 weeks after hatching], 40 were removed and killed in a dilute formalin solution. Only females were selected because the presence of ovisacs indicated both the sex and the state of maturity of individuals unambiguously. From these individuals, the right and left first antennae, the right and left maxillipeds, and the right and left third thoracic limbs were dissected from the body with modified 00 insect pins in a full-strength solution of PVA lactophenol (Clarke, Brand & Whitten, 1986). Limbs were generally removed as pairs to aid with positioning. After removal, the limbs were oriented on a glass slide

perpendicular to the plane of articulation as viewed from the posterior, except for the first antennae which were oriented as viewed dorsally. They were subsequently covered with a cover slip, and the mounting medium was allowed to harden. These limbs were chosen for their ease of mounting and because they included clearly defined and resolvable characters for measurement.

The culture we analyzed (initiated from San Juan Is., Washington, USA) was selected because it contained the largest number of individuals having a complete set of replicated measurements. Because of the small size of these copepods (< 2 mm total body length), limbs were occasionally damaged during dissection and hence could not be measured.

Measurement and analysis. A total of eight metrical traits were measured: three on the first antennae (Ant 1, Ant 2, and Ant 3), two on the maxilliped (Mxp 1, Mxp 2), and three on the third thoracic limb (Thc 1, Thc 2, Thc 3). The dimensions measured were: Ant 1- anterior margin of first article, Ant 2- anterior margin of second article, Ant 3- posterior margin of second article, Mxp 1- lateral margin of first article of exopodite, Mxp 2- medial margin of first article of endopodite, Thc 1- lateral margin of first article of exopodite, Thc 2- medial margin of first article of endopodite, Thc 3- from the central lobe of the proximal end to the distal-most tip of the third article of the endopodite. For Ant 1, Ant 2, Ant 3, and Mxp 1, measurements were made from the base of the article at the proximal joint to the distal-most edge of the article adjacent to the next joint. For Mxp 2, Thc 1, and Thc 2, measurements were made from the base of the article to the insertion point of the pronounced, solitary, articulated seta adjacent to the distal-most edge of the article.

All measurements were obtained by viewing the limbs under a compound microscope at 400X magnification and, via a camera lucida, digitizing the dimensions with a 500 × 500 dpi resolution graphics tablet (MacTablet, Summagraphics Corp.) attached to a microcomputer.

Measurement error. Measurement error was estimated by measuring each trait three times independently. In other words, one set of 16 measurements was completed (eight limbs, two sides) on all individuals before beginning a replicate set, hence measurement error included both digitizing error as well as the error in relocating landmarks.

Because differences between sides in studies of statistical asymmetries are often very small (1-2% of character size), these differences must be shown to be significantly larger than would be expected due to the error in measurement. Measurement error will, of course, give the same pattern of between-sides variation as FA. Following the procedure outlined in Palmer and Strobeck (1986), the magnitude of nondirectional asymmetry (which includes FA and all forms of covariant asymmetry) was tested relative to measurement error with a mixed-model, two-way analysis of variance (ANOVA; sides = fixed effect, individuals = random effect, repeat measures = replicates). This same analysis also yielded significance levels (relative to nondirectional asymmetry) for directional asymmetry and body size/character size variation among individuals.

Body size effects and departures from normality. To determine whether the between-sides variance varied with character size for a given trait, we examined scatterplots of R versus L. Variation appeared to be independent of size for each character examined (see Fig. 3 below), so no transformation was necessary to homogenize the variance before computing the conventional descriptors of statistical asymmetries.

To detect departures from normality, frequency distributions of $R - L$ were tested for skew and kurtosis. These tests will detect skewed covariant asymmetry and antisymmetry. To detect normal covariant asymmetry, we tested for negative correlations between R and L after factoring out body size variation (see next section). Replicate measurements were averaged for each side before conducting these tests.

Scaling variation to a common 'body size'. We realize that 'body size' does not correspond to any given entity that we may estimate, because it will always be a function of the particular, usually arbitry, subset of characters measured. We use the term here for convenience, even though we mean only the positive covariation among all the traits we examined. Over the rather narrow size range we examined, the between-sides variance in a particular character did not increase with the mean (see

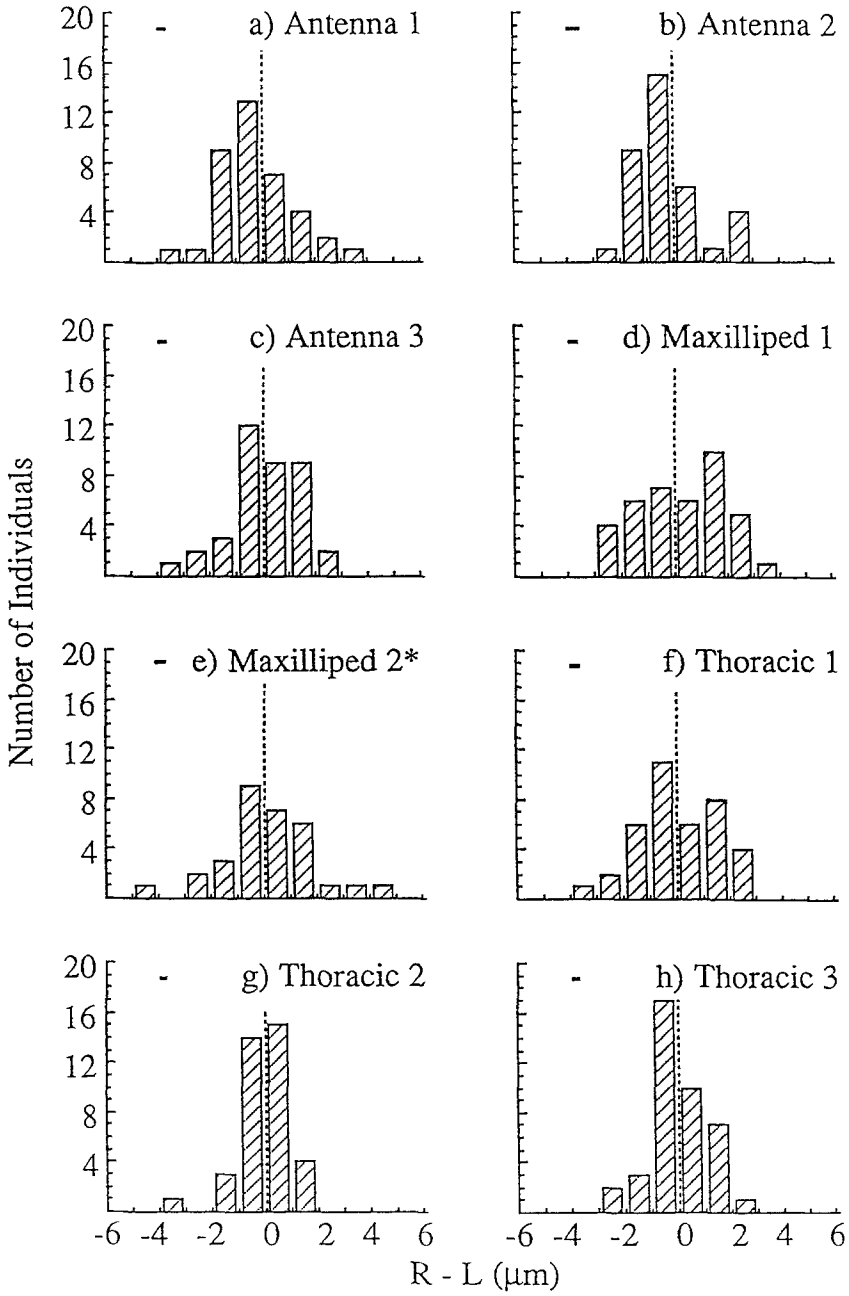

Fig. 2. Frequency distributions of *R* - *L* for eight traits in C-VI stage females of the marine harpacticoid copepod, *Tigriopus californicus*. Differences were computed after the three replicate measurements were averaged for *R* and *L*. Mean measurement error ranged from 0.20 to 0.31 μm among the eight characters, as indicated by small horizontal bars in each figure. Extreme values indicated by † in Fig. 3 were excluded from these distributions to improve the clarity of presentation. Dashed line indicates perfect symmetry. * Note that for Mxp 2, the units of deviation ranged from -12 to +12, but have been plotted to the same scale as the other characters for simplicity. The following five extreme values, some of which lie outside the bounds of these figures, were eliminated as statistical outliers using Grubb's test (Sokal & Rohlf, 1981): Ant 2: -7 and -8, Mxp 2: -15, Thc 1: +12, and Thc 2: -4.

Table 2. Significance tests for nondirectional asymmetry, body size/character size variation, and directional asymmetry for eight traits in the harpacticoid copepod *Tigriopus californicus*.

	Measurement error			Sides × Individuals (= nondirectional asymmetry)			Individuals (= both body & character size)		Sides (= directional asymmetry)	
Trait	df	MS(=σ_m^2)	σ_m^2/σ_i^2	df	MS	P*	MS	P†	MS	P†
Ant 1	152	0.194	0.225	37	2.78	< 0.001	52.31	< 0.001	2.42	0.36
Ant 2	152	0.280	0.146	37	6.04	< 0.001	33.32	< 0.001	18.14	0.09
Ant 3	152	0.145	0.180	37	2.57	< 0.001	24.11	< 0.001	2.69	0.31
Mxp 1	156	0.113	0.090	38	3.88	< 0.001	240.68	< 0.001	1.90	0.18
Mxp 2	128	0.240	0.024	31	29.78	< 0.001	379.63	< 0.001	4.68	0.69
Thc 1	156	0.194	0.072	38	8.30	< 0.001	51.77	< 0.001	6.44	0.38
Thc 2	148	0.119	0.310	36	1.27	< 0.001	32.81	< 0.001	0.58	0.50
Thc 3	160	0.162	0.285	39	1.87	< 0.001	62.16	< 0.001	0.0002	0.99

Note. Results are from a mixed model, two-way ANOVA with replication, following the procedure of Palmer and Strobeck (1986) ['Sides' = fixed effect, 'Individuals' = random effect, 'Sides × Individuals' = remainder whose expected MS is $\sigma_m^2 + M\sigma_i^2$ (where M = number of replicate measurements per side, three in this study)]. Computations were carried out prior to any correction for body size variation and prior to removal of outliers.

σ_m^2 = measurement error variance, σ_i^2 = nondirectional asymmetry variance [includes contributions due to FA and all forms of covariant asymmetry; from Table 3*B* of Palmer and Strobeck (1986)]. The ratio σ_m^2/σ_i^2 indicates the magnitude of measurement error relative to the between-sides variation.

df - degrees of freedom. The degrees of freedom for 'Individuals' is the same as that for 'Sides × Individuals', that for 'Sides' is one. MS - mean squares from ANOVA; P = exact probability; * tested over 'Measurement error' MS; † tested over 'Sides × Individuals' MS.

Fig. 3 below) so a linear transformation to remove 'size effects' seemed justified.

To test for normal covariant asymmetry, body size variation had to be factored out in order to examine the covariation of R and L about the parametric mean character size (μ in equations 1 and 2) for a given body size. Because some *positive covariation* between sides will be body-size independent (i.e. for some individuals, both limbs could be proportionally larger or smaller for the same body size), it was inappropriate to adjust each side simply by subtracting, or dividing by, the mean for each individual $[(R_i + L_i)/2]$. Furthermore, such a correction would merely create a perfect negative correlation between R and L (any departure of R_i in one direction from $[(R_i + L_i)/2]$ would precisely equal the departure of L_i in the opposite direction) and thus obscure any *offset variation*.

Body size was factored out by converting measurements for each side into deviations from a model II regression against a general size metric. To obtain this size metric, the data for each of the eight traits were first transformed to standardized normal deviates (SND) by subtracting the overall mean for the trait ($\Sigma[R_i + L_i]/2N$) and dividing by its standard deviation $[\mathrm{VAR}(R_i, L_i)^{-2}]$. This transformation was necessary because traits with a larger mean size had larger variances (Table 3). By converting each variate to an SND, the contribution to body size of each trait was thus weighted equally, independent of overall trait size. In addition, in contrast to a principal components analysis, this procedure permitted us to use all individuals in the analysis because the absence of one or more characters would not bias the size metric for that individual.

The size metric for a particular trait was computed as the arithmetic mean of the SNDs of all the *other* traits. In other words, for Antenna 1 of individual 1, the body size metric was computed as the mean SND for the remaining seven traits for individual 1. This resulted in a series of body size metrics for each individual, one metric for each trait. One advantage to this approach was that the body size metrics for a particular trait were thus independent of the variation in that trait.

Finally, body size was factored out of each trait via reduced major exis (RMA) regression of each side separately against the appropriate size metric

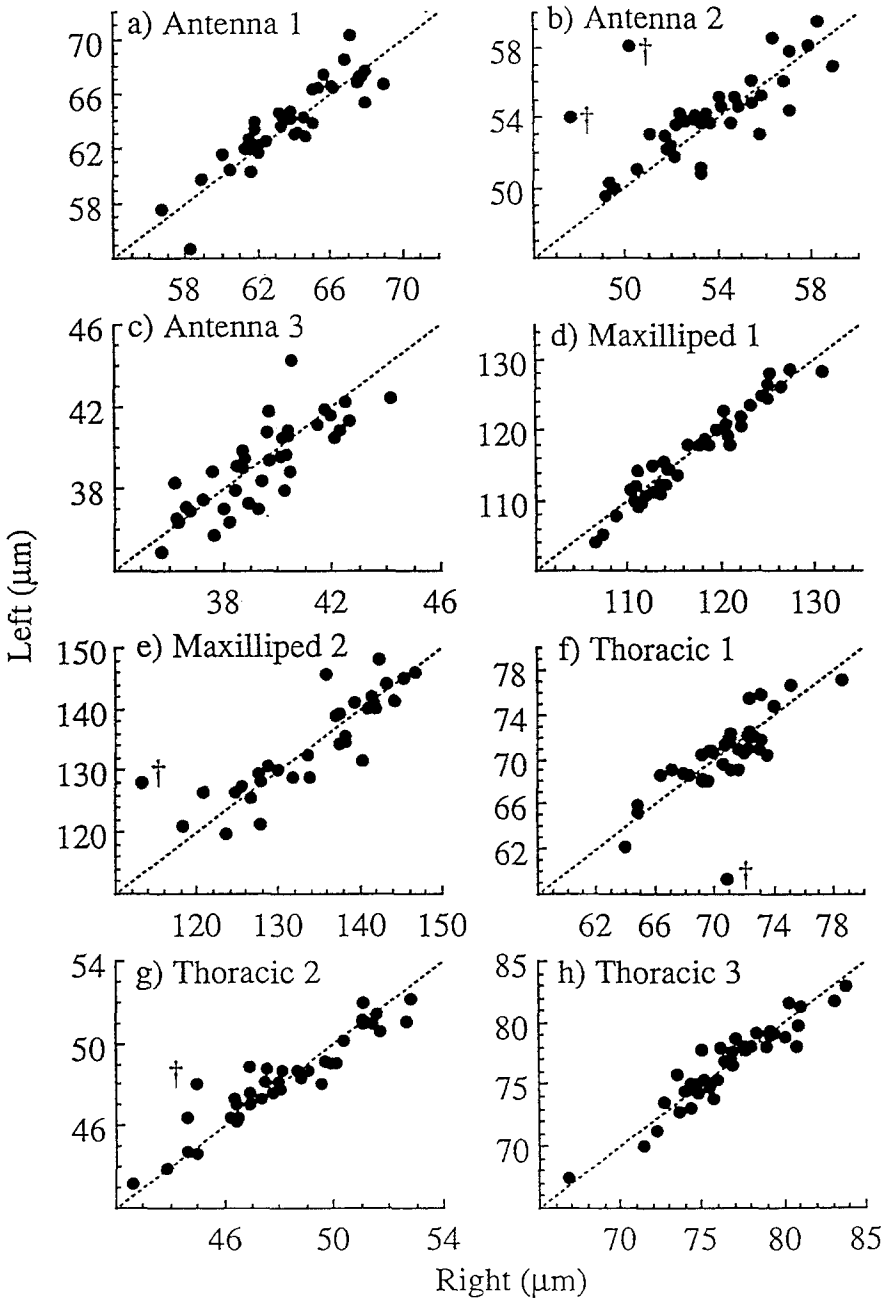

Fig. 3. Scatterplots of *R* versus *L*, uncorrected for body size variation, for eight traits in C-VI stage females of *Tigriopus californicus*. The mean measurement error ranged from 0.20 to 0.31 μm among the eight characters. Points indicated by † were excluded as extreme values from statistical analyses of asymmetry variation. Dashed line indicates perfect symmetry.

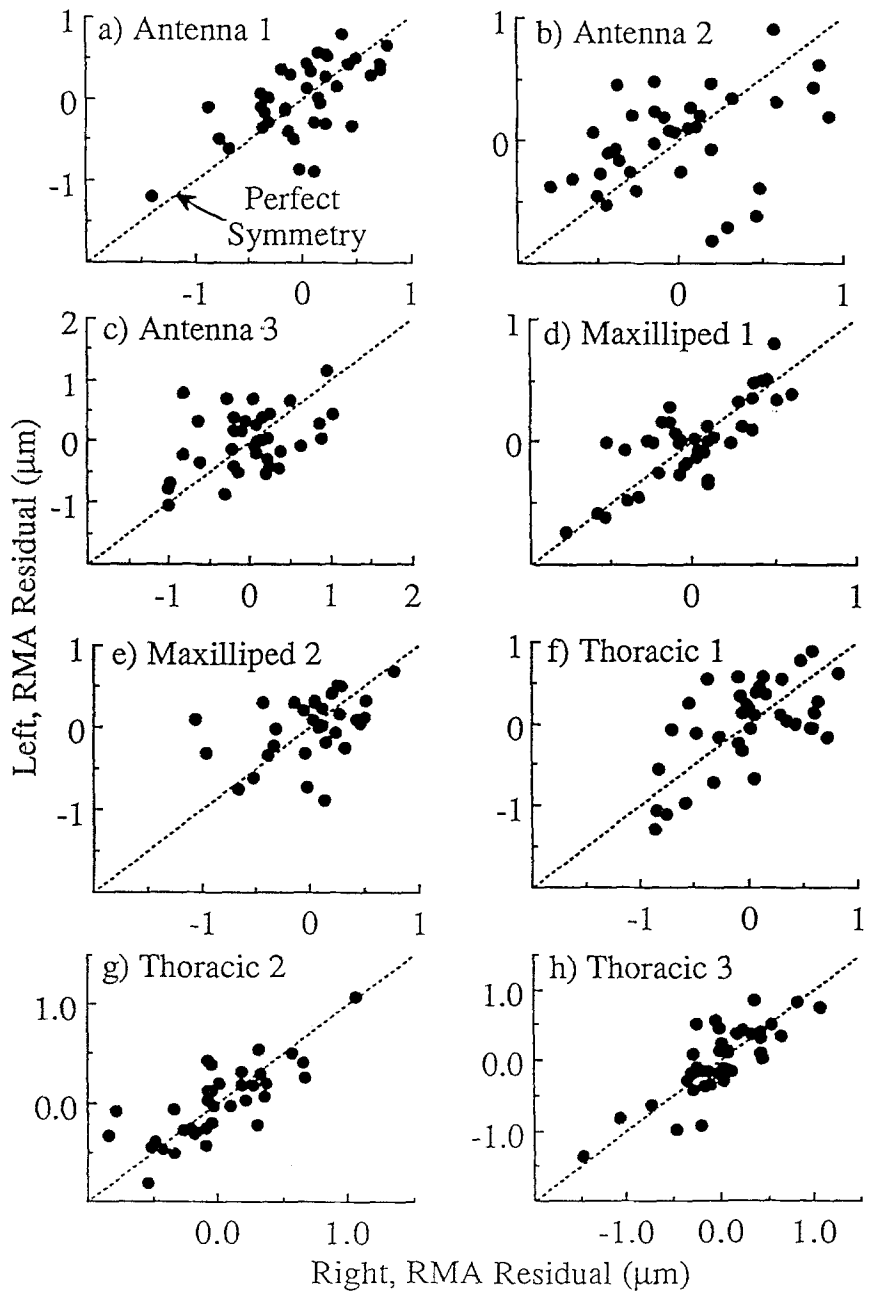

Fig. 4. Scatterplots of *R* versus *L*, after correction for body size variation, for eight traits in C-VI stage females of *Tigriopus californicus*. Data for each side are expressed as deviations from a model II regression on body size (see *Scaling vairation to a common body size* in text). Dashed line indicates perfect symmetry.

Table 3. Average character size, and statistics describing frequency distribution shape of $R - L$ in the harpacticoid copepod *Tigriopus californicus*.

		$(R + L)/2$	(R - L)						Other Indices**	
Character	N	Mean (SE)	Mean (SE)*	P	Skew (SE)	P	Kurtosis (SE)	P	σ_i^2	FA5
Antenna 1	38	63.8 (0.48)	-0.21 (0.221)	0.36	0.58 (0.383)	0.13	0.53 (0.750)	0.48	0.87	1.85
Antenna 2	38	53.9 (0.38)	-0.56 (0.325)	0.09	-1.54 (0.383)	<0.001	4.48 (0.750)	<0.001	1.92	4.23
Antenna 2 †	36	53.9 (0.39)	-0.20 (0.213)	0.35	0.89 (0.393)	0.026	0.14 (0.768)	0.85	0.73	1.63
Antenna 3	38	39.3 (0.33)	0.22 (0.212)	0.31	-0.66 (0.383)	0.088	0.69 (0.750)	0.35	0.81	1.71
Maxilliped 1	39	116.9 (1.01)	0.36 (0.257)	0.18	-0.30 (0.378)	0.43	-0.87 (0.741)	0.24	1.26	2.64
Maxilliped 2	32	134.1 (1.41)	-0.31 (0.788)	0.69	-1.05 (0.414)	0.012	2.45 (0.809)	0.003	10.01	19.33
Maxilliped 2§	31	134.5 (1.38)	0.16 (0.654)	0.81	-0.24 (0.421)	0.58	0.93 (0.821)	0.26	6.54	12.85
Thoracic leg 1	39	70.6 (0.47)	0.33 (0.377)	0.38	2.75 (0.378)	<0.001	11.46 (0.741)	<0.001	2.69	5.50
Thoracic leg 1§	38	70.7 (0.46)	0.04 (0.239)	0.88	-0.04 (0.383)	0.92	-0.76 (0.750)	0.31	1.02	2.12
Thoracic leg 2	37	48.2 (0.38)	-0.10 (0.151)	0.50	-1.00 (0.388)	0.011	1.89 (0.759)	0.014	0.38	0.84
Thoracic leg 2§	36	48.3 (0.39)	-0.02 (0.130)	0.89	-0.35 (0.393)	0.38	0.33 (0.768)	0.67	0.26	0.59
Thoracic leg 3	40	76.7 (0.51)	0.002 (0.176)	0.99	-0.14 (0.374)	0.72	0.44 (0.733)	0.55	0.57	1.21

Note. Computations were carried out prior to any correction for body size variation. The total number of individuals examined was 40, but some structures were missing or damaged on some individuals.
N - number of individuals measured; P - exact probability from a T-test comparing the observed values for the mean, skew, and kurtosis of the distribution of R - L versus the expected value of zero; * For comparison with other FA indices, Var($R - L$) will be N (SE)^2; ** σ_i^2 = non-directional asymmetry variance computed from ANOVA, and FA5 = index 5 =[$\Sigma(R - L)^2$]/N [from Tables 3b and 1 respectively of Palmer and Strobeck (1986)]; for a truly normal distribution these indices are related as follows: Var($R - L$) = FA5 = 2(σ_i^2); † re-computed excluding two extrema identified by Grubb's test for outliers (Sokal and Rohlf 1981); see Fig. 3). § re-computed excluding one extremum identified by Grubb's test for outliers (see Fig. 3).

for that trait (see LaBarbera (1989) for a nice overview and evaluation of various model II regression techniques). The size-standardized values for each side were computed as deviations perpendicular to the major axis of the RMA regression. Before performing the size-standardizations, five measurements were eliminated as anomalous (see Results).

All statistical analyses were conducted with Statview II (Ver. 1.04, Abacus Concepts).

Results

Measurement error and tests for normality. Average measurement error ranged from 0.20 to 0.31 μm (0.17 to 0.64% of mean character size) among the eight characters, and was independent of trait size. In addition, measurement error was only 17% as large as the nondirectional asymmetry variation on average (mean $\sigma_m^2/\sigma_i^2 = 0.17$), hence nondirectional asymmetry was highly significant for all characters ('Sides × Individuals', Table 2). We were thus justified in pursuing a more detailed analysis of this nondirectional asymmetry, which includes FA and all forms of covariant asymmetry (Palmer & Strobeck, 1986).

Directional asymmetry and departures from normality. Only one of the eight traits (Ant 2) exhibited a suggestion of directional asymmetry (P = 0.09, Table 2), but this was due to a single extreme specimen (Table 3, Fig. 2 legend). In the original data, four traits exhibited distributions of $R - L$ that departed significantly from normality (Ant 2, Mxp 2, Thc 1, and Thc 2; Table 3). A closer inspection of frequency distributions revealed that one extreme value (or in one case two; see Fig. 2 legend) was responsible for the apparent departure from normality. All five extrema were found to be significant outliers by Grubb's test (Sokal & Rohlf, 1981). When removed, none of the characters exhibited significant departures from normality except Ant 2 which exhibited a marginally significant skew (P = 0.026, Table 3). In summary, after eliminating a small number of extreme values (5 of a total of 301), the variation in $R - L$ for all traits was normally distributed about a mean of zero (Fig. 2). Thus no traits exhibited any statistical indication of

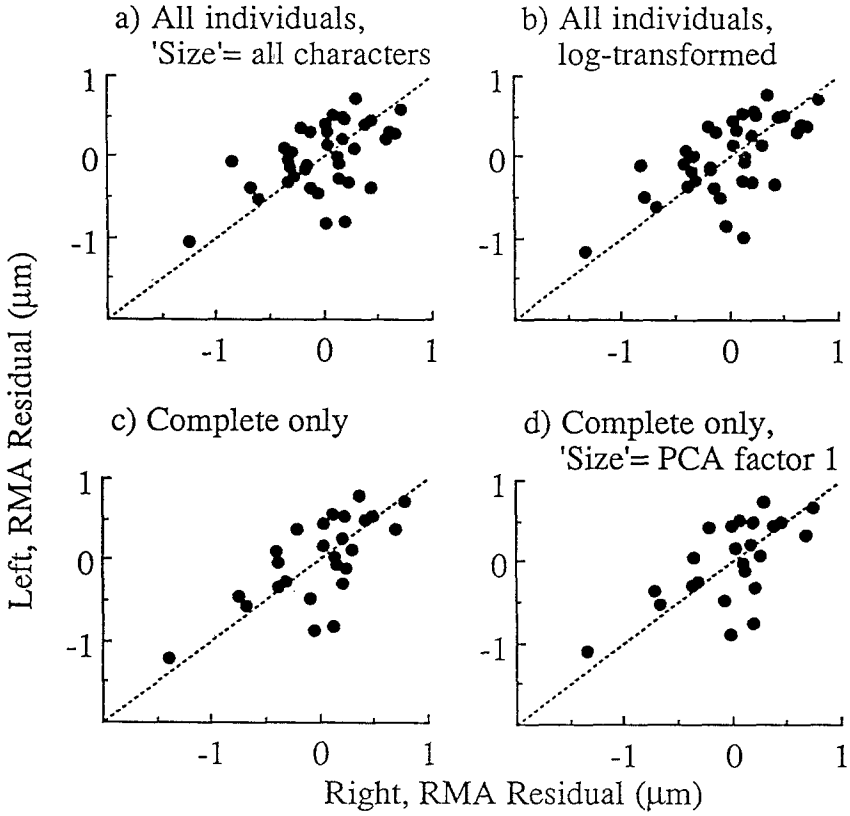

Fig. 5. Scatterplots of *R* versus *L* for Ant 1 of *Tigriopus californicus* only, illustrating the effects of using four differnt procedures to scale out body size variation (compare with Fig. 4a): (a) same procedure as for Fig. 4 except that 'size' was computed as the mean of *all* eight traits instead of all traits excluding Ant1, (b) same procedure as for Fig. 4 except that the raw data were log transformed before beginning the analysis, (c) same procedure as for Fig. 4 except that only individual with a complete set of measurements were used in the analysis (N = 26 vs. N = 31 - 40), (d) same procedure as for 5c except that scores for factor one from a principal components analysis were used as the body size metric.

directional asymmetry, skewed covariant asymmetry, or antisymmetry.

Tests for normal covariant asymmetry. In the original data, all eight traits exhibited highly significant positive correlations between *R* and *L* (Fig. 3). Some of this variation, however, was due to variation in overall body size, whereas some was due to *positive covariation* between sides at a given body size. When body-size was factored out, the predominant covariation remaining was still due to *positive covariation* between sides (Fig. 4). Hence, although we cannot exclude factors contributing to *offset variation* (σ_d^2 in equation 5) as the source of the variation perpendicular to the line of symmetry, the *Tigriopus* data revealed that this asymmetry was at least two to five times less than the *positive covariation* between sides at a given body size (σ_s^2 in equation 5).

To assess the sensitivity of this conclusion to our choice of procedure for scaling out overall size variation, we repeated the analysis four different ways on Ant 1. Although subtle differences were apparent (Fig. 5), the relative magnitudes of *positive covariation* between sides and *offset variation* did not appear to be sensitive to scaling procedure.

Discussion

Although conspicuously asymmetrical morphological traits occur in many otherwise bilaterally symmetrical organisms (Ludwig, 1932; Neville, 1976), the sequence by which they evolve from symmetri-

cal precursors is unknown. Rather than from sudden large mutations, macroscopic asymmetries seem most likely to have originated as small, statistical departures from symmetry that had a heritable basis. If true, studies of developmental stability that assume such statistical departures reflect only developmental noise must be aware of this potential concern.

Implications for studies of developmental stability based on fluctuating asymmetry

Studies of FA presume that differences between sides arise due to non-genetic *developmental noise* (σ_e^2 in equation 9). If individuals exhibit heritable variation for departures from symmetry, then differences in the extent of asymmetry variation among populations could arise via selection or drift. Furthermore, as outlined above, descriptions of FA based on frequency distributions of $R - L$ will not reveal certain forms of negative covariation between sides that may have a genetic basis (e.g. normal covariant asymmetry). These observations suggest caution when using statistical estimates of FA as a measure of developmental stability.

A note on terminology. The terms *developmental noise* and developmental stability are sometimes used as converses of each other in the FA literature, even though they refer to fundamentally different phenomena (Wright, 1920; Mather, 1953; Van Valen, 1962; Palmer & Strobeck, 1992). *Developmental noise* [= random developmental variability, or developmental disturbance of Zakharov (1989), p. 12] refers to the totality of non-genetic perturbations that deflect a developing system away from some ideal growth trajectory under a particular set of environmental conditions. In studies of FA, *developmental noise* refers to those non-genetic processes causing departures from the ideal of perfect symmetry (see *FA: Only developmental noise?* below). *Developmental noise* would seem unlikely to respond to selection.

Developmental stability, on the other hand, refers to the capacity of developing systems to resist or correct for the effects of *developmental noise*. Intrinsic phenomena influencing developmental stability (heterosis, genomic balance) do seem capable of responding to selection (Mather, 1953; Kindred, 1965; Rendel, 1967; Levin, 1970). When considering bilaterally symmetrical characters, *developmental noise* thus refers to the origin of deviations from symmetry whereas developmental stability refers to the capacity to correct such deviations.

Fluctuating asymmetry: The conventional metric for developmental stability. Fluctuating asymmetry has assumed prominence as a measure of developmental stability because of the intuitively appealing premises upon which this inference is based. Consider the paired first antennae of a copepod. In a sample of copepods, both the right and left antennae will exhibit variation in relative length. Some of this variation in an individual antenna will have a genetic basis. The remainder will have arisen as a product of perhaps several levels of environmental effects (see next section). By subtracting the length of the left antenna from that of the right, both the genetic and the environmental effects *common to both* will cancel out (Fig. 6*a*). For characters that exhibit a normal distribution of right-minus-left whose mean is zero, the variance in this distribution is routinely interpreted as a measure of developmental stability (Zakharov, 1992).

Fluctuating asymmetry: only developmental noise? Underlying all studies that relate levels of fluctuating asymmetry (FA) to levels of developmental stability is a common and crucial assumption: the subtle departure from symmetry in a given individual must *not* have been promoted by genetic factors (Palmer & Strobeck, 1992). For traits exhibiting FA, the difference between the right and left members of a bilateral trait-pair in an individual is assumed to have been caused exclusively by *developmental noise* or 'accidents during development' (Wright, 1920; Timoféeff-Ressovsky 1934; Mather, 1953; Waddington, 1957; Zakharov, 1989). Such accidents during development could arise, or be amplified, by non-genetic effects at several levels including: i) those at the level of the whole organism (e.g. extreme physical stress), ii) those having a greater impact on one side (e.g. injury, differential use), and iii) random cellular and sub-cellular noise [e.g. differences in cell division rates, thermal noise (Lewontin, 1983)].

The evidence cited most commonly to support the assumption that FA reflects only *developmental noise* is the apparent inability, under artificial selec-

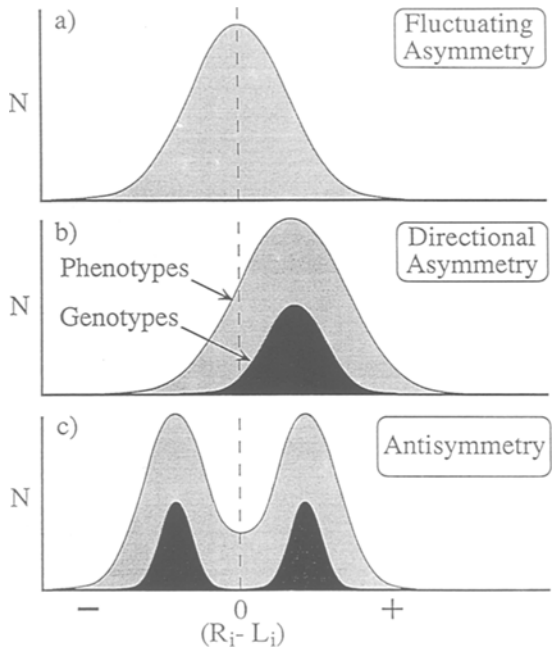

Fig. 6. Contributions of genetic and non-genetic factors to three forms of bilateral variation: (a) fluctuating asymmetry, (b) directional asymmetry, and (c) antisymmetry (from Palmer and Strobeck, 1992). Solid frequency distributions symbolize genetically induced variation, and stippled frequency distributions symbolize total phenotypic variation (including *developmental noise*). The exclusively stippled region under the curve is intended to symbolize environmentally induced variation only. Note that two aspects of these curves are not technically correct. First, the area under each should be the same (the area under any frequency distribution is one). Second, if environmental variation is added to underlying genetic variation, the combined distribution should be broader with a lower peak. We use this heuristic convention a) because we wish to emphasize that some subset of the total phenotypic variation has a genetic basis, and b) because it avoids introducing potentially distracting elements to the figures.

tion, to increase the extent of directional asymmetry (Palmer & Strobeck, 1986). But how strong is this evidence? Are there other ways in which gene action may cause subtle departures from symmetry and consequently compromise FA as a measure of developmental stability?

Directional asymmetry and all forms of covariant asymmetry: subtle asymmetries that may have a genetic basis. To draw conclusions about the extent to which stabilizing mechanisms can counter the effects of *developmental noise*, one must assume that no genes promote the departure of bilateral characters from symmetry. Should such genetic variation exist, differences among natural populations, or among laboratory lines exposed to selection, could arise due to changes in gene frequency at these loci influencing symmetry rather than due to differences in overall developmental stability.

If, for example, the antenna on the right side is larger on average than that on the left, the difference between them will, of course, not be zero (directional asymmetry, Fig. 6b). Within a sample, the variation in directional asymmetry seems likely to have at least some genetic basis (solid curve) although no studies to our knowledge have confirmed this by attempting to select for increased asymmetry of a trait already exhibiting some directional asymmetry.

If, alternatively, the antenna on one side is generally larger than that on the other, but the side which is larger varies randomly among individuals in a sample, then antisymmetry will result (Fig. 6c). As in the case of directional asymmetry, antisymmetry also seems likely to have at least some genetic basis (solid curve, Fig. 6c). See McKenzie and Clarke (1988) for very nice evidence for an antisymmetry polymorphism in the sheep blowfly, *Lucilia cuprina*. Similarly, Mather (1953, p. 332) suggests that some of the increased between-sides variance he obtained in response to selection for decreased developmental stability may in fact have been due to an increase in antisymmetry in his selected lines.

The difference between directional asymmetry and antisymmetry is not profound. It lies only in the *consistency* of the direction of departure for symmetry, not in its magnitude (note that the only difference between Fig. 6b and 6c lies in the value for the parameter p; see also equation 9). The only difference between them lies in how the side to be overdeveloped is specified: does overdevelopment always occur on the same side (directional asymmetry) or can it occur on either side (antisymmetry)?

Suggestions for studies of developmental stability. In many studies of FA a key goal is to compare differences in the levels of developmental stability in different populations. This is true in field studies, where differences in some measure of the variance of $R - L$ are used to infer different levels of environ-

mental or genetic stress (Leary & Allendorf, 1989; Parsons, 1990; Zakharov, 1992). It is also true in laboratory studies examining the effect of selection on developmental stability (Mather, 1953; MacKay, 1980; Leamy & Atchley, 1985). Such studies are particularly vulnerable to the confounding effects of genetically based asymmetries. However, experimental studies with genetically randomized treatment groups, such as those examining the effects of particular environmental stresses on developmental stability within the lifespan of an individual, should be less vulnerable to the confounding effects of genetically based asymmetries.

Because all forms of negative covariation between sides contribute to the variance in $R - L$ (equation 9; see also *A novel form of statistical asymmetry* above), and because the negative feedback implied by such negative covariation (see *Hypothetical pathways* below) seems likely to have a genetic basis, traits exhibiting various forms of covariant asymmetry should be given less weight or eliminated from consideration when drawing inferences about levels of developmental stability. Elsewhere we discuss in some detail the implications of other departures from normality for studies of developmental stability and suggest caution when drawing conclusions based on characters exhibiting such patterns of variation in $R - L$ (Palmer & Strobeck, 1992).

The same cautions should be exercised when traits are found to exhibit statistically significant directional asymmetry, since some of the between-sides variation may have a genetic basis (previous section). Figure 6b also makes clear why a 'correction' for directional asymmetry, by shifting the mean of the frequency distribution of $R - L$ to zero, is inappropriate in studies of developmental stability: such a correction is purely statistical and will not eliminate the fraction of the between-sides variation that may be genetically based.

The evolutionary origins of bilateral asymmetry

Hypothetical pathways. Purely statistical analyses of patterns of between-sides variation cannot, of course, indicate whether such variation has a heritable basis or not (e.g. see Lewontin, 1974), or what developmental mechanisms are involved. Whether a particular pattern of between-sides variation has a genetic basis, for example, requires an explicit attempt to measure heritability as in Mather (1953) and McKenzie and Clarke (1988). More studies of this type would be immensely informative about the potential evolutionary origins of bilaterally asymmetrical characters.

Table 4. Presumed developmental-genetic factors contributing to different forms of statistical asymmetries.

Form of statistical asymmetry	Side-bias control (G/E)	Bi-lateral offset (G/E)	Bi-lateral inhibi-tion (G/E)	Develop-mental noise (E)
Fluctuating asymmetry (see Figs. 1a,d)				X
Normal covariant asymmetry (see Figs. 1h,k)			X	X
Antisymmetry (see Figs. 1c,f)		X	X	X
Directional asymmetry (see Figs. 1b,e)	X	X	X	X

G - influenced by genetic factors; E - influenced by environmental or non-genetic factors (see *FA: Only developmental noise?* in text).

Although our model is only a simple one, we think three forms of bilateral variation may signal the presence of distinctly different heritable variation upon which natural selection could act: normal covariant asymmetry, antisymmetry, and directional asymmetry (Fig. 1h, 1c, and 1b respectively). However, some seem more likely than others as the starting point for the origin of macroscopic asymmetries because they would seem to require less developmental-genetic information.

Off all the major patterns of statistical asymmetries (Fig. 1, Table 4), fluctuating asymmetry (Figs. 1a, 6a) would seem to be the only one not likely to have a heritable basis (but see comments under *Normal covariant asymmetry: Confounding effects of positive covariation* above). Herein lies its appeal for studies of developmental stability (Palmer & Strobeck, 1986; Leary & Allendorf, 1989; Parsons, 1990; Zakharov, 1992). The remaining three would all seem to require some kind of genetic influence (Table 4).

On developmental grounds, we feel that normal covariant asymmetry (Fig. 1h) is the more likely first step in the evolution of macroscopic asymmetries. It would seem to require the least developmental-genetic information, because it could arise simply via some form of *bilateral inhibition* between sides (Table 4). Such inhibition is well documented in the development of conspicuous claw asymmetries in snapping shrimp (Mellon & Stephens, 1978) and lobsters (Govind, 1981), where it seems to involve action by the central nervous system. Whether asymmetry is regulated this way in other bilaterally asymmetrical organisms, or by some more complex developmental mechanism (Brown & Wolpert, 1990) remains to be seen.

Antisymmetry (Fig. 1c, 6c) would seem to be the next most likely precursor to macroscopic asymmetries. In addition to *bilateral inhibition*, however, some developmental mechanism would be required to specify the *bilateral offset*, or average departure from symmetry (Table 4). Finally, directional asymmetry (Fig. 1b, 6b) may be the least likely precursor of macroscopic asymmetries because it would seem to require an additional mechanism for consistently specifying which side was to become overdeveloped (*side-bias control*, Table 4).

This view of statistical asymmetries suggests several predictions that could be tested via a formal comparative study.

1. Among statistical asymmetries that depart from pure FA, the rank order of prevalence should be: normal covariant asymmetry > antisymmetry > directional asymmetry.
2. The number of independent evolutionary origins of macroscopic directional asymmetry should be fewer than those of either normal covariant asymmetry or antisymmetry.
3. Directional asymmetry should have evolved more frequently via ancestors exhibiting antisymmetry than directly from symmetrical ancestors (see also Mather, 1953, p. 334).
4. Among taxa with a clear plane of symmetry, macroscopic asymmetries in novel characters should arise more frequently in taxa with preexisting asymmetries in other characters, because a mechanism for the control of asymmetry would already exist.

Existing data. Our own study of statistical asymmetries in *Tigriopus* revealed no evidence for directional asymmetry or any form of covariant asymmetry. However, the observed residual *positive covariation* between sides in all traits, even after correcting for body size variation (Fig. 4), suggests that the genetic or environmental factors affecting limb development tend to affect both sides equally rather than each side independently or each side at the expense of the other.

Regrettably, although most studies of statistical asymmetries report whether directional asymmetry was detected in the characters examined, few report frequency distributions of $R - L$ or test for statistical departures of such frequency-distributions from normality (but see Ross & Robertson, 1990). Furthermore, as outlined above, such frequency distributions would not reveal normal covariant asymmetry even if it did exist. As a consequence, very little can be said about the prevalence of different forms of statistical asymmetries, in spite of the extensive literature on FA. We can only hope that future studies of FA will include a more detailed presentation and analysis of data. Without such information, generalizations about the prevalence of different patterns of statistical asymmetry will be slow in coming.

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