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FLUCTUATING ASYMMETRY: Measurement, Analysis, Patterns

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INTRODUCTION

"It might have been anticipated that deviations from the law of symmetry would not have been inherited."

Darwin (22:456)

With these words Darwin opened a brief paragraph citing observations antithetical to his supposition: anecdotal reports of the inheritance of characters missing from one side of the body. His initial hunch, however, has stood the test of time: Genetic studies have confirmed that where only small, random deviations from bilateral symmetry exist, the deviations in a particular direction have little or no measurable heritability (17, 47, 51, 65a, 73a, 74, 91). The genetic basis of bilateral symmetry thus appears to differ fundamentally from that of virtually all other morphological features.

Why, then, all the recent (and not so recent) interest in such minor, nondirectional deviations from bilateral symmetry [*fluctuating asymmetry* (FA); 60 cited in 99]? Four reasons. First, FA relates in a curious way to what is perhaps *the* major unsolved general problem in modern biology: the orderly expression of genotypes as complex, three-dimensional phenotypes. As was emphasized in a flurry of activity in the mid to late 1950s, and many times since, FA provides an appealing measure of 'developmental noise,' or minor environmentally induced departures from some ideal developmental program (101). Its appeal derives from an a priori knowledge of the ideal: perfect bilateral symmetry. For unilateral characters, the ideal is rarely known (but see 1, 2, and 59 for one possible approach). A second reason for interest in

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FA is that, even though deviations from symmetry *in a particular direction* of an otherwise symmetrical trait have little or no heritable basis, both the likelihood that an individual departs from bilateral symmetry and the degree to which it departs from bilateral symmetry *in either direction* often do appear to have a heritable basis (36, 48, 61, 65, 66, 76; but see 8, 51, 64, and 91 for negative results).

Third, over the last few years interest has been fueled by repeated observations of statistically significant associations between FA and protein heterozygosity: the more heterozygous the individual (49, 50) or the population (10, 44, 51, 53, 87, 100), the lower the FA. In other words, for reasons not yet clear, increased heterozygosity appears to provide increased 'buffering' against environmentally induced perturbations during development (55; reviewed in 67). Fourth, in apparant contradiction to the last pattern, two recent studies (32, 52) have reported increased FA in between-species hybrids compared to that of either parental species, even though the hybrids were more heterozygous than their parents (see also 105 cited in 32). Hence, whatever canalizing role heterozygosity may play within species, such canalization appears to be disrupted with the mixing of 'coadapted gene complexes' in interspecies hybrids (24).

Fluctuating asymmetry is one of three types of asymmetry, each characterized by a different combination of mean and variance of the distribution of right-minus-left ($R - L$) differences (99). *Directional asymmetry* reflects a consistent bias of a character within a species towards greater development on one side of the body than on the other; the coiling and associated anatomical asymmetry of gastropods or the asymmetry of flatfish (39) are examples of such. Directionally asymmetric traits exhibit normally distributed $R - L$ differences about a mean that is significantly either greater than or less than zero. *Antisymmetry* is distinguished by a platykurtic (broad peaked) or bimodal distribution of $R - L$ differences about a mean of zero. In male fiddler crabs, for example, the oversized signalling claw, which is much larger than the opposing one, occurs with approximately equal frequency on both the right and left sides in nearly all species (21a). *Fluctuating asymmetry* differs from the prior two because it has a normal distribution of $R - L$ differences whose mean is zero.

Variation in FA has been examined in a rather remarkable variety of traits and organisms including: teeth in humans (8, 33, 70, 73a; reviewed in 83), in rats and mice (6, 7, 79, 80, 81, 82, 99), and in extinct horses (99); palm ridge counts (42), fingertip ridge counts (63, 90), and ear lobe lengths (90) in humans, limb bones in humans (43, 78) and martens (43); cranial traits in species of large cats (103), rhesus macaques (66), muskrats (72) and kangaroo rats (90); various skeletal elements (34, 47, 48, 80, 91, 96) and mustachial vibrissae (90) in rats and mice; wing bones in birds (2, 90); numerous metric

and meristic traits in lizards (30, 40, 86, 87) and in fish (3, 5, 29, 32, 36, 39, 41, 49, 50, 51, 52, 53, 69, 97, 98, 100); sternopleural chaetae number (9, 12, 61, 65, 76, 90, 92, 93, 94, 99) and other features (10, 99, 102) in *Drosophila;* wing lengths in honeybees (13) and houseflies (90); wing and other features in butterflies (64, 89); antennal length in dipteran flies (46); and labial palps and siphonal papillae of freshwater bivalves (44).

Rather than detail the many specific contributions that have been made in this area (see reviews in 67, 71, 90) or discuss the causes (58, 83, 88), adaptive value (16) or relation to species formation (56) of variation in FA, we have chosen to discuss three issues that arose in our survey of the literature. The first is the diversity of FA indexes: In our survey we discovered an almost bewildering variety, which frustrated our attempts to compare results quantitatively across studies. We point out both the features common to and some of the important differences among these indexes. The second issue is statistical analysis of FA variation. We illustrate the use of analysis of variance for obtaining the best estimate of FA, and its use for separating FA from directional asymmetry, one form of antisymmetry, and measurement error. We also discuss a particularly thorny problem that arises when comparing FA among samples: How should differences in character size be handled?

The third issue we address is the implication of certain observed patterns of FA variation. Here we focus on the relationship between population structure (linkage disequilibrium, inbreeding, and population size) and correlations observed between FA and heterozygosity. These include: (*a*) the apparent relationship between FA and heterozygosity at individual loci (e.g. what can one conclude when individuals heterozygous at a particular locus are significantly less asymmetric than individuals homozygous at that locus within a population?), and (*b*) the apparent inconsistency between the patterns of concordance of asymmetry of several traits *among individuals within a population* versus such concordance *among populations*. (For example, if the supposed buffering effects of heterozygosity are organism-wide, why is asymmetry of one trait not in general a very good predictor of asymmetry in other traits *on the same individual?* This is so even though the average asymmetry of a trait is frequently a good predictor of the average asymmetry of other traits when compared *across populations*. See Table 4 for summary.). Finally, we review some novel potential applications of FA as an inferential tool.

MEASURING FLUCTUATING ASYMMETRY

Diversity of FA Indexes

A rather remarkable number of different indexes of FA have been used in previous studies; we have found at least 22. These 22 different indexes,

however, are all largely variants of 9 fundamental ones (Table 1). The asymmetry of a given character on an individual i (A_i) has been measured as either the absolute value of the difference between right and left ($|R_i - L_i|$), the signed difference ($R_i - L_i$), or the ratio of the measurements from each side of an individual (R_i/L_i). Each of these measures has served as the basis of one or more indexes used to analyze asymmetry differences among samples; the main difference is whether or not, or how, variation in overall character size is handled (Table 1). Some do not correct for differences in character size (Indexes 1, 4, and 5). Those that do correct for size do so either individual by individual (Indexes 2, 6, 8 and 9) or at the level of the population (Indexes 3 and 7). The most commonly used indexes are 1 and 4 (Table 1).

Discriminatory Ability of FA Indexes

TEST DISTRIBUTIONS Given their variety, how effective are these nine indexes at detecting true differences in FA among samples? To address this problem, we simulated samples for which we knew differences in the level of FA. Then we tested the ability of each index to discriminate these known differences. We assumed that FA arose as a product of uncorrelated, random disturbances experienced during development by each member of a bilateral pair of characters, as would be expected if FA was produced by thermal noise (58, 88) or by random accidents during development (65). These disturbances were assumed to cause each member to deviate from a mean character size determined by the size of the individual. We also assumed that directional asymmetry reflected an invariant, genetically fixed difference between the right and left sides. In these simulations we did not incorporate any antisymmetry. We did, however, consider two patterns of variation in FA with size: FA independent of size, and FA proportional to size.

For FA independent of character size, sides were simulated as

$$R_i = \mu + D/2 + s_i + r_i, \text{ and} \qquad 1.$$

$$L_i = \mu - D/2 + s_i + l_i, \qquad 2.$$

where R_i and L_i represent for individual i the sizes of the right and left members of a bilateral pair of characters respectively, where μ is the mean character size of the population, where D is the amount of directional asymmetry ($D/2$ was added to right and subtracted from left to keep character size independent of directional asymmetry), where s_i is a deviation in the character size of individual i from the population mean [drawn from an open interval, uniform distribution ($-S/2 < s_i < +S/2$), obtained from IMSL routine GGUBS], and where r_i and l_i are the deviations of the right and left sides respectively from the mean character size of individual i [$\mu + s_i$; each was

Table 1 Relationships among FA indices[a]

	Asymmetry measure on individual					
	$A_i = \lvert R_i - L_i \rvert$		$A_i = (R_i - L_i)$		$A_i = (R_i/L_i)$	
	Index Number	Index for population	Index Number	Index for population	Index Number	Index for population
No scaling by character size	1	$\frac{\sum (A_i)}{N}$	4	$\mathrm{var}(A_i)$	—	—
			5	$\frac{\sum (A_i)^2}{N}$		
Character size scaled by individual	2	$\frac{\sum \left[\frac{(A_i)}{(R_i + L_i)/2}\right]}{N}$	6	$\mathrm{var}\left[\frac{A_i}{(R_i + L_i)/2}\right]$	8	$\mathrm{var}[\log(A_i)]$
Character size scaled by population	3	$\frac{\left[\frac{\sum (A_i)}{N}\right]}{\left[\frac{\sum (R_i + L_i)}{2N}\right]}$	7	$\frac{\mathrm{var}(A_i)}{\left[\frac{\sum (R_i + L_i)}{2N}\right]}$	—	—

Index 9 = $1 - r^2$ (where r = correlation between R_i and L_i)

[a] A_i = Asymmetry of a particular character for individual i. R_i, size of right side. L_i, size of left side. N, number of individuals in a sample. var, variance of Users of each index (modifications noted in parentheses):

Index 1: 4, 5, 9(but not N^{-1}), 12, 40, 41, 44, 69, 76, 86, 87, 89, 92(but not N^{-1}), 93
Index 2: 41[but use $A_i/(R_i + L_i)$], 72, 77, 93 [but use $A_i/(R_i + L_i)$], 94, 100 [but use $A_i/(R_i + L_i)$]
Index 3: 86, 87
Index 4: 3, 4, 10, 29, 33, 47(except 2^{-1}), 48, 61, 79, 83 (SD), 98
Index 5: 6(exc. $2N^{-1}$), 12, 65, 75, 76, 97
Index 6: 2(but S.D. · 100), 3[but use $A_i/(R_i + L_i)$], 13, 29[but use $A_i/(R_i + L_i)$], 75, 91
Index 7: 4[exc. (population mean^2)$^{-1}$], 32, 98[exc. (population mean^2)$^{-1}$]
Index 8: 47, 91(except not log), 102(except larger/smaller), 103(exc. $\lvert \log R_i - \log L_i \rvert$)
Index 9: 4, 6, 42(r only), 47($1 - r$), 80

drawn from an independently created normal distribution of mean zero and variance $a/2$, from IMSL routine GGNML; a was divided by 2 so that var($R_i - L_i$), the underlying FA variance in the absence of any size variation, would equal a]. For FA increasing proportional to character size, sides were simulated as:

$$R_i = \mu + D/2 + s_i + [P(\mu + s_i)\, r_i], \text{ and} \quad 3.$$

$$L_i = \mu - D/2 + s_i + [P(\mu + s_i)\, l_i], \quad 4.$$

where P is a constant of proportionality.

To assess the relative discriminatory ability of the nine indexes at extracting true differences in FA from our computer-generated samples, we varied the range of character size (S), the proportionality of FA to character size (P), directional asymmetry (D), and FA (a). The mean character size of a sample was held constant throughout ($\mu = 100$). We calculated each index from the same sample of 20 individuals obtained for various combinations of S (0, 10, 20, 60, 100), P (0.01), D (0, 0.1, 1, and 5), and FA (a = 1, 2, 3, 4, and 5; thus, the FA standard deviation $\sqrt{a} = \sqrt{3}$ is used below to achieve dimensionless measures for comparing FA with size variation and directional asymmetry). A sample size of 20 was chosen because it was commonly used in the studies we examined; we note where variation in sample size may affect our qualitative conclusions. The above procedure was repeated 100 times using different distributions of random numbers. The ability of each index to distinguish among the 5 levels of FA was then evaluated separately for each combination of the remaining parameters by conducting a one-way analysis of variance [ANOVA Groups = level of FA (a), Within = 100 trials; BMDP7D 23] on log-transformed values. The average F value from 5 such one-way ANOVAs was used as a measure of the degree of separation each index achieved among FA groups; F values were used because they allowed us to assess power without assuming any a priori function relating each index to the parametric FA values. Since each index was essentially estimating a parametric variance, and estimates of variances are not normally distributed, all values were log transformed both to normalize and to homogenize them across FA groups (85).

SIZE VARIATION ABSENT The results of the analyses when no character size variation was present ($S = 0$; Figures 1a,b) were surprising. Except for Index 9, whose F was indistinguishable from zero, the F values for the various indexes, and hence their discriminatory powers, differed by less than 10% (Table 2). Index 5 exhibited consistently higher F values by 5–6%, while those remaining differed by only about 2% among themselves; those based on

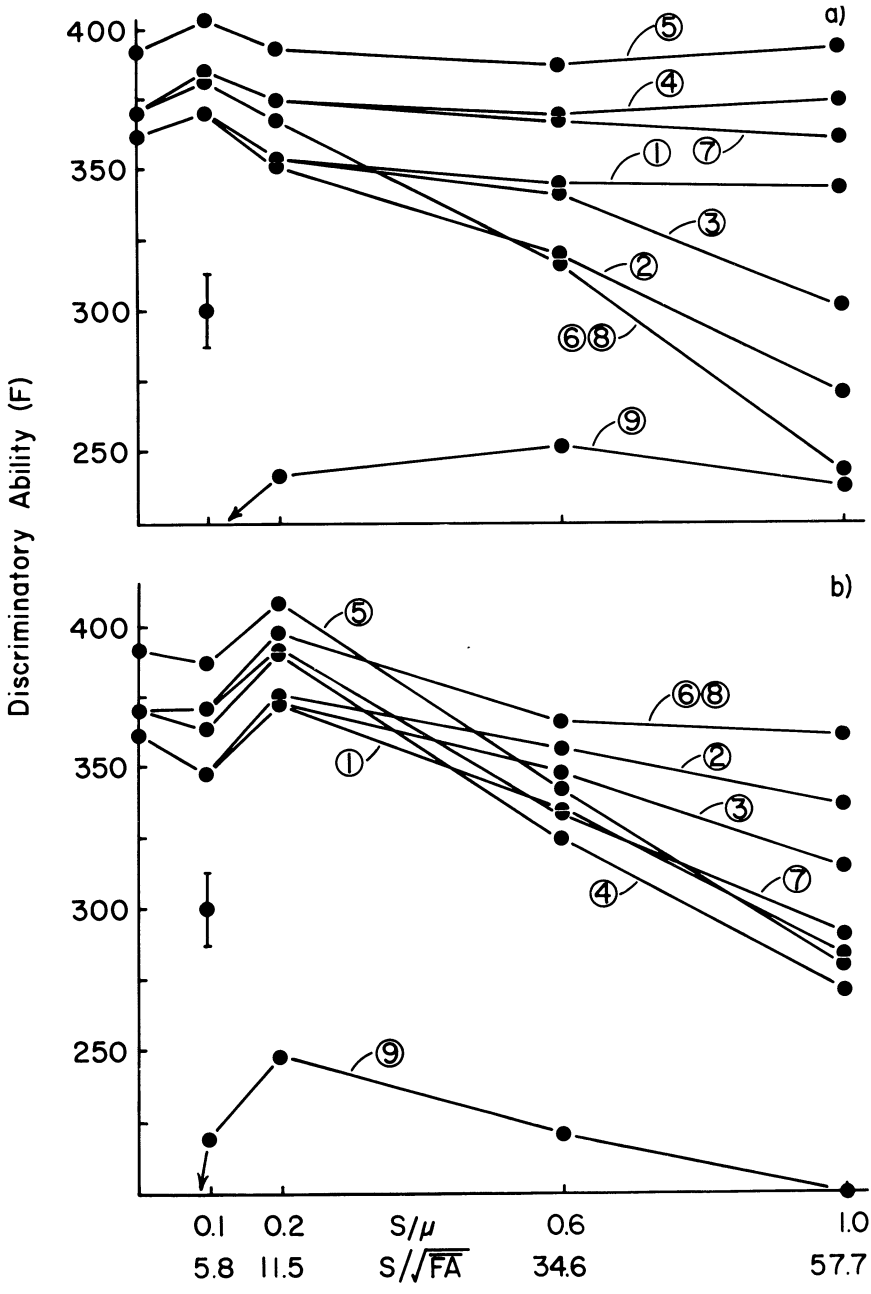

Figure 1 Discriminatory ability (mean F value from one-way ANOVA) of FA indices as a function of the range of size variation: (*a*) FA independent of character size (Equations 1 and 2), (*b*) FA increasing proportional to character size ($P = 0.01$, Equations 3 and 4). S, character size range (0, 10, 20, 60, 100); μ, mean character size (100); FA, fluctuating asymmetry variance (a; 1, 2, 3, 4, 5, hence $\sqrt{a} = \sqrt{3}$), circled numbers correspond to the numbered indices of Table 1. Solitary points with error bars illustrate ±SE for five runs. For these simulations, $D = 0$.

unsigned $R - L$ differences (Indexes 1–3) were on average the least able to distinguish true FA differences.

The higher discriminatory ability of Index 5 reflects the one additional degree of freedom it has over Index 4: Because Index 5 estimates the variance of a distribution whose true mean is zero, it does not lose (as Index 4 does) the one degree of freedom required to estimate this mean. Since the mean $R - L$ difference in these simulations was truly zero, and since sample sizes were 20, one additional degree of freedom is equivalent to a 5% increase in sample size. This yields a 5% increase in the accuracy with which FA is estimated; the larger the sample sizes, the smaller the difference between Indexes 4 and 5.

EFFECT OF SIZE VARIATION Differences among the indexes in their discriminatory ability became more apparent as the character size range (S) was

Table 2 Relative discriminatory ability of FA indices with increasing size variation[a]

	Relative character size variation ($S/\sqrt{a}$) with FA independent of size				
Index[b]	0	5.8	11.5	34.6	57.7
1	92	92	90	89	87
2	92	91	89	83	69
3	92	92	90	88	77
4	94	95	95	95	95
5	100	100	100	100	100
6	94	94	94	82	62
7	94	95	95	95	92
8	94	94	94	82	62
9	<1	54	62	65	60
	Relative character size variation ($S/\sqrt{a}$) with FA proportional to size ($P = 0.01$)				
Index[b]	0	5.8	11.5	34.6	57.7
1	92	90	91	91	79
2	92	90	92	97	93
3	92	90	91	95	87
4	94	94	95	89	75
5	100	100	100	93	77
6	94	96	97	100	100
7	94	96	96	91	80
8	94	96	97	100	100
9	<1	57	61	60	56

[a]Tabled values are the ratio (times 100) of the F of a particular index over the F for the index with the maximum discriminatory ability for each parameter combination; from Figures 1a,b.

[b]See Table 1 for indexes.

increased. First, as noted by others (4, 12), the correlation of R on L (Index 9) behaved fundamentally differently from all the others; over the parameter range tested, it never achieved power comparable to any other index (Figure 1a,b, Table 2). Further, Index 9 is very sensitive to size range (4) and hence is generally not very useful.

Not unexpectedly, of the remaining indexes, those scaling out character size variation (Indexes 2, 3, 6, and 8) became less reliable than those not scaling out size (Indexes 1, 4, and 5) when FA was independent of size (Equations 1 and 2, Figure 1a, Table 2). Index 7 appeared to be an exception, but this was an artifact of keeping the mean character size of the population (μ) constant (see Table 1). Similarly, with FA increasing proportional to character size ($P = 0.01$ in Equations 3 and 4), those indexes scaling out character size were more reliable than those not (Figure 1b). Although the results were not presented, increasing P to 0.05 had no effect on these qualitative conclusions.

The discriminatory ability of particular indexes in the presence of size variation depended on whether FA was independent of character size or not. With FA independent of size (Equations 1 and 2; Figure 1a), F values for Index 5 were consistently the highest, ranging from 5% to 38% above the remaining indexes, depending on the level of size variation (S). As in the absence of size variation, those indexes based on the variance of signed $R - L$ differences were, on average, more powerful than the equivalent ones based on the mean of the unsigned differences (Indexes 4 or 5 vs 1; Index 7 vs 3).

When FA increased with size ($P = 0.01$, Equations 3 and 4), Index 5 yielded the highest F values when size variation was small relative to FA (e.g. $S/\sqrt{a}$ less than 15; Figure 1b). With increased size variation, Indexes 6 and 8 became the most reliable for discriminating true differences in FA. However, over the parameter range tested, these latter two indexes surpassed the remaining ones by only 7–25% (Figure 1b, Table 2).

EFFECT OF DIRECTIONAL ASYMMETRY One of the more common causes of departures of bilateral variation from true fluctuating asymmetry is varying levels of directional asymmetry (6, 30, 43, 47, 64, 73, 99). Not all the indexes were sensitive to directional asymmetry, but some were very sensitive. As expected from their form (Table 1), Indexes 4, 6, 7, 8, and 9 remained unaffected by directional asymmetry in the absence of size variation ($S = 0$; Figure 2a). Indexes 4, 7, and 9 also remained unaffected at a high-size variation relative to FA ($S/\sqrt{a} > 50$) when FA was independent of character size (Equations 1 and 2; Figure 2b). Notably, Index 5 was very sensitive to directional asymmetry for these parameters (Figures 2a,b); even though it was 5%–38% more powerful than all the other indexes in the absence of directional asymmetry (Table 2). This higher discriminatory ability was quickly lost, however, as the ratio of directional asymmetry to FA ($D/\sqrt{a}$) exceeded

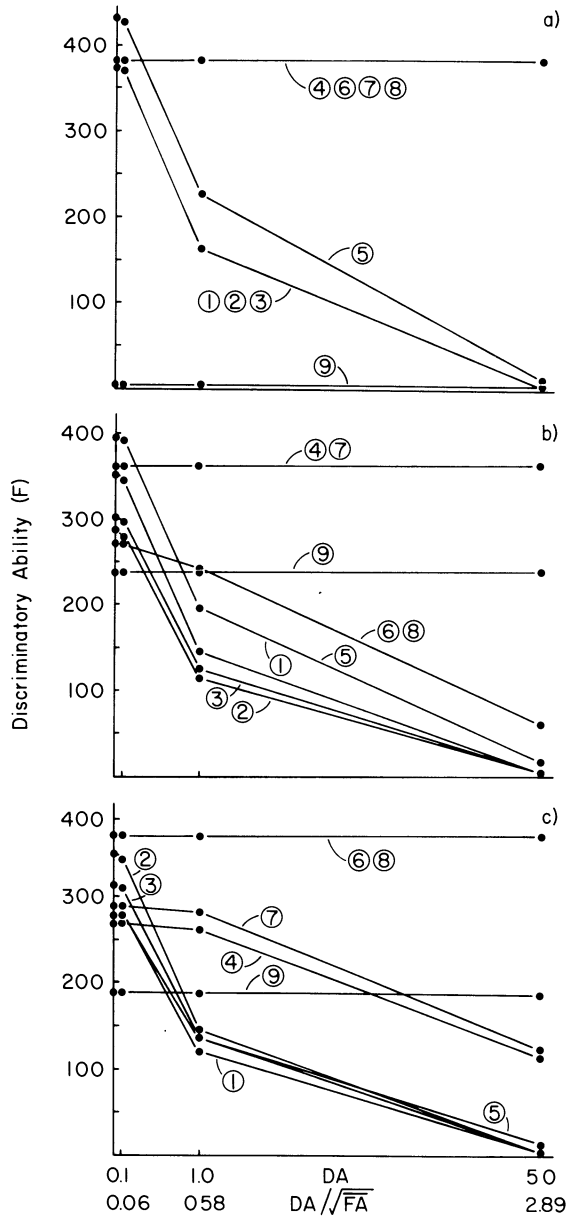

Figure 2 Discriminatory ability (F value from one-way ANOVA) of FA indices as a function of directional asymmetry (DA = D of Equations 1–4): (*a*) with no character size variation ($S = 0$), (*b*) high character size variation ($S = 100$) and FA independent of character size (Equations 1 and 2), (*c*) high character size variation ($S = 100$) and FA proportional to character size ($P = 0.01$, Equations 3 and 4). Other symbols as in Figure 1.

0.2. Thus, minor directional asymmetry may seriously weaken what is otherwise the most powerful index for distinguishing true differences in FA when FA is independent of character size.

With FA proportional to character size, most indexes lost power as directional asymmetry increased (Figure 1c). However, the two that were most effective (Indexes 6 and 8) in the absence of directional asymmetry remained unaffected.

Recommendations

The above analyses reveal that those indexes based on the variance of $R - L$ (Indexes 4, 5, 6, or 8) are more useful for detecting true differences in FA among samples than those based on the mean of the absolute values of $R - L$ (Indexes 1–3, Table 2). This is consistent with the conclusion of Kendall & Stewart (44a, pg. 20–21): Although the mean of the absolute values of deviations (multiplied by $\sqrt{\pi/2}$) is an unbiased estimator of the parametric standard deviation of a normal distribution, it is only 87.6% as efficient as the sample standard deviation. Because of its statistical power, we outline below a protocol using a modified analysis of variance that yields the best estimate of the FA variance.

In one situation, however, Index 5 may be preferred over this procedure: for small samples sizes. For small sample sizes ($N < 25$), *if* FA is independent of character size, and *if* directional asymmetry and antisymmetry are absent (see tests below), the index most able to distinguish true differences in FA among samples is Index 5 (Figure 1a, Table 2). Because of its one additional degree of freedom, the percentage increase in discriminatory ability of Index 5 over Index 4 will be approximately $100/N$, where N is the size of the sample for which FA is being estimated. This additional degree of freedom is lost in the analysis of variance because it is required to estimate the parametric mean.

ANALYZING VARIATION IN FLUCTUATING ASYMMETRY

The preceding discussion highlights the sensitivity of particular FA indexes both to character size variation and to directional asymmetry. It also underscores the overall greater discriminatory power of those indexes based on the variance of $R - L$. We thus suggest the following protocol, which yields the best estimate of $\text{var}(R - L)$ for a sample: It is essentially analogous to calculating Index 4 in the absence of any size dependence of FA or calculating Index 6 where FA is proportional to size and size is scaled out.

Testing for Size Dependence of FA Within Samples

As many have noted (3, 5, 6, 32, 51, 52, 65, 75, 76, 98), testing for a relationship between the magnitude of FA and character size is an essential

first step. Because of the many possible ways in which FA may vary with size, we recommend an inspection of plots of $(R_i - L_i)$ versus size (either $(R_i + L_i)/2$, or some other independent measure of body size). If the spread of $R - L$ differences varies (most likely, it increases) with character size, then some form of scaling is indicated; the transformation to be used will depend on the pattern of variation. If the spread increases linearly with size, we suggest dividing by size and replotting to see if size scaling is complete. If the spread increases nonlinearly, then the R_i and L_i values should be divided by size and transformed (e.g. log or square root) so that no relationship with size remains. Regression analysis of $|R_i - L_i|$ (31) or possibly of $\log|R_i - L_i|$ (to homogenize the variance) against size may also be used to detect size dependence. [But see the cautions of Gujarati (35:204).] For reviews of analyses of residuals and heteroscedasticity (unequal variances), with suggestions for transformations, see 20 and 35 respectively.

ASSUMPTIONS BEHIND SIZE SCALING The relationship of FA with character size is perhaps the most troublesome aspect of analyzing for differences in FA among samples. An important assumption behind the scaling out of size dependence is that such scaling does not reduce or eliminate desired information about real differences in FA among samples. However, since the variance for a character (and hence the FA if it were bilaterally paired) often increases with increasing size (45, 76), such size dependence could distort differences in FA among populations whose mean sizes or size ranges differed.

Correlations of FA with two factors, fitness and growth rate, may result in information being lost. Since both the level of FA (50, 51, 53) and heterozygosity (24, 25, 26, 57) can be correlated with fitness, larger individuals (those that have survived longer) in species with indeterminate growth may represent a sample biased towards higher heterozygosity. Similarly, since in some organisms the development rate (21) or growth rate (reviewed in 67) increases with increasing heterozygosity, larger individuals of a given age will be more heterozygous than smaller ones. For both cases, if increased heterozygosity in turn results in lower FA, as is often observed (e.g. see Table 4 below), then size scaling may reduce true differences between samples of different mean sizes or size ranges.

Assessing Measurement Error, Directional Asymmetry, and Antisymmetry Within Samples

Differences in FA among samples are generally small (7, 32, 43). Thus, confounding factors (measurement error, directional asymmetry, and antisymmetry) may make up a sizable fraction of the between-sides (within-individuals) variance in a sample. For example, although rarely reported, measurement error as a percentage of nondirectional asymmetry can be high: up to 10% and 22% for asymmetry of metric and meristic characters in a

centrarchid fish (3); up to 11% and 25% for fluctuating dental asymmetry in wild mice (7) and in human skeletons exhumed from Nubian cemeteries (33) respectively; and up to 33% and 76% in asymmetry of color patch dimensions and wing length respectively in a nymphalid butterfly (64). In addition, antisymmetry may also inflate the between-sides variance and hence may distort estimates of FA. To be confident of conclusions drawn about the presence or absence of differences among samples, these factors must be estimated or ruled out.

We suggest an approach modified from Leamy (47) which has several advantages: use a two-way, mixed-model analysis of variance [sides (fixed) × 'genotypes' (random)] with repeated measurements of each side (Table 3). In practice 'genotypes' would correspond to individuals except as outlined below. Note that this is *not* equivalent to a two-way ANOVA with replication: Repeat measurements of the same side are not replicates since they are not independent and do not provide any additional information about the variation of a character on a given side. True replicates (Table 3a) are only possible either: (*a*) where two or more individuals with identical genomes are available, as would occur with clonemates of parthenogenic species, or (*b*) where two or more units in modular organisms, such as colonial invertebrates or plants (77), are being compared. Caution must thus be exercised when testing MS.

Even if clonemates (individuals in Table 3a) are available, however, the interpretation of variance components assumes that character size differences among clonemates are not a product of environmentally induced differences in body size. In other words, 'developmental noise' is *not* calculated on $R - L$ differences; rather, it is calculated as the variance of a given side among clonemates, averaged across all sides and genotypes. Consequently, the apparent magnitude of developmental noise (nondirectional asymmetry or σ_i^2) may be inflated over its true value owing to environmentally induced variation in body size and hence character size. The significance of environmentally induced variation in overall body size may be tested by conducting a sides × individuals ANOVA without replication (Table 3b) for each genotype separately as outlined below. Here again, however, if size variation is significant, any dependency of FA on character size must be eliminated.

Two aspects of the expected mean squares (MS) from this ANOVA (Table 3) should be noted. First, all higher order MS include *both* variation due to measurement error (σ_m^2) and variation due to nondirectional asymmetry (σ_i^2; doubling MS_{sj} (Table 3b), in fact, actually yields Index 4 (Table 1). The expected MS in Table 3 thus make explicit how the confounding effects of both antisymmetry and measurement enter into the indexes of Table 1.

Second, if clonemates are available, two types of antisymmetry are revealed, only one of which may be partitioned out of nondirectional asymmetry. Antisymmetry I is the sides × genotypes (i.e. clones) interaction (σ_{sj}^2,

Table 3 Expected mean squares (MS) from ANOVA and the interpretation of their tests[a]

Source of variation	Label	*df*	Expected mean squares	Mean squares used to test for:
A. *I* replicate individuals per genotype (e.g. clonemates)				
Sides (*S*)	MS_s	$(S - 1)$	$\sigma_m^2 + M\left(\sigma_i^2 + I\,\sigma_{sj}^2 + \frac{IJ}{S-1}\sum \alpha^2\right)$	Directional asymmetry
Genotypes (*J*)	MS_j	$(J - 1)$	$\sigma_m^2 + M(\sigma_i^2 + IS\sigma_j^2)$	Size or shape variation
Interaction	MS_{sj}	$(S - 1)(J - 1)$	$\sigma_m^2 + M(\sigma_i^2 + I\sigma_{sj}^2)$	Antisymmetry I
Individuals (*I*)	MS_i	$SJ(I - 1)$	$\sigma_m^2 + M\sigma_i^2$	Nondirectional asymmetry (Antisymmetry II + FA/2)
Measurements (*M*)	MS_m	$SJI(M - 1)$	σ_m^2	Measurement error

B. Single individual per genotype (usual case; see Footnote b)

Sides (S)	MS_s	$(S - 1)$	$\sigma_m^2 + M\left(\sigma_i^2 + \frac{J}{S-1}\sum \alpha^2\right)$	Directional asymmetry
Genotypes (J)	MS_j	$(J - 1)$	$\sigma_m^2 + M(\sigma_i^2 + S\sigma_j^2)$	Size or shape variation
Remainder	MS_{sj}	$(S - 1)(J - 1)$	$\sigma_m^2 + M\sigma_i^2$	Nondirectional asymmetry (Antisymmetry I, II + FA/2)
Measurements (M)	MS_m	$SJ(M - 1)$	σ_m^2	Measurement error

[a] S, Number of sides (2 for a bilateral character); J, number of 'genotypes'; I, number of individuals per 'genotype' (e.g. clonemates), M, number of repeated measurements per side; $\Sigma\alpha^2$, added fixed variance component due to sides (directional asymmetry); σ_j^2, added random variance component due to 'genotypes' (normally this corresponds to variation among individuals except where clonemates allow replication as in 3A above); σ_{sj}^2, added variance component due to side X genotype interaction (antisymmetry I); σ_i^2, variance of a given side (R or L) among clonemates due to 'developmental noise' [antisymmetry II and FA/2; note that in the absence of clonemates (3B), σ_{sj}^2 in 3A is included in this term]; σ_m^2, measurement error variance (eliminated from all expected MS if only one measurement per side); FA/2, fluctuating asymmetry divided by two.

[b] The expected MS of 3B were derived from those in 3A for the case where only one individual exists per 'genotype' (i.e. no clonemates were measured). MS_i thus disappears in 3B. This derivation also assumes that σ_{sj}^2 (antisymmetry I) is zero. Consequently, if antisymmetry is present, MS_j (genotypes) can *not* be tested properly over MS_{sj} (remainder).

Table 3a) and is actually, from a functional standpoint, directional asymmetry *for a given clone*. This antisymmetry would have a genetic basis, and its contribution to the total variance may be partitioned out (see Antisymmetry below). Antisymmetry II, on the other hand, is actually a form of nongenetic developmental noise in which a character on one side of the body is consistently larger than its partner. Even for genetically identical individuals, however, the larger side may be either the right or the left. It is inseparable from FA in the variation due to nondirectional asymmetry (σ_i^2, Table 3a). Since an ANOVA cannot separate antisymmetry II from FA completely, some other test must be performed (see Antisymmetry below). Similarly, with no clonemates to provide replication (virtually always the case), ANOVA can not partition out either form of antisymmetry from FA since the variance components due to antisymmetry and FA are all indistinguishable as nondirectional asymmetry (σ_i^2, Table 3b).

FA RELATIVE TO MEASUREMENT ERROR Repeated measurements of each side are useful for two reasons even when, as is nearly always true, replicate individuals of a given genotype (clonemates) are not available. First, testing the MS due to variation among individuals (MS_i; Table 3a) or the remainder (MS_{sj}; Table 3b) over the MS due to measurement error (MS_m) reveals whether nondirectional asymmetry (σ_i^2) is making a significant contribution to the variation observed relative to measurement error. If it is not significant, of course, further analyses of variation in FA would be fruitless unless a larger number of measurements per side revealed significant differences.

Second, and perhaps more importantly for comparisons among samples, repeated measurements permit measurement error to be partitioned out of the estimate of FA. Subtracting the measurement error (MS_m) from the MS due to individual variation (MS_i; Table 3a) or the remainder (MS_{sj}; Table 3b), and dividing by the number of replicate measurements (M), leaves only the variance component due to nondirectional asymmetry (one or both types of antisymmetry, plus FA). This reveals how much of the nondirectional asymmetry is due to measurement error and hence how much would be gained by taking additional repeated measurements. It also yields a much better estimate of FA, which may then be compared among samples (see Detecting FA Differences Among Samples below). Given maximum measurement errors of from 10% to 76% of nondirectional asymmetry as indicated above, a substantial increase in the ability to distinguish small differences among samples may obtain from taking even two repeated measurements of a character on each side, instead of one.

DIRECTIONAL ASYMMETRY Since this is a mixed model ANOVA (sides = fixed, genotypes = random), the MS due to directional asymmetry or sides (MS_s) is tested over MS_{sj}, which corresponds to measurement error (σ_m^2) plus

the variance components due to nondirectional asymmetry. The test is the same regardless of whether replicate clonemates are available. Though sometimes significant for many characters (30, 47), directional asymmetry is more commonly significant statistically only for some characters (6, 43, 64, 73, 99), or it may be nonsignificant (2, 29, 32, 40, 44, 77, 79).

ANTISYMMETRY Because it may seriously confound interpretations of variation in FA, evident from the MS of Table 3, eliminating the possibility of antisymmetry is a very important but often ignored step. Should clonemates be available, one form of antisymmetry—antisymmetry I—may be estimated by subtracting the variation due to individuals (MS_i) from the sides × genotypes interaction (MS_{sj}) and dividing by the number of individuals per genotype (I; Table 3a). In the absence of clonemates, none of the contribution of antisymmetry may be partitioned out of nondirectional asymmetry. However, as emphasized above, even with replicate individuals only a portion of the contribution of antisymmetry to nondirectional asymmetry can be tested or partitioned out by ANOVA. Hence, an additional test must be performed to determine if significant antisymmetry is present.

Testing for platykurtic (broad-peaked or bimodal) departures from normality of the distribution of $(R_i - L_i)$ is the appropriate test for the presence of antisymmetry. Graphical tests (2, 99) or histograms of the signed $R - L$ differences will reveal departures from normality, and the Kolmogorov-Smirnov test (85), available on many statistical packages, will reveal the significance of those departures. If either form of antisymmetry is statistically significant, reliable conclusions about variation in FA among samples may not be drawn because they may be confounded by differences in antisymmetry. In those studies of FA that tested for antisymmetry, it was found to be either very rare ('no consistent trend'—29); significant in only 1 of 120 possible character distributions (40), or absent (not significant in a total of 20 characters measured for three different species—99). However, numerous examples of antisymmetry are known (21a, 43a, 46a, 69a).

EFFECTIVENESS OF SIZE SCALING The ANOVA outlined in Table 3 also permits an estimate of the effectiveness of size scaling, since the variance contribution of genotypes (MS_j, Table 3a) may now be tested over the variation due to nondirectional asymmetry. This tests whether the average size of a character varies more among individuals than would be expected if only FA were contributing to size variation. If not significant, size scaling is complete; this would occur necessarily if each side were divided by $(R_i + L_i)/2$ but would not necessarily occur if each side were scaled by some independent measure of body size. If the effect of genotypes is significant, $(R - L)$ differences should be plotted against size as above (see Testing for Size Dependence) and transformed if necessary. Note that in the absence of

clonemates, genotypes (MS_j) can only be tested over nondirectional asymmetry (MS_{sj}) if no antisymmetry is present (Table 3b).

Detecting FA Differences Among Samples

SINGLE TRAITS PER INDIVIDUAL The appropriate test for differences in FA among samples depends on which index is used. For indexes based on $|R_i - L_i|$ (Indexes 1 and 2, Table 1), a nonparametric ANOVA (e.g. Kruskall-Wallis) has been used (3, 41). Parametric ANOVAs have also been conducted with these indexes (9, 12, 76, 77, 92, 93), but this is not proper statistically for two reasons. First, the distributions are truncated at zero and hence highly skewed to the right; second, they have unequal variances.

The indexes with the highest discriminatory ability (Indexes 4, 5, 6, and 8; Tables 1 and 2) are variances. The more accurate estimate (the value of σ_i^2; Table 3) derived from the within-sample ANOVA outlined above, is also a variance. Thus, testing whether FA varies significantly among samples is essentially testing whether the variances are homogeneous among groups (for a review of such tests, along with estimates of their power see 19). Several tests allow such an analysis.

To compare FA between two samples, a standard F-test is not just appropriate; it is the most powerful parametric test for such differences *if* the two samples are normally distributed (54; examples of its application include 6, 13, 33, 79, 83, 91, 98). Even so, unless sample sizes are reasonably large, true differences in variance are difficult to detect; for example, to detect a true twofold difference between two variances even 75% of the time requires that the sample size of each group exceeds 40 (see Figure 4 in 83). The appropriate degrees of freedom for the F-test depend upon how FA is estimated. If no replicate measurements are taken, the degrees of freedom for each FA estimate will be those of the MS for nondirectional asymmetry (either MS_i of Table 3a, or MS_{sj} of Table 3b). If replicate measurements are taken, the Satterthwaite formula for the degrees of freedom of the *variance component* due to nondirectional asymmetry (σ_i^2) are (using the notation of Table 3):

$$\text{Table 3}a\text{:}\quad \frac{(MS_i - MS_m)^2}{\dfrac{(MS_i)^2}{SJ(I-1)} + \dfrac{(MS_m)^2}{SJI(M-1)}},$$

$$\text{or Table 3}b\text{:}\quad \frac{(MS_{sj}) - MS_m)^2}{\left[\dfrac{(MS_{sj})^2}{(S-1)(J-1)} + \dfrac{(MS_m)^2}{SJ(M-1)}\right]}$$

[from 'Approximate F-test' (77a:247–8)].

For comparing FA among three or more samples, several tests are available: *F*max test, Bartlett's test, Scheffé-Box test, Levene's test, and Box-Cox test. The *F*max test (37) is based on the ratio of the maximum sample variance to the minimum sample variance. Although quick, it is not as efficient as Bartlett's test at detecting true differences among variances (85:403). Bartlett's test, however, is very sensitive to nonnormality in general (85:403) and to long-tailed distributions in particular. Thus, Scheffé developed a test in which each sample is divided into two or more subsamples; the variance is calculated for each subsample, and an ANOVA conducted on the log-transformed variance estimates (62, 85). This test, or a minor modification of it, has been widely used in FA analyses (3, 29, 32, 47, 48, 103). Finally, Levene's test is an analysis of variance on the absolute deviations from the population means. This test has the advantage of being much less sensitive to nonnormality than Bartlett's test (see example 13.11 in 84).

A rather unusual test for heterogeneity of variances, the Box-Cox test offers a combination of the above analyses. It utilizes transformations that are commonly used to make variances homogeneous. For example, if the variance σ^2 is proportional to the mean, as in a Poisson distribution, then the square root transformation homogenizes the variances. If, on the other hand, the standard deviation σ is proportional to the mean, then the log transformation is correct. Box & Cox (11) provide a method to determine which value of the parameter λ yields the most appropriate power transformation from the family:

$$Y^{\lambda} = \begin{cases} \dfrac{Y^{\lambda} - 1}{\lambda}, & \lambda \neq 0 \\ \log Y, & \lambda = 0 \end{cases}.$$

This family contains both the square root transformation ($\lambda = 0$). One of the major advantages to such an analysis is that confidence levels on λ may be determined. However, this transformation has two serious limitations, one theoretical and one practical. First, a critical assumption of this test is that the *transformed* data are normally distributed; nonnormality may result in statistical significance even if the original data had equal variances. Second, from a practical perspective very few commercially available statistical packages contain this test; it may be found, for grouped data only, in the statistical package accompanying Biometry (85).

All of the above tests for single and multiple traits, however, determine only if FA differs significantly among samples; they do not test for the significance of an association between FA and any extrinsic factors. To determine if FA varies significantly as a function of some factor—for ex-

ample, stress (97) or heterozygosity (44, 50, 87, 100)—either regression analysis of FA (best weighted by sample size or degrees of freedom) or a nonparametric equivalent (Kendall's Tau or Spearman's rank correlation; 85, 18) may be used. Because each measure of FA for a sample estimates a variance (the variance of $R - L$), and because the confidence in such an estimate is a function of the sample size, each FA estimate should be weighted by its sample size (for discussions of weighted regressions, see 27).

MULTIPLE TRAITS PER INDIVIDUAL When multiple traits have been measured on each individual, both the Scheffé-Box test and Levene's test discussed above may be conducted as single classification ANOVAs for each trait. The additional power derived from measuring several characters as opposed to one is achieved by comparing the concordance of their variation among samples. Kendall's concordance test (85), on the values of FA ranked for each character across samples, allows the overall significance of FA variation to be determined. It has been the most commonly used test for the presence of Soulé's (86) 'population asymmetry parameter' (32, 40, 41, 50, 64, 69, 86, 87).

PATTERNS OF VARIATION IN FLUCTUATING ASYMMETRY

FA and Heterozygosity: Estimating Genomic Heterozygosity from Protein Variation

The level of FA has been correlated frequently with heterozygosity as that is determined from protein variation (see the summary in Table 4 below). Most commonly, populations of higher heterozygosity exhibit lower FA, but in some cases within populations, individuals heterozygous at more loci are less asymmetrical. Because genomic heterozygosity is argued to have a major influence on developmental stability (25, 26, 55, 88, 90, 93), the association of FA with protein heterozygosity raises an important question: To what degree does heterozygosity at a few protein loci predict genomic heterozygosity?

In general, an arbitrary sample of loci in an individual is a poor predictor of genomic heterozygosity (15, 68). For example, for an average genomic heterozygosity of 0.07, the correlation across individuals between the average heterozygosity in a sample of 20 loci and the genomic heterozygosity for a genome of 10,000 loci is only $r = 0.054$ (using Equation 8 in 15). Three conditions, however, permit some information on genomic heterozygosity of an individual to be inferred from the heterozygosity observed at one or a few loci of that individual: (*a*) the presence of large amounts of linkage disequilibrium, (*b*) the presence of inbreeding, and (*c*) the presence of nonrandom mating due to small population size.

ESTIMATING GENOMIC HETEROZYGOSITY WITH LINKAGE DISEQUILIBRIUM Consider an extreme example of linkage disequilibrium where the genome is arranged into large blocks of tightly linked genes with one set of alleles in one block and a different set in another. It is then intuitively obvious that the amount of heterozygosity at a few loci will be a strong predictor of genomic heterozygosity. Recent evidence suggests that electrophoretic markers on chromosome segments more distal to the centromere are more heterozygous than proximal ones. Indeed they may mark blocks of linked loci in salmonid fishes (50, 52). Rather provocatively, heterozygotes at these distal loci show a greater negative correlation with FA than do heterozygotes more proximal to the centromere (50).

Even the presence of linkage disequilibrium between two loci, however, does not necessarily mean that being heterozygous at one locus implies increased probability of being heterozygous at a second. For two loci A and B, such an inference may be drawn only if Prob(both A and B are heterozygous) / Prob(A is heterozygous) > Prob(B is heterozygous). With substitutions of the appropriate values and after some manipulations this reduces to:

$$p_1^2(X - Y)\,(2X - 1) > q_1^2(X - Y)\,(2Y - 1), \qquad 5.$$

where p_1 and q_1 are the frequencies of alleles A_1 and A_2 respectively, and X is the conditional frequency of carrying allele B_1, given that an individual carries A_1 and that Y is the conditional frequency of carrying B_1 given A_2 (the same conditional frequencies for allele B_2 are $1 - X$ and $1 - Y$ respectively). With this parameterization, the linkage disequilibrium is given by $D = p_1q_1(X - Y)$. The relevant point here is that this inequality may not be satisfied if the common allele at one locus is associated with the rare allele at the other. For example, if $p_1 = 0.8$, $q_1 = 0.2$, $X = 0.25$, and $Y = 0$, then the frequencies of alleles B_1 and B_2 will be $p_2 = 0.2$ and $q_2 = 0.8$, and the inequality of Equation 5 is not satisfied. Since Prob(both A and B are heterozygous) = 0.08 and Prob(A is heterozygous) = 0.32, the conditional probability that B is heterozygous given A heterozygous is 0.25, which is less than Prob(B is heterozygous) = 0.32.

ESTIMATING GENOMIC HETEROZYGOSITY WITH INBREEDING Inbreeding also allows inference of genomic heterozygosity from a few loci. If a population exhibits regular inbreeding, the more heterozygous an individual is at a few measured loci, the less likely that the individual is a product of inbreeding and, hence, the more likely it is heterozygous at other loci. More precisely, assume for two unlinked loci in a partial selfing population (104) a proportion $T = 1 - S$ of outcrossing and frequencies of alleles represented as B_1 and B_2 of p_2 and q_2 respectively. For these assumptions, the probability that the B

locus will be heterozygous in an individual given that the A locus is heterozygous is:

$$2p_2q_2\left[\frac{2(2-S)}{4-S}\right], \qquad 6.$$

This should be compared to:

$$2p_2q_2\left[\frac{2(1-S)}{2-S}\right], \qquad 7.$$

which is the expected probability of being heterozygous at locus B, under a partial selfing model, when nothing is known abut heterozygosity at other loci. Clearly, as $S \rightarrow 1$, the expected probability of being heterozygous at locus B, given that locus A is heterozygous, approaches $4p_2q_2/3$ (Equation 6), whereas in the absence of any information about other loci (Equation 7), the probability of being heterozygous at B approaches 0. Note, however, that an excess of homozygotes is expected at each locus in such a population; hence, in the absence of any deviation from Hardy-Weinberg equilibrium, inbreeding cannot be invoked to account for an increased ability to predict genomic heterozygosity from a small, arbitrary sample of loci.

ESTIMATING GENOMIC HETEROZYGOSITY WITH SMALL POPULATION SIZE If a population within which there is random mating is small, then information about genomic heterozygosity may also be gained from measuring heterozygosity at a few loci. In this situation, an individual within a particular population will be more closely related to some individuals than others. Consider an extreme example in which a population has been created with two unrelated hermaphroditic individuals taken at random from a larger population whose frequency of alleles B_1 and B_2 was p_2 and q_2 respectively. If gametes from these two individuals fuse at random (i.e. each individual may mate either with itself or with its partner), then on average across all such populations composed of two individuals, an offspring may with probability 1/2 be a product of outcrossing or with probability 1/2 be a product of selfing. Offspring that are selfed will be on average half as heterozygous (p_2q_2) as those that are outcrossed ($2p_2q_2$). Thus, if an offspring is heterozygous at locus A, it has a probability 2/3 of being a product of outcrossing and 1/3 of being a product of selfing. Consequently, if heterozygous at locus A, this offspring has a probability of being heterozygous at locus B of:

$$\left[\frac{2}{3}(2p_2q_2)\right] + \left[\frac{1}{3}(p_2q_2)\right] = \frac{5}{6}(2p_2q_2) \qquad 8.$$

compared to:

$$\left[\frac{1}{2}(2p_2q_2)\right] + \left[\frac{1}{2}(p_2q_2)\right] = \frac{3}{4}(2p_2q_2), \qquad 9.$$

which is the probability of being heterozygous at the *B* locus in the absence of any knowledge about the *A* locus.

CORRELATIONS BETWEEN ELECTROPHORETIC AND GENOMIC HETEROZYGOSITY IN NATURAL POPULATIONS The statistically significant negative association between the asymmetry of an individual and its heterozygosity as estimated by 11 protein loci in salmonid fishes (49, 50) offers an opportunity to test for the departures from multilocus equilibrium outlined above, since it seems unlikely that heterozygosity at the particular loci examined would be directly responsible for the observed differences in FA. An examination of some of the raw data from Leary et al (50) revealed no such disequilibrium: The heterozygosity at 5 of the 11 loci in Arlee rainbow trout did not predict the heterozygosity at the remaining 6 loci (N= 160). These 11 protein loci are unlinked (50), and as indicated above, the location of some of these loci more distally from the centromere may link them to blocks of more highly heterozygous loci (50, 52).

FA and Heterozygosity: Patterns in Natural Populations

WITHIN- VERSUS AMONG-POPULATION CORRELATIONS Assuming that the level of FA is a product of the level of heterozygosity at some subset of loci in the genome, are there any patterns of FA variation that can be predicted without actually estimating genomic heterozygosity? Using some rather simple assumptions about the distributions of heterozygosities within a population, we show below that differences in asymmetry may be more readily detectable between individuals drawn from different populations than between individuals drawn from the same population.

To illustrate, consider a sample of n loci from a genome, where the average heterozygosity per locus is H and where the variance of H across loci is σ^2. The heterozygosity H of these samples will be normally distributed with variance $[H(1 - H) - \sigma^2]/n$ (15). Also, if it is assumed that the heterozygosity at each locus is determined only by mutation (μ) and random drift, then the mean heterozygosity of an individual will be $\Theta/(1 + \Theta)$ with a variance $2\Theta/[(1 + \Theta)^2(2 + \Theta)(3 + \Theta)]$, where $\Theta = 4N\mu$, with N the population size and μ the mutation rate (28).

Under these assumptions, the distribution of heterozygosities in a sample of individuals as a function of genome size (Figure 3) reveals that if the level of FA in an individual was determined directly by the heterozygosity at 100 loci (equivalent to assuming a genome size of 100), and if the average heterozygosities differed by only 0.1 (e.g. 0.7 and 0.8, Figure 3), the asymmetries probably would not differ significantly between a pair of individuals, one drawn from each population. If, however, the level of FA was determined by the heterozygosity at 10,000 loci then the same two individuals would almost certainly exhibit different levels of asymmetry.

This analysis suggests that differences in FA should be detected more readily between a pair of individuals drawn from different populations than between a pair drawn from the same population unless the level of FA is determined by only a few loci. Such a pattern has frequently been found: Numerous studies have detected a significant concordance of FA of different characters across populations and yet detected little or no concordance across individuals (compare Patterns 4 and 5, Table 4). A particularly striking example of this pattern occurs in Mason et al's study (64) of wing pattern variation in a nymphalid butterfly (detailed in Palmer, 71). It is possible that this difference in the pattern of FA variation within versus among populations

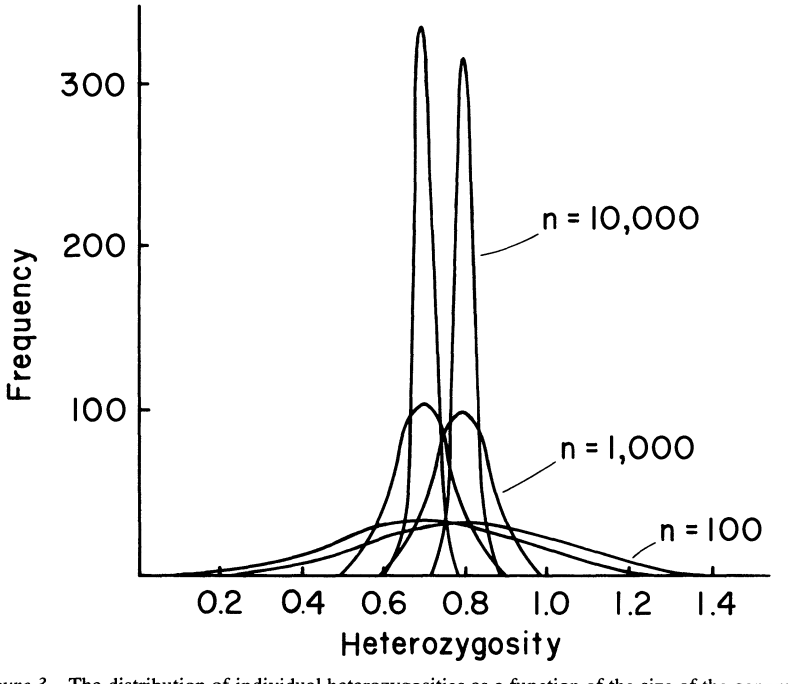

Figure 3 The distribution of individual heterozygosities as a function of the size of the genome determining the level of FA, for two mean heterozygosities and three genome sizes (n, number of loci per individual). Computed from Equation 8 of Chakraborty (15).

Table 4 Patterns of variation in fluctuating asymmetry

Pattern of variation	Study consistent with pattern?		
	Yes	No	Ambiguous
1. Decreased FA with increased heterozygosity among populations	10, 44[e], 51, 53, 87, 100	50[a]	
2. Decreased FA with increased heterozygosity among individuals	49, 50[a]	50[a]	50[a]
3. Significant FA variation among populations or species	2, 42, 46[f], 69, 77[h], 103[g]		
4. FA concordant for multiple characters among populations	29, 41[a], 33[j], 44[j], 69, 86, 89, 98[b], 100[a]	40, 41[b], 64[i], 98[a], 100[a]	3[f], 72, 77[h]
5. FA concordant for multiple characters among individuals within populations	91	3[f], 30, 39[e], 70, 77[h,k], 86, 89, 95[n], 96, 99[f], 100[e]	47[h], 64, 69
6. Increased FA in inbred versus wild populations	9, 80		5, 6, 91
7. Increased FA with increased inbreeding	9[h], 65[h], 91, 93, 94	8, 12[p], 102	76[h]
8. Increased FA in hybrid versus parental species	32, 52, 105[l]	29, 40	
9. Decreased FA in hybrid versus parental strains	47, 65, 75, 76	73, 93	6, 9, 92
10. Increased FA in phenotypically more extreme individuals	50, 90[a]	90[d]	90[d]
11. Increased FA in male vs female	13, 65[h], 76	9, 30[c], 33[h], 36, 43[e]	34, 47
12. Increased FA with strong directional selection	94		48
13. Increased FA with increased systemic stress	8[p], 79, 81[o], 82[o], 97	5	3[f], 9, 12, 38[m], 41[f], 80[f,h], 83, 93

[a] True for only one of species examined
[b] True for only two of species examined
[c] True for only three of species examined
[d] True for only four of species examined
[e] Two species examined
[f] Three species examined
[g] Five species examined
[h] Two or more strains examined
[i] Compared among years
[j] Calculated from data in tables
[k] Among leaves within a plant
[l] Cited in 32
[m] Cited in 41
[n] Cited in 42
[o] Cited in 80
[p] Cited in 87

is due to different environmental effects experienced among populations (for example 'stress'; see Pattern 13, Table 4). Unfortunately that possibility can not be ruled out with these data.

Other Patterns

A remarkable variety of patterns of FA variation have been reported (Table 4). In addition to the associations with heterozygosity (Patterns 1 and 2, Table 4) and the concordance of FA among versus within populations (Patterns 3–5) described above, several other patterns are worth noting. First, FA tends to increase with increased inbreeding (Patterns 6 and 7), although not all studies have yielded consistent results. Increased FA here is consistent with a decline in heterozygosity but could also have resulted from an increased expression of recessives alleles with lower canalizing ability. Second, FA increases in hybrid *species* compared to parental ones (Pattern 8), while at the same time hybrids between inbred *strains* exhibit lower FA than offspring from pure parental crosses (Pattern 9). The most likely explanation for these apparently contradictory results is that any increased buffering resulting from increased heterozygosity in between-species hybrids was outweighed by the disruption of the 'coadapted gene complexes' in each of the parental species (24, 32, 52). However, increased FA was *not* observed in natural hybrid populations of either bluegill sunfish (29) or an iguanid lizard (40). Third, a variety of organisms also exhibited increased FA among more phenotypically extreme individuals, but this pattern had numerous exceptions (Pattern 10). Finally, the effects of sex (Pattern 11; males expected to have higher FA due to hemizygosity of loci on the X chromosome), strong directional selection [Pattern 12; increased FA expected with increased directional selection for other characters because directional selection tends to eliminate genetic variability (14; 48)], and systemic stress (Pattern 13) were all quite variable among studies.

A notable feature of this summary is the widespread lack of consistency among studies. Although some patterns are moderately consistent (Patterns 1–5, Table 4), exceptions are present for nearly all. This lack of consistency among studies emphasizes what many authors have hinted at in discussions of their data: FA as a measure of developmental stability is a very small signal easily lost in a tumultuous sea of entropic forces. We hope our clarification of FA indexes and analyses helps reduce the entropy that is introduced by using analytical tools of variable precision.

POSSIBLE ADDITIONAL APPLICATIONS OF FLUCTUATING ASYMMETRY

In spite of the inconsistencies lamented above, are there any as yet untried applications of FA variation that might be of value as tools for evolutionary

inference? We note three worth exploring. First, if, by whatever causal mechanism, FA is correlated negatively with heterozygosity under most circumstances (Patterns 1 and 2, Table 4), then the possibility exists that differences in the level of FA may be used to infer *relative* differences in genetic variability among closely related contemporaneous populations or between ancestral and descendent ones of species in the fossil record (see 71).

Second, on a larger scale, D. Jablonski has suggested to us that if the level of FA reflects degree of canalization (65, 88, 101), then perhaps FA could be used to test for the evolution of increased canalizing ability within taxa (e.g. trilobites, brachiopods) over the first several million years of metazoan evolution. Finally, again assuming FA reflects degree of canalization, comparison of levels of FA among traits within single species may reveal differences in the strength of selection for canalization of different traits: presumably, characters of greater functional significance to the organism would be subject to stronger selection for canalization. In other words, differences in FA among characters may provide a way of ranking traits in terms of their functional significance to an organism with little or no a priori knowledge of how those characters actually function. These rankings could then be tested experimentally. Although hinted at (2, 43, 48, 51, 69, 90), an explicit application of this approach has not yet been found.

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