Waltzing with Asymmetry

Is fluctuating asymmetry a powerful new tool for biologists or just an alluring new dance step?

A. Richard Palmer

Remarkably few things escaped Aristotle's attention, or Darwin's for that matter. Aristotle noted regular patterns of bilateral asymmetries in animals: "In the Caribi and in the Carcini [true crabs] the right claw is invariably larger and stronger. In the Astaci [crayfish and lobsters] alone it is a matter of chance which claw is the larger, and this in either sex" (Herrick 1909, p. 149). Darwin too had a hunch about asymmetries while wrestling with mechanisms of inheritance. He believed that deviations from the "law of symmetry" (i.e., anomalous asymmetries in normally symmetrical organisms) would not be inherited, but the meager evidence at his disposal, dealing mainly with gross deformities, seemed to suggest otherwise (Palmer and Strobeck 1986). And so the history of the literature on morphological asymmetry progressed, fitfully and capriciously, from anecdotal observations and entertaining stories to extensive compilations of conspicuous asymmetries (Ludwig 1932, Neville 1976). Morphological asymmetries were but one of nature's many curiosities.

A. Richard Palmer is a professor in the Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9 Canada, and conducts his field research at the Bamfield Marine Station, Bamfield, British Columbia V0R 1B0 Canada. His research interests include the evolution of skeletal form, developmental precision, and conspicuous asymmetries in marine invertebrates.

When studied with care, subtle deviations from symmetry offer a unique measure of developmental precision for many organisms and traits, and may even yield insights into the evolution of conspicuous asymmetries

Rather recently, however, biologists have begun to realize that deviations from symmetry might be more than just a curiosity. One kind of asymmetry, fluctuating asymmetry, which refers to minute random deviations from perfect symmetry (VanValen 1962), has been advanced as a tool for inferring the health, quality, or developmental stability of organisms. The allure of this new and seemingly simple tool has attracted considerable attention (Markow 1995), but its uncritical application has also engendered much skepticism.

Just how powerful is this tool, and how reliable? Perhaps very, but those wishing to apply it must recognize that the biological signal is exceedingly small and that not all deviations from symmetry provide a useful signal. Without adequate attention to fundamental methodological and conceptual issues, statistical patterns of asymmetry variation, no matter how appealing, may be biologically meaningless. However, when used with care, fluctuating asymmetry offers a unique tool for quantitative comparisons of developmental precision among a wide range of organisms and traits.

General discussions of bilateral asymmetry pose a challenge because asymmetries exist on so many different scales. Technically speaking, all structures will be asymmetrical at some level of measurement. Some asymmetries are conspicuous, like the entire body of flatfish and snails and the large claws of lobsters and fiddler crabs. Most early work drew attention to such examples. Other asymmetries, including fluctuating asymmetry, are exceedingly subtle, on the order of 1% of trait size or less, and require careful measurement to detect.

Recent associations between environmental degradation and fluctuating asymmetry, and between mate choice and fluctuating asymmetry, have catapulted this rather obscure phenomenon into the limelight. (Those wishing a provocative potpourri of the many applications to which fluctuating asymmetry has been put will find much of interest in the proceedings of two recent meetings—in Moscow [Zakharov and Graham 1992] and in Tempe, Arizona [Markow 1994].)

Terminology and history

To appreciate the potential power and pitfalls of using fluctuating asymmetry as a tool for inferring health or quality, and to avoid confusion over what is being measured, the terms for patterns of variation must be kept distinct from those referring to underlying causes of the patterns (Palmer 1994). Thus, *fluctuating asymmetry* merely describes a specific pattern of bilateral variation—a frequency distribution of right minus left (R – L) differences whose mean is zero and whose shape does not depart from normal (bell-shaped). The term implies nothing definitive about causation. Similarly, *developmental precision* is a useful neutral term for referring to how closely a structure approaches its ideal for a particular genotype and growth environment. It too implies nothing definitive about causation. Thus, fluctuating asymmetry offers one measure of developmental precision.

Several other terms encountered in the fluctuating asymmetry literature apply most properly to the underlying causes of bilateral variation. *Developmental noise* refers to a suite of processes whose random variation during growth tends to cause a structure to depart from its ideal for a particular genotype and environment. *Developmental stability* and *developmental homeostasis* both refer to a suite of processes that somehow counteract or buffer the disruptive effects of developmental noise during growth. These phenomena ensure that a structure develops along a predetermined path in a particular environment. In contrast, as Zakharov (1992) cogently notes, the term *canalization* refers to the ability of a structure to develop along a predetermined path in a variety of different environments, so canalization and developmental stability represent different phenomena.

The notion that fluctuating asymmetry might be useful for drawing inferences about developmental homeostasis is comparatively recent. Although they did not use the term *fluctuating asymmetry*, Sumner and Huestis (1921) were among the first to draw attention to such variation when they observed considerably greater asymmetry variation in several skeletal traits of F_2 compared with F_1 progeny from crosses between inbred lines of mice. But their concern was with mechanisms of inheritance in an era when much was still being learned about polygenic effects: "In the present paper we are setting forth data which would seem to be much more baffling to the radical Mendelian. We are presenting cases in which the F_2 variability shows a marked increase, *in respect to characters that are not inherited at all*" (Sumner and Huestis 1921, p. 464).

The connection between fluctuating asymmetry and developmental stability did not fully blossom until the early 1950s. *Drosophila* geneticists such as K. Mather, E. C. R. Reeve, J. M. Thoday, and C. H. Waddington all contributed significantly to the idea that subtle deviations from symmetry might yield valuable insights into the developmental or genetic bases of developmental homeostasis (Palmer and Strobeck 1986).

The appeal: a seductively simple measure

What makes fluctuating asymmetry such an attractive tool for studying developmental precision? Quite simply, bilateral symmetry is one of the few morphological attributes for which we undeniably know the ideal—perfect symmetry. If we can somehow rule out other causes of departures from symmetry, such as the direct effects of genotype or external environment, then we have a tool for quantifying developmental precision. The trick, of course, is ruling out these other causes in a convincing way. Somehow, we must distinguish so-called well-behaved characters, in which the underlying cause of departures from symmetry is developmental noise, from so-called poorly behaved characters, in which departures from symmetry are caused by the direct action of genes or external environmental factors that affect one side more than the other.

In spite of the heightened interest in deviations from symmetry, we know little about the developmental origins of this subtle bilateral variation: What are the causes of fluctuating asymmetry? Fluctuating asymmetries are traditionally attributed to the rather elusive phenomenon of developmental noise. But developing systems are highly homeostatic and are capable of buffering or correcting for accidents or disruption. Thus, in the end, the subtle difference observed between sides in a symmetrical organism is but one manifestation of the outcome of two opposing forces: those tending to disrupt precise development (developmental noise) and those tending to stabilize it (developmental stability; Palmer 1994, Paxman 1956, VanValen 1962). However, for deviations from symmetry to offer a valid measure of developmental precision they must not be confounded by other causes of bilateral variation.

Developmental noise: origins

To most biologists, developmental noise refers to small, completely random accidents or errors of development and accounts for "the inability of organisms to develop in precisely determined paths" (VanValen 1962). In other words, it refers to a collection of processes that probably includes thermal noise at the molecular level and random variation in rates of physiological processes among cells, both of which may affect cell–cell communication and rates of cell growth, division, or elongation (Palmer 1994). Cell size variation, for example, accounts for a substantial fraction of both intra- and interspecific variation in *Drosophila* wing lengths (Partridge et al. 1994, Stevenson et al. 1995). These noiselike processes presumably promote the subtle differences we ultimately see between sides. Consequently, then, asymmetries arise because developmental noise affects the right and left sides of a bilateral pair of structures independently. This view of developmental noise explains why statistical considerations are so important: If noise is random and occurs independently on each side, as it should be to qualify as a noiselike process, then the average asymmetry (R – L) should be zero and the asymmetry variation should be normally distributed about zero (Figure 1, top; Palmer and Strobeck 1992).

Obviously, other factors might also cause the right side to differ from the left. Conspicuous asymmetries exist in many otherwise bilaterally symmetrical organisms, such as claws in lobsters and male fiddler crabs, teeth of narwhals, and beaks of crossbills (Neville 1976). These asymmetries are all presumably determined by the differential activity of genes on one side or the other (e.g., Yost 1995). We also know that vertebrate bone (Trinkaus 1994) and crustacean claws (Smith and Palmer 1994) remodel due to differential use, raising the distinct possibility that behavioral biases such as handedness may induce morphological differences between sides. Clearly, these kinds of asymmetry are not due to developmental noise.

Herein lies the source of all of the concern about statistical attributes of subtle asymmetries: If the mean of the R – L distribution departs from zero or its shape departs from normality, then the differences between sides probably reflect something more than simple developmental noise, and such deviations from symmetry may not be a valid measure of developmental precision (Palmer and Strobeck 1992). Common departures from ideal fluctuating asymmetry include directional asymmetry, such as when the right side is larger than the left on average (Figure 1, middle), and antisymmetry, when one side is consistently larger than the other but the larger side may be either the right or left, at random (Figure 1, bottom). Human limb bones, for example, exhibit directional asymmetry: on average, right arms are longer than left, whereas left legs are somewhat longer than right (Jolicoeur 1963). The impressive claws of lobsters and male fiddler crabs are extreme examples of antisymmetry: one claw is conspicuously larger than the other, but the right claw is larger only 50% of the time (Neville 1976). Antisymmetry is often referred to as "random asymmetry" in the literature on *situs inversus* (reversal of asymmetry in internal organs; Brown and Wolpert 1990). Developmentally, "antisymmetry may be defined as asymmetry due to negative interaction" (VanValen 1962, p. 126).

So before we can use subtle asymmetries as a reliable measure of developmental precision, we must reject these other possible kinds of asymmetry. Unfortunately, statistical tools are all that we have at our disposal, and the rejection of these other kinds of asymmetry is, in the end, probabilistic. We can never be certain that they are absent, only that we cannot detect them. Therefore, when trying to quantify something as subtle as fluctuating asymmetry, care in the application of statistics is essential.

To complicate matters further, discussions of these phenomena become muddled when terms for causation are synonymized with those for pattern. For example, in an otherwise interesting study of macaque monkeys Hallgrímsson (1993) considers the etiology of subtle deviations from symmetry (fluctuating asymmetry) to be synonymous with the etiology of developmental noise (a suite of random physiological or cellular processes). But variation in fluctuating asymmetry with growth reflects an interplay between noise and stability, and cannot provide an unambiguous measure of noise alone. Therefore, although subtle deviations from symmetry become proportionally larger with increasing size in the skulls of Hallgrímsson's (1993) macaque monkeys, they remain relatively unchanged in sign and magnitude with increasing size in the limbs of individual brachyuran crabs (Chippindale and Palmer 1993). Additional ontogenetic studies of subtle deviations from symmetry would reveal much about the actions of disruptive and stabilizing factors during development.

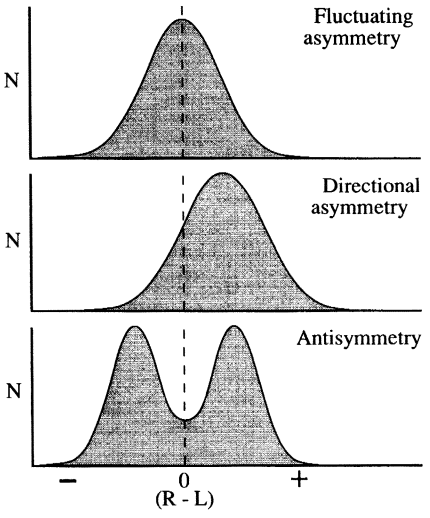

Figure 1. Frequency distributions of the signed difference between sides illustrating three commonly observed patterns of subtle deviations from bilateral symmetry. (top) Fluctuating asymmetry is defined as a distribution whose mean is zero and whose shape is statistically normal (bell shaped). (middle) Departures of the mean from zero signal directional asymmetry. (bottom) Departures of the shape of the distribution from normal in the form of platykurtosis (broad-peaked or bimodal) reveal antisymmetry. For comparisons among samples, the level of fluctuating asymmetry is quantified using some measure of the breadth of the right minus left (R – L) distribution (e.g., variance of (R – L) or mean of the absolute value |R – L|; see Palmer and Strobeck 1986). Subtle deviations from symmetry that take the form of directional asymmetry or antisymmetry may not be reliable measures of developmental precision.

Asymmetry and stability of development

Although the origins of developmental noise may seem obscure, the basis of developmental stability is even more so, even though it is by far the more impressive of the two. In the last decade, biologists have made tremendous strides toward understanding what is loosely called pattern formation—the specification in three dimensions of precisely what should happen when and where during development (see, for example, reviews in the 28 October 1994 issue of *Science*). In addition, many organisms, including crustaceans, amphibians, and reptiles, have remarkable powers of regeneration—entire limbs or tails may be replaced following loss.

Presumably, the same kinds of homeostatic mechanisms that ensure a regenerated limb is of the proper size and shape for its bearer also play a role in normal development (Bryant and Simpson 1984). What these mechanisms are, however, remains a mystery. Furthermore, regeneration is not always as precise as normal development. These and many other patterns suggest that the regulation of structure size, and hence symmetry, results from both long-distance communication as well as local cellular interactions (Bryant and

Simpson 1984). Thus the intriguing phenomenon of "compensating variations" (Schultz 1926), in which overdevelopment of one element of a multipart structure compensates for underdevelopment by another, is a form of developmental stability.

Although often unappreciated outside of developmental biology, bilateral symmetry is, for the most part, a fortuitous by-product of pattern formation, which does not imply that bilateral symmetry is not adaptive. It is simply a default: no special developmental mechanisms are required to specify bilateral symmetry per se (Morgan 1991). For example, if a cell makes a developmental decision based on its location in three dimensions, how does it "know" if it is on the right or left side? A simple coordinate system clearly provides insufficient information to distinguish what side it is on. The right and left sides become defined as soon as the first two primary developmental axes, the antero–posterior (A–P) and dorso–ventral (D–V), are established. The third axis is not a right–left (R–L) axis per se, as is often suggested, but rather a proximo–distal (P–D) one: cells on a given side of the body make developmental decisions based on their distance from the midline (Morgan 1991). If each cell on each side of the organism divides or changes shape in a particular direction, based purely on its distance from the midline (and its position along the A–P and D–V axes) then, within the limits of developmental precision, bilaterally symmetrical structures are the inevitable outcome.

The development of conspicuous asymmetry requires additional information (Palmer et al. 1993). One possibility is lateral inhibition, which apparently happens in some crustaceans in which the amplified master claw on one side suppresses amplification on the other via the central nervous system (Mellon and Stephens 1978). Although the molecular cues used to distinguish the primordial left and right sides early in development remain elusive, developmental geneticists have recently been able to trace the train of molecular interactions that direct the development of asymmetry in the internal organs of chickens back to the interplay among three genes (*activin receptor IIa*, *Sonic hedgehog*, and *nodal-related 1*; Yost 1995). Remarkably, these genes are also members of the same gene families implicated in intercellular signaling and in the definition of D–V and A–P axes. (See below for other hypothesized causes of conspicuous asymmetry).

Measuring heritability: what is in a sign?

One way of assessing whether the underlying causes of fluctuating asymmetry qualify as developmental noise is to ask whether deviations from symmetry in an individual are inherited. Most studies assume that they are not, because they are not exactly repeatable. If, for example, we could somehow raise the same individual many times under identical external environmental conditions, the subtle deviations from symmetry in that individual should vary at random from one trial to the next, because each time the unpredictable effects of developmental noise would cause a trait to be a little larger or smaller on one side or the other. If they are not repeatable, then they cannot be heritable. In fact, this assumption about the heritability of deviations from symmetry is absolutely essential to studies using fluctuating asymmetry as a measure of developmental precision. If this assumption were not true, subtle deviations from symmetry would not differ from any other kind of subtle morphological variation, and we would somehow have to factor out all of the possible effects of genotype and external environment on asymmetry to assess the level of developmental precision—clearly an impossible task.

To say that the deviations from symmetry are not inherited, however, is not to say that none of the factors that influence the development of fluctuating asymmetries is inherited. This rather subtle point is confusing, because whereas a noise-like process yields random variation, which should not be inherited, the breadth of this variation may differ because either noise levels or stabilizing abilities differ. That is, the factors that influence the extent of noise at the molecular or cellular level, or those homeostatic mechanisms that correct for errors during development, may have a genetic basis.

Here again, we see how important it is to distinguish between observable variation (e.g., fluctuating asymmetry) and hypothesized causes of that variation (e.g., developmental noise and stability). At present we cannot measure the levels of noise or stability directly, we can measure only their observable effects (e.g., fluctuating asymmetry). Separating the relative contributions of noise and stabilizing factors represents a significant challenge.

So how do we measure the heritability of subtle asymmetries like fluctuating asymmetry? As hinted at above, a complete answer is annoyingly complex (Palmer et al. 1993). However, in a well-behaved world—in which antisymmetry and directional asymmetry can be ignored—the answer is simple. If we think of how asymmetry might respond to selection, for example, we could potentially select for decreased asymmetry (Figure 2a), increased asymmetry (Figure 2b), or deviations toward one side only (Figure 2c). In this ideal world of pure fluctuating asymmetry, only three responses are possible: fluctuating asymmetry (the breadth of the R – L distribution) either decreases (Figure 2d), increases (Figure 2e), or remains the same. Were we to observe a change in the breadth of the distribution, we could legitimately conclude that variation in one or more of the factors influencing developmental precision is heritable: Developmentally unstable parents (those in the tails of the distributions, such as the shaded areas in Figure 2b) tend to produce more developmentally unstable offspring (Figure 2e).

But how is heritability quantified in this ideal world of pure fluctuating asymmetry? We had best heed the signs! Although other measures are possible, asymmetry in an individual is typically measured as the size of one side minus that of the other, and average asymmetry is computed in many ways (Palmer and Strobeck 1986). Fluctuating asymmetry in its strictest sense refers to the breadth of a frequency distribution of these deviations from sym-

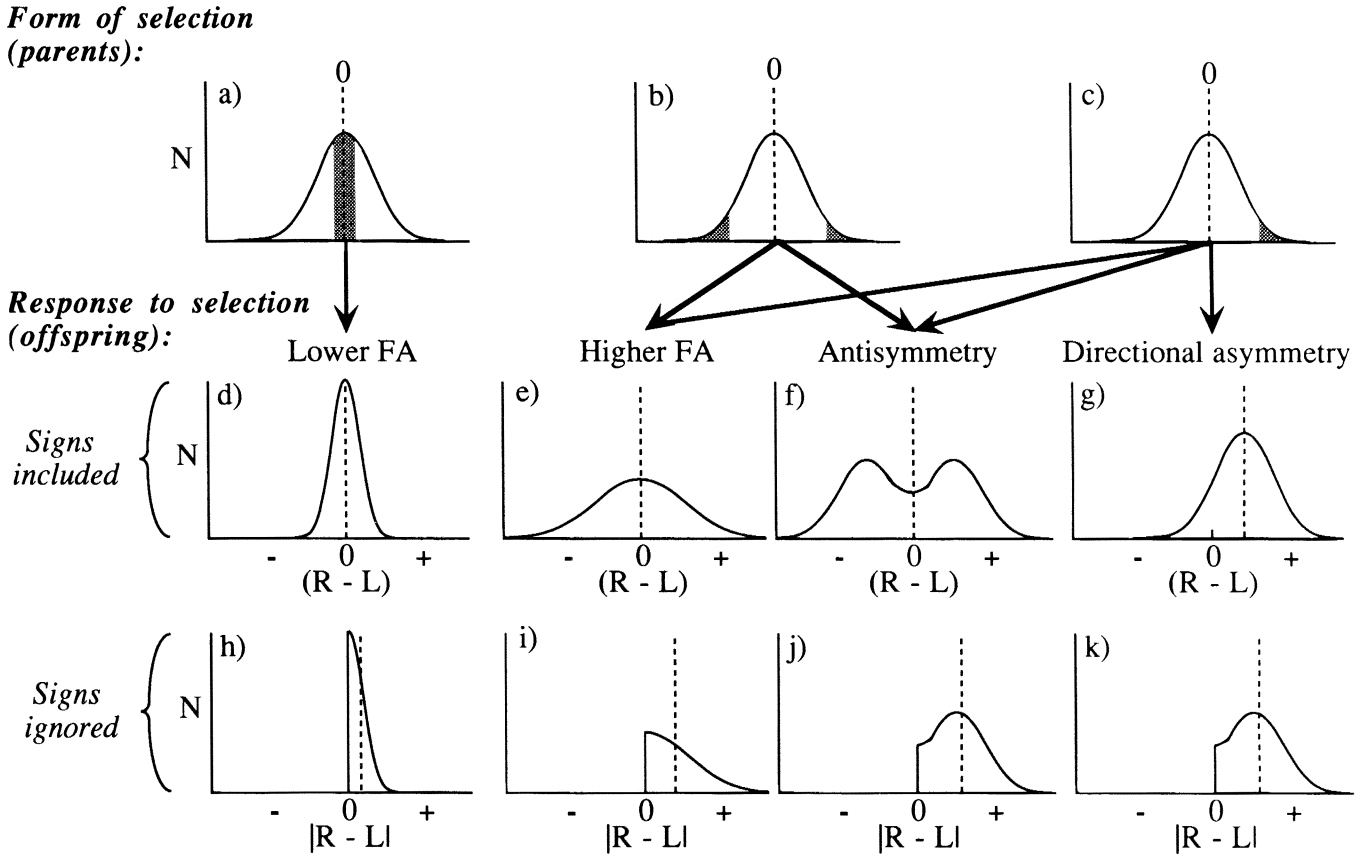

Figure 2. Frequency distributions of the difference between sides (R – L) illustrating forms of selection and responses to selection for subtle deviations from bilateral symmetry. Shaded areas in panels **a–c** indicate phenotypes selected from a parental population exhibiting fluctuating asymmetry (FA). Arrows indicate possible responses among the offspring for a particular type of selection. These responses are shown as either the signed value (**d–g**) or the absolute value (**h–k**) of the differences between sides. Vertical dashed lines indicate the mean for each distribution. If selection for decreased (**a**) or increased (**b, c**) asymmetry yielded only lower (**d, h**) or higher (**e, i**) fluctuating asymmetry, respectively, in the offspring, this would suggest a heritable basis to the factors influencing developmental precision. If selection for increased asymmetry (**b, c**) yielded antisymmetry (**f, j**) or directional asymmetry (**g, k**) in the offspring, then deviation from symmetry may not be a reliable measure of developmental precision. A regression of the absolute value of the R – L differences of the offspring (**h, i**) against that of the parents (that portion of the curve to the right of the dashed line in **a–c**) provides a convenient measure of the heritability of developmental precision only where fluctuating asymmetry (**d, e**) is the only form of asymmetry present. When either antisymmetry or directional asymmetry is present in the offspring (**f, g**), however, a regression of the absolute value of the R – L differences of the offspring (**j, k**) against that of the parents confounds the heritability of factors influencing developmental precision with those influencing asymmetry directly.

metry measured in a group of organisms (Figure 1, top). Regardless of the level of developmental precision in the offspring, the average difference between sides of all of the offspring is clearly zero if the sign is included (Figures 2d and 2e). This is, after all, how we define fluctuating asymmetry (Figure 1, top). A conventional heritability analysis (i.e., a regression of average offspring asymmetry against average parent asymmetry) that included the sign of those asymmetries would always yield a heritability of zero! This outcome occurs because, regardless of the magnitude of asymmetry in the parents, the average fluctuating asymmetry of the offspring will be zero provided that large enough samples are used.

A simple trick changes the answer completely: ignore the signs. This trick accomplishes a remarkable statistical feat, which accounts for why most people use it all of the time. It transforms each deviation from an estimate of the mean of a distribution (which is always zero in our ideal world; Figures 2d and 2e) into an estimate of the standard deviation or breadth of that distribution (compare Figures 2h and 2i with Figures 2d and 2e).

This trick underlies the preferred method for testing statistically for differences in fluctuating asymmetry among groups (univariate or multivariate Levene's test; Palmer and Strobeck 1992). It also reveals why we need not bother with two different kinds of selection for increased asymmetry in this idealized world. If the signs are ignored, selection for both tails (Figure 2b) is no different from selection for only one tail (Figure 2c). Indeed, this and related approaches have revealed a heritable basis to fluctuating asymmetry in some cases (e.g., bilateral bristles of fruit flies [Reeve 1960], teeth in inbred mice [Leamy 1986], pelvic spines in sticklebacks [Blouw and Boyd 1992]). However, other stud-

ies have obtained negative (e.g., nonmetric skull traits in macaque monkeys; McGrath et al. 1984) or mixed (meristic characters of salmonid fish; Leary et al. 1992) results.

Alas, there is no ideal world of pure fluctuating asymmetry. Antisymmetry and directional asymmetry (Figures 2f and 2g) are facts of life (Jolicoeur 1963, McKenzie and Clarke 1988), and we cannot ignore them. Sadly, they complicate efforts to estimate the heritability of developmental precision because two additional responses to selection are possible depending on the developmental or genetic origins of the initial subtle asymmetries.

Selection for individuals that are more asymmetrical (Figure 2b) could potentially yield antisymmetry (Figure 2f)—if asymmetries arose simply due to lateral inhibition—whereas before it yielded only increased fluctuating asymmetry (Figure 2e). Similarly, selection for deviations toward one side only (Figure 2c) could yield either antisymmetry (Figure 2f), if asymmetries arose only due to lateral inhibition, or directional asymmetry (Figure 2g), if asymmetries arose from both an inhibition between sides and a genetic bias toward a particular side. Both results contrast sharply with those in our original ideal world of pure fluctuating asymmetry, in which only one response is possible (Figure 2e).

When these other two kinds of asymmetry are present, ignoring the signs in a heritability analysis creates a muddle. First, if inhibition or negative covariation between sides is heritable, then we might be selecting either for antisymmetry or for directional asymmetry (Figures 2f or 2g). A conventional heritability analysis would yield a perfectly good regression of offspring versus parent asymmetry: parents that were more asymmetrical would produce offspring that were more asymmetrical (Figures 2j and 2k). However, the average unsigned deviation from symmetry (dashed line) now describes some unfathomable mix of the effects of developmental stability, developmental noise, and genetic predisposition toward directional asymmetry or antisymmetry. Second, we can no longer distinguish heritable variation for antisymmetry from heritable variation for directional asymmetry because Figures 2j and 2k will hardly differ. We can avoid this muddle only by testing the offspring for departures from ideal fluctuating asymmetry, in the form of antisymmetry or directional asymmetry, before we conduct our analysis. But rather large samples may be needed to detect antisymmetry.

Reassuringly for those who value fluctuating asymmetry as a measure of developmental precision, the few experimental attempts to select for directional asymmetry when starting with fluctuating asymmetry (i.e., transforming Figure 2c into 2g) have been unsuccessful (fruit fly ocelli and bristles; Tuinstra et al. 1990, and references therein). In addition, attempts to select for a behavioral bias toward one side when starting with what could best be called behavioral antisymmetry have also been unsuccessful in both fruit flies (Ehrman et al. 1978) and mice (Collins 1985). Simple heritability studies have also revealed no evidence for heritable deviations from symmetry in a particular direction (e.g., directional asymmetry in stickleback pelvic elements; Blouw and Boyd 1992), although a weak heritability of directional asymmetry has been reported in skeletal traits of inbred mice (Leamy 1984). Curiously, attempts to select for increased directional asymmetry when starting with some initial directional asymmetry have also been unsuccessful (e.g., polydactylous mutants in guinea pigs; Castle 1906), so variation in some forms of directional asymmetry may not have a heritable basis.

The bad news, though, is that in at least three studies, antisymmetry appears to have arisen from fluctuating asymmetry. Mather (1953) appeared to obtain antisymmetry as a consequence of selecting for high asymmetry (Figure 2b) in *Drosophila* sternopleural chaetae. Antisymmetry also arose in wing and head chaetae of sheep blowfly, where it was coupled with a pesticide resistance allele (*scalloped wings*; McKenzie and Clarke 1988), and in the forewings of mutants (*unstable micropterous*) in a phytophagous bug (Socha et al. 1993). Consequently, these last two studies suggest that the products of single genes are sufficient to induce antisymmetry.

What does all of this mean for studies that use fluctuating asymmetry as a measure of developmental precision? It is too early to say. Many bilaterally symmetrical organisms have evolved conspicuously asymmetrical structures (Neville 1976), so natural selection has clearly been able to amplify heritable variation for asymmetry. However, much of the subtle bilateral variation observed in studies of fluctuating asymmetry probably does arise from developmental noise, so long as it is not confounded by antisymmetry or directional asymmetry. Needless to say, with the present meager evidence we must still take a lot on faith. At the very least, those wishing to use fluctuating asymmetry as a measure of developmental precision must take great care to rule out other forms of asymmetry variation (Palmer 1994).

Biomonitoring and conservation biology

Because fluctuating asymmetry offers a measure of developmental precision, and because stress during development seems likely to influence that precision, fluctuating asymmetry has been advanced as a potentially useful tool for monitoring stress levels in natural populations (Graham et al. 1993a, Leary and Allendorf 1989). Although the strength of the effect varies, the magnitude of fluctuating asymmetry appears to correlate with a variety of stresses, which can generally be grouped into two categories: environmental, or extrinsic, stresses (temperature extremes, food shortage, pollution, pesticides, parasite load, population density), and genetic, or intrinsic, stresses (inbreeding, hybridization, chromosomal abnormalities, mildly deleterious recessive genes, disruptions of gene balance). Typically, the greater the stress, the greater the fluctuating asymmetry, both in natural populations and in controlled laboratory experiments (Parsons 1990, Zakharov and Graham 1992). Thus, fluctuating asymmetry does seem to offer promise as a biomonitoring tool.

For prudent use of fluctuating asymmetry as a management tool in biomonitoring and conservation bi-

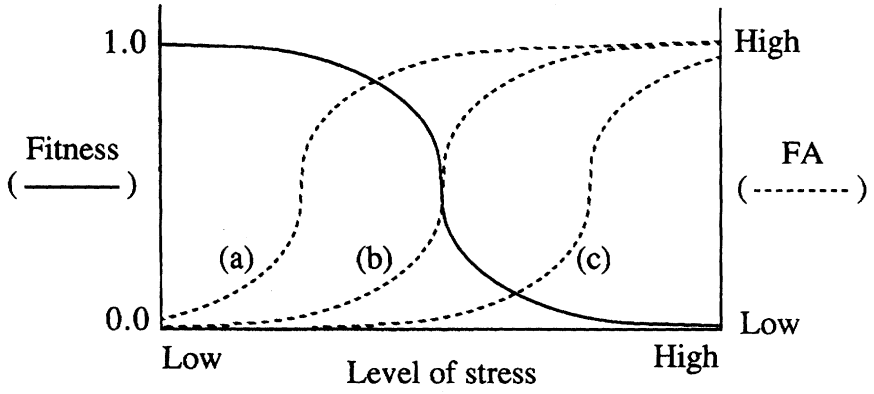

Figure 3. Possible relationships among stress, fitness, and fluctuating asymmetry (FA), illustrating how fluctuating asymmetry might increase (a) before, (b) concurrent with, or (c) after a substantial decline in fitness. Fluctuating asymmetry is most useful as a tool for biomonitoring when it increases before significant declines in fitness (a).

ology, however, two important questions still need to be addressed. First, how much must the level of fluctuating asymmetry increase before a natural population is considered to be significantly stressed? For example, although fluctuating asymmetry tends to increase in populations exposed to pollution, how much must it increase before pollution levels are deemed unusually or dangerously high? Only if the level of fluctuating asymmetry of a putatively stressed population lies significantly far from the background level of fluctuating asymmetry in unstressed populations can biologists answer this question with much confidence, and few studies have adequately sampled natural or unstressed populations. Additional surveys of many stressed and unstressed populations like those conducted by Zakharov and his colleagues (see Zakharov 1989) would be revealing.

Second, how valuable is fluctuating asymmetry as an early warning system? Although many studies report an association between increased fluctuating asymmetry and decreased fitness (Markow 1994, Zakharov and Graham 1992), few have assessed whether fluctuating asymmetry increases before fitness declines substantially (Figure 3a), whether it increases coincident with this decline (Figure 3b), or whether it increases only after this decline (Figure 3c). Experiments suggest that fluctuating asymmetry does increase before significant increases in mortality of laboratory-reared flies (Clarke and McKenzie 1992, Graham et al. 1993b), but these relationships clearly warrant more attention.

Heterozygosity: an ongoing controversy

Among the many inferences drawn from patterns of variation in fluctuating asymmetry, the relationship between fluctuating asymmetry and heterozygosity has generated more than its fair share of controversy for two reasons: inconsistent results and uncertainty about causal connections. The opposing views are perhaps best presented by Mitton (1993) and Clarke (1993a). In a nutshell, many studies have reported a negative association between fluctuating asymmetry and heterozygosity: the more heterozygous the sample, and sometimes the individual, the lower the level of fluctuating asymmetry. Mitton argues that heterozygosity as revealed by a sub-sample of enzyme loci cannot predict genome-wide heterozygosity and that the correlations with fluctuating asymmetry therefore probably arise as a by-product of direct effects of particular heterozygous loci on physiological efficiency. The perceptive studies of enzymatically nonfunctional null alleles in salmonid fishes (Leary et al. 1993) suggest that heterozygotes carrying one null allele actually exhibit higher levels of fluctuating asymmetry than homozygotes for functional alleles. These results certainly support the view that genotypes at particular enzyme loci can influence developmental precision, perhaps via their effects on metabolic efficiency.

Clarke (1993a), on the other hand, prefers to emphasize the negative or inconsistent results of several studies. He argues that genomic coadaptation—that is, the relational balance of alleles between chromosomes and the distribution of loci among chromosomes that result from ongoing selection for "harmoniously collaborating genes"—has a greater impact than heterozygosity on developmental precision. Unfortunately, he advances no mechanisms for such harmony and thus leaves us wondering how it might arise.

The debate becomes even more complex when studies of hybridization are included (reviewed in Graham 1992). Interspecies hybrids should theoretically be even more heterozygous than either of the two parental species, yet they often exhibit higher fluctuating asymmetry (i.e., lower developmental precision). But such hybrids may also experience disruption of coadapted gene complexes. Although fluctuating asymmetry could potentially increase or decrease in a hybrid zone depending on how genetically divergent the parental species were, hybrids seem to show only the same or increased levels of fluctuating asymmetry.

This debate is fuelled primarily by the discordant results of different studies of intraspecific variation. If the effect of heterozygosity on developmental precision is a general one, then why is it not observed in all studies? At the very least, such inconsistency suggests that the effects of heterozygosity are small compared to the effects of other factors. Even the tantalizing suggestion that fluctuating asymmetry correlates more closely with heterozygosity in poikilotherms than homeotherms may be due more to methodological differences than to biological ones (Novak et al. 1993). But if biologists were to smite all hypotheses with which occasional studies were inconsistent, we would be left with rather few after the carnage, particularly if the supposedly inconsistent studies did not provide a rigorous test. Perhaps the inconsistent patterns observed within species arise because the magnitude of the heterozygosity effect depends on some overlooked fac-

tors. Sensitivity to physiological stress seems a likely one.

Zakharov and his Russian colleagues have assembled one of the most comprehensive visions of the phenomenon of developmental homeostasis (Zakharov 1989, Zakharov and Graham 1992). One tantalizing feature of this vision is the observed association between metabolic efficiency (energy expended to accomplish a defined task) and distance from optimal conditions for growth. For two species of insect and three of fish, the total oxygen consumed between defined stages of development increases with increasing distance above or below some optimal temperature (e.g., see Figure 4a). Moreover, the effect of temperature on total oxygen consumption depends on the stage of development: earlier stages are more sensitive to temperature than later ones. Nevertheless, although the sensitivity changes, the optimal temperature remains the same. Such responses to stress may be widespread in biological systems (Alekseeva et al. 1992). Rather intriguingly, changes in metabolic efficiency of development parallel changes in developmental precision: although the organisms and temperatures are different, those reared under conditions further from their respective optima exhibit lower metabolic efficiency (Figure 4a) and developmental precision (Figure 4b).

Perhaps herein lies a clue that could account for the inconsistent associations between heterozygosity and fluctuating asymmetry. If metabolic efficiency decreases with increasing distance from optimal conditions, if allozyme genotypes affect metabolic efficiency, and if poor metabolic efficiency has a direct effect on developmental precision, then the dependence of fluctuating asymmetry on heterozygosity may itself depend on stress levels.

Considering how fluctuating asymmetry varies as stress levels increase away from optimal conditions, three effects of heterozygosity seem likely. Differences in heterozygosity might influence only the location of the curve (individuals that are more heterozygous would exhibit lower fluctuating asymmetry, but this effect would be most pronounced near the optimum; Figure 5a), they might influence only the shape of the curve (individuals that are more heterozygous would exhibit lower fluctuating asymmetry, but this effect would be most pronounced far from the optimum; Figure 5b), or they might influence both the shape and location of the curve (individuals that are more heterozygous would exhibit lower fluctuating asymmetry regardless of the level of stress; Figure 5c).

Thus, the association between fluctuating asymmetry and heterozygosity may depend on how much stress the organism experienced during development. For example, under near-optimal conditions (b') the association could be weak or nonexistent, whereas under more stressful conditions (a') the association could be pronounced (Figure 5b). Just such an association has been reported for mosquitofish reared under two different temperatures (Mulvey et al. 1994): no association was observed between fluctuating asymmetry and heterozygosity at the more normal temperature (25°C), but fluctuating asymmetry declined significantly with increasing number of heterozygous loci at the more stressful temperature (32°C). Metabolic efficiency may thus lie at the heart of the relationship between heterozygosity and fluctuating asymmetry, but the interaction with stress cannot be ignored (Hoffman and Parsons 1991).

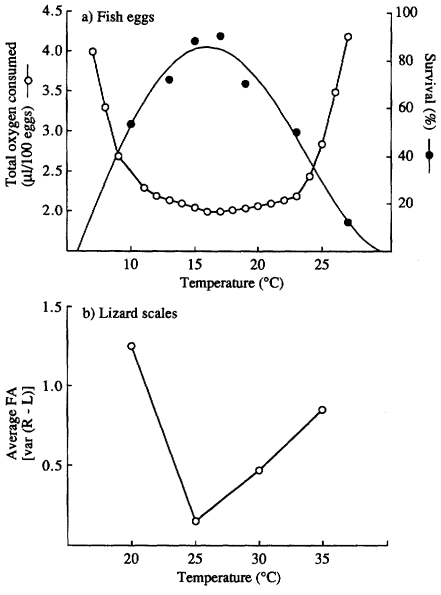

Figure 4. Relationships among metabolic efficiency (total oxygen consumed over a defined period of development), survival, fluctuating asymmetry, and temperature under laboratory conditions. (**a**) Total oxygen consumed during one entire cleavage division (a higher value means a lower metabolic efficiency) and percent survival as a function of temperature in loach eggs (*Misgurnus fossilis*; Ozernyuk 1989). (**b**) Average fluctuating asymmetry (based on the variance of (R–L) for 13 scale counts) as a function of temperature in the sand lizard (*Lacerta agilis*; Zakharov 1985). Both sets of data are from Alekseeva et al. (1992). Although the organisms and temperature ranges are different, departures from optimal conditions during development result in lower metabolic efficiency (**a**) or lower developmental precision (**b**).

Sexual selection: the latest dance step?

Most early work on fluctuating asymmetry explored its use as a biologically informative measure of developmental precision: How did it respond to environmental and genetic stress during development? In addition, this early work focused almost exclusively on the average effects on groups of organisms. Only recently have deviations from symmetry been used to predict the genetic or phenotypic quality of individuals. The enthusiasm of behavioral ecologists for this potential tool has been remarkable.

Something about sexual selection seems to generate more excitement, controversy, and sometimes vitriol than most other areas of biology. Perhaps biologists all believe that they have a right to comment on the subject based on personal experience. Regardless of the opinion one may hold about rigor in the burgeoning literature on the subject, some of which does seem rather naive and superficial, the connection to sexual selection has brought fluctuating asymmetry to the attention of a wide audience (e.g., see Angier 1994, Concar 1995). Who would not find Anders Pape Møller's studies of tail-feather asymmetry and mate selection in barn swallows appealing (Møller 1994a)? He found that females appear to mate preferentially with more symmetrical males, that asymmetry varies with parasite load during ontogeny, that asymmetrical

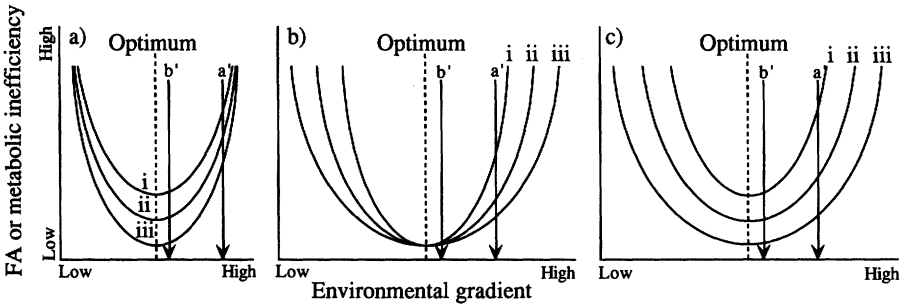

Figure 5. Three ways in which differences in heterozygosity might influence the effect of stress (deviation from the optimum) on fluctuating asymmetry (FA) or metabolic inefficiency: (a) the location of the curve at the optimum varies, (b) the shape of the curve varies but not its location at the optimum, and (c) both the shape of the curve and its location at the optimum vary. Each graph illustrates curves for three heterozygosity levels: (i) low, (ii) intermediate, and (iii) high. Arrows indicate levels of high (a') and low (b') stress. The effect of heterozygosity on fluctuating asymmetry may depend not only on the level of stress (a' versus b') but also on the effect of heterozygosity on the shape or location of the curve describing the relationship between fluctuating asymmetry and stress (compare the relation between heterozygosity and fluctuating asymmetry at high stress [a'] versus low stress [b'] in panels a–c).

males are less likely to survive from one year to the next, and that females cue directly on this asymmetry. These results suggest that deviations from symmetry may serve as a measure of fitness and as a cue for choosy females.

The temptation to apply these ideas to humans is substantial. Thornhill and Gangestad (1993), for example, predicted that human facial symmetry should correlate positively with attractiveness, heterozygosity, overall health, growth rate, physiological efficiency, longevity, lifetime reproductive success, number of copulatory partners in both sexes, and development of secondary sexual characters (body and genital hair, strength, shoulder breadth, cheekbones, prominent chin, square jaw) and testosterone levels in men. Some data appear to suggest that women have more frequent orgasms when their male partner is more symmetrical (Thornhill et al. 1995). More is sure to come.

The behavioral ecology literature has exploded over the last five years as many have rushed to apply this new approach. Asymmetry has now been correlated with sexual selection in a surprisingly wide variety of organisms (Tomkins and Simmons 1995 and references therein).

However, much debate rages over whether deviations from symmetry provide cues used by females or whether they simply correlate with other, more conspicuous measures of quality. "People are embracing this idea of symmetry because it's something you can go out and measure, but it's a black box as to what it means, and what it's indicating to the female" (Zuk quoted in Angier 1994). "Even if symmetry is correlated with heritable fitness, that's far from saying that the preference evolved to allow females to pick the fittest males" (Kirkpatrick cited in Concar 1995, p. 43). Furthermore, given the proportionally small size of some of these asymmetries (Table 1), direct cueing seems unlikely for most traits.

Nevertheless, clever experiments suggest that females can cue on certain feather or color asymmetries if these are conspicuous enough (Swaddle and Cuthill 1994 and references therein). Whether females of organisms other than birds respond to similar cues remains to be seen, because fluctuating asymmetry appears to be unrelated to the size of secondary sexual characters in other organisms, as expected if they function as "honest advertising" (Tomkins and Simmons 1995).

Even in birds, though, the connection between fluctuating asymmetry and size in extravagant tail feathers may be influenced by other factors, such as aerodynamic considerations (Balmford et al. 1993). Alternatively, preferences for symmetry might be a simple by-product of neural architectures that respond more strongly to the redundant information symmetric characters provide (Johnstone 1994), rather than an outcome of natural selection.

Sometimes the enthusiasm for correlations with asymmetry in the sexual selection arena seems to have gone a bit far. Typically, individuals departing from some norm are considered less well off than those closer to it. Yet, curiously, Møller (1994b) suggests that increased asymmetry for traits that are on average asymmetrical (e.g., testes of male birds) can also be interpreted as evidence for higher quality. This result contrasts with some fascinating studies in humans suggesting that extreme right-handedness is not necessarily a good thing. Not only do the 5%–10% of the population who are left-handers exhibit a higher incidence of immunological and neuroanatomical disorders, but extreme right-handers suffer many of the same maladies (Yeo and Gangestad 1993). Thus, contrary to Møller's suggestion, deviations from the norm may matter more than deviations from symmetry (Zakharov 1992).

Developmental precision in plants

At first glance, plants would seem ideally suited for studies of developmental precision. Deviations from symmetry could potentially be measured on multiple developmentally equivalent parts (e.g., leaves or petals of separate flowers) and thus yield a much more robust estimate of developmental precision for a single plant than a single measurement of asymmetry for a single pair of structures (e.g., legs, wings) as in most animals. Freeman et al. (1993) have argued further that peculiarities of plant growth offer other ideal forms, such as fractal dimension and radial regularities, about which deviations might be measured in the same way they are for symmetry. Deviations from any of these ideal forms could, in principle, be used to measure developmental precision.

Unfortunately, the remarkable developmental plasticity of plants (e.g., Solangaarachchi and Harper 1989) renders deviations from an ideal form exceedingly difficult to

Table 1. Selected examples of subtle asymmetries as a percent of average character size (linear dimensions or counts). Average right – left (|R – L|) includes both underlying bilateral variation and measurement error. Therefore, tabulated values overestimate the true level of fluctuating asymmetry.

Species	Organism	Trait	Number of samples	Sample size(s)	Asymmetry (average |R – L|) as % trait size	
					Mean	Range
Mammals						
*Homo sapiens**	Humans	Tibia lengths (male/female)	1	79	0.2/0.2	
		Humerus lengths (male/female)	1	79	1.2†/1.8†	
Rattus norvegicus‡	Lab rat	First mandibular molar size	4	32–40	1.6	(1.1–1.8)
Birds						
Aratinga pertinax§	Parakeet	Three leg bones	1	28	0.4†	(0.1–0.7)
Various bird species‖		Wing feather lengths	31	10	0.9	(0.3–1.8)
		Tail feather lengths	14	10	1.4	(0.1–7.1)
		Ornament feather lengths	16	10	3.5	(0.7–8.9)
Falco sparverius¶	Kestrel	Three leg and four wing bones	1	> 30	0.3	(0.2–0.4)
Fish						
Gasterosteus aculeatus#	Stickleback	Pelvic girdle length (normals)	1	125	1.0	
*Cottus bairdi***	Sculpin	Otolith length	20	6–75	2.9	(1.0–11.5)
Insects						
Chrysopa perla††	Lacewing	Number of forewing cells	8	35–49	5.8	(5.1–6.1)
Coenagrion puella‡‡	Damselfly	Forewing length	12§§	20–36	0.42	(0.29–0.93)
Dermaptera (26 spp.)‖‖	Earwigs	Forceps length	26	12–44	0.10	(0.02–0.25)
Crustaceans						
Tigriopus californicus¶¶	Copepod	Three antennae and five leg segments	1	31–40	1.70	(1.10–2.65)
Hemigrapsus nudus##	Shore crab	Four posterior leg segments	1	40	3.91	(2.25–5.61)

*Ruff and Jones 1981.
†Includes small but statistically significant directional asymmetry.
‡Siegel and Smookler 1973.
§McNeil et al. 1971.
‖Møller and Höglund 1991.
¶Bortolotti and Gabrielson 1995.
#Blouw and Boyd 1992.
**Downhower et al. 1990.
††Clarke 1993b.
‡‡Harvey and Walsh 1993.
§§Restricted to samples with n ≤ 20.
‖‖Tomkins and Simmons 1995.
¶¶Palmer et al. 1993.
##Chippindale and Palmer 1993.

interpret. As noted above, a central concept must be kept in mind: to serve as a measure of developmental precision, deviations from an ideal form must not arise as a result of the direct and repeatable effects of genes or external environmental factors. Differential growth between sides of individual leaves in a strong light gradient, for example, could yield asymmetries that have little to do with the random contributions of developmental noise because they could easily be repeated simply by growing a leaf in the same conditions again. Similarly, environmentally induced differential growth could also yield deviations from ideal phyllotaxies (spacing patterns) of leaves or leaflets.

Early studies of developmental precision in plants recognized these limitations. For example, although he did not examine fluctuating asymmetry, Paxman (1956) took advantage of repeated structures (flowers and leaves) to measure developmental precision in a thoughtful and elegant study of different varieties of tobacco. But even he found that "[flower] position [on the plant] has a definite effect on [pistil length, which] is not constant throughout the five varieties used" (Paxman 1956, p. 334). Similarly, Dormer and Hucker (1957, p. 385) concluded, based on their study of holly leaves, that "the number of prickles upon the edge of a leaf is related by complex laws to the size of the leaf, the number of prickles on the other edge, the number of prickles on other leaves of the same shoot, the phyllotaxy of the shoot, and the intrinsic asymmetry of the leaf."

Thus, to use asymmetry variation or variation about some other ideal as a measure of developmental precision in plants, the repeatable variation due to developmental plasticity or position must somehow be minimized or ruled out. Otherwise, measures of variability are likely to represent an uninterpretable mix of developmental noise and developmental plasticity.

Methods: yes, the choreography does matter

By virtue of its methodological and conceptual simplicity, fluctuating asymmetry seems an alluring tool—simply measure the difference between sides in your favorite bilateral character and, voilà, you have a measure of developmental precision. However, several pitfalls await the casual user of this seemingly simple tool. The most important of these pitfalls are: insufficient measurement accuracy, potentially confounding

effects of body size and age, and the low statistical power for detecting fluctuating asymmetry differences among samples and for detecting departures from ideal fluctuating asymmetry (Palmer 1994).

Only rather recently has the nontrivial problem of measurement error been given sufficient respect in studies of fluctuating asymmetry (reviewed in Palmer and Strobeck 1986). Given the small difference between sides of many bilateral structures (Table 1), it simply cannot be ignored. Errors in measurement give rise to beautiful fluctuating asymmetry because they are noiselike (i.e., random and independent). Therefore, reporting the accuracy or repeatability of measurements of wing or feather length can be misleading, because what matters is the accuracy or repeatability of measured differences between sides. Wing lengths measured to an accuracy of 1% are not likely to help detect differences in fluctuating asymmetry among groups of birds, where the differences themselves are only approximately 1% or less of wing size. Thus, for example, in one study (Fields et al. 1995) even though the repeatability of ten anthropometric traits ranged from 90% to 99%, the repeatabilities of the bilateral differences for those same traits ranged from 24% to 43%, and for nearly all traits the variation between repeat measurements was significantly greater than that due to real bilateral differences.

Furthermore, if measurement error is not reported in a useful way, such as the average difference between pairs of repeated measurements, then we can never know what fraction of the between-side variation is biologically meaningful and what is meaningless. Consequently, progress toward understanding the causes and hence the biological significance of fluctuating asymmetry will be slowed substantially. Needless to say, if the level of fluctuating asymmetry does not correlate with some factor of interest, conclusions are pointless without a careful error analysis.

However, although measurement errors normally will not create patterns of biological interest, they can do so when scaled by overall trait or body size. For example, an average difference between repeat measurements $|M_1 - M_2|$ of 1 mm will yield an average difference between sides $|R - L|$ of roughly 1.5 mm. If trait size varies from 10 mm to 100 mm with increasing body size, the average difference between sides (fluctuating asymmetry) will decrease from 15% to 1.5% with increasing body size when expressed as a percentage of trait size. An uncritical biologist might conclude that such a pattern supports the inference that fluctuating asymmetry is a measure of fitness because the decline in fluctuating asymmetry with increasing size is predicted to occur due to the differential mortality of those individuals with higher fluctuating asymmetry (lower quality or fitness), but in this example the decline is a simple artifact.

Even if measurement error has been minimized, variation in body size introduces another challenge. As a rule, variability in related traits increases with increasing trait size: the right and left femur of a mouse will differ less than those of an elephant, even though the difference will be small relative to femur size in both cases. Logarithmic transformations, such as $\log(R) - \log(L) = \log(R/L)$, or ratios, such as $(R - L)/[(R + L)/2]$, allow differences between sides to be expressed as a proportion of trait size. This transformation, however, provides only a partial solution to the problem of size dependence because the precise form of the dependence of variability on trait size may not be a simple linear relationship. If applied improperly, corrections for body size variation can either create artificial associations or obscure underlying patterns of biological interest (Palmer and Strobeck 1986).

Low statistical power is another pitfall encountered in studies of fluctuating asymmetry. First, measures of fluctuating asymmetry are measures of variability, and tests for differences in variability between samples are notoriously less powerful than tests for differences between sample means. Although many tests of relative variability are possible, the ratio of two variances (F-test) is one of the most powerful statistically. Even with this test, though, sample sizes must be at least 20 to detect a twofold difference in variances only 50% of the time, or 40 to detect such a difference 75% of the time. Few studies of fluctuating asymmetry include sample sizes greater than 40; thus, most have the statistical power to detect only rather large differences in fluctuating asymmetry (more than a twofold difference in variance). Second, tests for departures from normality are even less powerful than tests for variance differences among samples. Thus, for a what might seem like a conspicuous example of antisymmetry (Figure 2f), the sample size must be at least 60 for the shape (kurtosis) to be considered significantly different from a normal curve at the 5% level.[1] Consequently, the conclusion that a frequency distribution does not depart significantly from a normal curve is not a strong one for the sample sizes used in most studies of fluctuating asymmetry.

None of these problems is insurmountable. But lack of vigilance by authors, reviewers, and editors can lead to publication of superficially attractive studies that offer little substance.

The curious case of antisymmetry

Some of the most fascinating and provocative work on subtle deviations from symmetry is that by John McKenzie and colleagues on the sheep blowfly, *Lucilia cuprina* (McKenzie and Batterganm 1994 and references therein). Although mainly concerned with the evolution of insecticide resistance, they combine studies of fitness, biochemical function, genetics, and asymmetry variation in a remarkably comprehensive program that promises to yield considerable insight into the phenomena influencing developmental precision.

Within 12 years of the introduction of Diazinon, blowflies evolved resistance to this pesticide. Initially, the resistance allele was associated with a substantial increase in deviations from symmetry in three bristle characters (frontal head stripe, outer wing margin, one wing vein) and a correlated decrease in fitness as com-

[1] A. R. Palmer and C. Strobeck, 1996, manuscript in preparation.

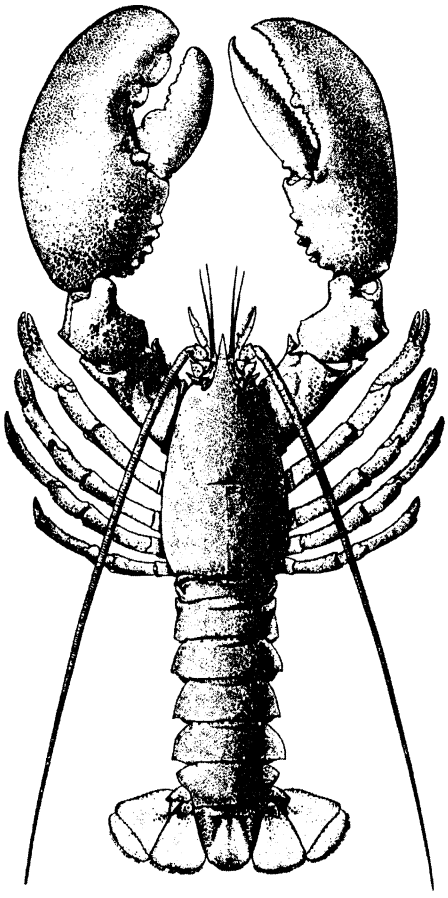

Figure 6. A mature male lobster, *Homarus americanus,* exhibiting a pronounced bilateral asymmetry in the claws that has evolved many times in decapod crustaceans. In postlarval lobsters, the claw that is used more frequently transforms into the crusher claw and, in turn, inhibits transformation of the other claw (Govind 1989).

pared to strains lacking the resistance allele when both were reared in the absence of pesticide. Resistance thus appears to have been purchased initially at the price of reduced developmental precision and lowered fitness. Subsequently, an allele at a *modifier* locus arose and returned the levels of both asymmetry and fitness to their preresistance levels.

McKenzie's studies are all the more remarkable because of the form of subtle asymmetry exhibited in resistant flies lacking the stabilizing allele at the *modifier* locus. For both Diazinon and Malathion, elevated asymmetry in resistant flies took the form of weak to modest antisymmetry as opposed to simple fluctuating asymmetry (McKenzie and Clarke 1988, McKenzie and O'Farrell 1993). Thus, rather than providing a clear-cut demonstration of single-locus control of developmental precision (as measured by fluctuating asymmetry), these workers uncovered a gene that influences antisymmetry.

Socha and his colleagues (1993) reported an even more dramatic example of a genetically based antisymmetry in their study of unstable forewing polymorphisms in a phytophagous bug, *Pyrrhocoris apterus*. The *unstable micropterous* locus induces a high incidence, not only of micropterous (short-winged) individuals but also of individuals in which one forewing is significantly shorter than the other at random. These pronounced asymmetries are not expressed in legs or antennae. Hence, for two different insects, genetic differences at one or a few loci may induce antisymmetry.

Much is now understood about the biochemistry of pesticide resistance in *Lucilia* (McKenzie and Batterganm 1994), and some persistent genetic sleuthing suggests that the *modifier* locus of *Lucilia*, which returns blowflies to normal levels of asymmetry, may be homologous with the *Notch* locus of *Drosophila*, which controls development of the peripheral and central nervous system. This putative homology raises the intriguing possibility that the nervous system may play an important role in regulating symmetry, much like it may regulate the striking bilateral asymmetries in some crustacean claws (Mellon and Stephens 1978).

Distinguishing direct genetic effects from epigenetic effects, some of which result from idiosyncrasies of morphogenetic mechanisms, is a major challenge to developmental biologists. Not everyone believes, for example, that antisymmetries in the above examples represent genetically determined asymmetries. Graham et al. (1993c, p. 123) argue, based on theoretical studies of morphogenesis using Rashevsky–Turing reaction–diffusion models, that both antisymmetry and directional asymmetry may also result from epigenetic phenomena because they can be induced by "symmetry-breaking phase transitions [in which] concentrations of morphogen on [the] right and left sides can be induced to undergo transitions from phase-locked periodicity, to phase-lagged periodicity, to chaos, by simply changing the levels of feedback and inhibition in the model." They further suggest that stress could induce antisymmetry or directional asymmetry. If this conclusion is true, such effects would be worth exploring further because of the insights they might yield into the developmental genetic mechanisms that affect symmetry.

However, because antisymmetry and directional asymmetry are the normal state in many organisms, one cannot conclude that an organism exhibiting such asymmetries has been stressed without additional information about the normal state. Unfortunately, the Graham et al. (1993c) model extrapolates from cell–cell interactions, in which reaction–diffusion mechanisms may work among nearby cells, to side–side interactions, which must occur at a distance. Consequently, the relevance of this model to bilateral asymmetry remains unclear.

Conspicuous asymmetries: the grand waltz

Often overlooked in the fluctuating asymmetry fray is a simple observation: many bilaterally symmetrical organisms have evolved conspicuously asymmetrical structures (Neville 1976). For example, claw asymmetry has evolved multiple times within decapod crustaceans (Figure 6; e.g., see Martin and Abele 1986), and ear asymmetry has evolved at least five times in owls (Norberg 1977). Presumably these asymmetrical structures arose from initially symmetrical ones (Palmer et al. 1993), so heritable variation for subtle deviations from symmetry clearly must have arisen at *some* point in their evolutionary history.

Although biologists have unraveled some of the developmental and genetic bases of conspicuous asymmetries (Wolpert 1991, Yost 1995), we still know little about their evolutionary origins. These origins are more problematical than those of conventional traits because, for natural selection to yield any evolutionary change, the phenotypic variation must have an effect on performance. Given the small size of the differ-

ences between sides normally observed for fluctuating asymmetry (Table 1), these differences would seem unlikely to affect performance favorably. The connection between handedness—a behavioral preference for one side—and morphological asymmetry offers an appealingly simple mechanism for amplifying potentially functional asymmetries.

A surprising number of organisms with paired limbs for feeding or manipulation develop a handedness that is stereotyped for an individual but varies randomly among them (Morgan 1991). In addition, in both crustacean claws (Smith and Palmer 1994) and human limbs (Trinkaus 1994) increased use leads to increased skeletal development. Thus, differential use could easily induce or amplify morphological differences between sides. The correlation between handedness and limb asymmetry in both humans (Trinkaus 1994) and birds (McNeil et al. 1971) certainly suggests such an effect.

If these facultative asymmetries improve performance, selection would favor genes that enhance the ease with which use influences form. Eventually, such asymmetries could become fixed via genetic assimilation (Waddington 1953). The coupling of behavioral biases (handedness) with phenotypic plasticity provides one way in which conspicuous asymmetrical external structures might arise evolutionarily.

Coda

When a new approach to a problem initially becomes popular, the competition for visibility tends to be won by the best stories rather than by the best data. Where the biological signal is so tiny and prone to confounding factors as in studies of fluctuating asymmetry, the good stories are those that report a positive association. If enough studies are done, some will be significant statistically just due to chance. Thus, we see published reports in which statistically significant results arise due to one or two points in a scatter plot of many (e.g., see Figure 2a in Witter and Swaddle 1994 and Figure 3 in Møller 1994c). Because negative results are rarely published—they are harder to make convincing and often lack appeal—the literature becomes overburdened with positive results, a disproportionate number of which may be spurious. Presumably, in time, as others try to repeat earlier work, we will be able to sort the wheat from the chaff. For now, though, the sheer enthusiasm for new and exotic good stories lends an almost surreal aura to the field. Who can blame those outside it for occasional "bemused incredulity" (Concar 1995)?

Phil Hedrick's concluding remarks, as a respected population geneticist at the Tempe conference (14–15 June 1993) on developmental instability, should sound a clarion call to those in the field: "Personally, I am skeptical of the generality of the use of asymmetry, and at times during this [conference] I felt like I was an atheist attending a church revival.... The true believer of asymmetry must realize that most scientists are skeptics, and the claim that [fluctuating asymmetry] is the greatest thing since sliced bread will be met in most quarters with derision. If the use of asymmetry is to be accepted in the mainstream of evolution and population biology, then a great deal of persuasive work remains, and the rabid claims made by some at this meeting must be carefully evaluated so that they will not discredit every asymmetry study or researcher" (Hedrick quoted in Markow 1994, p. 434). Similar doubts have been raised by other respected biologists such as Robert May, who stated: "Personally, I find the evidence [of genetic impoverishment in cheetahs] from skin grafts and from fluctuating asymmetries a bit dodgy" (May 1995, p. 309).

Although Hedrick's comments may seem harsh, and the pitfalls inherent to fluctuating asymmetry analyses daunting to the uninitiated, the number of studies yielding positive results is too great to dismiss the phenomenon and its potential value as a tool. In addition, fluctuating asymmetry has one thing to offer over many other phenomena—quantitative measures of developmental precision that can be compared among several organisms. As is the case with quantitative measures of heritability (Mousseau and Roff 1987), one can legitimately ask whether some organisms or traits exhibit higher developmental precision than others.

One may even begin to ask how developmental precision itself has evolved by looking back in the fossil record (Erwin 1993). But such questions will remain outside our reach, despite the immense effort invested to produce the data for particular studies, so long as the results are reported with information insufficient to determine exactly what the published numbers represent. If we are ever to separate defendable results from wishful thinking, more care must be taken in analysis and presentation. Guides are available (Graham et al. 1993a, Palmer 1994) for those interested in presenting their data in a way that will outlast the brief interest shown by others who care only for the punch line.

Acknowledgments

I thank Gary Freeman, Lois Hammond, Curtis Strobeck, and four anonymous reviewers for their comments on the manuscript. Vladimir Zakharov and Larry Leamy provided stimulating discussions of some of the issues raised in this article. I also thank the Natural Sciences and Engineering Research Council of Canada for their sustained support of this research. Because of limits on the number of references, I have been constrained to cite many overview papers. The authors of the original papers should not feel slighted.

References cited

Alekseeva TA, Zinichev VV, Zotin AI. 1992. Energy criteria of reliability and stability of development. Acta Zoologica Fennica 191: 159–165.

Angier N. 1994. Sex appeal: beauty in the beasts. The New York Times (Feb. 19).

Balmford A, Jones IL, Thomas ALR. 1993. On avian asymmetry: evidence of natural selection for symmetrical tails and wings in birds. Proceedings of the Royal Society of London B Biological Sciences 252: 245–251.

Blouw DM, Boyd GJ. 1992. Inheritance of reduction, loss, and asymmetry of the pelvis in *Pungitius pungitius* (ninespine stickleback). Heredity 68: 33–42.

Bortolotti GR, Gabrielson JR. 1995. Fluctuating asymmetry in the skeleton of the American kestrel, *Falco sparverius*: a test of the consequences of sexual size dimorphism. Canadian Journal of Zoology 73: 141–145.

Brown NA, Wolpert L. 1990. The development of handedness in left-right asymmetry. De-

velopment 109: 1–9.
Bryant PJ, Simpson P. 1984. Intrinsic and extrinsic controls of growth in developing organs. Quarterly Review of Biology 59: 387–415.
Castle WE. 1906. The origin of a polydactylous race of guinea-pigs. Publications of the Carnegie Institution of Washington 49: 17–29.
Chippindale AK, Palmer AR. 1993. Persistence of subtle departures from symmetry over multiple molts in individual brachyuran crabs: relevance to developmental stability. Genetica 89: 185–199.
Clarke GM. 1993a. The genetic basis of developmental stability: I. Relationships between stability, heterozygosity and genomic coadaptation. Genetica 89: 15–23.
_____. 1993b. Patterns of developmental stability of *Chrysopa perla* L. (Neuroptera: Chrysopidae) in response to environmental pollution. Environmental Entomology 22: 1362–1366.
Clarke GM, McKenzie LJ. 1992. Fluctuating asymmetry as a quality control indicator for insect mass rearing processes. Journal o Economic Entomology 85: 2045–2050.
Collins RL. 1985. On the inheritance of direction and degree of asymmetry. Pages 41–71 in Glick SD, ed. Cerebral lateralization in non-human species. New York: Academic Press.
Concar D. 1995. Sex and the symmetrical body. New Scientist April 22: 40–44.
Dormer KJ, Hucker J. 1957. Observations on the occurrence of prickles on the leaves of *Ilex aquifolium*. Annals of Botany (New Series) 21: 385–398.
Downhower J, Blumer L, LeJeune P, Gaudin P, Marconats A, Bisazza A. 1990. Otolith asymmetry in *Cottus bairdi* and *C. gobio*. Polski Archiwum Hydrobiologii 37: 209–220.
Ehrman L, Thompson J, Perelle I, Hisey B. 1978. Some approaches to the question of *Drosophila* laterality. Genetical Research (Cambridge) 32: 231–238.
Erwin DH. 1993. The origin of metazoan development: a palaeobiological perspective. Biological Journal of the Linnean Society 50: 255–274.
Fields SJ, Spiers M, Herschkovitz I, Livshits G. 1995. Reliability of reliability coefficients in the estimation of asymmetry. American Journal of Physical Anthropology 96: 83–87.
Freeman DC, Graham JH, Emlen JM. 1993 Developmental stability in plants: symmetries, stress and epigenesis. Genetica 89: 97–119.
Govind CK. 1989. Asymmetry in lobster claws. American Scientist 77: 468–474.
Graham JH. 1992. Genomic coadaptation and developmental stiability in hybrid zones. Acta Zoologica Fennica 191: 121–132.
Graham JH, Freeman DC, Emlen JM. 1993a. Developmental stability: a sensitive indicator of populations under stress. Pages 136–158 in Landis WG, Hughes JS, Lewis MA, eds. Environmental toxicology and risk assessment. Philadelphia (PA): American Society for Testing and Materials.
Graham JH, Roe KE, West TB. 1993b. Effects of lead and benzene on developmental stability of *Drosophila melanogaster*. Ecotoxicology 2: 185–195.
Graham JH, Freeman DC, Emlen JM. 1993c. Antisymmetry, directional asymmetry, and dynamic morphogenesis. Genetica 89: 121–137.
Hallgrímsson B. 1993. Fluctuating asymmetry in *Macaca fascicularis*: a study of the etiology of developmental noise. International Journal of Primatology 14: 421–443.
Harvey IF, Walsh KJ. 1993. Fluctuating asymmetry and lifetime mating success are correlated in males of the damselfly *Coenagrion puella* (Odonata: Coenagrionidae). Ecological Entomology 18: 198–202.
Herrick FH. 1909. Natural history of the American Lobster. Fishery Bulletin (USA) 29: 149–408.
Hoffman AA, Parsons PA. 1991. Evolutionary genetics and environmental stress. New York: Oxford University Press.
Johnstone RA. 1994. Female preference for symmetrical males as a by-product of selection for mate recognition. Nature 372: 172–175.
Jolicoeur P. 1963. Bilateral asymmetry and asymmetry in limb bones of *Martes americana* and man. Revue Canadienne du Biologie 22: 409–432.
Leamy L. 1984. Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. American Naturalist 123: 579–593.
_____. 1986. Directional selection and developmental stability: evidence from fluctuating asymmetry of dental characters in mice. Heredity 57: 381–388.
Leary RF, Allendorf FW. 1989. Fluctuating asymmetry as an indicator of stress in conservation biology. Trends in Ecology and Evolution 4: 214–217.
Leary RF, Allendorf FW, Knudson KL. 1992. Genetic, environmental, and developmental causes of meristic variation in rainbow trout. Acta Zoologica Fennica 191: 79–95.
_____. 1993. Null alleles at two lactate dehydrogenase loci in rainbow trout are associated with decreased developmental stability. Genetica 89: 3–13.
Ludwig W. 1932. Das Rechts-Links Problem im Teirreich und beim Menschen. Berlin (Germany): Springer.
Markow TA, ed. 1994. Developmental instability: its origins and evolutionary implications. Dordrecht (the Netherlands): Kluwer.
_____. 1995. Evolutionary ecology and developmental stability. Annual Review of Entomology 40: 105–120.
Martin JW, Abele LG. 1986. Phylogenetic relationships of the genus *Aegla* (Decapoda: Anomura: Aeglidae), with comments on anomuran phylogeny. Journal of Crustacean Biology 6: 576–616.
Mather K. 1953. Genetical control of stability in development. Heredity 7: 297–336.
May RM. 1995. The cheetah controversy. Nature 374: 309–310.
McGrath JW, Cheverud JM, Buikstra JE. 1984. Genetic correlations between sides and heritability of asymmetry for nonmetric traits in rhesus macaques on Cayo Santiogo. American Journal of Physical Anthropology 64: 401–411.
McKenzie JA, Batterganm P. 1994. The genetic, molecular and phenotypic consequences of selection for insecticide resistance. Trends in Ecology and Evolution 9: 166–169.
McKenzie JA, Clarke GM. 1988. Diazinon resistance, fluctuating asymmetry and fitness in the Australian sheep blowfly, *Lucilia cuprina*. Genetics 120: 213–220.
McKenzie JA, O'Farrell K. 1993. Modification of developmental stability and fitness: malathion-resistance in the Australian sheep blowfly, *Lucilia cuprina*. Genetica 89: 67–76.
McNeil R, Rodriguez JR, Figuera DM. 1971. Handedness in the brown-throated parakeet *Aratinga pertinax* in relation with skeletal asymmetry. Ibis 113: 494–499.
Mellon DF, Stephens PT. 1978. Limb morphology and function are transformed by contralateral nerve section in snapping shrimp. Nature 272: 246–248.
Mitton JB. 1993. Enzyme heterozygosity, metabolism, and developmental stability. Genetica 89: 47–65.
Møller AP. 1994a. Sexual selection and the barn swallow. New York: Oxford University Press.
_____. 1994b. Directional selection on directional asymmetry: testes size and secondary sexual characters in birds. Proceedings of the Royal Society of London B Biological Sciences 258: 147–151.
_____. 1994c. Sexual selection in the barn swallow (*Hirundo rustica*). IV. Patterns of fluctuating asymmetry and selection against asymmetry. Evolution 48: 658–670.
Møller AP, Höglund. 1991. Patterns of fluctuating asymmetry in avian feather ornaments: implications for models of sexual selection. Proceedings of the Royal Society of London B Biological Sciences 245: 1–5.
Morgan MJ. 1991. The asymmetrical genetic determination of laterality: flatfish, frogs and human handedness. Pages 234–247 in Wolpert L, ed. Biological asymmetry and handedness. Toronto (Canada): Wiley & Sons.
Mousseau TA, Roff DA. 1987. Natural selection and the heritability of fitness components. Heredity 59: 181–197.
Mulvey M, Keller GP, Meffe GK. 1994. Single- and multiple-locus genotypes and life-history responses of *Gambusia holbrooki* reared at two different temperatures. Evolution 48: 1810–1918.
Neville AC. 1976. Animal asymmetry. London (UK): Edward Arnold.
Norberg RÅ. 1977. Occurrence and independent evolution of bilateral ear asymmetry in owls and implications on owl taxonomy. Philosophical Transactions of the Royal Society of London B Biological Sciences 280: 375–408.
Novak JM, Rhodes OE Jr., Smith MH, Chesser RK. 1993. Morphological asymmetry in mammals: genetics and homeostasis reconsidered. Acta Theriologica 38: 7–18.
Palmer AR. 1994. Fluctuating asymmetry analyses: a primer. Pages 335–364 in Markow TA, ed. Developmental instability: its origins and evolutionary implications. Dordrecht (the Netherlands): Kluwer.
Palmer AR, Strobeck C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 17: 391–421.
_____. 1992. Fluctuating asymmetry as a measure of developmental stability: implications of non-normal distributions and power of statistical tests. Acta Zoologica Fennica 191: 57–72.
Palmer AR, Strobeck C, Chippindale AK. 1993.

Bilateral variation and the evolutionary origin of macroscopic asymmetries. Genetica 89: 201–218.
Parsons PA. 1990. Fluctuating asymmetry: an epigenetic measure of stress. Biological Reviews (Cambridge) 65: 131–145.
Partridge L, Barrie B, Fowler K, French V. 1994. Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. Evolution 48: 1269–1276.
Paxman GJ. 1956. Differentiation and stability in the development of *Nicotiana rustica*. Annals of Botany 20: 331–347.
Reeve ECR. 1960. Some genetic tests on asymmetry of sternopleural chaetae number in *Drosophila*. Genetical Research (Cambridge) 1: 151–172.
Ruff CB, Jones HH. 1981. Bilateral asymmetry in cortical bone thickness of the humerus and tibia: sex and age factors. Human Biology 52: 69–86.
Schultz AH. 1926. Fetal growth of man and other primates. Quarterly Review of Biology 1: 465–521.
Siegel MI, Smookler HH. 1973. Fluctuating dental asymmetry and audiogeneic stress. Growth 37: 35–39.
Smith LD, Palmer AR. 1994. Experimental evidence for adaptive phenotypic plasticity in the claws of brachyuran crabs. Science 264: 710–712.
Socha R, Nedved O, Zrzavy J. 1993. Unstable forewing polymorphism in a strain of *Pyrrhocoris apterus* (Hemiptera: Pyrrhocoridae). Annals of the Entomological Society of America 86: 484–489.
Solangaarachchi SM, Harper JL. 1989. The growth and asymmetry of neighbouring plants of white clover (*Trifolium repens* L.). Oecologia (Berlin) 78: 208–213.
Stevenson RD, Hill MF, Bryant PJ. 1995. Organ and cell allometry in Hawaiian *Drosophila*: how to make a big fly. Proceedings of the Royal Society of London B Biological Sciences 259: 105–110.
Sumner JL, Huestis RR. 1921. Bilateral asymmetry and its relation to certain problems in genetics. Genetics 6: 445–485.
Swaddle JP, Cuthill IC. 1994. Female zebra finches prefer males with symmetrical chest plumage. Proceedings of the Royal Society of London B Biological Sciences 258: 267–271.
Thornhill R, Gangestad SW. 1993. Human facial beauty: averageness, symmetry and parasite resistance. Human Nature 4: 237–269.
Thornhill R, Gangestad SW, Comer R. 1995. Human female orgasm and male fluctuating asymmetry. Animal Behaviour 50: 1601–1615.
Tomkins JL, Simmons LW. 1995. Patterns of fluctuating asymmetry in earwig forceps: no evidence for reliable signalling. Proceedings of the Royal Society of London B Biological Sciences 259: 89–96.
Trinkaus E. 1994. Postcranial robusticity in Homo. II: humeral bilateral asymmetry and bone plasticity. American Journal of Physical Anthropology 93: 1–34.
Tuinstra EJ, Dejong G, Scharloo W. 1990. Lack of response to family selection for directional asymmetry in *Drosophila melanogaster*: left and right are not distinguished in development. Proceedings of the Royal Society of London B Biological Sciences 241: 146–152.
VanValen L. 1962. A study of fluctuating asymmetry. Evolution 16: 125–142.
Waddington CH. 1953. Genetic assimilation of acquired characters. Evolution 7: 118–126.
Witter MS, Swaddle JP. 1994. Fluctuating asymmetries, competition and dominance. Proceedings of the Royal Society of London B Biological Sciences 256: 299–303.
Wolpert L, ed. 1991. Biological asymmetry and handedness. New York: Wiley & Sons.
Yeo RA, Gangestad SW. 1993. Developmental origins of variation in human hand preference. Genetica 89: 281–296.
Yost HJ. 1995. Vertebrate left–right development. Cell 82: 689–692.
Zakharov VM. 1989. Future prospects for population phenogenetics. New York: Harwood Academic Publishers.
_____. 1992. Population phenogenetics: analysis of developmental stability in natural populations. Acta Zoologica Fennica 191: 7–30.
Zakharov VM, Graham HJ, eds. 1992. Developmental stability in natural populations. Acta Zoologica Fennica 191: 1–200.