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The biological relevance of testing for perfect symmetry

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In a recent Commentary, Pomory (1997) suggests that researchers interested in quantifying fluctuating asymmetry (differences between left and right sides of bilaterally symmetric traits, Ludwig 1932) should first test for the presence of asymmetry within their data, that is, a deviation from perfect bilateral symmetry. We are concerned that some of the analyses he employs are inappropriate but, more so, by the biological relevance of the issue he raises.

Pomory's point was illustrated with four sets of 'artificial' data ($N=4$ within each), designed to illustrate antisymmetry, perfect bilateral symmetry, and two levels of fluctuating asymmetry, plus one real data set of posterior petal lengths in sand dollars, *Mellita tenuis* ($N=20$). Pomory (1997) starts by testing for directional asymmetry (is the right side different from the left?), by means of *t*-tests, one-way ANOVA and two-way (i.e. repeated measures) ANOVA. The *t*-tests he employs are two-sample tests, so these, and the one-way ANOVA, would be appropriate only if each left and right measure came from a different animal. However, it is usual, and sensible, to measure both left and right sides from each animal, in which case the data are necessarily paired. Only paired *t*-tests (exactly equivalent to a one-sample *t*-test on the signed differences between the sides), or repeated measures ANOVA are suitable for such data. Indeed, using the correct *t*-test on Pomory's fourth data set reveals a significant difference from zero asymmetry (paired *t*-test: $t_4=3.23$, $P<0.05$; Minitab 1994), undetected by the erroneous two-sample *t*-test (Table I in Pomory 1997). However, Pomory's main point is not that of testing for directional asymmetry, but the importance of testing whether asymmetry exists at all.

Pomory (1997) proposes a method consisting of measuring left and right components of a trait, assigning the larger values to one column and the smaller values to another column irrespective of whether the measures are obtained from left or right sides, and performing a two-way ANOVA, blocked by individual, to see if bigger values are consistently larger than smaller values. The problem is that this method can generate apparent asymmetry when none exists, and fails to detect asymmetry when it does exist. First, finding a significant asymmetry using this technique (or indeed any of the many relevant techniques that could be used) does not mean that the trait under assessment shows any asymmetry at all. Measurement error can give rise to a 'significant asymmetry' even though all traits within a population are perfectly symmetric. As measurement error will often exhibit a normal distribution, this can be demonstrated by generating a series of normally distributed random data ($N=50$, $X=100$, SD$=1$) for left and right components of a trait, then repeating the process for subsequent repeated measures. In this example we have generated five sets (repeats) of random data for left and right trait components for 50 individuals, where all traits on all individuals 'truly' measure 100 units but there is normally distributed measurement error associated with these values ($X \pm$ SE measurement error expressed as percentage of trait size$=0.63 \pm 0.063\%$). Therefore all individuals possess left and right traits of the same size (i.e. there is zero asymmetry across the whole population) and the small between-individual variation in the data represents random measurement error. Application of the ANOVA technique suggested by Pomory reveals 'significant asymmetry' in the data even when all five repeated

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measures are taken into account, that is, an average of the five 'repeats' is entered into the analysis ($F_{1,49}=108.01$, $P<0.0001$; SPSS 1988). As the Pomory technique assumes that zero asymmetry exists in nature, for measurement error not to create deviations from symmetry it must also be zero. As measurement error is often of the same magnitude as fluctuating asymmetry, within-subject replicates are essential (Palmer & Strobeck 1986; Swaddle et al. 1994; Merilä & Björklund 1995). The important question to ask when investigating fluctuating asymmetry is therefore whether asymmetry exceeds that attributable to measurement error. This can be done using a mixed-model ANOVA (Palmer & Strobeck 1986; Swaddle et al. 1994; Merkilä & Björklund 1995).

However, even if one could attain zero measurement error, we argue that it is not useful to ask whether a trait exhibits 'significant asymmetry'. If you define sides by their relative size, and then test to see whether the larger side is bigger than the smaller side, then the only way you can fail to get a significant result is if (1) measurement accuracy is insufficient to define one side as larger than the other, (2) the sample size is too small, or (3) no asymmetry exists (as Pomory surmises). However, this latter situation cannot occur and it is vital to realize that real bilateral structures will never exhibit zero asymmetry. The detection of deviations from perfect symmetry is merely a matter of scale as all structures are developmentally unstable to some degree. Exactly identical translation of genotype to phenotype on both sides of bilateral traits in all individuals within a population simply cannot occur. If the scale of the measurement device used is small enough, then an asymmetry will be detected. In meristic traits, symmetry can occur, but even they are subject to some elements of measurement error (see Palmer 1994). Whilst it is valid to compare relative levels of fluctuating asymmetry across individuals, populations, or traits once measurement error has been accounted for (Møller & Swaddle, in press), it is not useful to test whether a trait is 'significantly asymmetric' or not.

The title of Pomory's Commentary, 'Fluctuating asymmetry: biological relevance or statistical noise?' suggests to us that he feels that testing for a population deviation from zero asymmetry is biologically relevant; we would beg to differ. As zero asymmetry does not exist and measurement error will always be present to some degree, testing for a difference from perfect symmetry in metric traits does not tell us any more than previous guidelines set out in detail by Palmer & Strobeck (1986), Palmer (1994) and Swaddle et al. (1994), and in some cases could be misleading.

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