doi: 10.1111/j.1420-9101.2005.01011.x

On hidden heterogeneity in directional asymmetry – can systematic bias be avoided?

L. C. STIGE, B. DAVID & P. ALIBERT

Biogéosciences, Equipe Différenciation et Espèces, UMR-CNRS 5561, Université de Bourgogne, Dijon, France

Keywords:

Abatus cordatus;
case study;
developmental stability;
directional asymmetry;
echinoid;
fluctuating asymmetry;
methodology.

Abstract

Directional asymmetry (DA) biases the analysis of fluctuating asymmetry (FA) mainly because among-individual differences in the predisposition for DA are difficult to detect. However, we argue that systematic bias mainly results from predictable associations between signed right–left asymmetry and other factors, i.e. from systematic variation in DA. We here demonstrate methods to test and correct for this, by analysing bilateral asymmetry in size and shape of an irregular sea urchin. Notably, in this model system, DA depended significantly on body length and geographic origin, although mean signed asymmetry (mean DA) was not significant in the sample as a whole. In contrast to the systematic variation in DA, undetectable, random variability in the underlying DA mainly leads to reduced statistical power. Using computer simulations, we show that this loss of power is probably slight in most circumstances. We recommend future studies on FA to routinely test and correct for not only as yet for mean DA, but also for systematic variation in DA.

Introduction

Fluctuating asymmetry (FA) (Van Valen, 1962; Palmer & Strobeck, 2003) refers to small, random deviations from perfect morphological symmetry. For bilaterally symmetric traits showing 'ideal' FA (see below), individual asymmetries cannot be explained by genetic or environmental differences between the sides, but by imprecision of development (Palmer & Strobeck, 1986). Consequently, the unsigned asymmetry (|right-left|) is widely used to estimate the developmental instability (DI) of individuals or populations (Polak, 2003).

Fluctuating asymmetry can be separated from two other forms of bilateral asymmetry based on the distribution of signed asymmetry values in the population (Van Valen, 1962; Palmer, 1994). For a trait showing 'ideal' FA, the right–left differences are normally distributed around a mean of zero. *Directional asymmetry* (DA) is characterized by a normal distribution with a mean different from zero. Conspicuous examples of DA include the position of the mammalian heart and the anatomy of many species of flatfish. *Antisymmetry* (AS) is characterized by a platykurtic or bimodal distribution with a mean of zero. AS can be exemplified by the American lobster that has one large crusher claw and one slender cutter claw, and right- and left-biased individuals are equally frequent in the population. DA and AS are generally thought to have adaptive bases (Palmer & Strobeck, 1986). Because asymmetry is then the norm and not just a result of imprecise development, the unsigned asymmetry should not be used as an index of DI (Palmer & Strobeck, 1986; Palmer, 1994; Palmer & Strobeck, 2003; but see Graham *et al.*, 2003).

Statistical corrections for DA (Graham *et al.*, 1998; van Dongen *et al.*, 1999; Palmer & Strobeck, 2003) and AS (Graham *et al.*, 1998) have therefore been suggested. We will in this paper focus on DA, which is probably of more general occurrence than AS. Most DA corrections essentially consist in considering FA as deviations around the mean signed right–left asymmetry (*mean DA*) in the sample instead of as deviations around zero. For example, mean DA can be subtracted from the individual asymmetry values (Palmer & Strobeck, 2003), or corrected for by ANOVA or regression procedures, using the fixed side effect to quantify DA (Palmer & Strobeck, 1986; van Dongen *et al.*, 1999). However, these corrections provide unbiased estimates of DI only in the situation where the underlying DA is the same for all specimens. With the *underlying DA*, we refer to the

Correspondence and present address: Dr Leif Christian Stige, Department of Biology, Centre for Ecological and Evolutionary Synthesis, University of Oslo, P.O. Box 1066 Blindern, N-0316 Oslo, Norway.
Tel.: + 47 22 85 46 48; fax: +47 22 85 40 01;
e-mail: l.c.stige@bio.uio.no

'targeted' right–left asymmetry for a given genotype in a given environment (cf. the 'creode' of Zakharov, 1992). We thus consider the underlying DA as a biological property of an individual, not a population. But because observed asymmetries differ from the targeted because of DI, the underlying DA of a single individual is not directly measurable (except perhaps for clonal organisms). As a consequence, we can never exclude the possibility that individuals differ in the underlying DA. Because of this problem, it has been argued that traits displaying significant DA are best excluded from FA analyses (Palmer & Strobeck, 1992; Palmer, 1994; but see: Graham *et al.*, 1998; Palmer & Strobeck, 2003).

However, we argue that parts of the among-individual variation in the underlying DA can in fact be detected and corrected for statistically. If the underlying DA depends predictably on another factor, such as sex, body size or geographic origin, we can expect an association between the observed right–left asymmetry and this factor. We here demonstrate how such systematic variation in DA can be tested and corrected for within the methodological framework already developed for FA analyses. If not corrected for, systematic variation in DA can systematically bias FA estimates, and this can lead to false conclusions about the relationship between FA and these factors. On the other hand, variation in the underlying DA that is not associated with any other factor can in effect be considered as random. Such variation, like random measurement error, mainly reduces the precision with which FA estimates DI (but see Discussion). This reduces the power of a study, but is not likely to lead to falsely positive conclusions.

As model system, we study bilateral asymmetry of the test of *Abatus cordatus*. *A. cordatus* is an irregular sea urchin endemic to the subantarctic Kerguelen Islands (70°E; 49°S). Irregular sea urchins possess bilateral symmetry imposed on a pentaradial body plan, which allows the analysis of FA. The body plan also includes directionally asymmetric components, which make these animals good model organisms for the analysis of DA. Both shape and size data are considered. Specifically, we test and correct for systematic variation in DA as evidenced by the associations between signed asymmetry and geographic origin or body size. Using a simulation approach, we also explore the possible loss of precision from uncorrected variation in the underlying DA.

Methods

Model system and morphological measurements

We investigated a total of 420 *A. cordatus* from four locations 15–30 km apart in the Kerguelen Islands (Halage des Swains: 'POP_{HDS}' $n = 175$, Ile Haute: 'POP_{IH}' $n = 128$, Port-aux-Français: 'POP_{PAF}' $n = 91$, Ile Sûhm: 'POP_{IS}' $n = 26$). The samples were collected in the period 1989–2003 by scuba-diving and dredging and conserved in 70% ethanol. Spines were removed with a toothbrush after a 5-min immersion in 0.1–0.3% Cl-solution. The plate pattern was revealed by applying a 40 : 60 solution of ethanol : glycerol after drying.

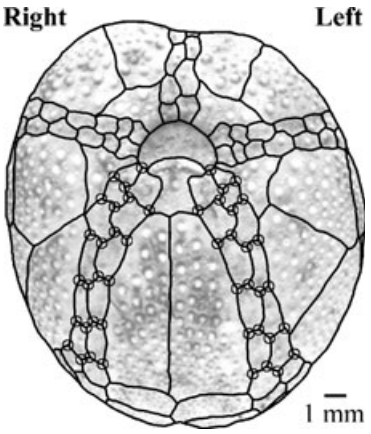

Fig. 1 Plate pattern of *A. cordatus*, oral view. Circles: landmarks used for asymmetry analyses.

In each of the two posterior ambulacral zones, 23 landmarks corresponding to boundaries between test plates were defined (Fig. 1). Two-dimensional landmark coordinates were scored to the nearest 0.001 mm using a Nikon MM-60 measuring microscope. To assess measurement error, two independent sets of measurements were made of all specimens. Total length of the test was measured to the nearest 0.01 mm with a digital calliper.

Procrustes superimposition

The data were analysed by generalized Procrustes superimpositions of right and mirror-reflected left halves of the landmark configurations as described in Klingenberg & McIntyre (1998). By this procedure, asymmetry in shape is separated from asymmetry in centroid size (c.s.: the square root of the sum of squared distances of a set of landmarks from their centroid, Slice *et al.*, 1996). While specialized procedures are needed for the subsequent analyses of the shape data (Klingenberg & McIntyre, 1998; Klingenberg *et al.*, 2001), the centroid size data can be analysed similarly to asymmetries of ordinary metric traits (e.g. van Dongen *et al.*, 1999; Palmer & Strobeck, 2003).

Modelling systematic variation in DA

Fluctuating asymmetry data are traditionally analysed by mixed-effects models in which DA is represented by a fixed side effect (Palmer & Strobeck, 1986; Klingenberg & McIntyre, 1998; van Dongen *et al.*, 1999). The fixed side effect then estimates the mean signed asymmetry (mean DA) in the sample. These models can be extended to include systematic variation in DA by adding fixed-effect

interactions between side and other variables. These terms represent predictable associations between signed asymmetry, i.e. DA, and other factors. Thus, the fixed-effect interaction between side and body size can be used to represent an allometric change in DA. The fixed-effect interaction between side and population represents population differences in DA. By including such interaction terms in FA models, systematic variation in DA can be specifically tested, and if found significant, statistically corrected for. This extension of the basic FA model was also demonstrated by van Dongen *et al.* (1999), yet the potential to correct for systematic variation in DA seems later largely to have been overlooked.

Size analyses

Centroid size data were analysed using a restricted maximum likelihood (REML) mixed regression approach (van Dongen *et al.*, 1999). By this method, side is coded as a numeric variable with the possible values −0.5 and 0.5, the fixed side effect represents DA and the random individual side effect represents FA (see van Dongen *et al.*, 1999, for details). Systematic variation in DA was included by adding fixed-effect interaction terms between side and body size (measured as test length) and/or population. All fixed effects were tested by *F*-tests (Pinheiro & Bates, 2000). Individual FA-estimates ('$FA_{C.S.}$') corrected for effects of DA and heterogeneous measurement error were provided by the random side estimates from the best model (van Dongen *et al.*, 1999). This procedure thus allowed simultaneously testing and correcting for DA, systematic variation in DA and heterogeneous measurement error.

Shape analyses

Shape data were analysed by Procrustes ANOVA (Klingenberg & McIntyre, 1998; Klingenberg *et al.*, 2001). Systematic variation in DA was included by adding interaction terms between side and body size (measured as centroid size) and/or population. In addition, allometric effects on shape were included by adding centroid size (both as a linear and a quadratic term) to the model (Klingenberg *et al.*, 2001). Terms were entered and tested sequentially by permutation tests using 10 000 iterations (Klingenberg & McIntyre, 1998; Klingenberg *et al.*, 2001). After choosing a model in which all terms were significant, the following procedure was used to calculate individual absolute asymmetry in shape (FA_{SHAPE}) corrected for effects of allometry and population-specific DA: (i) Right–left Procrustes-aligned landmark configurations were calculated after averaging across repeat measurements. (ii) These configurations, representing signed shape asymmetry, were used as response in a multivariate regression with the predictor variables population (representing population differences in DA) and right–left centroid size (representing allometry). (iii) FA_{SHAPE} of each individual was calculated as the square root of the sum of squared residuals from this model. DA in shape was visualized by plotting population-specific shape and DA using coefficients from a multivariate regression model with right and left Procrustes scores as response and the explanatory variables determined by the Procrustes ANOVA.

Simulating the loss of precision caused by undetected DA heterogeneity

The proposed methods correct for systematic variation in DA. However, there may also be among-individual variation in the underlying DA that is not associated with any measured factor, and is therefore not detectable. Such variation reduces the precision with which DI is estimated. To quantify this loss of precision, we estimated the correlation between FA and the underlying DI in response to: (i) the amount of DA heterogeneity, and (ii) the amount of DI heterogeneity. For a given level of DA heterogeneity and DI heterogeneity, we randomly generated 100 000 signed FA-values from normal distributions with individual-specific means and SDs, DA_i and DI_i, respectively. DA_i- and DI_i-values were randomly generated from a normal and a gamma distribution, respectively. The characteristics of these two distributions determined the level of DA heterogeneity and DI heterogeneity. It can be shown that the coefficient of variation (CV; SD over mean) of DI is $a^{-0.5}$, and the proportion of the total asymmetry variance originating from variation in DA is $\sigma^2 \, (a^2 \, s^2 + a \, s^2 + \sigma^2)^{-1}$, where a and s are the shape and scale parameters of the DI gamma distribution and σ^2 is the variance of the DA normal distribution. For each level of DA heterogeneity and DI heterogeneity, we calculated the Pearson's coefficient of correlation between DI and unsigned FA.

All statistical analyses were performed with the program R (R Development Core Team, 2003). A critical level of 5% is used in all tests.

Results

Preliminary analyses

Because of a positive scaling between unsigned asymmetry and trait size, centroid size was transformed by taking the natural logarithm of the square root of the original values. Asymmetry calculated from the transformed values was unrelated to trait size ($F_{1,415} = 0.06$, $P = 0.81$, analysis of covariance, with population as a categorical covariate). Both size and shape FA was more than an order of magnitude larger than measurement error and highly significant in each population (all $P < 0.001$, REML mixed regression or Procrustes ANOVA). Measurement error for centroid size depended negatively on the total length of the test ($\chi^2_1 = 79.3$, $P < 0.001$, likelihood ratio test) and it differed between

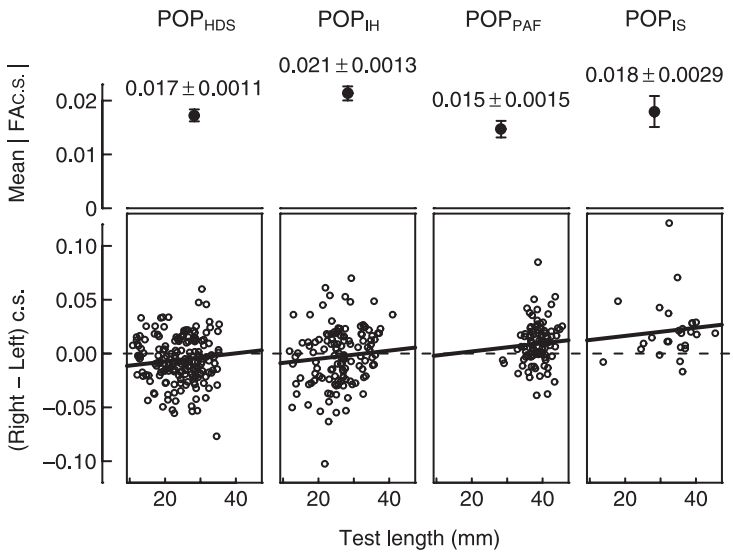

Fig. 2 Unsigned and signed asymmetry in centroid size (c.s.). The lower panels show right–left c.s. as a function of test length for each population. Bold lines show predictions from a global mixed REML regression model. These lines represent systematic variation in DA. The upper panels show mean ± SE for unsigned $FA_{C.S.}$. $FA_{C.S.}$ are individual random side effect estimates from the regression model and represent FA corrected for effects of DA and heterogeneous measurement error.

populations ($\chi^2_3 = 47.5$, $P < 0.001$, likelihood ratio test). In the subsequent analyses of centroid size asymmetry, models with heterogeneous error structure were therefore used. There was no indication of AS in any population (Klingenberg & McIntyre, 1998; Palmer & Strobeck, 2003). Two outlier centroid size asymmetry values were identified (Palmer & Strobeck, 2003) and it was controlled that the conclusions of the study were robust to the exclusion of these.

Directional asymmetry

Directional asymmetry in centroid size varied in both sign and magnitude among populations (Side × Pop: $F_{3,1249} = 16.7$, $P < 0.001$), and DA depended allometrically on test length (Side × Test length: $F_{1,1249} = 4.4$, $P < 0.05$) (Fig. 2). Overall mean DA was not significant, however (Side: $F_{1,1249} = 0.004$, $P > 0.5$). The population differences and the allometric changes in DA are partly confounded because test lengths differed between populations, but both effects contribute independently towards explaining DA variation. After accounting for measurement error, 12% of the variation in signed asymmetry could be explained by systematic variation in DA, while 88% was attributed to FA.

Mean DA in shape across all populations was highly significant, but there were also significant differences in DA between the populations (Table 1). Plots of population-specific DA suggested that the DA pattern was similar across populations; it was mainly the magnitudes that differed ($POP_{HDS} > POP_{IH} > POP_{PAF} > POP_{IS}$; this ranking was confirmed by Procrustes ANOVAS of each population separately). There were no significant allometric changes in shape DA (Table 1). As for centroid size DA, the population differences and the allometric changes in DA are partly confounded, but for shape DA only the population effect contributes independently towards explaining DA variation (Table 1). Population differences in shape DA accounted for 2.9% of the total variance in signed asymmetries across all landmarks.

Table 1 Procrustes ANOVA for asymmetry in shape.

Effect	d.f.	MS	*F*	*P*-value	Interpretation
C.s.	42	41031	129.9	<0.001	Allometric change in shape (linear)
C.s.^{2}	42	18792	59.5	<0.001	Allometric change in shape (quadratic)
Pop.	126	9738	30.8	<0.001	Population differences in shape
Ind.	17598	316	3.1	<0.001	Individual differences in shape
Side	42	7299	72.1	<0.001	Mean DA
Side × Pop.	126	417	4.1	<0.001	Population differences in DA
Side × C.s.	42	251	2.5	>0.1	Allometric change in DA
Side × Pop. × C.s.	126	5.1	0.1	>0.1	Pop. diff. in allometric change in DA
Side × Ind	17598	101	78.2	<0.001	FA
Residual	35280	1.3			Measurement error

All populations are analysed in one model. Effects are tested sequentially by permutation tests. The Side × Pop. effect is also significant if Side × C.s. is accounted for first ($F_{126,17598} = 3.4$, $P < 0.01$). Sums of squares are multiplied by 10^6. C.s., centroid size.

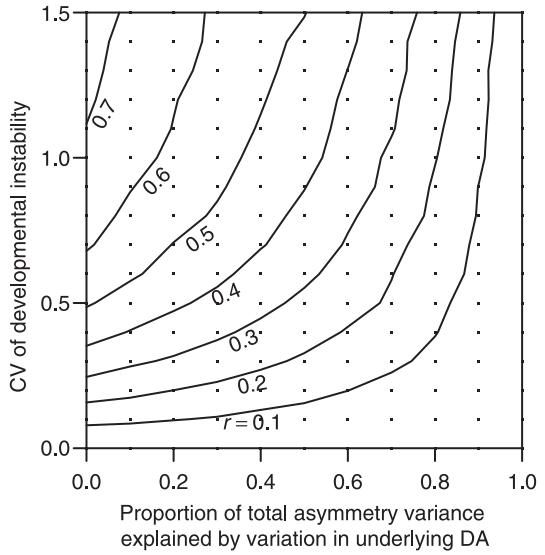

Fig. 3 Contour plot showing the estimated Pearson's coefficient of correlation (r) between unsigned FA and DI in response to heterogeneity in DA (x-axis) and heterogeneity in DI (y-axis). The correlations are estimated from randomly generated data sets of $n =$ 100 000. Data sets differ in the amounts of heterogeneity in DA and DI (points). Heterogeneity in DA is represented by among-individual differences in the statistical expectation of signed asymmetry. Heterogeneity in DI is represented by among-individual differences in the SD of signed asymmetry (measured as the coefficient of variation, CV).

Fluctuating asymmetry – with and without correction for systematic variation in DA

For comparison, we calculated FA both with and without correction for systematic variation in DA. The uncorrected estimates of mean centroid size FA (mean $|FA_{C.S.}|$) in each population were 2–34% higher than the corrected estimates (POP_{HDS}: +4%, POP_{IH}: +2%, POP_{PAF}: +12%, POP_{IS}: +34%). For shape (FA_{SHAPE}), the uncorrected estimates were 1–10% higher than the corrected ones (POP_{HDS}: +1%, POP_{IH}: +1%, POP_{PAF}: +5%, POP_{IS}: +10%). In our case, these differences did not alter the conclusions that centroid size FA varied significantly among populations ($F_{3,415} = 3.87$, $P < 0.01$, ANOVA; Fig. 2) while shape FA did not ($F_{3,416} = 0.97$, ANOVA, $P = 0.4$, permutation test). However, when using uncorrected centroid size FA estimates, none of the pairwise comparisons between populations reached significance, but when using corrected estimates, POP_{IH} was found to be significantly more asymmetric than POP_{PAF} (Tukey's 'Honest Significant Difference' method).

We also compared individual-level FA estimates. On average uncorrected individual unsigned $FA_{C.S.}$-estimates differed in absolute value by 0.0061 from corrected estimates, that is by 34% compared with mean unsigned $FA_{C.S.}$ (=0.018). The corresponding average difference for individual FA_{SHAPE}-estimates was 0.0031, which is 5.8% of mean FA_{SHAPE}.

Effect of DA heterogeneity on precision

The isoclines in Fig. 3 are relatively flat in the lower left part of the figure. This means that if the heterogeneity in DI is at a low to intermediate level, the introduction of some heterogeneity in the underlying DA does not lead to a drastic loss of precision. For example, if the coefficient of variation of DI is 0.2 (which is typical for many species according to estimates of Gangestad & Thornhill, 2003) and there is no heterogeneity in DA, the correlation between FA and DI is 0.25. If the heterogeneity in DA increases to a level where 20% of the total asymmetry variance is caused by among-individual differences in the underlying DA, the correlation between FA and DI is only reduced to 0.20. The curves are steeper at higher DA heterogeneity as well as at higher DI heterogeneity, but the relevance of this to real populations may be questionable.

Discussion

Directional asymmetry in *A. cordatus*

The results show that DA in *A. cordatus* depends on body size as well as on population. This suggests an ontogenetic change in DA, in addition to genetic and/or environmental effects on DA. Because populations differed genetically (Poulin & Féral, 1998) as well as environmentally (Poulin & Féral, 1995), it is not possible to separate the two effects in our study. Note that genetic differentiation in *A. cordatus* is comparatively high across short geographic distances because of the absence of planktonic stages in the life cycle.

A likely cause of DA in the tests of irregular sea urchins is asymmetry in the digestive system (Lawrence *et al.*, 1998). In spatangoids, the intestine, which forms the main part of the digestive system, comprises two circuits (loops) coiled in opposite direction. This may cause DA in the test either directly by bilaterally asymmetric pressure of the gut contents, or indirectly by genetic control to accommodate the more spacious intestine circuit on the right side. The observed association between size and DA, which has also been found in the irregular sea urchin *Mellita tenuis* (Lawrence *et al.*, 1998), may be because of an allometric effect of the intestine. Population differences in DA could also be related to differences in the digestive system. In addition, DA may be a consequence of asymmetric plate formation pattern, as plate formation at the left and right sides are not exactly mirrored by each other (see David *et al.*, 1995 for details). Note that the two posterior ambulacral zones retained for the present study are ontogenetically perfectly symmetric, and therefore homologous, but because the test is an

integral entity asymmetric plate formation pattern in other parts of the test may have contributed to the observed DA.

Heterogeneous DA in other organisms

Systematic variation in DA has been reported in a wide range of organisms (e.g. Pither & Taylor, 2000; Mazzi & Bakker, 2001; Kellner & Alford, 2003; Oleksyk *et al.*, 2004; Stige *et al.*, 2004). In some cases, variation in DA may be related to differential adaptive function of DA (Windig & Nylin, 1999). In other cases, environmental stress appears to cause a transition from 'ideal FA' to DA (Teather, 1996; Collin, 1997; Pither & Taylor, 2000; Kellner & Alford, 2003). But in many cases the reasons for the differences are not apparent, and would have been impossible to predict *a priori*. Notably, several studies in which the growth of single individuals was monitored have reported ontogenetic changes in DA (Teather, 1996; Collin, 1997; Pither & Taylor, 2000; Kellner & Alford, 2003).

We suspect that systematic variation in DA may be more common than evident in the literature, as it is typically not tested for. The fact that the heritability of signed asymmetry is usually estimated to be low, though occasionally significant (Coyne, 1987; Tuinstra *et al.*, 1990; Leamy *et al.*, 1997, 1998; Leamy, 1999; Roff & Réale, 2004; Santos *et al.*, 2005; Stige *et al.*, 2005), suggests that there is generally little additive genetic variation for DA. However, differences in DA could also be linked to age variation (as demonstrated in the present study), environmental variation or nonadditive genetic variation. Therefore, until more tests for systematic variation in signed asymmetry are made, we cannot know what the general occurrence of such variation is.

Why correct for systematic variation in DA?

The results on centroid size asymmetry in *A. cordatus* demonstrate the importance of testing for systematic variation in signed asymmetry. If all data had been pooled, no significant DA had been detected, as mean signed asymmetry was close to zero. Still, systematic differences in DA between populations and size classes existed.

If not corrected for, systematic variation in signed asymmetry can systematically bias FA estimates. In the present study, uncorrected vs. corrected population-level FA estimates differed by up to 34%. Although the final conclusions remained the same, this would obviously not always be the case. With different baseline levels of FA or DA, undetected population differences in DA could easily lead to false conclusions about differences in FA. Also, associations between DA and continuous factors could lead to systematic bias. For example, if a linear relationship between DA and body size goes undetected, FA of the smallest and largest individuals will tend to be over-estimated. By statistically correcting for systematic variation in signed asymmetry, such bias is avoided. One then controls that inferences about differences in unsigned asymmetry, i.e. FA, cannot be explained by differences in signed asymmetry, i.e. DA.

Statistical correction for systematic variation in DA leads to more precise estimation of DI, and therefore also to increased power to detect associations between FA and other factors. However, this is probably less important than the avoidance of systematic bias.

An important advantage of the approach presented here is that DA is analysed by one global model instead of separately for each subsample in a data set. If a study comprises many groups, the statistical power to detect DA in each group separately may be very low, and DA may easily be overlooked. In contrast, a global approach allows precise estimation of both mean DA and systematic variation in DA using information from all the data.

Limitations of the corrections

The proposed DA corrections are unlikely to remove absolutely all systematic bias that can result from DA. Especially, the amount of among-individual variation in underlying DA may differ between groups. This will have the same effect as heterogeneous measurement error, and is in practice impossible to detect or correct for. If mean DA or the systematic variation in DA is large, FA comparisons across groups should therefore be made with caution.

An unavoidable limitation is the fact that we cannot correct for among-individual variation in the underlying DA that is not correlated with any of the measured variables. Undetected DA variation reduces the precision with which DI is estimated by FA. The simulation analysis suggests that this loss of precision is probably not a very serious concern unless a considerable proportion of the total asymmetry variance is caused by differences in the underlying DA. For *A. cordatus*, we can conclude that if the unknown variation in the underlying DA is not of much larger magnitude than the systematic component of the signed asymmetry variance (12% and 2.9%), the loss of precision is small.

Conclusion

We suggest that future FA studies should test and, if necessary, correct for systematic variation in DA. We here demonstrate methods applicable for metric traits (represented by centroid size) and for shape data. Finally, we think that a strict separation between 'ideal FA' and DA should be abandoned – at least some very slight difference in developmental condition between sides may be the norm rather than the exception (Kraak, 1997) and whether or not DA is actually detected depends to a great deal on sample size and measurement precision. Consequently, instead of omitting from FA

studies all traits that show significant DA, information is gained if we include these traits, making the best possible statistical corrections for DA, but interpret the results with the limitations of these corrections in mind.

Acknowledgments

This paper is a contribution to the team 'Différenciation et Espèces' of the UMR-CNRS 5561 Biogéosciences and of the GDR-CNRS 2474 'Morphométrie et Evolution des Formes'. The study was financed by a post-doctoral grant from Région Bourgogne to L.C.S. Samples were collected by P. Mespoulhé, P. Schatt, J.P. Féral, E. Poulin and B. David. We thank C.P. Klingenberg for commenting on an earlier version of the manuscript.

References

Collin, R. 1997. Ontogeny of subtle skeletal asymmetries in individual larvae of the sand dollar *Dendraster excentricus*. *Evolution* **51**: 999–1005.

Coyne, J.A. 1987. Lack of response to selection for directional asymmetry in *Drosophila melanogaster*. *J. Hered.* **78**: 119.

David, B., Mooi, R. & Telford, M. 1995. The ontogenetic basis of Lovén's rule clarifies homologies of the echinoid peristome. In: *Echinoderm Research* (R. Emson, A. Smith & A. Campbell, eds), pp. 155–164. Balkema, Rotterdam, Netherlands.

van Dongen, S., Molenberghs, G. & Matthysen, E. 1999. The statistical analysis of fluctuating asymmetry: REML estimation of a mixed regression model. *J. Evol. Biol.* **12**: 94–102.

Gangestad, S.W. & Thornhill, R. 2003. Fluctuating asymmetry, developmental instability, and fitness: toward model-based interpretation. In: *Developmental Instability. Causes and Consequences* (M. Polak, ed.), pp. 62–80. Oxford University Press, Oxford.

Graham, J.H., Emlen, J.M., Freeman, D.C., Leamy, L.J. & Kieser, J.A. 1998. Directional asymmetry and the measurement of developmental instability. *Biol. J. Linn. Soc.* **64**: 1–16.

Graham, J.H., Shimizu, K., Emlen, J.M., Freeman, D.C. & Merkel, J. 2003. Growth models and the expected distribution of fluctuating asymmetry. *Biol. J. Linn. Soc.* **80**: 57–54.

Kellner, J.R. & Alford, R.A. 2003. The ontogeny of fluctuating asymmetry. *Am. Nat.* **161**: 931–947.

Klingenberg, C.P. & McIntyre, G.S. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes method. *Evolution* **52**: 1363–1375.

Klingenberg, C.P., Badyaev, A.V., Sowry, S.M. & Beckwith, N.J. 2001. Inferring developmental modularity from morphological integration: analysis of individual variation and asymmetry in bumblebee wings. *Am. Nat.* **157**: 11–23.

Kraak, S.B.M. 1997. Fluctuating around directional asymmetry? *Trends Ecol. Evol.* **12**: 230.

Lawrence, J.M., Pomory, C.M., Sonnenholzner, J. & Chao, C.-M. 1998. Bilateral symmetry of the petals in *Mellita tenuis*, *Encope micropora*, and *Arachnoides placenta* (Echinodermata: Clypeasteroida). *Invert. Biol.* **117**: 94–100.

Leamy, L. 1999. Heritability of directional and fluctuating asymmetry for mandibular characters in random-bred mice. *J. Evol. Biol.* **12**: 146–155.

Leamy, L.J., Routman, E.J. & Cheverud, J.M. 1997. A search for quantitative trait loci affecting asymmetry of mandibular characters in mice. *Evolution* **51**: 957–969.

Leamy, L.J., Routman, E.J. & Cheverud, J.M. 1998. Quantitative trait loci for fluctuating asymmetry of discrete skeletal characters in mice. *Heredity* **80**: 509–518.

Mazzi, D. & Bakker, T.C.M. 2001. Acid stress increases pelvic spine asymmetry in juvenile three-spined sticklebacks. *J. Fish Biol.* **59**: 582–592.

Oleksyk, T.K., Novak, J.M., Purdue, J.R., Gashchak, S.P. & Smith, M.H. 2004. High levels of fluctuating asymmetry in populations of *Apodemus flavicollis* from the most contaminated areas in Chornobyl. *J. Env. Radioactiv.* **73**: 1–20.

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

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

Palmer, A.R. & Strobeck, C. 1992. Fluctuating asymmetry as a measure of development stability: implications of non-normal distributions and power of statistical tests. *Acta Zool. Fennica* **191**: 57–72.

Palmer, A.R. & Strobeck, C. 2003. Fluctuating asymmetry analyses revisited. In: *Developmental Instability. Causes and Consequences* (M. Polak, ed.), pp. 279–319. Oxford University Press, Oxford.

Pinheiro, J.C. & Bates, D.M. 2000. *Mixed-Effects Models in S and S-PLUS*. Springer, New York.

Pither, J. & Taylor, P.D. 2000. Directional and fluctuating asymmetry in the black-winged damselfly *Calopteryx maculata* (Beauvois) (Odonata: Calopterygidae). *Can. J. Zool.* **78**: 1740–1748.

Polak, M. (ed.) 2003. *Developmental Instability. Causes and Consequences*. Oxford University Press, Oxford.

Poulin, É. & Féral, J.-P. 1995. Pattern of spatial distribution of a brood-protecting schizasterid echinoid, *Abatus cordatus*, endemic to the Kerguelen Islands. *Mar. Ecol. Prog. Ser.* **118**: 179–186.

Poulin, É. & Féral, J.-P. 1998. Genetic structure of the brooding sea urchin *Abatus cordatus*, an endemic of the subantarctic Kerguelen Island, and the origin of the diversity of antarctic echinoids. In: *Echinoderms. Proc. 9th Intn. Echinoderm Conf., San Francisco, USA* (R. Mooi & M. Telford, eds), pp. 793–795. Balkema, Rotterdam.

R Development Core Team. 2003. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org.

Roff, D. & Réale, D. 2004. The quantitative genetics of fluctuating asymmetry: a comparison of two models. *Evolution* **58**: 47–58.

Santos, M., Fernández Iriarte, P. & Céspedes, W. 2005. Genetics and geometry of canalization and developmental stability in *Drosophila subobscura*. *BMC Evol. Biol.* **5**: 7.

Slice, D.E., Bookstein, F.L., Marcus, L.F. & Rohlf, F.J. 1996. Appendix I: a glossary for geometric morphometrics. In: *Advances in Morphometrics* (L. F. Marcus, M. Corti, A. Loy, G. J. P. Naylor & D. E. Slice, eds), pp. 531–551. Plenum Press, New York and London.

Stige, L.C., Hessen, D.O. & Vøllestad, L.A. 2004. Severe food stress has no detectable impact on developmental instability in *Daphnia magna*. *Oikos* **107**: 519–530.

Stige, L.C., Slagsvold, T. & Vøllestad, L.A. 2005. Individual fluctuating asymmetry in pied flycatchers (*Ficedula hypoleuca*) persists across moults, but is not heritable and not related to fitness. *Evol. Ecol. Res.* **7**: 381–406.

Teather, K. 1996. Patterns of growth and asymmetry in nestling tree swallows. *J. Avian Biol.* **27**: 302–310.

Tuinstra, E.J., de Jong, G. & Scharloo, W. 1990. Lack of response to family selection for directional asymmetry in *Drosophila melanogaster*: left and right are not distinguished in development. *Proc. R. Soc. Lond. B* **241**: 146–152.

Van Valen, L. 1962. A study of fluctuating asymmetry. *Evolution* **16**: 125–142.

Windig, J.J. & Nylin, S. 1999. Adaptive wing asymmetry in males of the speckled wood butterfly (*Pararge aegeria*)? *Proc. R. Soc. Lond. B* **266**: 1413–1418.

Zakharov, V.M. 1992. Population phenogenetics: analysis of developmental stability in natural populations. *Acta Zool. Fennica* **191**: 7–30.

Received 18 July 2005; accepted 22 July 2005