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The distribution of individual fluctuating asymmetry: Why are the coefficients of variation of the unsigned FA so high?

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It has been established for a long time that rigorous statistical analyses of fluctuating asymmetry (FA) requires great caution. The reason for this is twofold. Firstly, the magnitude of FA is often small and measurement error (ME) may substantially bias asymmetry estimates, and secondly, there are other forms of asymmetry that are sometimes difficult to distinguish from real FA. To avoid bias due to ME, within-subject repeats and mixed model analysis are required. The model assumptions, however, are that both FA and ME are normally distributed. In this paper I show that in many cases these assumptions are not met. In particular, available data in the literature and these presented here suggests that FA (signed) often has a leptokurtic distribution, as indicated by relative high coefficients of variation of the absolute value of the signed FA. This may be due to patterns of antisymmetry and/or heterogeneity of FA within the sample.

1. Introduction

Fluctuating asymmetry (FA, i.e. small random deviations from perfect bilateral symmetry) has been of interest to many biologists as it may measure developmental stability of individuals. The latter can be defined as an individuals ability to correct for developmental noise (i.e. the cumulative effect of small random developmental perturbations or accidents of environmental origin) (Palmer & Strobeck 1992). It has long been recognised that FA requires careful measurement and statistical analysis (Palmer & Strobeck 1986). There are two reasons for this. Firstly, degrees of FA are often of the same magnitude as measurement error (ME). Therefore, within-subject repeats and mixed model analysis are required to separate 'real' FA from ME. Secondly, there are other forms of asymmetry, most commonly directional asymmetry (DA) and antisymmetry, which may have a genetical rather than environmental origin (Palmer & Strobeck 1992, but see Graham *et al.* 1993). While statistical tests for DA can be performed with relatively high power, antisymmetry is more likely to go undetected (Palmer & Strobeck 1992).

Two measures of individual FA are commonly

used, the signed (left–right) and unsigned (absolute value of left–right) FA (e.g., Björklund & Merilä 1997). If the asymmetry of individuals of a particular sample confirms to ideal FA, in the sense that it reflects developmental noise of a homogeneous sample, the signed FA is expected to have a normal distribution (with mean (μ) equal to zero and variance s^2_{FA}), and the unsigned FA a half-normal distribution. For a half-normal distribution, μ and s^2 are closely related: $\mu \approx (4/3) \times s^2$ (Whitlock 1996). The coefficient of variation [$CV = (s^2_{|FA|}/\mu^2_{|FA|})^{1/2}$], therefore, is a constant ≈ 0.76. However, a review of available data that permit the calculation of CV showed that in most of those studies CV was much higher than the expected 0.76 (Whitlock 1996, Björklund & Merilä 1997). Björklund and Merilä (1997) ascribed this to variation in degree of measurement error (see also Whitlock 1996, but see Rowe *et al.* 1997). In this short paper, I investigate the origin of high CVs and show by means of data simulation that they are likely to be the result of a leptokurtic distribution of the signed FA. Possible implications are discussed.

2. Measurement error and the value of CV

First, I will show that the high CV values are not the result of higher ME. To do that, I will adopt the model from Whitlock (1996). Let us assume that a trait develops many times on both sides within a single individual. The mean trait value on both sides equals M (no DA) with variance s^2_{DS}. The variance, s^2_{DS}, is the result of developmental noise and the individuals ability to buffer its development, and thus presents a measure of that individuals developmental stability (DS). The distribution of the signed FA (left–right) is normal with $\mu = 0$ and $s^2 = 2 \times s^2_{DS}$ (see Whitlock 1996 for details and figures) if all individuals have the same developmental stability. The CV of the unsigned FA equals approximately 0.76. After adding ME, which can be assumed to be normally distributed with mean zero and variance s^2_{ME}, the distribution of the signed FA (uncorrected for ME) will have $\mu = 0$ and $s^2 = 2 \times (s^2_{DS} + s^2_{ME})$. The CV of the unsigned FA will still be ≈ 0.76 as the same relationship between mean and variance holds. This can also be easily seen from the calculations presented in Björklund and Merilä (1997). They show that the μ and s^2 of the unsigned FA increase with ME of 5% as compared to the situation where ME is absent, for a low degree of FA (between side correlation = 0.95 in their notations). The mean increased from 1.09 to 1.33, whereas the variance increase from 0.67 to 1 when ME equalled 5% compared to no measurement error. In both cases, CV approximated 0.76. Thus, ME cannot account for the higher CVs observed in several studies assuming normality.

3. Deviations from normality of signed FA and the value of CV

CVs of the unsigned FA that differ from 0.76 indicate that the distribution of the signed FA is not normal, and thus that the observed asymmetry may not be 'ideal' FA (*sensu* Palmer & Strobeck 1992). In this section, I will investigate how deviations from normality of the signed FA may influence the CV of the unsigned FA. The following forms of deviations from normality have been studied in the context of FA. The distribution of the signed FA may be skewed, platy- and leptokurtic, or bimodal (an extreme form of platykurtisis). Palmer and Strobeck (1992) discussed in great detail which underlying processes may results in these deviations from normality. Bimodal distributions may originate from strong antisymmetry, while platykurtic distributions may reflect weak antisymmetry relative to measurement error. Skewness of signed FA can be generated as a distribution of a mixed sample with some individuals exhibiting DA and others antisymmetry. Finally, Palmer and Strobeck (1992) differentiated three types of leptokurtic distributions of which the numbering is adopted here. Type I leptokurtisis originated from a mixed sample of individuals with real FA and a minority of individuals exhibiting antisymmetry. Type II leptokurtisis arises from a mixture of individuals with different degrees of real FA, and Type III leptokurtisis was defined as the result of a relationship between character growth and degree of asymmetry in early ontogeny (see Palmer & Strobeck 1992 for more details).

I generated datasets (left and right trait value without repeats) of 500 individuals with SAS

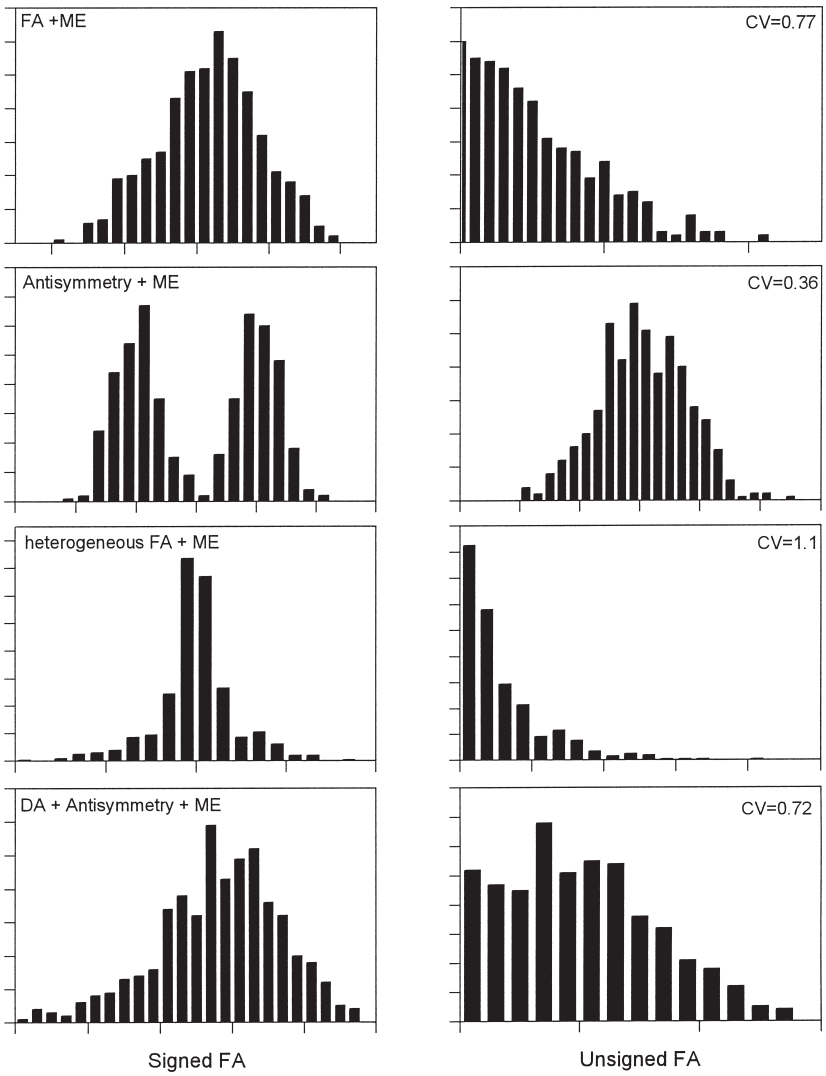

Fig. 1. Frequency distributions for signed (left) and unsigned (right) FA for four simulated datasets of 500 individuals generated in SAS under different conditions. See text for details.

(Ver 6.12) of which the distribution of the signed FA varied in degree of skewness, kurtosis, antisymmetry and DA. Fig. 1 shows a few examples of how these deviations from normality of the signed FA influenced the distribution and the CV of the unsigned FA. The distribution generated by real FA and ME (top) was normal and yielded a CV of the unsigned FA close to the expected 0.76 (see also above). The leptokurtic distribution of the signed FA was generated from an un derlying distribution with heterogeneity in FA (i.e. type II leptokurtisis with 2 different levels) and ME. It resulted in a CV value of the unsigned FA which was higher than 0.76. The binomial (high antisymmetry relative to measurement error) and skewed (mixture of DA, antisymmetry and ME), signed FA both showed CV values lower than 0.76. Additionally, DA also appeared to lower CV values of the unsigned FA (data not shown). In general, the analyses of the generated distributions (results not shown) indicated that CV of the unsigned FA increased from platy- to leptokurtic distributions of the signed FA, where platykurtisis lead to relative low CVs (< 0.76) and leptokurtisis to relative high CVs (> 0.76). Increasing skewness and DA of the distribution of the signed FA lowered CV below 0.76. Thus, the relative high CVs found in many studies may very well be attributable to leptokurtic distributions of the signed FA.

Fig. 2 illustrates the relationship between CV

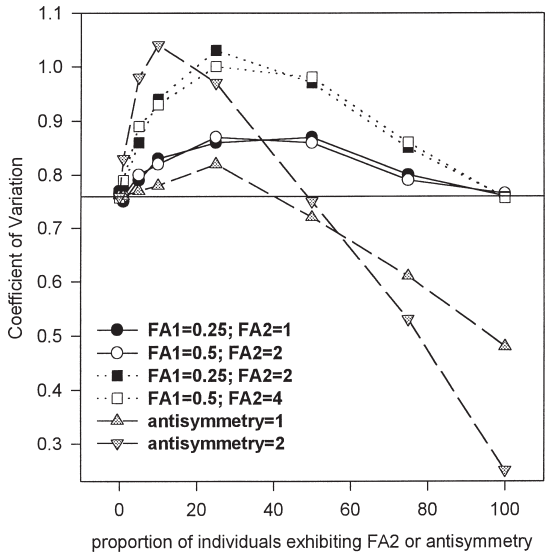

Fig. 2. Effects of between individual heterogeneity in the underlying developmental stability and the presence of antisymmetrical individuals on the value of CV. Samples of 5 000 individuals were generated with SAS. The higher size compared to those in figure one were required to reduce sampling variation. I simulated different degrees of heterogeneity (FA1 and FA2, squares and circles) and antisymmetry (= 1 or 2, triangles and inverse triangles) for a range of population compositions. For the antisymmetry simulations, FA and ME both had variance equal 0.25. Note that the overall variance in FA did not affect the values of CV (compare black vs. white squares and circles). The expected value of CV when the signed FA is normally distributed (i.e. 0.76) is indicated as well (solid horizontal line).

of the unsigned FA and both the level of between individual heterogeneity in developmental stability and the degree of antisymmetry. CV sharply increases when the population consists of a majority of individuals exhibiting low FA (thus high developmental stability) and a small proportion of developmentally instable or antisymmetrical individuals. Higher heterogeneity or stronger antisymmetry relative to ME resulted in higher values of CV. As the population becomes dominated by the developmentally unstable or antisymmetrical individuals, CV drops again and becomes even lower than 0.76 (i.e. platykurtisism) in the case of antisymmetry. The value of CV does not seem to depend on the total degree of developmental stability but on the degree of heterogeneity only (Fig. 2).

4. The effect of the distribution of measurement error

Measurement error may bias single sample as well as individual FA estimates when no within-subject repeats are available (Palmer & Strobeck 1986, Merilä & Björklund 1995, Van Dongen *et al.* 1998). Similarly, one can expect that deviations of the distribution of ME from normality will cause individual FA estimates to become non-normal. To illustrate this, I simulated two datasets of 500 individuals each (left and right trait value without repeats) (see datasets FAME1 and FAME2 in Table 1). FAME1 was generated with $s^2_{FA} = s^2_{ME} = 0.5$, while for FAME2, $s^2_{FA} = 0.5$ and s^2_{ME} equalled either 1 or 0.25 with 50% probability. The latter, thus, contained heterogeneity in ME (i.e. leptokurtisis of the overall ME). While for FAME1 signed FA was normally distributed, and the unsigned FA had CV close to the expected 0.76, for FAME2 it was significantly leptokurtic and CV equalled 0.93 (Table 1). Like heterogeneity in ME, other deviations from normality of ME can also be expected to influence the distribution of individual FA estimates.

While in this simulated example it is possible to distinguish between the effects of deviations from normality of ME and FA, in practise this is not possible without within-subject repeats. In the case that repeated measures are present, heterogeneity in ME can be modelled, obviously providing that the groups with different ME can be identified (Van Dongen *et al.* 1998). Nevertheless, the mixed-model analysis of repeats also allows further investigation of the distribution of ME in any case. Unfortunately, these analyses are rarely done or at least rarely published. Table 1 contains data on the analysis of the distribution of both individual FA and ME for 5 real datasets. The full analyses of the different datasets will be published elsewhere. Three datasets come from the winter moth (*Operophtera brumata* L.). OBRUM1 and OBRUM2 consist of asymmetry data of the shape (circularity) of a wing area (con-

tained within 3 veins) and the length of the front wing for 72 male individuals collected in a large forest area near Antwerp, Belgium. Three independent within-subject repeats were taken for both traits. Wing lengths were measured with callipers, while the shape of the selected area was obtained with an image analyser (Optimas Ver. 5.2) (Van Dongen 1997). OBRUM3 consists of tibia length asymmetry in the winter moth. Two hundred and nine males, originating from the first generation of a lab reared culture, were measured twice under a microscope. The olive sunbird (OS) (*Nectarina olivacea*) dataset contains tarsus length measures (2 repeats) of 188 individuals collected in a number of habitat fragments in the Taita Hills, Kenya. Finally, the blue tit (BT) (*Parus caeruleus* L.) analysis was based on asymmetry (2 repeats) in weight of outer rectrices of the tail for 32 individuals collected in the Antwerp region.

Data were analysed with mixed regression models, which yields identical results as a mixed two-way ANOVA, but has the advantage that unique estimates of individual FA are obtained. These estimates represent a slope but are comparable to the signed FA used in many other studies (left–right trait value), provided that only one ME variance is modelled (see Van Dongen *et al.* 1998 for more details). In three cases, OBRUM1, OBRUM2 and BT, the CV of the unsigned FA was close to the expected 0.76. In these cases, the signed FA and ME were normally distributed. The significant deviation from normality of the ME for OBRUM2, as tested with the Shapiro-Wilk test, still has a high W indicating approximate normality. Furthermore, kurtosis and skewness were close to zero (Table 1). On the other hand for both OBRUM3 and OS significant deviations of normality of the signed FA, and leptokurtisis in particular, appeared to co-occur with a relative high CV of the unsigned FA. While ME was approximately normally distributed for OBRUM3, for OS the measurement error was leptokurtic as well (Table 1). However, this did not explain the leptokurtisis of the signed FA. A selection of observations where the repeated measurements within both sides were identical ($s^2_{ME} = 0$; OS2 dataset in Table 1) still showed a leptokurtic signed FA, and relative high CV for the unsigned FA (Table 1). The deviation of normality of ME for OS is likely to be the result of the discreteness of the error of measurement. The tarsus lengths were obtained by bending the toes back to 90° to the tarsus and measuring from this point to the notch of the intertarsal joint. Because this procedure is highly repeatable (Table 1) within-subject repeats will be identical in many cases. Relatively few differed by 1 and even less differed by 2 or more units of measurement. ME therefore can take only a limited number of values resulting in a discrete distribution where 0 is highly abundant. It is also interesting to note

Table 1. Summary statistics of the distribution of the both signed and unsigned fluctuating asymmetry and of the measurement error; s^2_{FA} and s^2_{ME} represent the variance components as obtained from a mixed regression model analysis (*see* Van Dongen *et al.* 1998 for details) or the simulation model values for datasets FAME1 and FAME2. *W*, *K*, *S* and CV represent the Shapiro-Wilk statistic, kurtosis, skewness and the coefficient of variation respectively. For a description of the datasets see text. A dash indicates that this parameter could not be estimated for that dataset. The significance of *W* was tested in SAS (*** = $p < 0.0001$).

Dataset	Signed FA					Unsigned FA	ME		
	s^2_{FA}	s^2_{ME}	*W*	*K*	*S*	CV	*W*	*K*	*S*
FAME1	0.5	0.5	0.99	0	0	0.74	–	–	–
FAME2	0.5	0.25-1	0.97***	1.65	0.3	0.93	–	–	–
OBRUM1	2.86	0.7	0.97	0	– 0.3	0.80	0.99	0.5	0.2
OBRUM2	0.07	0.02	0.97	– 0.5	– 0.2	0.65	0.95***	– 0.9	0.1
OBRUM3	0.10	0.002	0.81***	19.3	– 0.2	1.6	0.94***	0.9	0
OS	0.04	0.008	0.71***	16.6	– 2.6	1.7	0.82***	12	0.2
OS2	–	–	0.74***	12.8	– 1.2	1.6	–	–	–
BT	1.53	0.47	0.97	0.4	0.1	0.79	0.98	– 0.2	0

that the two datasets with the lowest ME relative to FA (OBRUM3:0.02; OS:0.2; relative to OBRUM1:0.24; OBRUM2:0.29; BT:0.31) showed inflated CV values, again indicating that increased ME is not responsible for the relative high CV values found in many studies.

5. Discussion

Because the magnitude of FA is generally relatively small, and as FA may be confounded with other forms of asymmetry its statistical analysis and interpretation requires great caution. The examination of the distribution of the signed FA may reveal deviations of the asymmetry from real FA. Unfortunately, statistical power for detecting deviations from normality with the Kolmogorov-Smirnov test and the Shapiro-Wilk test (two test commonly used) is relatively low (Palmer & Strobeck 1992). Alternatively, a combination of tests for skewness and kurtosis may be more useful. However, most studies devote relatively little attention to the investigation of the distribution of the signed FA so that many deviations from normality may have gone undetected. In his recent paper, Whitlock (1996) reviewed values of coefficients of variation (CV) of the unsigned FA. Björklund and Merilä (1997) noted that most of these values were higher than 0.76; the value expected under normality of the signed FA. Although the direct investigation of the raw data of the different studies is impossible, the simulation-results presented in this note show that these high CV values may be attributed to leptokurtisis of the signed FA, and not to different degrees of measurement error as suggested earlier (Whitlock 1996, Björklund & Merilä 1997, but see Rowe *et al.* 1997). A preliminary analysis of five real datasets also indicated that the high CV values could not entirely be by explained by deviations of ME from normality.

Palmer and Strobeck (1992) distinguished three types of leptokurtisis depending on the underlying origin of the distribution (see also above). Types I and II originated from mixed samples, while type III resulted from the dependency of allometric growth on the degree of asymmetry. While I am unaware of any empirical support of the latter, types I and II may be more common that previously thought. Rowe *et al.* (1997) recently showed that several studies reporting a negative correlation between FA and individual condition for signalling [but not nonsignalling traits (only 1 tested)], in fact suggested size dependent antisymmetry. However, not all traits, of which a relative high CV value is listed in Whitlock (1996), were signalling ones. This might be due to the fact that also non-signalling traits show some degree of antisymmetry as well. Furthermore, FA has been argued to be genetically determined, at least to some extend (Møller & Thornhill 1997). Therefore, populations with genetic variation for FA will consist of individuals with different degrees of FA. The distribution of the signed FA from such a population will be a mixed distribution from several normal distributions with mean zero and variances s^2_{FAi} (where $i = 1$ to k, and $k =$ number of genotypes; see also Van Dongen 1998). This can be expected to lead to a leptokurtic distribution (type II in Palmer & Strobeck 1992).

Rowe *et al.* (1997) listed two alternative explanations to the pattern of size dependent antisymmetry they found. Unconscious or conscious bias and/or subtle wear or damage may result in antisymmery as well. These two factors can be rejected for the OBRUM3 data. An examination of the tibia under the microscope during measurement has never revealed signs of damage, and blind repeats showed consistent results. Thus, at least for this dataset, the deviations from normality seem to indicate deviations from 'ideal' FA. Whether this is due to antisymmetry or heterogeneity in FA is not clear at this point.

Mixed model analyses of within-subject repeats to obtain unbiased estimates of FA is only correct and useful if both the signed FA (i.e. random factor) as ME (i.e. random error) are normally distributed (Verbeke & Molenberghs 1997). If not, the model assumption that the random effect [side × individual interaction in the two-way mixed ANOVA or side in the mixed regression (Palmer & Strobeck 1986, Van Dongen *et al.* 1998)] and the random error term follow a normal distribution is violated. The high CV values of the unsigned FA found in many studies indicate that either antisymmetry is more abundant and/or that many samples contain a heterogeneous group of individuals. Careful examination of the validity of model assumptions is required in order to per-

form proper statistical analyses of FA. The use of restricted maximum likelihood estimation of a mixed regression model allows to both model and test heterogeneity in FA and ME (Van Dongen *et al.* 1998). Additional examination of the distribution of individual FA and of the residual values is indispensable as model validation. Rowe *et al.* (1997) suggest a graphical examination of the relationship between trait size and the unsigned FA to be able to differentiate between increased FA or antisymmetry.

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