The repeatability of fluctuating asymmetry: a revision and extension

Michael Whitlock

Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4
(whitlock@zoology.ubc.ca)

Fluctuating asymmetry (FA) is an imperfect measure of the developmental stability of an individual. Estimates of the heritability of FA will underestimate the heritability of developmental stability; the extent of this bias can be estimated and corrected by estimating the repeatability of developmental stability. This note corrects an error in a previous derivation of this repeatability using the absolute value of the difference between sides as the measure of FA, and derives the repeatability of developmental stability for another common measure of FA, the variance among sides within an individual.

Keywords: fluctuating asymmetry; developmental stability; repeatability; heritability

1. INTRODUCTION

The repeatability of a trait measures the reliability with which it can be measured. Traits with low repeatabilities will give underestimates of important quantitative genetic parameters such as the heritability and correlations among characters. Estimates of developmental stability using bilateral fluctuating asymmetry (FA) have very low repeatabilities (Whitlock 1996; see also Houle (1997) for an independent statement of the problem), because we are trying to estimate the variance due to developmental noise with only two data points, the left and right side measures. A variance estimate with only two data points is unreliable, and therefore individuals with exactly the same degree of sensitivity to developmental noise can express very different levels of FA. As a result, estimates of the heritability of developmental stability made using FA are substantial underestimates. In a previous note (Whitlock 1996), I described this bias and attempted to provide a mechanism for correcting for it. As has been shown by Van Dongen (1998), there was an error in that correction term. This brief note will serve to correct that lapse.

The observed variance among individuals in FA is due to at least three sources: measurement error, variance in expression of FA due to the sampling of two sides, and real variance among individuals in developmental stability, which I will call V_{me} , V_{err} and V_{DS} , respectively. V_{DS} is a measure of the intrinsic variance among individuals in their developmental stability, expressed on the scale of FA. In other words, if each individual were somehow replicated an infinite number of times, but with the same developmental stability function, then V_{DS} would be the variance among individuals of each individual's FA averaged over all its replicates. The error in the previous paper came from using V_p , the total phenotypic variance of FA, which would also include V_{err} , in place of V_{DS} . I show here that the coefficient of variation of FA allows estimation of the repeatability from typical data. Further-

more, I provide an estimate of the repeatability of developmental stability when FA is measured by the variance among sides.

The repeatability, R , is defined as the proportion of the variance measured for a trait which is due to real differences among individuals in that trait. Let V_{tot} be the total measured variance in FA among individuals, i.e. $V_{tot} = V_{DS} + V_{err} + V_{me}$. The repeatability is then $R = V_{DS} / V_{tot}$.

2. FA AS THE ABSOLUTE VALUE OF THE DIFFERENCE BETWEEN SIDES

Whitlock (1996) showed that the mean and variance of FA among individuals with the same developmental stability function could be calculated. If FA is measured as the absolute value of the difference between the left and right sides of a trait, then the mean and variance of FA over all hypothetical replicates of the same individual i are $2\sqrt{(V_{N,i}/\pi)}$ and $2V_{N,i}[(\pi-2)/\pi]$ respectively, where $V_{N,i}$ is the variance due to developmental error in the expression of a trait on a side for the i th individual (Whitlock 1996). We can take this further by realizing that the measurement error of the sides themselves also should follow a normal distribution with mean zero and some variance V_e , and therefore the measured FA would be a function of the added variance $V_{N,i} + V_e$. The mean and variance among hypothetical replicates of an individual of its FA including measurement error are $M_{FA,i} = 2\sqrt{[(V_{N,i} + V_e)/\pi]}$ and $V_{FA,i} = 2(V_{N,i} + V_e)[(\pi-2)/\pi]$, respectively. Thus, we can find (similar to equation (3) in Whitlock (1996)):

$$\begin{aligned} V_{err} + V_{me} &= E[V_{FA,i}] = \frac{\pi-2}{2} E[M_{FA,i}^2] \\ &= \left(\frac{\pi-2}{2}\right) [Var[M_{FA,i}] + \overline{FA}^2], \end{aligned} \quad (1a)$$

where $\overline{FA}$ is the mean value of FA in the measured distribution, and $E[]$ and $Var[]$ denote the expectation and

variance over all individuals. $Var[M_{FA,i}]$ is approximately equal to V_{DS} if V_e is small relative to V_N , or if V_{DS} is small. V_e can be made small relative to V_N by repeated measures of each side, decreasing in proportion to the inverse of the number of measurements made per side. Therefore, we have, approximately,

$$V_{err} + V_{me} \cong \left(\frac{\pi - 2}{2} \right) [V_{DS} + \overline{FA}^2]. \quad (1b)$$

It is impossible to measure V_{DS} directly. The measured variance among individuals also includes V_{err} and V_{me} , and we cannot in reality replicate the genetic and environmental influences on the phenotype of an individual to estimate V_{err} . Fortunately, we can use equation (1b) with an estimate of the total variance in FA to calculate V_{DS} .

Using equation (1b) and the definition of V_{tot} and solving for V_{DS} , we find

$$V_{DS} \cong \frac{2V_{tot} - (\pi - 2)\overline{FA}^2}{\pi}, \quad (2)$$

which leads directly to

$$R \cong \frac{2V_{tot} - (\pi - 2)\overline{FA}^2}{\pi V_{tot}} = \frac{2}{\pi} - \frac{\pi - 2}{\pi} \left(\frac{1}{C_v^2} \right), \quad (3)$$

where C_v is the observed coefficient of variation of FA (expressed as a proportion) (see figure 1). These calculations have been checked by simulations. Note that the coefficient of variation, being the result of real variance in developmental stability, measurement error and variability in the actual outcome of development, is not constrained to be $\sqrt{[(\pi - 2)/2]}$, as suggested by Björklund & Merilä (1997). The approximation in equation (3) only requires knowing the coefficient of variation, which is easily calculated from data. The approximation is most accurate with low variance in developmental stability or low measurement error, and can therefore be easily improved by multiple measurements of each side. The maximum repeatability for developmental stability is $2/\pi \cong 0.64$, when C_v is very large. The minimum value of C_v is $\sqrt{[(\pi - 2)/C_v]}$, which gives $R=0$. Measured values of C_v that are less than this are either due to estimation error (C_v is difficult to estimate accurately even with moderate replication) or to a non-Gaussian mode of developmental error, in which case the analysis presented here does not apply.

The major conclusions from Whitlock (1996) remain unchanged: estimates of heritability of FA can be large underestimates of the heritability of developmental stability of a character. The C_v of FA is the important parameter for correcting this bias.

3. FA AS THE VARIANCE AMONG SIDES

Another measure of developmental stability which is often used is the variance among sides of an individual (Palmer & Strobeck 1985). The variance among individuals in FA measured in this way also includes variance due to the details of development, so that the variance in FA would be large even if there is no variance in the underlying developmental stability function. This section will

Figure 1. The repeatability of developmental stability as a function of the coefficient of variation of FA.

develop a measure of the repeatability of developmental stability when measured with this version of FA. The derivation here allows for there to be more than two 'sides'.

Again, imagine that the developmental function of an individual i could be replicated many times. The true FA of individual i , i.e. the variance among an infinite number of sides of this individual, is $V_{N,i}$. Let the actual sample variance among the sides of this individual be s_i^2 . The repeatability is the variance among individuals' $V_{N,i}$ values (V_{DS}) divided by the total variance among individuals in their measured FA (V_{tot}). The total variance in FA is the sum of the true variance in FA (V_{DS}), the variance in FA due to the particular outcome of the developmental process (V_{err}), and variance in FA due to measurement error (V_{me}). The real variance among individuals in FA is $V_{FA} = V_{DS} + V_{err}$. Note that these terms carry the same meaning as with the other measures of FA, but their mathematical meaning is different. V_{err} is the expected value of the variance in s_i^2 among replicates of each individual.

V_{tot} , V_{FA} and V_{me} can be estimated from analysis of variance on repeated measures of s_i^2 from each individual. The expected value of s_i^2 can be estimated from the data by another nested ANOVA on the values of the sides; the within-individual component of variance is the best estimate of $E[s_i^2] = E[V_{N,i}] \equiv \overline{FA}$. These estimates are sufficient to find the repeatability.

To estimate V_{err} , observe that the distribution of $\nu s_i^2 / V_{N,i}$ among replicates of individual i is χ^2 distributed, where ν is one less than the number of sides measured. For bilateral traits with only two sides, $\nu=1$. Therefore, the mean and variance of s_i^2 among hypothetical replicates of the same individual are $V_{N,i}$ and $2(V_{N,i})^2/\nu$, respectively. V_{err} is the expectation across individuals of the within-individual variance in s_i^2 . Thus,

$$V_{err} = E[V[s_i^2]] = E\left[\frac{2}{\nu} (V_{N,i})^2\right] = \frac{2}{\nu} [V_{DS} + (\overline{FA})^2] \quad (4)$$

where $V[s_i^2]$ is the variance among hypothetical replicates of individual i in s_i^2 .

Note that $V_{DS} = V_{FA} - V_{err}$. Solving for V_{DS} and dividing by V_{tot} to find R , we can find

$$\begin{aligned} R &= \frac{V_{FA} - \frac{2}{\nu} (\overline{FA})^2}{\left(1 + \frac{2}{\nu}\right) V_{tot}} \\ &= \frac{V_{FA}}{\left(1 + \frac{2}{\nu}\right) V_{tot}} - \frac{2}{\left(1 + \frac{2}{\nu}\right) \nu C_v^2}, \end{aligned} \quad (5)$$

where C_v is the coefficient of variation of the measured FA. Therefore, for bilateral traits,

$$R = \frac{V_{FA}}{3V_{tot}} - \frac{2}{3C_v^2}. \quad (6)$$

In the case where there is no variance among individuals in $V_{N,i}$, $V_{FA} = 2FA^2$, leaving a zero repeatability. Note that even when the C_v is very large and there is no measurement error, so that $V_{FA} = V_{tot}$, the repeatability of developmental stability is at most 1/3 when the measure of FA is the variance among two sides. R can approach one if there are a large number of 'sides' to measure per individual.

4. ERRATA: CORRELATIONS IN DEVELOPMENTAL STABILITY AMONG CHARACTERS

I would also like to take this opportunity to point out a typographical error in Whitlock (1996) in the section on estimating the correlation of developmental stability among traits. The variance of the underlying stability of trait i is in fact $V_{tot,i}R$, and the correlation among characters in the amount of developmental noise, β_{DN} , is therefore given by

$$\beta_{FA} \leq \beta_{DN} \leq \beta_{FA} / \sqrt{R_1 R_2}. \quad (7)$$

The actual magnitude of β_{DN} depends on whether the asymmetry values are correlated because of the particulars of development (in which case β_{DN} is well approximated by β_{FA}), or because of similarities in the efficiencies of the developmental process (in which case β_{DN} will approach the upper limit given in equation (7)).

The verbal conclusions in Whitlock (1996) were based on the correct equations: 'the correlation given by FA is a substantial underestimate of the true relation of the developmental patterns of different characters.'

The estimates of the heritability of developmental stability, and of the correlation among characters in developmental stability, can be substantial underestimates of their true value. These biases can be corrected with information about the C_v of FA measures, with a relatively simple equation. I apologize for any inconvenience these errors may have caused.

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