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Consequences of reduced genetic variance on developmental instability estimators

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ABSTRACT

Several studies have failed to find the negative relationship between genetic variability and developmental instability hypothesized by Lerner in 1954. Developmental instability is often assessed using its common estimator, fluctuating asymmetry. In this study, we use a computer simulation procedure that enables us to quantify the effect of a loss of genetic variability on average fluctuating asymmetry. We simulated a systematic loss of genetic variability, which in populations can be generated by a process of stabilizing selection or by a reduction in population size. Average fluctuating asymmetry decreased during the simulated process of a systematic loss of genetic variability, even though developmental instability was held constant and equal for all the individuals comprising the simulated populations. The error associated with the use of fluctuating asymmetry to estimate developmental instability of the simulated population is assessed and discussed. This study is the first to apply a theoretical rationale to the relationship between genetic variability and developmental instability as estimated by fluctuating asymmetry. The results presented here should be taken into account in future studies of fluctuating asymmetry, as they may help to explain some of the contradictory results reported in the literature regarding the correlation between genetic variability and fluctuating asymmetry. Furthermore, the results of this study also show how difficult it can be when comparing fluctuating asymmetry among populations with different population size and/or different genetic variability. In fact, the observed positive relationship between genetic variability and fluctuating asymmetry can counteract the hypothesized negative relationship between genetic variability and developmental instability hypothesized by Lerner. The results of the present study will have an impact on research into the application of fluctuating asymmetry as an indicator of genetic and environmental stress.

Keywords: fluctuating asymmetry, genetic drift, genetic variance, phenotypic variance.

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INTRODUCTION

The development of a trait in a given environment is disturbed by random processes that cause it to deviate from its expected phenotype. It is believed that an individual's ability to buffer its development against these random perturbations is influenced by genotype, environment and/or genotype × environment interactions (Van Dongen and Lens, 2000). Developmental instability results when stress affects the buffering capacity of the processes that provide stability to an organism's development (Lens *et al.*, 2000). The theoretical argument that stressed individuals have greater developmental instability is supported by some research showing a positive relationship between developmental instability and the intensity of stress (Møller and Swaddle, 1997). Despite the potential use of developmental instability in conservation work, empirical studies supporting its general adequacy for monitoring endangered species or populations are generally lacking or contradictory (Leary and Allendorf, 1989; Fowler and Whitlock, 1994; Clarke, 1995; Vøllestad *et al.*, 1999; Gilligan *et al.*, 2000). In some cases, developmental instability tends to increase under environmental stress, such as pollution (Østbye *et al.*, 1997) and extreme temperatures (Imasheva *et al.*, 1997), or because of genetic factors, such as loss of genetic variation, inbreeding (Leary *et al.*, 1983; Mitton, 1993; Vøllestad *et al.*, 1999) and episodes of directional selection (Markow and Ricker, 1992).

The negative relationship between genetic variability and developmental instability was first hypothesized by Lerner (1954). Many investigators promoted the existence of the hypothesized relationship based on empirical results. For example, Clarke and McKenzie (1987) demonstrated that homozygous individuals were developmentally less stable than their heterozygous counterparts. Another common observation is that progeny in the F_1 generation after hybridization exhibit enhanced fitness and decreased developmental instability relative to their parents. This effect is generally believed to originate from increased heterozygosity (Ferguson *et al.*, 1987). As a result of the above findings, the relationship between developmental instability and heterozygosity has largely been accepted and promoted (for reviews, see Mitton, 1993; Britten, 1996). However, many studies have failed to support the existence of this relationship. These latter contradictory results have not been fairly represented in reviews of developmental instability. A meta-analysis by Vøllestad *et al.* (1999) cites a representative sample of these 'pro' and 'con' studies, but is still slightly biased in favour of those reporting significant relationships.

Other approaches have been used to determine the relationship between genetic variability and developmental instability. These include an analysis of differences in developmental instability between males and females of haplo-diploid taxa, or between parthenogenetic and sexually reproducing individuals. However, these studies have been limited to a few species only (Clarke *et al.*, 1992; Clarke, 1997; Crespi and Vanderkist, 1997; Pertoldi *et al.*, 2001a) or to outbreed strains versus completely homozygous strains and their hybrids (Ross and Robertson, 1990; Andersen *et al.*, 2002). No clear patterns have emerged. Hence, the debate about the relationship between developmental instability and genetic variability is still raging. In this paper, we present a model that may help us to understand why several studies of the relationship between genetic variability and developmental instability and its common estimator, fluctuating asymmetry, have provided controversial results (for a review, see Gilligan *et al.*, 2000). Fluctuating asymmetry can be defined as random deviations from perfect symmetry in a bilaterally symmetrical trait (Palmer and Strobeck, 1986).

Phenotypic variance can also be considered to be an estimator of developmental instability, but only if phenotypic variance is estimated among genetically identical individuals (genetic variability, $\sigma_g^2 = 0$) that are reared under identical environmental conditions (environmental variability, $\sigma_e^2 = 0$). This, of course, is very difficult to assess empirically (Palmer and Strobeck, 1986; Pertoldi *et al.*, 2001b; Kristensen *et al.*, 2003). In populations composed of genetically different individuals in a constant environment ($\sigma_e^2 = 0$), the phenotypic variance is roughly correlated with the genetic variability.

Whereas it is generally accepted that phenotypic variance is influenced by genetic and environmental variability (Falconer and Mackay, 1996), fluctuating asymmetry at the individual level has the advantage that dissimilarity in the expression of a given character on the left and right sides cannot be explained by either differences in genotype or environment. However, because the estimation of individual developmental instability by single-trait asymmetry is an attempt to estimate a variance with two data points (i.e. left and right trait values), the correlation between individual asymmetry and the presumed underlying developmental instability is weak (Van Dongen *et al.*, 1999). Extending the estimation of developmental instability by means of fluctuating asymmetry at the population level, the problems associated with the among-individual heterogeneity of the extent of developmental instability further clouds the picture. The among-individual heterogeneity of developmental instability can be due to the presence of environmental variability within the population sampled and/or different susceptibilities to stress of the genotypes comprising the population under study (Kristensen *et al.*, 2003). Whitlock (1998) proposed a method, based on the hypothetical repeatability of individual asymmetry, to convert patterns in fluctuating asymmetry into patterns in the presumed underlying developmental instability. The repeatability estimates the proportion of the total variation in the unsigned fluctuating asymmetry that results from between-individual heterogeneity in the underlying developmental instability.

The model presented here allows quantification of the impact on the fluctuating asymmetry associated with a reduction in genetic variability. We show how a systematic loss of genetic variability (estimated by phenotypic variance) changes the fluctuating asymmetry. We discuss the implications of our findings.

METHODS

Model assumptions

Given the above-mentioned complications associated with the estimation of developmental instability by fluctuating asymmetry, several simplifying assumptions were necessary in our model to obtain a clear interpretation of the model results: additivity of the gene effects ($\sigma_g^2 = \sigma_a^2$); the absence of additional variability produced by environmental variability ($\sigma_e^2 = 0$); and the assumption that the mean trait value in the simulated population did not change considerably during the simulation. These assumptions do not, however, constitute serious violations of the 'real-world' scenario.

Simulation studies

We simulated a systematic loss of phenotypic variance (and, consequently, additive genetic variance, σ_a^2) that could be produced by a process of stabilizing selection or by a reduction

in population sizes. The simulation was conducted with the software program StatView SE+Graphics™ (SAS System) by generating two groups of values for the right and left side of a bilateral trait, respectively. Each group consists of a series of eight discrete values ranging from 16 to 23 (numbers commonly observed for sternopleural bristles in *Drosophila mercatorum*). The eight discrete values represent the means of the simulated trait of the eight theoretical genotypes that constitute the population. Around each of the eight discrete values, 1000 normally distributed discrete values were generated, representing developmental instability. In our simulation, all eight normal distributions had the same standard error (= 0.0323), since we assumed that all eight genotypes had the same developmental instability (DI = constant). Therefore, the distribution of, for example, the genotype that has a mean trait value of 19 is constituted by 1000 discrete values with a mean ± standard error of 19.0 ± 0.0323, which is equivalent to 19.0 ± 0.5 DI. We simulated a meristic trait; however, metric traits could also be simulated.

Theoretical background

The decrease in phenotypic variance due to stabilizing selection was simulated by systematically removing the extreme genotypes at the two tails of the distribution (the Bulmer effect; see Bulmer, 1985, chapter 9). The genotype removal process was alternated on the right and left sides of the trait distribution. If a trait is selectively neutral, the reduction in phenotypic variance could eventually be caused by a reduction in genetic variability due to a reduced population size and increased mating among relatives (Fowler and Whitlock, 1999). The neutral models have been shown to be of considerable value as null models, as they allow tests to be made about the nature of selection (Hey, 1999). The assumption of neutrality will not be seriously violated in the 'real world' if we consider a polygenic trait for our simulation, where the selective pressure on specific loci is small enough to be overwhelmed by random genetic drift (Lynch, 1996).

We assume that gene action is purely additive ($\sigma_g^2 = \sigma_a^2$). Genetic variability consists of two other components with non-additive gene action, the dominance variance (σ_d^2) at a locus and the epistatic variance (σ_i^2) (Blows and Sokolowski, 1995). Assuming that genes are purely additive, $\sigma_d^2 = 0$ and $\sigma_i^2 = 0$:

$$\sigma_g^2 = \sigma_a^2 \tag{1}$$

The additive gene action implies that the mean value of a trait in an inbred population will be the same as the mean value of the same trait in an outbred population. Therefore, if $\sigma_d^2 = 0$, the mean will not change with inbreeding (Falconer and Mackay, 1996). However, additive genetic variance within the inbred population will decrease: $\sigma_a^2 = \sigma_{ai}^2(1 - F)$ (Whitlock and Fowler, 1996), where σ_{ai}^2 is the initial σ_a^2 before the inbreeding event and F is the inbreeding coefficient.

In the non-selective neutral model, we also expect the mean size of a trait to be constant during the simulated process of stabilizing selection. Eventual violations of these assumptions are related to the amount of 'symmetry' of the selective forces acting on the population. We assumed no environmental variability ($\sigma_e^2 = 0$) during the simulated process of stabilizing selection. The assumptions of additive gene action, $\sigma_g^2 = \sigma_a^2$, and the absence of environmental variability, $\sigma_e^2 = 0$, are not seriously violated in 'real-world' scenarios if we assume that the trait being simulated is not directly related to fitness. In fact, non-fitness-related traits may be influenced to a greater extent by additive genetic variance than traits

related to fitness, which are assumed to be influenced mainly by dominance and epistatic interactions (Fowler and Whitlock, 1994). Furthermore, fitness-related traits are typically more sensitive to environmental variability, as these traits' genetic variability tends to be depleted (Fowler and Whitlock, 1994).

In the process of loss of additive genetic variance in the complete absence of environmental variability, there will be no interactions among different genotypes and the environment [(G × E) = 0 = cov(GE)]. Therefore, phenotypic variance (σ_p^2) can be described as follows:

$$\sigma_p^2 = \sigma_a^2 + \mathrm{DI} \tag{2}$$

[For further details on the equation describing the relationship between phenotypic variance and its components, see Pertoldi *et al.* (2001b).]

As developmental instability was assumed to be constant (DI = constant), it can be seen from equation (2) that there is a constant difference (DI) between phenotypic and additive genetic variance; hence phenotypic variance will decrease when additive genetic variance decreases, meaning that phenotypic variance in a constant environment can be considered as a reliable estimator of additive genetic variance. Fluctuating asymmetry (FA) is estimated following Palmer and Strobeck (1986) as:

$$\mathrm{FA} = \sigma^2(\mathrm{r} - \mathrm{l}) \tag{3}$$

where r and l are the trait values on the right and left side, respectively.

During the simulation, we did not observe considerable change in the mean trait size; therefore, no scaled FA index was utilized, as the estimate of fluctuating asymmetry will not change (Palmer and Strobeck, 1986). $\sigma^2(\mathrm{r} - \mathrm{l})$ is equal to:

$$\mathrm{FA} = \sigma^2(\mathrm{r} - \mathrm{l}) = \sigma_r^2 + \sigma_l^2 - 2\mathrm{cov}(\mathrm{r}, \mathrm{l}) \tag{4}$$

As we are dealing with bilateral traits, we assume that

$$\sigma_r^2 = \sigma_l^2 = \sigma_p^2$$

and

$$\mathrm{cov}(\mathrm{r}, \mathrm{l}) = R\sigma_r\sigma_l$$

where R is the correlation coefficient between r and l. Because $\sigma_r^2 = \sigma_l^2 = \sigma_p^2$, $\sigma_r = \sigma_l$ and, consequently, $\sigma_r^2\sigma_l^2 = \sigma_p^2$. Therefore,

$$2\mathrm{cov}(\mathrm{r}, \mathrm{l}) = 2R\sigma_p^2 \tag{5}$$

Substituting this into equation (4) we get:

$$\mathrm{FA} = \sigma_r^2 + \sigma_l^2 - 2R\sigma_p^2 \tag{6}$$

Reorganizing equation (6) we get:

$$\mathrm{FA} = 2\sigma_p^2(1 - R) \tag{7}$$

We can see from equation (7) that the relationship between fluctuating asymmetry and R is linear under the assumptions stated above. Hence, we expect from equation (7) that if phenotypic variance remains constant, an increase in fluctuating asymmetry can only occur if R decreases.

RESULTS AND DISCUSSION

In total, the simulation generated 8000 normally distributed discrete trait values for the right and for the left side (see Table 1, Fig. 1). Fluctuating asymmetry was calculated from equation (3); as expected, no significant differences in fluctuating asymmetry or phenotypic variance were noted among the eight genotypes, because we assumed developmental instability to be constant among genotypes (the homogeneity of fluctuating asymmetry and phenotypic variance was assessed using a Bartlett test; results not shown). The overall $FA = \sigma^2(r - l)$ was 2.211, which corresponded with the fluctuating asymmetry values and was similar to those found in an outbred strain of *Drosophila mercatorum* reared under laboratory conditions (D.H. Andersen, unpublished data). The systematic alternate removal of genotypes from the two tails of the population's right and left side trait distribution progressively reduced phenotypic variance (and, consequently, additive genetic variance).

From the simulation results (see Table 1), we can see that R and fluctuating asymmetry are progressively reduced with a progressive reduction in phenotypic variance. In our simulation, R was reduced by 96.4% when phenotypic variance was reduced (see Table 1). From equation (7), we would have expected the reduction in R to have increased fluctuating asymmetry, as the two variables are inversely correlated. However, since phenotypic variance (which is positively correlated with fluctuating asymmetry) showed a steeper decrease than R, we instead observed a reduction in fluctuating asymmetry.

The reduction in fluctuating asymmetry produced by the loss of phenotypic variance is considerable if compared with normal values for fluctuating asymmetry and which correspond to that in our simulation: FA = 2.211. The reduction in fluctuating asymmetry (calculated using equation 3) seen in our simulation (comparing our starting population with max. σ_p^2 and the final population with min. σ_p^2) was:

$$FA = (\text{max. } \sigma_p^2) - (\text{min. } \sigma_p^2) = 2.211 - 1.888 = 0.323$$

Table 1. Number and range of genotypes present in the simulated population

No. of genotypes (range of genotypes)	Max. range of genotypes ± 0.5 DI	No. of discrete values generated (n)	R	σ_p^2	FA $\sigma^2(r - l)$
8 (16–23)	13–27	8000	0.825	6.318	2.211
7 (16–22)	13–25	7000	0.795	5.013	2.055
6 (17–22)	13–25	6000	0.741	3.934	2.038
5 (17–21)	13–25	5000	0.661	3.017	2.046
4 (18–21)	14–25	4000	0.555	2.263	2.014
3 (18–20)	14–23	3000	0.399	1.647	1.980
2 (19–20)	15–23	2000	0.215	1.256	1.972
1 (19–19)	16–22	1000	0.030	0.973	1.888

Note: The maximum range of values is generated by adding developmental instability (DI) on all the genotypes. Number of values generated (n). The correlation coefficient (R) is the correlation between the right (r) and the left (l) sides of the generated traits. The phenotypic variability (σ_p^2) is calculated by averaging (σ_p^2) of the right and left sides of the simulated traits. Fluctuating asymmetry (FA) is estimated as $\sigma^2(r - l)$. The data have been obtained by simulating a reduction in σ_p^2 due to the effect of stabilizing selection with increasing intensity, or to a progressive reduction of population size (highest at the top of the table and lowest at the bottom).

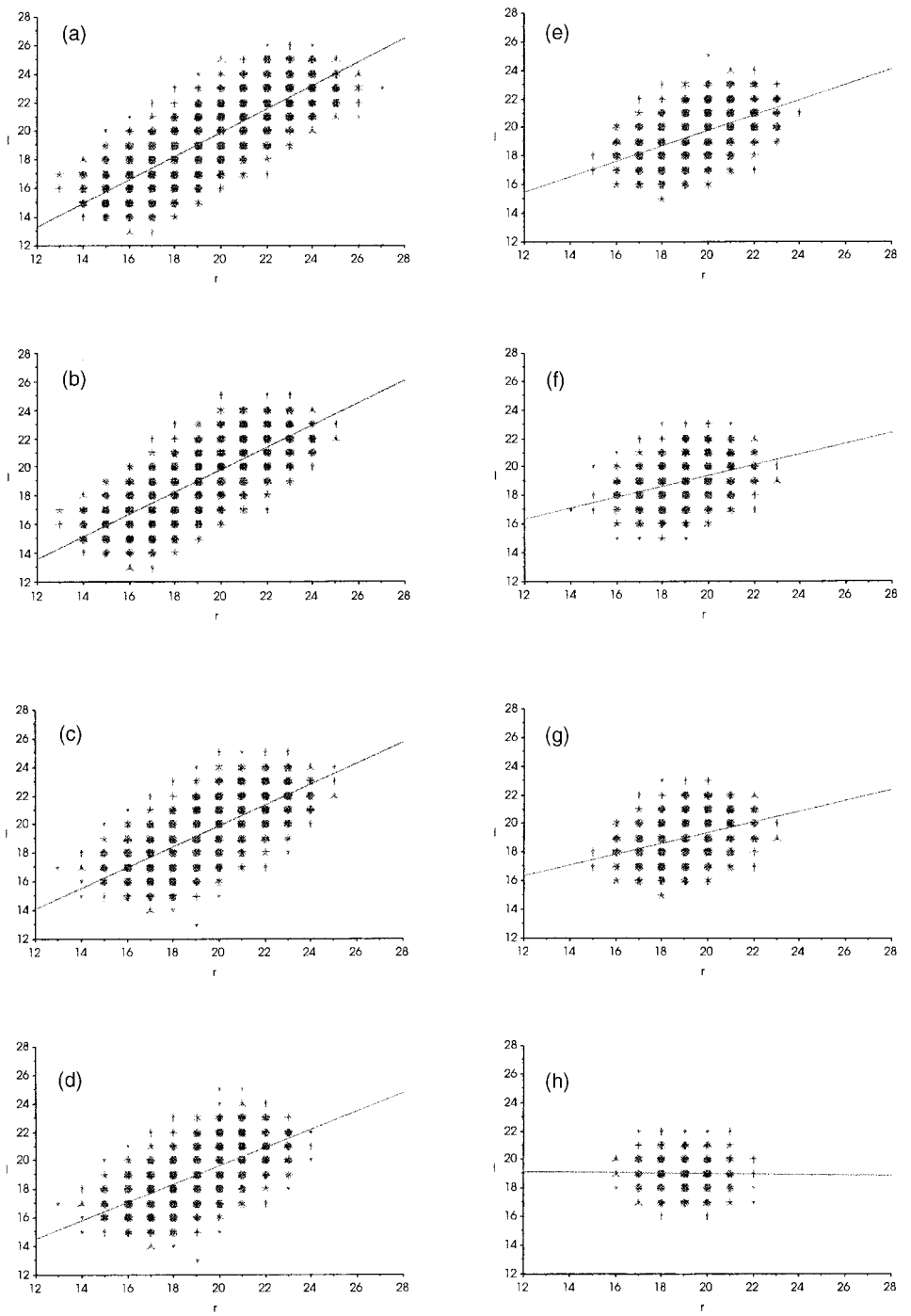

Fig. 1. Simulations showing the distribution of right (r) and left (l) side traits: constant developmental instability with increasing loss of phenotypic variance (σ_p^2) and, consequently, additive genetic variance (σ_a^2) (see Table 1 for details).

This corresponds to a reduction of 14.6% (see Table 1). Hence, in a theoretical comparison of fluctuating asymmetry between our starting population (max. σ_p^2) and our final population (min. σ_p^2), we should take into account this effect when making comparisons of fluctuating asymmetry among populations. Before proceeding with further analysis, the estimate of fluctuating asymmetry should be corrected for the bias estimated in our simulation.

The present study is the first to apply a theoretical rationale to the relationship between genetic variability and fluctuating asymmetry. Our simulation has clearly shown that we should be cautious when interpreting the results from comparisons of fluctuating asymmetry among populations, with the intention of estimating environmental or genetic stress. In fact, the observed positive relationship between genetic variability and fluctuating asymmetry can counteract the hypothesized negative relationship between genetic variability and developmental instability hypothesized by Lerner (1954). Our simulation results confirm that fluctuating asymmetry can be problematic when used to monitor genetic stress. Similarly, problems can arise when using fluctuating asymmetry as an indicator of environmental stress. Indeed, populations often have different amounts of genetic variability, which can make comparisons of fluctuating asymmetry difficult because of the bias demonstrated above. Hence, we recommend molecular genetic studies to ascertain the genetic homogeneity among and within populations before proceeding with comparative estimates of environmental stress experienced by those populations. Caution should at least be taken when comparing different amounts of phenotypic variance. To overcome the need for molecular genetic analysis in studies using fluctuating asymmetry to estimate environmental stress factors, investigations could be conducted on monoclonal organisms under controlled environmental conditions. This would eliminate the genetic variability among individuals ($\sigma_g^2 = 0$) and would allow the estimation of bias produced by environmental variability on developmental instability. If $\sigma_e^2 = 0$, then fluctuating asymmetry reflects the real developmental instability of the population under investigation (Pertoldi *et al.*, 2001b; Kristensen *et al.*, 2003).

Our model results suggest an alternative explanation for the lack of a consistent association between fluctuating asymmetry and heterozygosity. It is generally believed that more heterozygous individuals have lower fluctuating asymmetry than their more homozygous counterparts (Mitton, 1993). However, our results show that this hypothesized relationship can be obscured by the bias in fluctuating asymmetry produced by a reduction in genetic variability. The scenario can be further complicated if the assumption of additivity is violated. Indeed, genetic bottlenecks can also increase phenotypic variance. Non-additive genetic variance (dominance and epistatic variance) can be converted into additive genetic variance (Fowler and Whitlock, 1999). Consequently, the expected total genetic variability and thus phenotypic variance can be greater in inbred populations than in outbred ones. The presence of recessive deleterious alleles can further be revealed during inbreeding, thereby increasing the developmental instability counteracting the bias in fluctuating asymmetry produced by the reduction in genetic variability. The violation of the assumption of additivity and the presence of recessive deleterious alleles implies the presence of dominance variance, which is associated with inbreeding depression (Hartl and Clark, 1989). In our simulation, the mean trait size was not changed considerably. However, a reduction in mean trait size during an inbreeding event is very common (Hartl and Clark, 1989). As phenotypic variance is known to be positively correlated with trait size, a reduction in the mean trait size is accompanied by a reduction in phenotypic variance. Therefore, the presence of dominance variance will reinforce the observed bias.

In conclusion, the idea presented here is substantially different from explanations for the lack of a consistent relationship between fluctuating asymmetry and genetic variability made in the literature (e.g. Mitton, 1993; Klingenberg and Nijhout, 1999). Furthermore, it may help to explain why analyses of differences in fluctuating asymmetry between males and females of haplo-diploid taxa have not shown a clear pattern (Clarke *et al.*, 1992; Clarke, 1997; Crespi and Vanderkist, 1997).

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