Detecting genetic variation in developmental instability by artificial selection on fluctuating asymmetry

R. C. FULLER & D. HOULE

Department of Biological Sciences, Florida State University, Tallahassee, FL, USA

Keywords:

additive genetic variation;
canalization;
developmental stability;
heritability;
repeatability;
selection differential;
sexual selection.

Abstract

Fluctuating asymmetry (FA) is frequently used as a measure of developmental instability (DI). Assuming a genetic basis to DI, many have argued that FA may be a good indicator of genetic quality to potential mates and to human managers of populations. Unfortunately FA is a poor indicator of DI, making it very difficult to verify this assertion. A recent review of the literature suggests that previous studies of the inheritance of FA and DI using half-sib covariances and parent–offspring regression have been unable to put meaningful limits on the heritability of FA and DI because of the extremely low power of the experiments performed. In this study, we consider the power of artificial selection on FA as an alternative approach to studying the inheritance of FA and DI. Using simulations, we investigate the efficacy of selection for both increased and decreased FA for detecting genetic variation. We find that selection for increased FA has much more power to detect the presence of genetic variation than does selection for decreased FA. These results hold when realistic sample sizes are employed. Artificial selection for increased FA is currently the most powerful approach for the detection of genetic variation in DI.

Introduction

Fluctuating asymmetry (FA) (deviation from perfect bilateral asymmetry) is frequently used as a measure of developmental instability (DI), under the assumption that organisms possess homeostatic mechanisms that control trait development (Van Valen, 1962). If these hypothetical homeostatic processes are costly, it is then reasonable to assume that high quality individuals have low DI, and tightly controlled development, whereas low quality individuals have higher DI and more loosely controlled development. Studies showing female choice for low levels of FA (Møller, 1994; Møller & Swaddle, 1997; Martin & Lopez, 2000) and studies proposing the use of FA as an assay for genetic quality (Parsons, 1992; Clarke, 1995) rely on the assumption of a tight relationship between FA and DI.

Correspondence: Rebecca Fuller, Department of Biological Sciences, Biomedical Research Facility, Tallahassee, FL 32306-4340, USA.
Tel.: 850 6449820; fax: 850 6440989;
e-mail: fuller@neuro.fsu.edu

Unfortunately, the relationship between our measures of FA and DI is expected to be weak (Whitlock, 1996, 1998; Houle, 1997, 2000; Van Dongen, 1998). DI is essentially the variance of possible trait values around a trait mean. This point is obvious once we recall that in order for asymmetry to be considered fluctuating, signed asymmetry must be normal with mean equal to zero (Palmer & Strobeck, 1986; Palmer, 1994; Swaddle *et al.*, 1994). Estimating DI from the FA of a single character is estimating a variance with two data points. Such estimates themselves have very high variances, as the majority of data points for any value of DI occur close to the mean resulting in small values of FA. Thus, even if DI is quite heritable, FA is expected to show little resemblance among relatives.

The expected loose relationship between FA and DI makes the detection of genetic variation problematic (Fuller & Houle, in press). In a recent literature review, we found that the number of studies reporting statistically significant genetic variation in FA did not differ from the type 1 error rate (Fuller & Houle, in press).

Furthermore, data simulations indicated that parent–offspring regressions have essentially no power to detect additive genetic variation in FA and DI. Paternal half-sib breeding designs had more power than parent–offspring regressions but still required unrealistically large sample sizes to achieve high power for the most common conditions. These findings cast a pall of uncertainty over the question of whether FA and DI are heritable; there is little convincing evidence of heritability of FA on the one hand, but on the other hand the vast majority of studies that have been performed have no power to detect biologically important levels of inheritance.

In our previous paper, we did not consider the use of selection experiments to estimate additive variance in FA and DI. Selection experiments have been infrequently used for this purpose (Reeve, 1960; Breuker, 2002) but could be useful by allowing the accumulation of small effects over many generations. In this paper, we ask how effective is selection on FA at changing DI using a simulation model.

Model FA and DI

This model used to simulate selection on FA follows that developed in Fuller & Houle (in press), where the SAS code that implements a very similar model is available. Briefly, variation in DI was assumed to reflect log-normally distributed additive genetic, maternal and environmental deviations. We assume that the distribution of DI is log-normal. The log-normal distribution is well suited for traits such as DI near a limiting boundary, where genetic effects are likely to be proportional, rather than additive.

We assume that FA is measured from the difference between measurements of a bilaterally symmetrical character on each side of the body. Trait values for the two sides of an individual are drawn from a normal distribution with variance that equals DI, and a trait mean that does not differ among individuals. FA is the absolute value of the difference between two such trait values (i.e. left and right sides). In these simulations we set the trait mean to an arbitrary value of 10, and the coefficient of variation of the trait to 0.0665, a value that is close to the mean value found in a literature review (Fuller & Houle, in press). Our implementation of this model was thoroughly tested with simulations (data not shown).

We used our model to generate populations in which we selected for increased and decreased FA. We then attempted to detect genetic variation in DI from the observed response. We address the following questions: (1) Does selection on FA effectively generate selection on DI? (2) Does the direction of selection (i.e. increased FA vs. decreased FA) produce qualitatively different results? (3) How do the following parameters affect our ability to detect genetic variation in DI: mean-standardized variation in DI (CV_{DI}), heritability of DI (h^2_{DI}), and strength of selection (B)?

We simulated selection in populations of 1000 individuals. Selection was applied to this population, and the selection differential for DI calculated on the log scale. The mean DI of the next generation was assumed to follow from the breeder's equation (Falconer & Mackay, 1996). We then simulated another 1000 individuals with this new value of DI. We calculated the response to selection for FA and DI as the difference in those variables between generations.

We simulated three levels of mean-standardized variation in DI, labelled low variation ($CV_{DI} = 0.1$), medium variation ($CV_{DI} = 0.5$), and high variation ($CV_{DI} = 1.0$). We also simulated three heritabilities of DI on the log scale: $h^2_{DI} = 0.1$, 0.4, or 0.8. Finally, we simulated strengths of selection (B), the proportion of individuals chosen as parents, of 1, 5 and 10%. For each combination of parameters we simulated five replicate lines selected for increased FA and five replicate lines selected for decreased FA.

We analysed selection differentials and responses to selection separately for lines selected for increased and decreased FA. We used ANOVA to examine the effects of parameters. All simulations and analyses were performed using SASv.8 (SAS Institute, Inc., Cary, NC, USA).

Results

Selection differentials

Fluctuating asymmetry selection differentials were on average 2.5 times higher in lines selected for increased FA (hereafter referred to as up lines) than in lines selected for decreased FA (hereafter referred to as down lines). Figure 1a–c shows mean selection differentials on FA for both up and down lines. For up lines, selection differentials were 1.89 and 1.28 times higher under high ($B = 0.01$) and intermediate ($B = 0.05$) strengths of selection than under low selection ($B = 0.10$) ($F_{2,126} = 838.73$, $P < 0.001$). Similarly, selection differentials for FA were highest when there was high variation in the base population. The selection differentials for FA when variation in DI was high and medium were 1.45 and 1.08 times higher than when variation in DI was low ($F_{2,126} = 196.57$, $P < 0.001$).

Differences in FA selection differentials between strengths of selection and levels of variation in DI were much lower in down lines. Selection differentials from down lines under high and intermediate selection strengths were 1.09 and 1.03 times larger in magnitude than differentials from the low strength of selection treatment ($F_{2,126} = 84.38$, $P < 0.001$). Selection differentials were higher in magnitude variation in DI was low ($S = -0.71$) than when variation in DI was medium ($S = -0.70$) or high ($S = -0.68$) ($F_{2,126} = 115.47$, $P < 0.001$). However, these differences are quite small in magnitude.

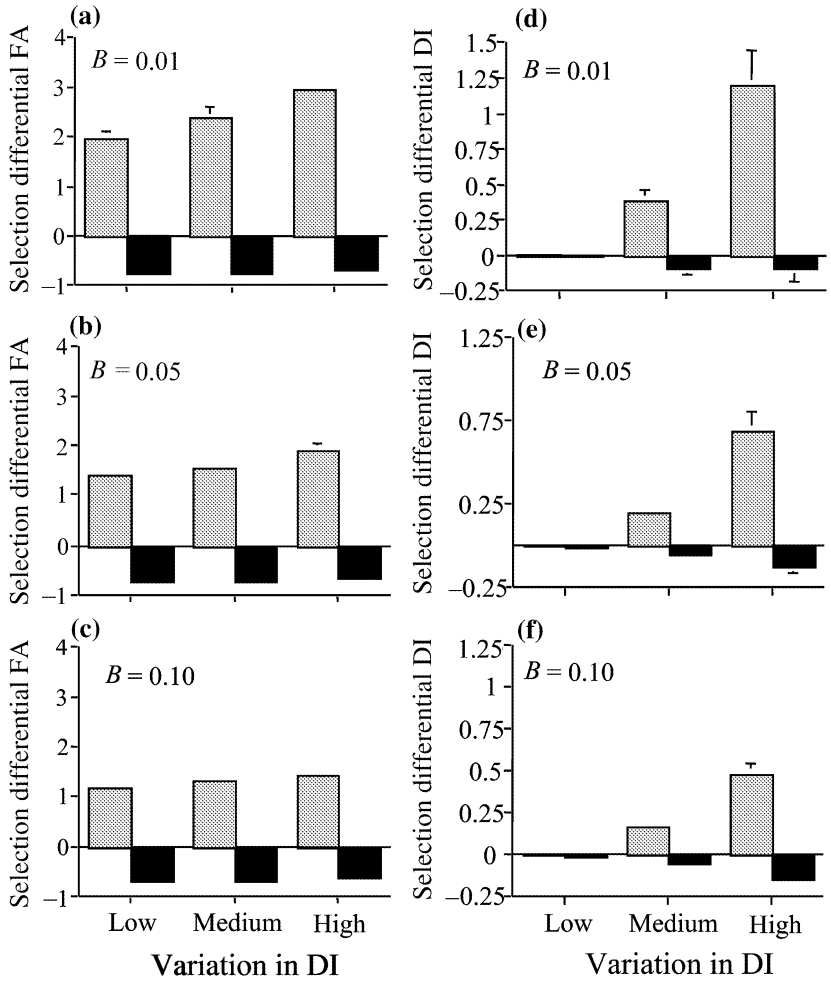

Fig. 1 Selection differentials for both up (stipled bars) and down (black bars) selection on FA. Means and SD are shown. $n = 15$ for each mean. Graphs a, b, and c show the results for selection differentials on FA. Graphs d, e, and f show the results for the selection differentials on DI. Results are shown for three different strengths of selection ($B = 0.01$, 0.05, or 0.10). This means that 1, 5 or 10% of the animals in the base population are used to create the next generation.

The purpose of selecting on FA is to indirectly select on DI. The differences between up and down lines, strengths of selection, and levels of variation in DI were much larger for DI selection differentials than those for FA. Figure 1d–f shows mean DI selection differentials as a result of selection for increased and decreased FA. The absolute magnitude of DI selection differentials in up lines was 5.5 times greater than those in down lines. For up lines, high and intermediate strengths of selection generated DI differentials that were 2.56 and 1.43 times those under low strengths of selection ($F_{2,126} = 133.943$, $P < 0.001$). Variation in DI had particularly large effects on DI differentials ($F_{2,126} = 821.844$, $P < 0.001$). DI differentials from up lines with high and medium levels of variation in DI were 89 and 24 times higher than differentials from lines with low levels of variation in DI.

Variation in DI also had effects on DI differentials from down lines ($F_{2,126} = 98.012$, $P < 0.001$). DI differentials were higher in magnitude when variation in DI was high ($S = -0.07$) or medium ($S = -0.05$) than when variation in DI was low ($S = 0.001$). Note that this occurred despite the fact that FA differentials from down lines were slightly higher when variation in DI was low. Strength of selection had no appreciable effect on DI differentials from down lines (Fig. 1, $F_{2,126} = 0.144$, $P = 0.866$).

Response to selection

In order to detect a significant response to selection on FA, there must first be a significant response in DI. The response to selection in DI largely mirrored the DI selection differentials. The response to selection in DI was 5.3 times higher on average for up lines than for down lines (data not shown). DI responses to selection in up lines differed from 0 in 22 of 27 cases. In contrast, DI response to selection differed from zero in 12 of 27 cases. In general, responses were greatest when selection was strong, heritability was high, and variation in DI in the population was high.

In order to detect a response to selection, the response in DI must be manifested through FA. The probability of detecting genetic variation is further weakened by this

last step. Figure 2 shows the FA responses to selection for both increased and decreased FA. The FA response to selection was 3.5 times greater for up lines than for down lines (Fig. 2). As with DI responses, the strength of selection ($F_{2,108} = 100.58$, $P < 0.001$), the variation in DI ($F_{2,108} = 777.17$, $P < 0.001$), DI heritability ($F_{2,108} = 394.12$, $P < 0.001$), and the interaction between DI heritability and variation in DI ($F_{4,108} = 135.59$, $P < 0.001$) had the strongest effects. In addition, all of the possible combinations between these three factors accounted for significant amounts of variation. FA responses to selection differed from zero in 16 of 27 cases (Fig. 2). Note that a significant response in FA was only detected for treatments from which a significant response was detected for DI. Furthermore, six treatments that had a significant response in DI did not have a significant response in FA.

Among down lines, DI heritability ($F_{2,108} = 9.42$, $P < 0.001$), variation in DI ($F_{2,108} = 20.29$, $P < 0.001$), and the interaction between DI heritability and variation in DI ($F_{4,108} = 3.869$, $P < 0.001$) had the strongest effects on FA response to selection. However, strength of

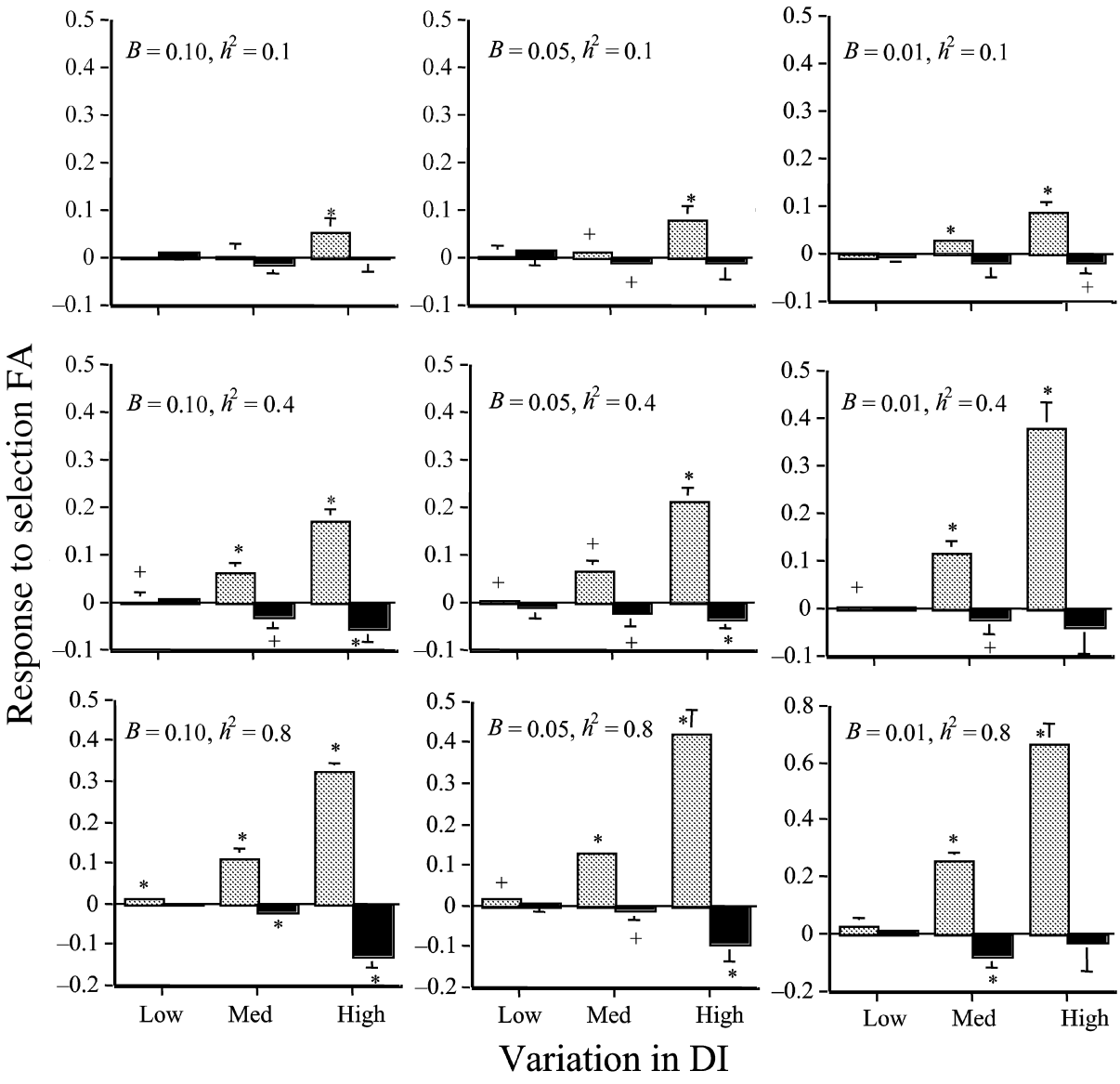

Fig. 2 Response to selection for FA. Results are shown for all combinations of selection strength, h^2_{DI} and variation in DI. Note the change in scale on the *y*-axis. Means and SD are shown. $n = 5$ in all cases. Stars over bars indicate responses to selection that differ from zero at $P < 0.05$. Crosses over bars indicate treatments where there was a significant response to selection for DI but not for FA.

selection had no significant effect ($F_{2,108} = 0.388$, $P = 0.679$). The FA response to selection in down lines differed from zero in only 6 of 27 cases (Fig. 2).

FA and power

The previous results suggest that genetic variation in FA (and therefore DI) is detectable when the true heritability of DI and population variation in DI are not low. However, these results were obtained for simulated selection experiments involving 1000 individuals per line. We next wanted to address the question of whether these results hold when more realistic sample sizes are employed. We repeated the simulations used above, but reduced the number of individuals in each line to 100. We simulated five lines selected for increased FA and five lines selected for decreased FA under each combination of strength of selection (0.01, 0.05, 0.10), DI heritability (0.1, 0.4, 0.8), and variation in DI (low, medium, high). We report the realized trait heritabilities for FA only.

Table 1 shows the realized trait heritabilities from lines for increased and decreased FA. Strength of selection had little effect on the realized trait heritabilities for either up or down FA lines. Six of nine realized FA heritabilities from up lines differed significantly from zero, and these occurred when variation in DI was medium or high. In contrast, realized FA heritabilities from down lines differed significantly from zero in only one of nine sets of parameters. This occurred when both variation in DI and the heritability of DI were high. There were no statistically significant differences in realized trait heritabilities between up and down lines, but this occurred because the variance in heritabilities from down lines was high.

Discussion

The main conclusion of this study is that selection for decreased FA should be largely ineffective whereas selection for increased FA is much more likely to produce a significant increase in mean FA of a population provided that variation in DI and heritability of DI are not low. These results stem largely from the fact that high values of FA are more informative than are low levels of FA. Under most conditions, an individual with a high level of FA is likely to have higher than average DI because an individual with low DI is unlikely to produce such a phenotype. In contrast, an individual with a low level of FA can have almost any value of DI. Low quality individuals with high levels of DI have a relatively high probability of producing traits with low FA. This happens because the expected distribution of traits is normal with the majority of trait realizations occurring close to the mean.

There are few selection experiments on FA that can be used to examine this prediction. Reeve (1960) performed two selection experiments on *Drosophila melanogaster*. In one experiment, he found that selection for decreased FA had a higher response than selection for increased FA, but this result is suspect because mean FA was decreasing in both lines. In the other experiment, Reeve found no changes in FA until generation 4 when the two lines diverged. This was a transient effect, and the difference was not maintained throughout subsequent generations. Interpreting these experiments is difficult primarily because the base population was quite small (about 70) and the strength of selection was somewhat weak (15–20%). Breuker (2002) selected for decreased FA in eyespots of *Bicyclus anynana* and found no significant response.

Selection for increased FA in a single trait should allow investigators to examine the question of whether genetic variation in DI exists at all. Some have assumed that FA is indicative of an organism-wide trait (or coadapted gene complex) that controls homeostasis of trait development (Clarke, 1994; Lens & Van Dongen, 1999; Leung *et al.*, 2000). If such a trait exists, then it is critically important for the function of individual traits as well as the overall functioning of the organism. However, skeptics have doubted this idea pointing out that FA of different traits expressed in a single individual are usually not positively correlated, as well as the fact that physiologists cannot point to any known mechanisms which would provide DI across many traits (Markow, 1995; Klingenberg &

Table 1 Realized heritability for FA.

DI h^2	Variation in DI	Realized h^2 FA (up)	Realized h^2 FA (down)
0.1	Low	0.0023 (0.0118)	–0.0077 (0.0285)
0.4	Low	0.0019 (0.0059)	0.0017 (0.0181)
0.8	Low	0.0093 (0.0071)	0.0093 (0.0289)
0.1	Medium	**0.0176 (0.0077)**	0.0119 (0.0258)
0.4	Medium	**0.0541 (0.0144)**	0.0196 (0.0350)
0.8	Medium	**0.0826 (0.0167)**	0.0579 (0.0406)
0.1	High	**0.0315 (0.0110)**	0.0092 (0.0418)
0.4	High	**0.1056 (0.0217)**	0.1001 (0.0569)
0.8	High	**0.2679 (0.0251)**	**0.127 (0.031)**

Up and down lines are indicated in parentheses. Simulations were performed using five replicate lines with 100 individuals per line. Mean and SD are shown. $n = 15$ for all cases. Means in bold differ significantly from zero.

Nijhout, 1999; Bjorksten *et al*., 2000a). Selecting on increased FA in a single trait will allow us to finally answer this question. If there is an organism-wide property controlling the stability of trait development, then increases in FA in one trait as a result of selection should be correlated with increases in FA in other traits that have not been selected upon. Such an experiment might find suites of traits that have correlated responses to selection, but other traits which are unresponsive (e.g. increases in overall feather asymmetry, but not increases in skeletal asymmetry).

Selection for increased FA should also help to resolve the controversy of whether FA is a meaningful measure of genetic stress (Clarke, 1994; Bjorksten *et al*., 2000b; Lens *et al*., 2000). The assumption has been that individuals with good genes should have low DI, and therefore low FA. However, it is conceivable that FA is not linked to genetic quality and can evolve independently of fitness (Nijhout & Emlen, 1998; Klingenberg & Nijhout, 1999). Support for the link between FA and genetic quality will be found if selection for increased FA results in selection for deleterious alleles and decreased fitness. It should be noted that several studies have approached this question by examining the relationship between inbreeding and FA (Fowler & Whitlock, 1994; Sheridan & Pomiankowski, 1997; Roldan *et al*., 1998; Gomendio *et al*., 2000). The rationale here is that inbred individuals should have lower fitness as a result of the expression of deleterious alleles. This should result in individuals with decreased homeostatic buffering abilities and increased FA. These two approaches (selection for increased FA vs. inbreeding) tackle the same problem from different directions. Selection for increased FA asks whether lines with high FA also have low fitness. Inbreeding studies ask whether inbred individuals or inbred lines with low fitness also have high FA.

These results also have important implications for mate choice involving asymmetry. The assumption is that females choose symmetrical males in order to obtain offspring with low levels of DI (Møller, 1992, 1993). Assuming a link between FA and male quality, what is the optimum level of choosiness? This study suggests that spending time and energy in attempts to mate with the most symmetrical males in a population (e.g. the upper 5%) is largely a waste because males with low FA need not have low DI. In contrast, avoiding matings with the most asymmetrical males in the population (e.g. the lower 5%) should decrease DI for offspring provided that there is heritability in DI and an intermediate amount of variation in DI in the population.

Can a response to increased selection on FA ever be detected when variation in DI is low and when the heritability of DI is low? Our results indicate that selection for increased FA in a single trait is unlikely to generate a significant response in FA in one generation when both variation in DI and the heritability of DI are low. The same result is also obtained when larger populations are simulated. Even when the base population is comprised of 100 000 individuals (an unrealistic size for nearly any organism on which FA can be measured), a significant response cannot be detected in our simulations after a single generation of selection, although DI is heritable. Similar results were obtained in a simulation study where we tried to determine the minimum sample size needed to detect additive genetic variation in DI and FA (Fuller & Houle, in press). We found that when variation in DI is low and DI heritability is low, nearly 1 000 000 individuals are needed to detect additive genetic variation in a standard half-sib design. Of course, over multiple generations of selection, a response may be detectable (Endler, 1985). However, simulations of five generations of selection on populations of 1000 individuals were unable to detect a response in FA, although significant responses in DI were observed. The noisy relationship between FA and DI easily obscures small differences in DI.

Finally, it is worth noting that selection experiments for decreased FA are not without value. Both natural and sexual selection are expected to favour low FA (Van Valen, 1962; Watson & Thornhill, 1994; Blows & Sokolowski, 1995). Selection experiments for decreased FA examine the degree to which such a phenomenon can readily occur. This is an important question. However, if one is interested in knowing whether or not heritable variation in FA (and DI) exists, then one should select for increased FA. Our simulations indicate that under most conditions the power of detecting the presence of additive genetic variation in FA (and DI) is increased when selecting for higher values of FA. Selection for decreased FA is less effective because low values of FA are not as informative about DI. These results hold even when more realistic sample sizes (100 individuals per selection line) are used. However, no selection regime can readily detect additive genetic variation when variation in DI and the true heritability of DI are low.

Acknowledgments

We thank Casper Breuker for giving us access to unpublished data. R.C. Fuller was supported by a University Fellowship from Florida State University.

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Received 2 July 2002; accepted 17 July 2002