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FLUCTUATING ASYMMETRY IN *SCABIOSA CANESCENS* AND *SCABIOSA COLUMBARIA*: ASSOCIATION WITH GENETIC VARIATION AND POPULATION SIZE

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Developmental instability and fluctuating asymmetry (FA) have become important topics in evolutionary biology. For example, it has been suggested that FA could be a useful tool for identification of genetic and environmental stress factors. This study used plants from each of six populations of *Scabiosa canescens* and *Scabiosa columbaria* grown under greenhouse conditions. I tested whether there was a relationship between petal FA and allozyme heterozygosity, the heritabilities of eight traits, and population size. Flowers displayed no directional asymmetry or antisymmetry. The rare species *S. canescens* had significantly higher FA values than *S. columbaria*, but only the latter demonstrated interpopulation differentiation for the expression of FA levels. There was no evidence for an association between population-level FA and genetic variation when compared with the allozyme heterozygosity or with the heritabilities of the quantitative traits. A tendency for a negative association between FA and population size was found for both species, but it was not significant when adjusted for multiple comparison. Hence, flower FA should not be considered a reliable indicator of the amount of genetic variation in populations of *S. canescens* and *S. columbaria*.

Keywords: fluctuating asymmetry, allozyme heterozygosity, heritability, population size, *Scabiosa*.

Introduction

Developmental instability is defined as the inability of an organism to correct for random disturbances under development and is generally detected by studying differences between repeated structures within organisms (Møller and Swaddle 1997). Deviations from perfect symmetry of a bilateral trait are referred to as "fluctuating asymmetry" (FA) (Van Valen 1962; Palmer and Strobeck 1986). Many studies have examined how FA responds to environmental and genetic alterations. Adverse environmental factors can have substantial effects on FA. In plants, radioactive and electromagnetic radiation, soil quality, elevation, temperature, pollution, herbivory, parasitism, and competition have been found to increase developmental instability and FA (Møller and Shykoff 1999). Inbreeding and hybridization have increased FA levels in some studies (Waldmann 1999), but the genetic basis of FA is still debated (see Møller and Thornhill 1997 and the following discussion).

Given the recent focus on population fragmentation, loss of genetic diversity, and inbreeding in conservation biology, it has been advocated that studies of FA could not only provide insights into evolutionary processes but also may serve as an important tool to monitor both environmental and genetic stress of threatened species (Leary and Allendorf 1989; Parsons 1992). However, for FA to be useful in conservation of genetic diversity, it is necessary to show empirically that there is a connection between FA and genetic variation (Fenster and Galloway 1997). Vøllestad et al. (1999) performed a meta-analysis of published results and found no evidence for a clear association between single-locus heterozygosity and FA. The majority of samples in the meta-analysis came from animals, a common pattern in the study of developmental stability despite the suitability of plants for investigating the relevance of FA (Freeman et al. 1993). Hence, further plant studies are certainly needed before any general conclusion can be reached.

Unfortunately, neutral single-locus studies may give a relatively biased estimate of the additive genetic variation behind quantitative characters, especially if the traits are subjected to selection (Waldmann and Andersson 1998; Lynch et al. 1999; Reed and Frankham 2001). Hence, association studies between heritabilities of quantitative traits and FA should provide additional important information regarding the usefulness of FA as a predictor of genetic variation behind adaptive traits. Several studies have estimated heritabilities of FA and genetic correlations between FA of different traits (reviewed in Møller and Thornhill 1997; Van Dongen and Lens 2000), but studies that investigated associations between FA and heritabilities of non-FA traits are still scarce.

Both mean single-locus heterozygosity and neutral additive genetic variance are expected to decline in small populations because of genetic drift. Therefore, considerable effort has been devoted to testing for a relationship between population size and genetic diversity in wild species, although no conclusive results have emerged (Ellstrand and Elam 1993; Waldmann and Andersson 1998; Montgomery et al. 2000). Few studies have investigated whether there is a correlation between population size and FA. In a study of *Phlox pilosa*, Heard et al. (1999) found that three measures of developmental instability increased with increasing population size.

In a previous study of two *Scabiosa* species, Waldmann and

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Table 1

Population Sizes, Heritabilities (h^2), Expected Heterozygosities (H_e), and Median Fluctuating Asymmetry (FA) for Six *Scabiosa columbaria* Populations

	Sm	Bj	Kl	Bi	Gr	Ma
Population size	55	59	236	600	1500	5000
h^2:						
Leaf number	0.108	0.177	0.753	0.221	0.174	0.414
Leaf area	0.386	0.315	0.470	0	0.184	0.129
Leaf length	0.098	0.154	0.176	0	0.078	0
Main stem height	0.447	0.109	0.110	0	0.325	0.630
Maximum stem height	0.677	0.082	0.432	0.147	0.360	0.880
Head number	0.438	0.098	0.091	0.405	0.300	0.338
Flowering date	0.405	0.198	0.450	0.222	0.319	0.437
Flower size	0.526	0.557	0.328	0.112	0.499	0
Average	0.386	0.211	0.351	0.138	0.280	0.354
H_e	0.445	0.221	0.327	0.179	0.422	0.224
FA	0.0285	0.0338	0.0252	0.0212	0.0223	0.0246
CI[a]	0.0248–0.0322	0.0284–0.0401	0.0204–0.0309	0.0181–0.0246	0.0189–0.0257	0.0210–0.0284

Note. Abbreviations for population names are as follows: Sm = Smygehuk; Bj = Bjärred; Kl = Klagshamn; Bi = Billebjär; Gr = Grödby; Ma = Margaretevall.

[a] 95% confidence intervals of FA based on 5000 bootstrap samples.

Andersson (1998) used progenies from maternal families to estimate the heritability and the level of population divergence (Q_{ST}) for eight phenotypic characters and to compare these estimates with the corresponding measures for allozyme markers (F_{ST}). The resulting observations indicated that spatially varying selection played a greater role in *Scabiosa columbaria* than in *Scabiosa canescens* (Waldmann and Andersson 1998). Natural selection was also invoked to explain some of the differences in the genetic (co)variance matrices between populations of *S. columbaria* (Waldmann and Andersson 2000).

In this study, the data from the study of Waldmann and Andersson (1998) is used to test additional hypotheses regarding flower FA. Defining FA as within individual microenvironmental variance (Gavrilets and Hastings 1994), I assume no additive genetic basis of FA itself, just that it may reflect epigenetic properties of the genome. First, I tested whether there is a relation between allozyme heterozygosity and FA. Second, the associations between the heritabilities and FA were investigated. Finally, the different population sizes allowed me to investigate whether this parameter influenced the FA levels. In FA studies, it is common to subject the data to an absolute transformation before analysis (Palmer and Strobeck 1986), but the resulting approximate half-normal distribution would have violated the assumptions behind parametric statistics, e.g., ANOVA. Therefore, I also used general and robust randomization and bootstrap methods to avoid violating those assumptions (Manly 1997).

Material and Methods

Scabiosa columbaria L. and *Scabiosa canescens* W. & K. (Dipsacaceae) are diploid (2n = 16) perennials with blue, bisexual, self-compatible, and insect-pollinated flowers arranged in dense, terminal inflorescences (heads). The marginal flowers have an upper lip divided into two small lobes and a lower lip with three large lobes (one middle lobe and two side lobes). *Scabiosa columbaria* occurs in various grassland habitats in large parts of Europe and is locally common in most of southern Sweden (Skåne). *Scabiosa canescens*, however, is more restricted to open grasslands (steppe-heaths) in eastern Europe, extending northward to Skåne, and is classified as "vulnerable" in the Swedish red list of threatened plant species (Aronsson 1999). The plants and the experimental design in the present investigation are the same as in Waldmann and Andersson (1998).

Seeds from open-pollinated plants were collected from 25 randomly chosen individuals per population. Six different-sized populations from each species were studied (tables 1, 2). Plants were grown under controlled greenhouse conditions, and a quantitative genetic analysis was performed on eight traits. Heritabilities for each trait were estimated assuming a full-sib relation for both species. A North Carolina II (NCII; Lynch and Walsh 1998) experiment with two *S. canescens* populations (Hä and ÅH; table 2) has shown that most of the genetic variance is additive (Waldmann 2001*a*). Moreover, the NCII study showed that no maternal genetic effects were present and that the full-sib assumption in Waldmann and Andersson (1998) was reasonable. Estimates were based on data from 751 and 619 individuals of *S. columbaria* and *S. canescens*, respectively. *Scabiosa canescens* in general had higher heritabilities than *S. columbaria* (tables 1, 2). Allozyme electrophoresis was carried out on leaves from maternal plants. Five loci were polymorphic for *S. columbaria*, and two were polymorphic for *S. canescens*. Expected mean heterozygosities ranged from 0.18 to 0.45 for *S. columbaria* and from 0.04 to 0.39 for *S. canescens* (tables 1, 2).

One randomly chosen marginal flower from the first flowering head, preserved in 70% ethanol and measured under a stereomicroscope, was scored for the distance from the base of the corolla to the tip of each side lobe (left and right side referred to as *L* and *R*, respectively). Fluctuating asymmetry was estimated as the absolute difference between the sides

Table 2

Population Sizes, Heritabilities (h^2), Expected Heterozygosities (H_e), and Median Fluctuating Asymmetry (FA) for Six *Scabiosa canescens* Populations

	Hä	Ve	De	Vi	Yn	Åh
Population size	25	100	138	2000	4000	5000
h^2:						
Leaf number	0.009	0.002	0.500	0.734	0.411	1.010
Leaf area	0.603	0.672	0.409	0.988	0.452	0.583
Leaf length	0.548	0.236	0.192	0.546	0.212	0.570
Main stem height	0.608	0.213	0.350	0.408	0.610	0.068
Maximum stem height	0.691	0	0.395	0.327	0.778	0.270
Head number	0.414	0	0.056	0.014	0.713	0.739
Flowering date	0.557	1.49	0.511	0.418	0.293	0.098
Flower size	0.470	0	0.677	0.403	0.107	0.176
Average	0.488	0.327	0.386	0.480	0.447	0.439
H_e	0.249	0.363	0.040	0.390	0.225	0.373
FA	0.0452	0.0409	0.0361	0.0379	0.0335	0.0393
CI[a]	0.0376–0.0540	0.0300–0.0546	0.0308–0.0418	0.0319–0.0443	0.0279–0.0398	0.0326–0.0468

Note. Abbreviations for population names are as follows: Hä = Hällestad; Ve = Veberöd; De = Degeberga; Vi = Vitemölla; Yn = Yngsjö; Åh = Åhus.

[a] 95% confidence intervals of FA based on 5000 bootstrap samples.

divided by the mean flower size (Palmer and Strobeck 1986):

$$\mathrm{FA} = \frac{|R - L|}{(R + L)/2}.$$

To test for directional asymmetry, I pooled the data over populations, bootstrapped 5000 samples of the signed FA values, and determined whether the 95% confidence interval (CI) of the mean excluded 0. A similar approach was used to test for skewness and kurtosis, quantified by the moment statistics g_1 and g_2, respectively (Waldmann 1999). A previous study showed that measurement error of flower FA of *S. canescens* was of minor importance under the current experimental set up (Waldmann 2001*b*).

The absolute transformation of the signed FA values yields an approximate half-normal distribution with a heavy tail to the right because of the leptokurtic distribution ("Results"). The assumption behind parametric statistics, e.g., homoscedasticity in ANOVA, would then be violated (Sokal and Rohlf 1995). Instead, to test for differences in FA between the species and the populations, I constructed a randomization test based on a standard ANOVA. This test would equal a randomization version of the commonly applied Levene's test but with better statistical properties (Manly 1997). First, the F value (i.e., mean $\mathrm{square}_{\mathrm{between}}$/mean $\mathrm{square}_{\mathrm{error}}$) from the ANOVA was obtained as a test value. A random distribution of F values was then generated by randomly assigning the FA values between the groups 5000 times. The P value was then estimated by dividing the number of values from the random F distribution that was larger than the test value with the number of permutations.

The FA for each population was estimated by bootstrapping using the mean as test statistic. Medians and 95% CI were obtained from 5000 bootstrap samples (Manly 1997). The median FA estimates were then used in a randomization version of a standard linear regression analysis to test for associations with expected mean heterozygosity, single-trait and mean heritabilities, and population size (logarithm transformed). The significance test of the regression analyses was constructed as follows (Manly 1997). First, the test t value (i.e., regression coefficient β/standard error of β) was calculated using FA as the response variable. Then a random distribution of t values was created from 5000 permutations of the FA values (keeping the explanatory variable intact). If β initially was positive, a P value was obtained by dividing the number of values from the random t distribution that was larger than the test value with the number of permutations. Similarly, if β initially was negative, the P value was calculated as the number of values from the random t distribution that was smaller than the test value divided by the number of permutations.

Results

There was no significant directional asymmetry for *Scabiosa canescens* (95% CI: −0.0074 to 0.0009) or for *Scabiosa columbaria* (95% CI: −0.0014 to 0.0039). Moreover, there was no evidence for skewness of the unsigned distribution (95% CI of g_1 overlapped 0 for both species). None of the species showed any evidence for antisymmetry because the g_2 values were significantly larger than 0 (95% CI: 0.86–2.6 and 1.6–6.9 for *S. canescens* and *S. columbaria*, respectively).

The ANOVA-based randomization test showed that *S. canescens* had significantly higher FA values than *S. columbaria* ($F = 57.9$, $P = 0.0002$). It also showed that the FA levels of the populations of *S. columbaria* were significantly different ($F = 9.58$, $P = 0.0024$). However, the populations of *S. canescens* did not display any significant differences in FA ($F = 2.49$, $P = 0.124$). Median estimates of the FA levels of the populations varied between 0.0211 and 0.0338 for *S. columbaria* (table 1) and between 0.0335 and 0.0452 for *S. canescens* (table 2).

From the regression analyses, it was evident that FA was

Table 3

Fluctuating Assymetry Associations with Expected Mean Heterozygosity (H_e), Single-Trait and Mean Heritabilities (h^2), and Population Size (Logarithm Transformed)

	Scabiosa canescens			*Scabiosa columbaria*		
	α	β	P	α	β	P
Population size	0.046	−0.001	0.079	0.037	−0.002	0.044
h^2:						
Leaf number	0.041	−0.005	0.195	0.027	−0.004	0.418
Leaf area	0.036	0.005	0.289	0.022	0.015	0.113
Leaf length	0.034	0.012	0.111	0.023	0.038	0.090
Main stem height	0.039	−0.001	0.455	0.026	−0.001	0.486
Maximum stem height	0.040	−0.002	0.388	0.029	−0.002	0.402
Head number	0.039	−0.002	0.390	0.030	−0.014	0.166
Flowering date	0.037	0.003	0.216	0.029	−0.008	0.350
Flower size	0.038	0.001	0.443	0.022	0.011	0.104
Average	0.035	−0.008	0.428	0.024	0.006	0.444
H_e	0.036	0.010	0.248	0.026	0.001	0.495

Note. α and β are the regression coefficients for the intercept and the slope, respectively. P values were obtained from a randomization procedure based on 5000 permutations. No values remained significant after adjustment for multiple comparisons.

not significantly associated with any of the variables (table 3). The sign of the β regression coefficient showed no particular pattern, with both positive and negative estimates for the heritabilities and the expected heterozygosity.

Discussion

Petal asymmetry of *Scabiosa canescens* and *Scabiosa columbaria* displayed no directional asymmetry or antisymmetry and could therefore be considered to consist of pure FA. The unsigned FA values showed leptokurtic distributions, which is a natural outcome from the mixing of distributions with different variances and therefore constitute no problems for the analyses here (Gangestad and Thornhill 1999). The rare species *S. canescens* had significantly higher FA values than *S. columbaria*, but only the populations of *S. columbaria* proved to have different FA levels. There was no evidence for an association between population-level FA and genetic variation when compared with the allozyme heterozygosity or with the heritabilities of the quantitative traits. A tendency for a negative association between FA and population size was found for both species, but it was not significant when adjusted for multiple comparison. Given that this experiment was performed on plants grown from seeds and raised under controlled greenhouse conditions, the contribution to FA from differences in the natural environments should be minimal. Hence, flower FA should not be considered a reliable indicator of the amount of genetic variation in populations of *S. canescens* and *S. columbaria.*

However, because this study is based on a relatively small number of populations and allozyme loci for both species, the results should be interpreted with some caution. Quantitative genetic parameters estimated from small sample sizes almost always have very large standard errors (Lynch and Walsh 1998; Waldmann and Andersson 1998). Moreover, the sampling variance of the heterozygosity between different allozyme loci is considerable because of the stochastic nature of genetic drift (Gillespie 1998), and ca. 50 loci would probably be needed to give robust mean estimates. The number of populations needed for accurate estimates of regression parameters would also be large, probably at least around 20. Consequently, to find statistically relevant associations between FA and population genetic parameters, such as the heterozygosity and the heritability, sample sizes on the order of 10,000 individuals would be needed. Unfortunately, this would be practically unmanageable for most wild organisms, and no studies have so far been based on sample sizes of that magnitude. The conclusions that can be drawn from most studies of the reliability of FA as an indicator of genetic variation are therefore limited and should only be used as rough guidelines. Thus, if there is any relationship between genetic variation and FA, it is likely to be very weak.

Bearing those statistical problems in mind, it is not surprising that different studies yield different results and that no clear patterns have been found. In a recent meta-analysis based on 118 samples, Vøllestad et al. (1999) found no convincing evidence for an association between heterozygosity and FA (the number of loci in the included studies varied between two and 27). However, the two plant studies included in the meta-analysis found a positive association between the level of allozyme homozygosity and developmental instability (*Clarkia tembloriensis* [Sherry and Lord 1996*b*]; *Lychnis viscaria* [Siikamäki and Lammi 1998]). It should be pointed out that the meta-analysis did not include the companion article by Sherry and Lord (1996*a*), which found no effect of homozygosity on FA when looking at flower FA instead of leaf FA. Several other contrasting results have also been found (see Møller and Swaddle 1997).

Neutral theory predicts that mean single-locus heterozygosity and additive genetic variance should decline in small populations because of genetic drift. Therefore, considerable effort has been devoted to testing for a relationship between population size and genetic diversity in wild species, although no conclusive results have emerged (Ellstrand and Elam 1993; Waldmann and Andersson 1998; Montgomery et al. 2000). The same biasing factors mentioned above also apply to those studies, with additional uncertainty introduced from estimating effective population sizes. In 13 populations of *Phlox pilosa*, Heard et al. (1999) tested whether small populations had higher levels of developmental instability by estimating correlations between population size and developmental stability of both leaves and petals. Unexpectedly, they found that three measures of developmental instability increased with increasing population size. However, because this study was intended to capture both environmental and genetic causes of FA, no simple interpretation of the results in a purely genetic context is possible. To elucidate the roles of genetic parameters and population size on FA, it is necessary to perform controlled experiments in common environments.

In a previous study, we found that genetic drift played a greater role in *S. canescens* than in *S. columbaria* (Waldmann and Andersson 1998). One interpretation could be that genetic drift contributes to the higher levels of developmental instability in *S. canescens*. However, the current study was based on seeds collected from wild parents. Therefore, it is possible that maternal FA effects could be present, but it seems unlikely that there would be systematic effects over the whole species because some populations occur in similar habitats. Another explanation could be that the habitat-specific *S. canescens* experienced the greenhouse environment as more stressful than *S. columbaria*. This factor is difficult to control for and can be investigated only by performing additional experiments under different environmental conditions.

Controlled crossing experiments are still scarce, and the results are somewhat contrasting. Some studies have found that FA increases with inbreeding and hybridization (Hochwender and Fritz 1999; Siikamäki 1999; Waldmann 1999), whereas others have found no effect on FA (Fowler and Whitlock 1994). A recent large-scale experiment by Whitlock and Fowler (1999) with *Drosophila melanogaster* showed that inbreeding significantly altered the environmental variance between different lines, and the authors interpreted this as a genetic basis for developmental stability, in contrast to their earlier result. In a controlled inbreeding experiment with the smallest *S. canescens* population, a significant increase in FA and a reduction of fitness was detected for the inbred individuals (Waldmann 2001*b*). It is therefore likely that nonadditive genetic components in the form of deleterious mutations or hybrid incompatibilities are involved in the expression of FA (Klingenberg and Nijhout 1999; Waldmann 2001*b*). The low heritabilities of FA that have been found in many experiments further support these ideas (Møller and Thornhill 1997).

In conclusion, it seems as if FA is not a good predictor of the general level of genetic variation of specific populations. Hence, if the aim is to conserve high levels of genetic variation, FA seems to be an inaccurate tool that certainly should be used with caution. However, if the aim is to monitor effects of inbreeding and hybridization, it is too early to rule out the usefulness of FA because more results from large experiments based on controlled crosses are required.

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