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VARIATIONAL AND GENETIC PROPERTIES OF DEVELOPMENTAL STABILITY IN *DALECHAMPIA SCANDENS*

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Abstract.—Because low developmental stability may compromise the precision with which adaptations can be reached, the variability and genetic basis of developmental stability are important evolutionary parameters. Developmental stability is also an important clue to understanding how traits are regulated to achieve their phenotypic target value. However, developmental stability must be studied indirectly through proxy variables, such as fluctuating asymmetry, that are suggested to have noisy and often nonlinear relationships to the underlying variable of interest. In this paper we first show that mean-standardized measures of variance and covariance in fluctuating asymmetry, unlike heritabilities, repeatabilities, and correlations, are linearly related to corresponding measures of variation in underlying developmental stability. We then examine the variational properties of developmental stability in a population of the Neotropical vine, *Dalechampia scandens* (Euphorbiaceae). By studying fluctuating asymmetry in a large number of floral characters in both selfed and outcrossed individuals in a diallel design, we assemble strong evidence that both additive genetic and individual variation and covariation in developmental stability are virtually absent in this population.

Key words.—*Dalechampia scandens*, developmental stability, evolvability, fluctuating asymmetry, genetic variation, heritability, homozygosity.

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Adaptation of a biological trait is often equated with the fit of the population mean to a fitness optimum. However, this does not guarantee that the average individual is well adapted (Orzack and Sober 1994). In fact, the target of selection is not the population mean, but rather the adaptive accuracy, that is, the average closeness of individuals to the fitness optimum (Armbruster et al. 2004). The adaptive accuracy itself has two components: (1) the closeness of the target phenotype (i.e., the phenotype that would be reached from a given genetic and environmental background without noise of any kind; Nijhout and Davidowitz 2003) to the adaptive optimum; and (2) the adaptive precision, the reliability with which the individual is able to attain its target phenotype in the face of environmental and developmental disturbance. Because developmental noise may represent a substantial part of the total phenotypic variance, it can seriously hamper the precision with which the target phenotype is reached and negatively affect the accuracy of adaptation. This underscores the evolutionary relevance of developmental stability, the ability of an individual to buffer disruptions of the developmental trajectory and reduce developmental noise in a particular environment (Palmer 1994).

Trait canalization, selection for increased developmental precision across variable genetic and environmental backgrounds, may also mask additive genetic variance and thus reduce the evolvability of characters (Gibson and Wagner 2000). Ultimately, this may lead to reduced adaptive accuracy, because target phenotypes are not able to evolve to new optima. These observations reveal the importance of developing a predictive theory for how developmental stability and other variational properties vary among characters, individuals, and species. Toward such a theory, we need to understand the evolutionary potential of developmental sta-

bility itself. One important question is, then, what is the level of additive genetic variation in developmental stability? Addressing this question will help us to understand whether developmental stability is evolvable and what forces may be important in shaping its genetic architecture.

Fluctuating asymmetry (FA), subtle nondirectional departures from perfect bilateral symmetry (Van Valen 1962), has been widely used as a measure of developmental noise and to assess developmental stability (Palmer and Strobeck 1986). Fluctuating asymmetry has the conceptual advantage of having a clear developmental optimum (perfect symmetry); because both sides of bilateral traits are expected to be influenced by the same genes and share the same macroenvironment, deviations from perfect symmetry are thought to reflect microenvironmental disturbances and be negatively related to developmental stability. It is, however, inherently difficult to assess the variational properties of developmental stability, as variation in FA due to individual differences in developmental stability tend to be swamped by variation due to developmental noise (Whitlock 1996, 1998; Houle 1997, 2000; Van Dongen 1998; Fuller and Houle 2003).

The above limitation notwithstanding, evolvability of FA, and therefore of developmental stability, has been directly demonstrated in the Australian blowfly (*Lucilia cuprina*; Clarke and McKenzie 1987; Davies et al. 1996). In this case, the initial increase in developmental noise, following a genetic modification caused by a pesticide-resistance gene, was rapidly eliminated by selection. Recent reviews of the heritability of FA and developmental stability, however, have shown conflicting results. Some authors have concluded that additive genetic components of FA and developmental stability exist (Møller and Thornhill 1997; Polak and Starmer 2001), whereas others remain skeptical (Houle 1997; Markow

and Clarke 1997; Whitlock and Fowler 1997). In a meta-analysis using hierarchical modeling to take estimation accuracy into account, Van Dongen (2000) showed that the heritability of FA was extremely low on average. Several authors have argued that the heritability of FA stands in a sigmoidal relationship to underlying genetic variation in developmental stability (Whitlock 1996, 1998; Houle 1997, 2000; Van Dongen 1998). This implies that small or moderate amounts of genetic variation in developmental stability are difficult to detect and can remain hidden when measured as heritability.

In this paper, we first present theoretical observations on the relationship between variation in developmental stability and variation in FA. Based on models by Whitlock (1996, 1998) and Houle (1997, 2000), we show that mean-standardized variances and covariances of FA map linearly to corresponding mean-standardized measures of variation in developmental stability. We suggest that mean-standardized variances may thus be easier to interpret than heritabilities, repeatabilities, and correlations, which map nonlinearly onto underlying variation. We then estimate the additive genetic variance and evolvability of developmental stability of floral traits of *Dalechampia scandens* (Euphorbiaceae), measuring FA on a large number of traits in both selfed and outcrossed individuals. Variance components in FA were estimated using a diallel design that is more powerful than regression-based methods (Fuller and Houle 2003).

THEORY

Here we present some simple theoretical relationships between underlying developmental variation and organism-level variational properties, such as FA. The aim of this section is to provide a background for interpretation of the experimental results presented below.

Model

Following Whitlock (1996, 1998) and Houle (1997, 2000), the right (R) and left (L) side of a bilateral trait can be modeled as

$$R = g + e + e_R \quad \text{and} \quad (1a)$$

$$L = g + e + e_L, \quad (1b)$$

where g and e represent the genetic and environmental components of the trait common to both sides and e_R and e_L are the developmental errors in each side. We assume that each developmental error is normally distributed with mean zero and variance $\sigma^2/2$, such that

$$R - L = e_R - e_L \sim N(0, \sigma^2). \quad (2)$$

From this it follows that

$$FA = |R - L| = |e_R - e_L| \sim RN(0, \sigma^2), \quad (3)$$

where RN is the normal distribution reflected around zero (i.e., a scaled χ -distribution). The parameter σ^2 is a measure of developmental stability (called V_N by Whitlock 1996). Conditional on a value of σ , the expectation, variance, and coefficient of variation for this distribution are

$$E[FA | \sigma] = \sigma\sqrt{2/\pi}, \quad (4)$$

$$\text{Var}[FA | \sigma] = \sigma^2(1 - 2/\pi), \quad \text{and} \quad (5)$$

$$\text{CV}[FA | \sigma] = \sqrt{\pi/2 - 1} \approx 3/4, \quad (6)$$

respectively. Note that the mean FA is proportional to σ , whereas the variance in FA is proportional to σ^2 . The FA is thus a direct measure of developmental stability on the σ -scale; in the following, we will assume that developmental stability is measured on a σ -scale. The unconditional expectation, variance, and coefficient of variation are

$$E[FA] = E[E[FA | \sigma]] = E[\sigma]\sqrt{2/\pi}, \quad (7)$$

$$\begin{aligned} \text{Var}[FA] &= E[\text{Var}[FA | \sigma]] + \text{Var}[E[FA | \sigma]] \\ &= \text{Var}[\sigma] + (1 - 2/\pi)E[\sigma]^2, \quad \text{and} \end{aligned} \quad (8)$$

$$\text{CV}[FA] = \sqrt{(\pi/2)\text{CV}[\sigma]^2 + \pi/2 - 1}, \quad (9)$$

respectively. Note that a $\text{CV}[FA]$ larger than $3/4$ indicates individual variation in susceptibility to developmental noise. Individual variation may also manifest itself as kurtosis in the distribution of signed FA (Rowe et al. 1997; Gangestad and Thornhill 1999). The component of variation in FA that is due to individual differences in degree of developmental stability is $\text{Var}[E[FA|\sigma]] = (2/\pi)\text{Var}[\sigma]$. Van Dongen (1998) and Whitlock (1998) defined the hypothetical repeatability, \mathfrak{R} , as the proportion of variation in FA due to real differences in developmental stability, that is:

$$\begin{aligned} \mathfrak{R} &= \frac{\text{Var}[E[FA | \sigma]]}{\text{Var}[FA]} = \frac{(2/\pi)\text{Var}[\sigma]}{\text{Var}[\sigma] + (1 - 2/\pi)E[\sigma]^2} \\ &= \frac{(2/\pi)\text{CV}[\sigma]^2}{\text{CV}[\sigma]^2 + 1 - 2/\pi}. \end{aligned} \quad (10)$$

Note that the repeatability links the phenotypic variation in FA to the phenotypic variation in underlying developmental stability as $\text{CV}[\sigma^2] = \mathfrak{R}\text{CV}[FA]^2$. The variance in signed FA is

$$\text{Var}[R - L] = E[\sigma^2] = \text{Var}[\sigma] + E[\sigma]^2. \quad (11)$$

The variation in one side of the trait due to all sources of developmental stochasticity (including developmental noise and developmental variation across individuals) is $E[\sigma^2]/2$. This is most efficiently estimated as half the variance of the signed FA (e.g., Palmer 1994). The variance in developmental stability, $\text{Var}[\sigma]$, can be estimated by obtaining two of the following: mean FA, variance in FA, or the variance in signed FA.

Genetic and Environmental Variation in Developmental Stability

The component of variation in FA due to additive genetic variation in developmental stability is

$$\text{Var}_A[FA] = \text{Var}_A[E[FA|\sigma]] = (2/\pi)\text{Var}_A[\sigma]. \quad (12)$$

Assuming only additive genetic variation, the heritability and the coefficient of additive genetic variation of FA are

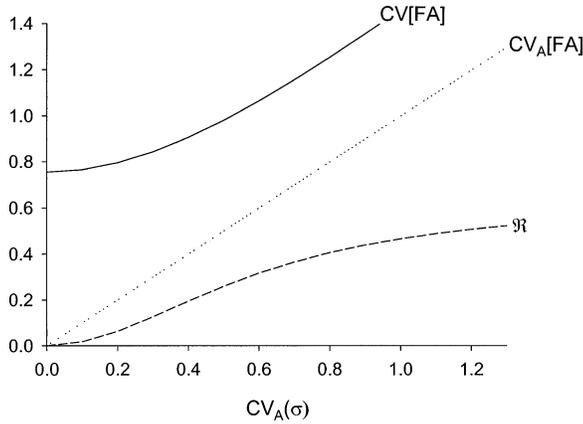


FIG. 1. Relationship between $CV[FA]$, $h^2[FA]$, $CV_A[FA]$ and $CV_A(\sigma)$. This model includes no environmental variation. The hypothetical repeatability, h^2 , is the proportion of variance in asymmetry due to the real differences in developmental stability. In the case of no environmental variation in developmental stability, the repeatability equals the heritability.

$$h^2[FA] = \frac{\text{Var}_A[FA]}{\text{Var}[FA]} = \frac{(2/\pi)CV_A[\sigma]^2}{CV[\sigma]^2 + 1 - 2/\pi} = h^2[\sigma] \quad (13)$$

and

$$CV_A[FA] = \frac{\sqrt{\text{Var}_A[FA]}}{E[FA]} = CV_A[\sigma]. \quad (14)$$

Thus, the additive genetic coefficient of variation of FA is identical to the additive genetic coefficient of variation in developmental stability, whereas the heritability of FA is nonlinearly related to heritability of developmental stability on the σ -scale, because repeatability is itself a function of variation in developmental stability (eq. 10; Fig 1). Mean-standardized variances such as the CV_A or I_A ($= CV_A^2$) are thus theoretically preferable statistics for gauging the genetic variance and evolutionary potential of developmental stability. We note, however, that the statistical problem of accurately estimating genetic variation in developmental stability is not thereby solved, as a low repeatability, or low signal-to-noise ratio, makes estimates of mean-standardized variances very imprecise.

Furthermore, even very low heritabilities in FA are compatible with high evolvabilities of developmental stability. Using I_A as a measure of evolvability (Houle 1992; Hansen et al. 2003a), we find

$$I_A[\sigma] = I_A[FA] = h^2[FA]CV[FA]^2. \quad (15)$$

This is derived by use of equation (14) and the definition of heritability as the ratio of additive genetic and phenotypic variances. Because $CV[FA]$ has to equal or exceed $\sqrt{\pi/2 - 1}$ (eq. 9), the evolvabilities of FA and σ must exceed $(\pi/2) - 1 \approx 0.57$ times the heritability of FA. This means that even a heritability of 0.1 implies an evolvability in excess of 5%. The interpretation of the I_A evolvability as a percent refers to the expected percent response per generation in the trait if exposed to a mean-standardized selection gradient of slope one, which equals the strength of selection on relative fitness itself (for details see Hansen et al. 2003a). Thus, to

correctly assess the evolutionary potential of developmental stability, it is preferable to evaluate evolvabilities directly in the form of mean-standardized additive genetic variances.

We add the caveat that developmental stability is a complex property that can be expected to have many sources of both genetic and environmental variance. The complexity of the trait may lead to nonlinear interactions among the various sources of variation (Klingenberg 2003). Epistasis and genotype \times environment interactions may be expected, and the above relationships, which are built on an additive model, are thus best seen as approximations that are most likely to be valid when the variation in developmental stability is not large relative to the mean.

Covariance in Fluctuating Asymmetry

An important question regarding the genetic basis of developmental stability is whether an organism-wide buffering capacity exists or, alternatively, whether developmental stability is trait specific (Leamy 1993). If developmental stability corresponds to an organism-wide property, sometimes called an individual asymmetry parameter (Leamy 1993; Clarke 1998; Polak et al. 2003), some correlation of FAs among traits of the same individual is expected. However, correlation among traits in FA may also result from a correlation in developmental noise, due, for example, to structural relationships between traits (Klingenberg et al. 2001).

Consider two traits with fluctuating asymmetries FA_1 and FA_2 , underlying developmental stabilities σ_1 and σ_2 , respectively, and a developmental covariance σ_{12} .

We may write the covariance between the FAs of the two traits as

$$\begin{aligned} \text{Cov}[FA_1, FA_2] &= E[\text{Cov}[FA_1, FA_2 | \sigma_1, \sigma_2, \sigma_{12}]] \\ &+ \text{Cov}[E[FA_1 | \sigma_1], E[FA_2 | \sigma_2]]. \end{aligned} \quad (16)$$

(This is analogous to the decomposition of variance in eq. 8; for derivation of this equation in a different context see Hansen and Martins 1996.) From this, we see that the covariance in FA can be divided into two components, which we may interpret as due to covariance in developmental noise and covariance in developmental stability, respectively.

The component due to covariance in developmental stability is the term

$$\text{Cov}[E[FA_1 | \sigma_1], E[FA_2 | \sigma_2]] = (2/\pi)\text{Cov}[\sigma_1, \sigma_2], \quad (17)$$

which we obtained by using equation (4). Thus, if the covariance in developmental noise can be removed, there is a direct relationship of covariance in FA to covariance in developmental stability. We can control for covariance due to correlated developmental noise by estimating the term $E[\text{Cov}[FA_1, FA_2 | \sigma_1, \sigma_2, \sigma_{12}]]$ in equation (16). There is no simple analytical expression for this term, but it can easily be computed numerically. In Figure 2, we describe this computation and show how the correlation in FA relates to the correlation in developmental noise (i.e., to $\rho = \sigma_{12}/\sigma_1\sigma_2$).

Provided there is no individual variation in directional asymmetry (i.e., a constant difference between the left and the right side), the covariance in signed FA can be taken as a direct estimator of the covariance in developmental noise, σ_{12} . Using this estimate with equations (16) and (17), we

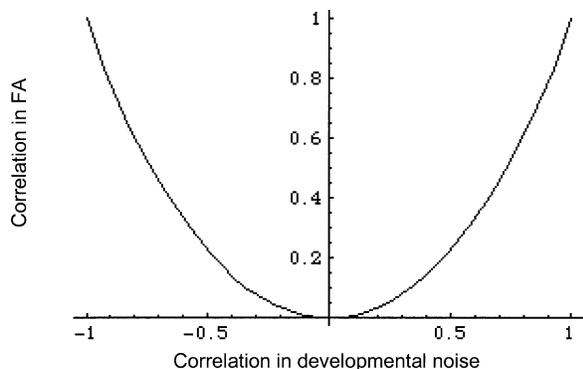


FIG. 2. Mapping of correlation in developmental noise to correlation in fluctuating asymmetry (FA). This is based on computation of the integral $\text{Cov}[\text{Abs}[x], \text{Abs}[y] | \sigma_1, \sigma_2, \sigma_{12}]$ under the assumption that x and y follow a joint normal distribution with means zero, variances σ_1^2 and σ_2^2 , and covariance σ_{12} . If there is variation in these parameters, the population covariance is the expectation of this function over their distribution. The integration was computed numerically by use of Mathematica 4.0 (Wolfram 1999).

obtain an estimator of the covariance in developmental stability as follows

$$\text{Cov}[\sigma_1, \sigma_2] \approx \frac{\pi}{2} \{ \text{Cov}[\text{FA}_1, \text{FA}_2] - f(\text{Cov}[\text{R}_1 - \text{L}_1, \text{R}_2 - \text{L}_2]) \}, \quad (18)$$

where f is the above-mentioned mapping from covariance in developmental noise to covariance in FA that is illustrated in Figure 2. This mapping is an approximation, as it is based on the assumptions that there is no individual variation in directional asymmetry or in the covariance of developmental noise (i.e., in σ_{12}).

Correlations in FA behave in a way that resembles the heritability. Using equations (4) and (8), the correlation in FA due to covariance in developmental stability is

$$\begin{aligned} & \text{Corr}[E[\text{FA}_1 | \sigma_1], E[\text{FA}_2 | \sigma_2]] \\ &= \frac{\text{Corr}[\sigma_1, \sigma_2]}{\sqrt{\pi/2 + (\pi/2 - 1)/\text{CV}[\sigma_1]} \sqrt{\pi/2 + (\pi/2 - 1)/\text{CV}[\sigma_2]}}. \end{aligned} \quad (19)$$

Thus, just like the heritability, the correlation stands in a nonlinear relationship to the variance in developmental stability. Therefore, the low correlation across traits and the low heritability of FA may have a common mathematical basis in the nonlinear relationship with the underlying variation in developmental stability. It is therefore inherently difficult to determine whether developmental stability is systemwide or trait specific. Even if developmental stability is a completely systemwide property, we will not expect to see strong correlations in FA across traits unless there is substantial component of individual variation in developmental stability. We therefore need to combine FA measures from many characters to obtain more precise information on developmental stability (e.g., Zhivotovsky 1992; Leung et al. 2000). Note that this is true whether we assume that there is global asymmetry parameter or whether we see the combination of measures as a meta-analysis that aims at estimating average developmental stability of the organism.

MATERIALS AND METHODS

Study Organism

Dalechampia scandens is a Neotropical vine with unisexual flowers aggregated into bisexual, pseudanthial inflorescences, or blossoms (Webster and Webster 1972; Webster and Armbruster 1991). Each blossom typically contains 10 staminate flowers arranged in three groups of three flowers with an additional central flower. Three pistillate flowers are present under (abaxial to) the staminate flowers. Associated with the staminate subinflorescence is a gland composed of bractlets that secrete resin (Armbruster 1984). Two large, showy involucre bracts subtend the pistillate flowers, staminate flower, and resin gland. Bees that collect the resin for nest construction pollinate the flowers. The blossoms of *D. scandens* are bilaterally symmetrical (Fig. 3; for detailed descriptions of the blossom see also Webster and Webster 1972; Webster and Armbruster 1991). The area of the gland, which affects the production of resin reward, determines the subset of the resin-collecting bee fauna that will visit the blossoms. In combination with the placement of anthers and stigma, this determines which bees will be effective pollinators (Armbruster 1986, 1988, 1991).

Experimental Design and Rearing Conditions

We estimated the additive genetic variance in FA using a diallel analysis where 12 sets of five parental individuals were crossed in complete 5×5 diallels, with both reciprocals and selfed offspring. The parental individuals used in this study were derived from seeds collected near Tulum, Territorio de Quintana Roo, Mexico ($20^\circ 13' \text{N}$, $87^\circ 26' \text{W}$) early in 1998. Seeds were collected and stored by maternal family. Several seeds from each family were germinated in March–May 1998. At full flower (September–December 1998), these plants were crossed. The experiment was conducted at the Department of Biology greenhouse, Norwegian University of Science and Technology (Trondheim, Norway). Two individuals were raised from each mating. Consequently, four full-sibs from each parental pair were present in the experimental population. Initial parents were not close relatives. Crossing methods and rearing conditions are reported in more detail in Hansen et al. (2003a). Plants were haphazardly repositioned in the greenhouse during the growth and measurement phases. Measurements were made between November 1999 and September 2000.

Measurements and Definitions

Two observers made the measurements. In the first dataset (observer CP), a selection of traits (see Fig. 3, Table 1) was measured on two different blossoms per plant ($n = 1042$). Blossoms were collected haphazardly from each plant. All measurements in this dataset were made using an optical binocular magnifier (Optivisor, Donegan Optical, Kansas City, MO; $5\times$ and $10\times$ magnification). In the second dataset (observer TFH), blossoms were dissected under a stereomicroscope. This second set of measurements was performed to measure more detailed structures and was made on only one blossom per plant per type of cross ($n = 392$). All measurements were made at 0.01-mm precision using digital cal-

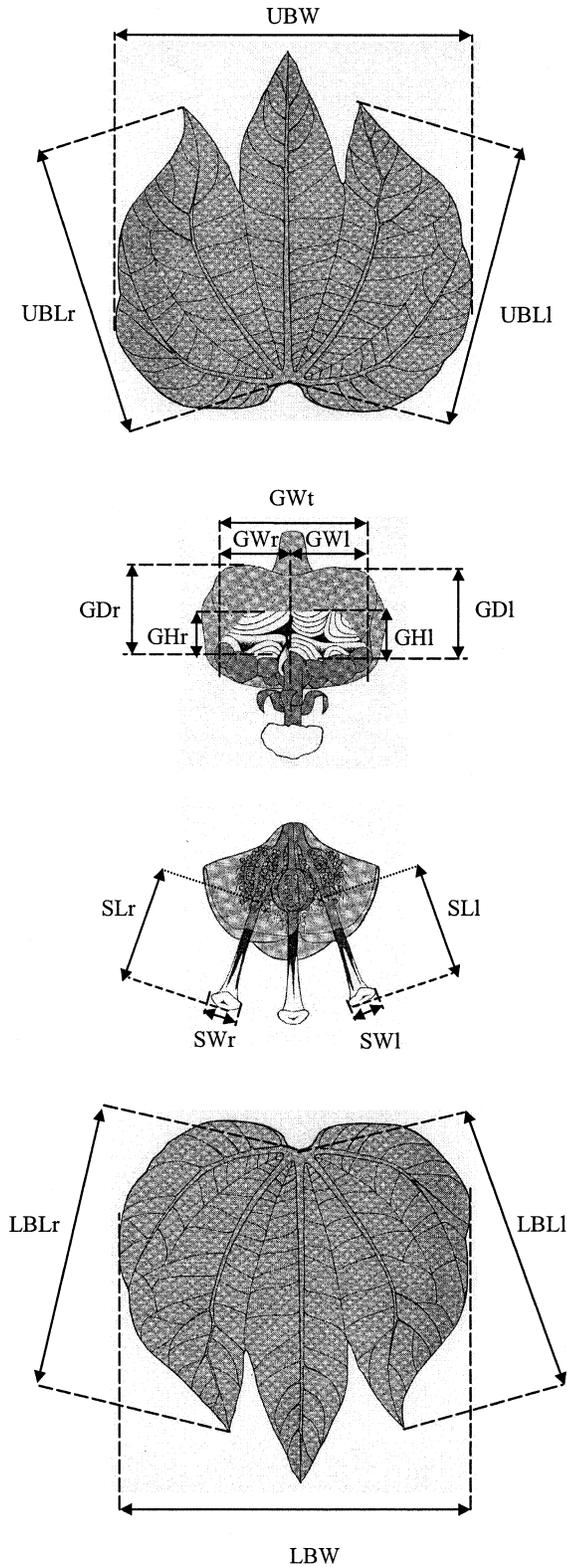


FIG. 3. Exploded view of the *Dalechampia* blossom displaying the different traits measured (see Table 1 for definition).

TABLE 1. Definition of the traits measured on the blossoms of *Dalechampia scandens* (see Fig. 3 for a visual representation of the traits). Gland-stigma distance is measured as the minimum distance between the tip of each stigma and the gland. For all traits signed FA was calculated as $100[\ln(\text{trait}_L) - \ln(\text{trait}_R)]$. The σ_m^2 FA is the variance in FA due to measurement errors (see eq. 20–22). Measurement variances were estimated as follow: $\sigma_m^2(\text{FA}) = \text{Var}(m1 - m2)$, where $m1$ and $m2$ are the signed FAs calculated from the first and second measurements on (n) repeated measures. Measurement error for gland number could not be estimated due to the necessary destructive dissection of the structure to count the number of bractlets forming the gland, but is probably near absent.

Trait	N outcrossed	N selfed	σ_m^2 FA (n)
Upper bract length (UBL)	816	224	4.92 (54)
Lower bract length (LBL)	816	224	3.00 (54)
Gland-stigma distance (GSD)	813	223	26.78 (140)
Gland width (GW)	816	224	26.59 (97)
Gland height (GH)	816	224	19.06 (97)
Gland depth (GD)	288	107	43.32 (54)
Style length (SL)	287	107	6.15 (54)
Style width (SW)	813	223	10.83 (118)
Gland number (GN)	287	107	—
Gland area (GA)	816	224	43.06 (97)

ipers. Both observers performed repeated measures to assess measurement errors for each trait.

FA was measured on a set of traits showing bilateral symmetry (Table 1, Fig. 3 for trait definitions). Following Clarke (1998), we measured FA on a log scale to allow direct comparison of FA across traits and remove potential allometric relationship between FA and trait size. Signed FA was estimated as $100[\ln(L) - \ln(R)]$ and FA as $100|\ln(L) - \ln(R)|$.

Measurement Error and Bias Correction

Fluctuating asymmetry is inherently biased by measurement error (Palmer 1994; Whitlock 1996, 1998; Van Dongen 1998). We can model this by adding an estimated measurement variance σ_m^2 to σ^2 in the above equations. Remember that the measurement variance in a unilateral trait is then $\sigma_m^2/2$. The conditional (on a value of σ) mean and variance in FA become:

$$E[\text{FA} | \sigma] = \sqrt{2/\pi} \sqrt{\sigma^2 + \sigma_m^2} \quad \text{and} \quad (20)$$

$$\text{Var}[\text{FA} | \sigma] = (\sigma^2 + \sigma_m^2)(1 - 2/\pi). \quad (21)$$

Furthermore, the coefficient of variation is unaffected by measurement error, and a CV elevated above 3/4 cannot be due to the effect of measurement error (provided it is normally distributed). The mean and variance are, however, biased. If the measurement error is known, a bias-corrected estimate of the mean FA can be obtained as

$$\text{FA} = \sqrt{\text{FA}_{\text{obs}}^2 - 2\sigma_m^2/\pi}, \quad (22)$$

where FA_{obs} is the observed FA. Similarly, the variation in FA due to measurement error is equal to $\sigma_m^2(1 - 2/\pi)$, and this term should be subtracted from the observed variance to obtain an unbiased estimate of the real variance. Measurement variances (σ_m^2) estimated from replicated measurements are given in Table 1. As explained above, we used these to correct our estimates of mean and variance in FA for bias. Repeatabilities and heritabilities can also be bias corrected

by adding this term to their denominators (Van Dongen 1998; Whitlock 1998). These corrections are exact when there is no variation in developmental stability. If there is variation in σ , the corrections becomes more complicated, but still relatively unimportant as long as σ_m is much smaller than σ (not shown).

Statistical Analysis

Although we found some small differences in the level of asymmetry measured by the two observers, there was no consistency in the direction of the differences (not shown). Most analyses in this study are based on the measurements from the first observer (CP) supplemented by the measurements from the second observer (TFH) for the traits gland number (GN), gland depth (GD), and style length (SL).

The pedigree consisting of full-sibs, half-sibs, and some other types of relatives was analyzed statistically as described in Lynch and Walsh (1998) with a mixed-model implemented into PROC MIXED in SAS 6.12 by use of the TYPE = LIN general linear variance structure and restricted maximum likelihood estimation of variance components (SAS Institute, Inc., Cary, NC; described in detail in Hansen et al. 2003a,b). We included an additive genetic effect in all analyses. In the analyses involving FA for dataset 1, but not dataset 2, we also included stage (i.e., one, two, or three staminate flowers open) as a fixed effect and a random effect representing the individual, as there were two blossoms from each individual in this set. Stage effects were always small and are not presented.

FA measures are far from normally distributed and need to be transformed to make residuals comply with the assumptions of the mixed-model. The choice of transformation is not purely a statistical consideration, however. Fuller and Houle (2003) argued that Box-Cox transformation might adversely affect the estimation of the additive genetic variance in developmental stability, because it magnifies small errors and compresses large differences in the right tail of the distribution. However, large FA at the right end of the distribution may also result from major instead of minor disturbances of developmental process such as physical injuries, and leaving the FA untransformed may give these an overly large effect on the outcome. For most of our traits a cube-root transformation appeared to give the best fit to a normal distribution. However, because the square-root transformation also gave very good fit and is much less extreme in its magnification of small errors, we chose this as a compromise, and square-root transformed all FA measures for the genetic analysis. We verified that no result was qualitatively changed by use of square-root versus cube-root transformation.

RESULTS

Descriptive Statistics of Fluctuating Asymmetry

Descriptive statistics for FA in outcrossed and selfed individuals are presented in Table 2. Directional asymmetries were extremely small, but sometimes statistically significant, except for gland-stigma distance (GSD), where the directional asymmetry represented 3% of the mean trait size. Note also that for the other directional asymmetries, the directions

TABLE 2. Summary statistics for fluctuating asymmetry (FA) in outcrossed and selfed individuals. Basic statistics were calculated using the largest dataset available for each trait. Mean unsigned FA was corrected for measurement error (using σ_m^2 FA) as described in eq. (22). The 95% confidence interval of the mean signed FA was obtained by bootstrapping. Kurtosis of the distribution in signed FA correspond to the Fisher's G2. The last column gives the P-values for the comparison of the FA level between outcrossed and selfed individuals (t-test on square-root-transformed data). The hypothetical repeatability, \mathfrak{R} , was estimated following Whitlock (1998). For trait abbreviations, see Table 1.

Trait	Outcrossed						Selfed						
	Signed FA			Unsigned FA			Signed FA			Unsigned FA			
	Mean (95% CI)	Variance	Kurtosis	Mean	CV	\mathfrak{R}	Mean (95% CI)	Variance	Kurtosis	Mean	CV	\mathfrak{R}	P
UBL	-0.18 (-0.56, 0.27)	32.94	3.46	4.245	0.825	0.103	-0.28 (-1.01, 0.38)	28.30	1.70	3.756	0.913	0.201	0.021
LBL	-0.33 (-0.58, -0.07)	14.36	3.63	2.783	0.807	0.079	-0.28 (-0.78, 0.23)	15.87	3.07	2.804	0.897	0.185	0.933
GSD	3.40 (2.52, 4.29)	164.37	0.79	9.554	0.783	0.044	4.79 (3.08, 6.31)	147.16	0.72	9.596	0.83	0.109	0.349
GW	-1.61 (-2.49, -0.63)	188.84	1.49	10.245	0.828	0.107	-1.43 (-3.26, 0.74)	245.45	0.11	12.008	0.785	0.047	0.021
GH	0.16 (-0.69, 1.14)	174.81	3.80	9.212	0.957	0.240	-0.48 (-1.99, 1.76)	208.31	1.46	10.352	0.915	0.203	0.089
GD	0.92 (0.22, 1.52)	35.15	7.38	4.581	0.841	0.123	-0.09 (-1.35, 1.17)	43.61	0.81	3.445	0.820	0.096	0.482
SL	-0.82 (-1.26, -0.38)	15.33	0.65	2.810	0.790	0.054	-0.72 (-1.55, 0.11)	18.96	0.15	3.132	0.825	0.103	0.733
SW	0.48 (0.08, 0.83)	32.35	1.91	3.912	0.864	0.150	0.545 (-0.11, 1.31)	29.83	0.36	3.826	0.813	0.087	0.559
GN	1.73 (-1.44, 3.96)	561.41	0.66	16.776	1.004	0.276	-0.091 (-4.66, 4.48)	572.85	1.53	17.642	0.922	0.209	0.406
GA	-1.39 (-3.23, -0.30)	398.65	3.80	14.876	0.833	0.113	-1.802 (-4.93, 1.20)	531.01	0.32	17.668	0.797	0.065	0.017

were not consistent across observers (not shown). None of the distributions of signed FA showed significant skewness, but several showed some kurtosis.

The differences in FA between selfed and outcrossed individuals were always small and inconsistent in direction across traits (Table 2). Thus, there appears to be no effect of homozygosity on developmental stability in this population. This is expected from the mixed mating system of *D. scandens*, which is self-compatible (Armbruster 1988) with most deleterious recessive alleles likely having been purged. These results should not be extrapolated to organisms with different mating systems.

Genetic Variance in Fluctuating Asymmetry

Mean-scaled additive genetic variances (I_A) and heritabilities (h^2) of square-root-transformed FA (estimated from the outcrossed dataset) were small and symmetrically distributed around zero (six negative and four positive, Table 3). These estimates indicate that there were very low levels of additive genetic variance in developmental stability. The same was true for family variances (σ_{FS}^2) in the selfed dataset (four negative—set to zero in a table 3—and six positive). The maximum heritability, h^2 , of FA was 0.03. The I_A evolvabilities were generally low, but some approached the evolvability of the trait itself (Hansen et al. 2003a). Note also that the square-root transformation is likely to somewhat reduce the absolute value of I_A . They were never statistically significant, however, and the low precision of these estimates means that we expect to see this level of scatter around zero.

The individual variation in FA (σ_{ind}^2) was as small and as symmetrically distributed around zero as was the genetic variance (Table 3). Thus, the FAs of the two blossoms on an individual were not correlated (for all traits where two blossoms per individual were measured: $-0.046 < r < 0.040$; all $P > 0.30$; $n = 510$).

Components of Variance and Covariance in Fluctuating Asymmetry

The importance of developmental precision varied considerably from trait to trait: from 1% to 25% of the phenotypic variation in the trait can be explained by lack of developmental precision (Fig. 4). Only a small proportion of variance appears to be due to variation in degree of developmental stability across individuals (Fig. 4; Table 4).

Covariances in FA were generally small. The decomposition into covariances due to developmental noise and developmental stability revealed that covariances due to developmental noise are practically zero for all trait combinations except in structurally related gland characters (Table 4). Due to the rather small variances of developmental stability for most traits, the estimates of covariance in developmental stability are not very informative. Although some of the correlations in Table 4 are large, the covariances are in fact very close to zero (the variances are also very small). The absence of covariance in developmental stability is also suggested by the fact that some correlations were negative. Only the developmental stabilities of the gland characters were all strongly positively correlated with each other. Because these characters also showed the most variation in de-

TABLE 3. Genetic components of fluctuating asymmetry (FA; results of genetic analysis of square-root transformed FA). The model includes an additive genetic and an individual random effect, and developmental stage as a fixed effect. Shown are estimates with standard errors of additive genetic and individual variance components. Only the mean effect of stage 1 is shown. For illustration, we have also given the mean FA; additive genetic variation scaled with the mean, I_A ; heritability, h^2 ; and total phenotypic variation, σ_{β}^2 (not including stage variance), all on the square-root scale. Selfed-sib variance, σ_{FS}^2 , and corresponding individual effect, σ_{ind}^2 , are also shown. This was computed in a separate analysis, which also included an individual random effect and a fixed stage effect. Three traits (gland depth, gland number, and style length) were computed from the smaller dataset and do not include a stage or an individual effect. None of the random effects were statistically significant at the 0.1 level (joint likelihood-ratio tests for genetic and individual effects). For trait abbreviations, see Table 1.

Trait	\sqrt{FA}	I_A	h^2	$\sigma_A^2 \pm SE$	$\sigma_{ind}^2 \pm SE$	σ_{β}^2	$\sqrt{FA_{self}}$	$\sigma_{FS}^2 \pm SE$	$\sigma_{ind}^2 \pm SE$	$\sigma_{\beta, self}^2$
UBL	1.93 ± .03	-0.40%	-0.02	-0.015 ± .021	-0.020 ± .039	0.744	1.74 ± .07	0.035 ± .047	-0.124 ± .082	0.785
LBL	1.62 ± .03	0.04%	0.002	0.001 ± .017	0.003 ± .025	0.474	1.57 ± .05	0	-0.021 ± .049	0.509
GSD	2.98 ± .06	0.38%	0.02	0.034 ± .050	-0.127 ± .089	1.695	2.91 ± .11	0	0.071 ± .165	1.799
GW	3.01 ± .05	-0.41%	-0.02	-0.037 ± .034	-0.055 ± .097	1.885	3.25 ± .12	0.030 ± .133	0.089 ± .237	2.083
GH	2.74 ± .07	0.87%	0.03	0.065 ± .065	0.031 ± .099	1.937	2.89 ± .11	0.060 ± .132	-0.023 ± .227	2.029
GD	1.96 ± .05	-0.91%	-0.05	-0.035 ± .047	—	0.772	1.45 ± .07	0.154 ± .066	—	0.441
SL	1.61 ± .04	-1.08%	-0.05	-0.028 ± .032	—	0.536	1.67 ± .08	0.022 ± .094	—	0.659
SW	1.87 ± .04	0.69%	0.03	0.024 ± .025	0.006 ± .045	0.888	1.85 ± .06	0	0.005 ± .067	0.750
GN	3.33 ± .13	-2.32%	-0.04	-0.257 ± .387	—	5.755	3.53 ± .22	0	—	5.264
GA	3.63 ± .05	-0.69%	-0.04	-0.091 ± .046	-0.045 ± .132	2.554	3.84 ± .14	0.070 ± .205	0.025 ± .341	2.992

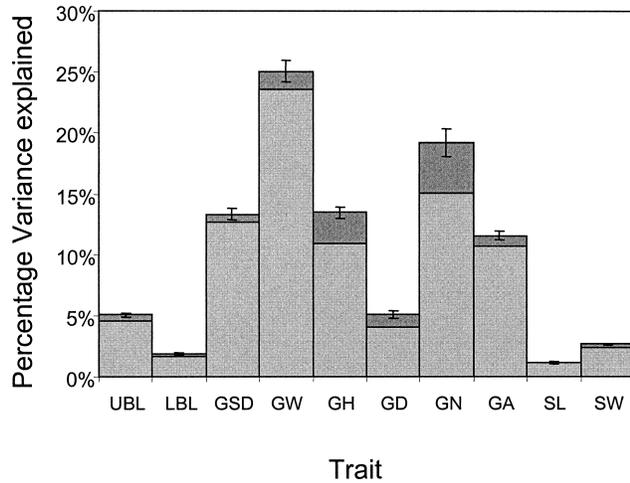


FIG. 4. Percentage of trait phenotypic variance (one side) explained by developmental stochasticity (± 2 SE). This percentage is divided into two components, one due to developmental variance (light gray) and one due to variance in developmental stability across individuals (dark gray). The developmental stochasticity is estimated as half the variance in signed FA. The standard errors are based on the standard formula for the relative error of a variance estimator, $\sqrt{2/(n - 1)}$, where n is number of blossoms. The component due to variation in developmental stability is computed from $(\text{Var}[R - L] - (\pi/2)E[\text{FA}]^2)/2$. For trait abbreviations, see Table 1.

developmental stability (Fig. 4), joint control of developmental stability is suggested. This is not surprising, as the gland characters are structurally related to each other. For example, variation in the position or number of bractlets in the gland can simultaneously affect gland width, height, and depth.

DISCUSSION

Developmental instability can be a significant source of adaptive imprecision. Figure 4 shows that as much as 25% of the phenotypic variation in adaptively important blossom traits such as gland dimensions may be ascribed to a failure to reach the target phenotype. Similar values can be found in other organisms (Lajus et al. 2003). Despite the obvious importance of developmental imprecision in the *Dalechampia* blossoms, there appears to be little variation in developmental stability itself across blossoms or individuals. In particular, there is no evidence for any additive genetic variation in developmental stability. This is consistent with similar results in several previous studies of the heritability of FA (for plants: Perfectti and Camacho 1999; Wilsey and Saloniemi 1999; Andalo et al. 2000; Rao et al. 2002; for animals: Blanckenhorn et al. 1998; Woods et al. 1999; Bjorksten et al. 2000; Cadee 2000; Kruuk et al. 2003).

Several theoreticians have argued that we should not be surprised by such results, because genetic (or other) variation in FA will be almost impossible to detect (Whitlock 1996, 1998; Houle 1997, 2000; Van Dongen 1998). For example, Houle (1997) argued that heritabilities of FA on the order of 0.18, the median value found in the meta-analysis of Møller and Thornhill (1997), were “implausible, if not impossible.” Houle based his argument on a two-genotypes model in which he showed that the ratio between the two variances of developmental stability, σ^2 , must be >5 to produce heritabilities

TABLE 4. Above the diagonal are the correlation in fluctuating asymmetry (FA) due to developmental noise estimated from the correlation in signed FA (as read from Fig. 2). On the diagonal are reported (left) the variance in FA due to developmental noise, computed as the square of the variance in signed FA multiplied by $\pi/4$, and (right) the variance in FA due to variance in developmental stability, calculated as $\text{Var}_{\text{ds}} = (E[\text{FA}]^2/2) [\text{CV}(\text{FA})^2 - (\pi/2 - 1)]$. Below the diagonal are the correlations in developmental stability ($\text{Corr}[\sigma_1, \sigma_2]$). Only outcrossed individuals were included in this analysis. For trait abbreviations, see Table 1.

	UBL	LBL	GSD	GW	GH	GD	SL	SW	GN	GA
UBL	14.15/0.98									
LBL	-0.42	6.08/0.31								
GSD	-0.01	0.33	71.69/1.93							
GW	-0.03	0.23	-0.39	82.43/6.02						
GH	-0.15	0.29	0.03	0.52	66.65/14.64					
GD	0.86	-0.61	-0.91	0.59	0.88	16.48/1.43				
SL	0.61	0.32	-1.15	0.08	0.25	0.84	6.20/0.21			
SW	0.13	-0.36	0.19	0.28	0.25	-0.39	-0.54	12.02/1.34		
GN	-0.26	0.37	-0.15	0.66	0.61	1.09	0.05	-0.06	221.04/61.52	
GA	-0.20	0.59	-0.13	0.47	0.56	0.89	0.31	0.32	0.80	173.81/13.62

as high as this. He later extended this into a more realistic model, in which σ^2 was assumed to follow a gamma distribution, and reached the same conclusion (Houle 2000).

We question this theoretical position for two reasons. First, the question of what constitutes an implausibly high level of variation in developmental stability is a biological judgment call. A five-fold change in σ^2 translates into a $\sqrt{5}$ -fold change in σ or in FA, which does not seem implausible. Second, even heritabilities of FA considerably smaller than 0.18, as found in the careful meta-analysis of Van Dongen (2000), are compatible with high evolvability of developmental stability. As seen from equation (15), even a heritability of FA of 0.04 is compatible with an I_A evolvability in excess of 2%, which is large enough to fuel a reasonable evolutionary response (Hansen et al. 2003a).

Therefore, we regard the question of the evolvability of developmental stability as still open. Although it is almost impossible to assess evolutionary potential through a consideration of the heritability of FA in a single trait, we suggest that the question is accessible through the use of mean-standardized variances and covariances of FA, which are more directly related to variation in developmental stability. Estimation of these parameters is still very imprecise, and an assessment of genetic variation in developmental stability requires either extremely large experiments or the simultaneous assessment of FA in many traits.

Thus, although we cannot exclude a substantial evolvability of developmental stability in any of the individual traits we studied, consideration of the 10 traits together shows that the best estimates of evolvability are centered on zero in both selfed and outcrossed individuals. A similar result was found when estimating genetic variance components of FA computed across two blossoms on each plant, for a total of 12 size-corrected traits (not shown). This yielded seven positive and five negative estimates in outcrossed individuals and five positive and seven negative estimates in selfed individuals. Also, the fact that the estimates of evolvability are not consistent across selfed and outcrossed offspring indicates strongly that the deviations from zero are due to estimation error. Thus, we regard our results as a genuine demonstration of extremely low evolvability in developmental stability.

It should be noted that our results comes from a population that has been previously shown to have rather low (although highly statistically significant) levels of quantitative genetic variation in the characters examined here (Hansen et al. 2003a,b). Thus, we may not expect high levels of genetic variation in developmental stability. Nevertheless, this is an example of genetic variation in developmental stability being lower than genetic variation in the morphological traits themselves.

A genuine lack of additive genetic variation in developmental stability would be theoretically surprising. Developmental stability is a complex character that would be expected to have a large mutational target size (but see Monedero et al. 1997). Furthermore, developmental stability evolves (Clarke and McKenzie 1987; Davies et al. 1996), and if not based on additive genetic variation, its evolvability must be explained in other ways. Below, we consider some possibilities based on canalization, epistasis, and genotype \times environment interaction.

Genetic canalization, the reduction of the effects of new mutations and segregating alleles on the phenotype, is a predicted outcome of stabilizing selection on the trait (Wagner et al. 1997). A long history of selection for reduced developmental noise may lower the expressed genetic variation in developmental stability under normal conditions (Rutherford 2000). Hidden genetic variation may, however, be released following the breakdown of the canalizing system (Waddington 1959; Levin 1970; Eshel and Matessi 1998; Rutherford 2000). Increased levels of FA may thus result from drastic genetic changes, such as after hybridization events (Graham 1992; but see Pélabon et al. 2004), or rapid adaptive responses to drastic environmental changes, such as the response to a novel pesticide in the Australian blowflies (Clarke and McKenzie 1987; Davies et al. 1996).

Recent theoretical work on epistatic models suggests that equilibria may exist in which additive genetic variation is absent in mutation-selection balance (Hermisson et al. 2003). Surprisingly, this occurs when stabilizing selection on the trait is relatively weak compared to the strength of epistasis. Trait symmetries are prime candidates for such equilibria because epistatic interactions are expected due to the complex functional architecture and stabilizing selection on the underlying genetic value is likely to be weak. If s is the strength of stabilizing selection on a trait, the strength of the stabilizing selection on the underlying genotype will be $s_G = s / (1 + 2V_E/s)$. Therefore, s_G will strongly decrease with increasing effect of the environmental variance (V_E) on the trait. Because the relation of the selected phenotype to developmental stability is inherently noisy and, as demonstrated by numerous studies, developmental stability is particularly sensitive to environmental disturbances, the strength of stabilizing selection on the underlying genetic basis should be weak. The epistatic variance could, however, be large in such situations. Leamy et al. (2002) and Leamy (2003) recently provided evidence for epistatic genetic variance for FA in centroid size in the mandible of mice. The possibility of large amounts of epistatic variance in FA needs further investigation.

Alternatively, because developmental noise often increases under stressful conditions (Parson 1990), it is possible that developmental stability captures (sensu Rowe and Houle 1996) the genetic variability associated with stress resistance. In this case, individual variation in resistance to stress and, consequently, in developmental stability will only be expressed in stressful environment. Both the genetic canalization and the stress-dependent-response hypotheses imply that the genetic basis of developmental stability should be analyzed under situations sufficiently stressful to provoke drastic changes in the expression of the genetic information and reveal the hidden genetic variation (but see Waddington 1961; Woods et al. 1999; Andalo et al. 2000).

Through the use of unbiased measures of variance in developmental stability on a large number of floral traits, we have assembled strong evidence that levels of additive genetic variation in developmental stability are extremely low in our study population. These results are consistent with the apparent absence of additive genetic variance in developmental stability observed in many previous studies, but they

do not preclude the possibility of more subtle genetic effects through, for example, epistatic interactions.

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