IS THERE A GENETIC BASIS FOR FLUCTUATING ASYMMETRY AND DOES IT PREDICT FITNESS IN THE PLANT *LOTUS CORNICULATUS* GROWN IN DIFFERENT ENVIRONMENTAL CONDITIONS?

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Fluctuating asymmetry (FA) is considered to be a good measure of developmental stability. We measured the asymmetry of leaves and flowers of 16 different genotypes of *Lotus corniculatus* grown in four different experimental environments to estimate the plasticity or developmental stability of asymmetry itself. We found that an index of FA (absolute difference between size of left and right sides, corrected for trait size) differed significantly across environments, with the treatment $CO_2+/N+$ inducing the greatest FA for both flowers and leaves. Genotypes did not differ in FAs. Individual plants showed significantly different FAs only for flowers. At the individual level, we found no significant relationship between flower FA and fitness. Previous work indicates that change in asymmetry in a poor or perturbing environment versus a good environment could reflect the intrinsic quality of a particular genotype. However, in our experiment, genotype effect was significant only for change in asymmetry of leaves, and this last trait was not significantly correlated with our fitness estimate for each genotype in either the most or the least perturbing environment.

Keywords: Birdsfoot Trefoil, CO_2, developmental stability, genotypic quality.

Introduction

Developmental stability reflects the ability of an individual to buffer its development against disturbances and is often considered to be an integral component of an individual's fitness, revealing perturbations of genetic or environmental origins (reviewed in Møller and Swaddle 1997). Thus, developmental stability may be a useful instrument in monitoring plant population acclimatization to environmental changes (Parsons 1990; Clarke 1995).

Many organisms develop symmetrical structures, and deviation from perfect symmetry is often used to estimate developmental instability (Freeman et al. 1993). Fluctuating asymmetry represents slight deviations from perfect symmetry, with the signed differences between the size of left and right sides for a population being normally distributed around a mean of 0 (Møller and Swaddle 1997). Individual asymmetry for traits that have a distribution of fluctuating asymmetry at the population level is commonly used to quantify the response of a genotype to environmental or genetic perturbations (Møller and Swaddle 1997). In plants, convincing evidence exists for a positive correlation between the degree of individual or fluctuating asymmetry and the level of environmental stress, including herbivory and disease (Møller 1995b; Shykoff and Kaltz 1998), radiation (Møller 1998), chemical pollutants (Freeman et al. 1993; Kozlov et al. 1996), level of competition

(Rettig et al. 1997), and elevation (Wilsey et al. 1998); see Møller and Shykoff 1999 for a recent review.

The genetic basis or heritability of fluctuating asymmetry is controversial (see Møller and Thornhill 1997 and commentaries) and has been debated for a long time (Mather 1953; Waddington and Robertson 1966; Clarke et al. 1992; Clarke 1997). If fluctuating asymmetry is a characteristic of a genotype, then good genotypes will buffer environmental insult better during development and will show less individual asymmetry than will inferior genotypes. Asymmetry could thus reflect the intrinsic quality of a particular genotype (Møller and Swaddle 1997). On the other hand, symmetrical individuals may have developed in a high-quality environment, because under nonperturbing environmental conditions even poor genotypes should be able to produce symmetrical phenotypes. Therefore, we suggest that the more important trait to measure is the increase in fluctuating asymmetry for a particular genotype in a poor or perturbing environment versus a good environment. This change in fluctuating asymmetry should be a better predictor of genetic quality than fluctuating asymmetry measured in any one environment. Thus, genotypes that increase their asymmetry the least when confronted with poor environments should have higher fitness, both in the poor environment, on account of their better, more symmetrical phenotypes, and in the good environment, on account of their superior genetic quality (Shykoff and Møller 1999).

Plants grown in elevated CO_2 atmospheres generally modify their growth because net photosynthetic carbon-gain increases (Bazzaz 1990; Bowes 1993). Many components of growth, including plant morphogenesis, biomass allocation, and phenology, are altered. In *Lotus corniculatus*, Carter et al. (1997) found that doubling CO_2 concentration increased growth rate

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and shoot biomass and advanced flowering time but reduced specific leaf area (surface area/mass). Erhardt and Rusterholz (1997) found an increase in the number of flowers under high CO_2 conditions.

Few studies exist on the shape of organs grown under elevated CO_2. Some studies found no effect of CO_2 on leaf shape (Leadley 1988; Sasek and Strain 1988), whereas Thomas and Bazzaz (1996) found pronounced modification of leaf shape of *Taraxacum officinale* at high CO_2. To date, no study has investigated the shape of flowers under various CO_2 concentrations, though flower size and shape have pronounced effects on pollinator preference (Møller 1995a).

For many different traits, significant differences in the response to CO_2 have been found among populations or genotypes of the same species (Garbutt and Bazzaz 1984; Wulff and Alexander 1985; Curtis et al. 1994; Leadley and Stöcklin 1996; Schmid et al. 1996). Furthermore, nitrogen availability interacts significantly with CO_2 in determining plant growth among other variables (Field et al. 1992). We, therefore, investigated the influence of environmental change on developmental instability of different genotypes of the plant *L. corniculatus* by varying CO_2 concentration and nitrogen availability. We further relate our measures of developmental instability with some components of plant fitness in the different environments.

In this article, we address three general questions: (1) Is individual asymmetry modified by environmental conditions (CO_2 concentration or nitrogen availability) differently for different plant genotypes? Clonal organisms provide an opportunity to distinguish between genetic and environmental factors. (2) Is fluctuating asymmetry in different environmental conditions, or is the change in fluctuating asymmetry between more and less perturbing environments a characteristic of the individual or the genotype? and (3) Does individual asymmetry or change in asymmetry between more and less perturbing environments correlate negatively with fitness at the level of the plant individual or the plant genotype, where clones contain several individuals per genotype?

Material and Methods

Plant Culture

Sixteen Birdsfoot Trefoil (*Lotus corniculatus* [Fabaceae]) genotypes were collected from a meadow on the campus of the University of Paris (XI) at Orsay, France, in 1995. The plants were maintained in the greenhouse and were multiplied vegetatively. In April 1997, new cuttings were made. In May, after root formation, 16 of these new cuttings from each genotype were planted in 1-L pots filled with vermiculite. Each pot was inoculated with *Rhizobium lotii*, the nodule-forming nitrogen-fixing bacterium of this species, diluted in water. Four plants of each genotype were randomly assigned to each of four treatments—ambient (350 ppm) or elevated (700 ppm) CO_2 and nitrogen-free or nitrogen-addition (10.7 mmol/L NO_3^-), in a factorial design. All plants were watered with modified half-strength Hoagland's solutions (Kinney et al. 1997) every 2 d. Once a week, the plants in the N+ treatment received supplementary NO_3^- in their watering solution. The plants were placed in four growth tunnels ($2 \times 0.5 \times 1$ m), two with ambient air and the other two with CO_2-enriched air, with a constant flow of industrial CO_2 pumped in from one side. All tunnels had slightly higher than ambient pressure because of the constant air inflow and air escaped through large holes at the opposite end of the tunnels, through which insect pollinators also entered. The plants were re-randomized 10 times over the course of the experiment by moving them from one tunnel to the other, and the CO_2 treatment was changed among tunnels to limit a tunnel effect.

Measurements

We measured plants in the same developmental stage: peak flowering with at least 10 umbels. Of the 256 plants in this experiment, only 179 could be measured. From these plants, three flowers (one from each of three different umbels) and three leaves (without insect damage) were randomly sampled. The two lateral petals of each flower and the two lateral leaflets of each leaf were spread on a Plexiglas surface and digitized, and the pictures were analyzed with an image-analyzing computer program (Images Tools, University of Texas, San Antonio, Health Science Center). For the leaves, we measured the surface and major axis length. For the flowers, we measured only the major axis length of the petal because of their concave forms and deformation when they were spread. Digitized measures were very precise but, unfortunately, destructive, so that individual petals and leaves could not be repositioned on the Plexiglas for a second independent measurement. The best we could do was to estimate the measurement errors associated with the digitization process itself. To this end, we redigitized 50 flowers and leaves four times and calculated repeatabilities of the measurements (length and surface area) and of asymmetry, following Swaddle et al. (1994).

In order to determine whether digitized measurements corresponded to measurements carried out by hand, a second experimenter performed two repeated measurements of the length of the right and left lateral leaflets and lateral petals of 51 leaves and flowers, which were subsequently prepared and digitized as above, using digital callipers. Trait size and asymmetry were then compared between the two methods of measuring.

From an additional two flowers per plant, we collected nectar by gently squeezing the base of the corolla after petals had been removed and by collecting the droplet of nectar that emerged in a 1-μL microcapillary tube. This nectar was then blotted onto a small piece of Whatman no. 1 filter paper and the diameter of the blot was measured once. From this value, nectar volume of each flower was calculated.

From the same flowers, fresh pollen was collected from the anthers situated within the corolla tube and dusted onto a slide where the pollen was stained with Alexander's stain (Alexander 1969), which differentially stains the pollen exine green and the cytoplasm magenta. These slides were examined under a light microscope, and the proportion of empty inviable pollen grains per hundred grains was estimated. For 75 slides, we counted two randomly chosen positions and calculated the repeatability of the two counts. Because the repeatability (cal-

culated as r_I, intraclass correlation coefficient; Zar 1984, p. 323) was high ($r_I = 0.86$, $F_{75,74} = 13.31$, $P < 0.0001$), for the rest of the slides only a single count was made.

All plants were harvested after 3 mo. The vegetative parts (roots, shoots, and leaves) were dried at 40°C for 3 d, and dry mass was recorded. For each plant, the number of fruits produced and the number of flowers that remained at the time of harvest were recorded as an estimate of total reproduction. Reproductive effort was calculated as the residuals from a linear regression of the total number of fruits and flowers per plant on its total vegetative dry mass. This provided an estimation of reproductive effort, controlling for the size of the plant.

Statistical Analysis

For the floral and vegetative traits in each of the four environments, we tested whether the asymmetry for each measure of flower and leaf was distributed as fluctuating asymmetry (Palmer and Strobeck 1986; Møller and Swaddle 1997). To test for directional asymmetry, we used one-sample t-tests on the signed differences (right-minus-left) to determine whether the mean value differed from 0. The normality of the distribution was tested by determining whether skewness and kurtosis coefficients deviated from 0 (Sokal and Rohlf 1995). Kurtosis tested for antisymmetry. We calculated the repeatability of our asymmetry measures, following the method of Swaddle et al. (1994), by partitioning variance components from a mixed-model ANOVA. Further, we investigated the relationship between trait size and symmetry at the trait and individual levels for both leaves and flowers.

We tested the difference between relative asymmetry, measured as asymmetry divided by trait size, for flowers and leaves of the same plants, using a paired t-test. To test whether the two traits differed in their levels of overall asymmetry, we compared the variances of the signed asymmetry distributions with an F-test. For this test, both asymmetries of the major axis were used.

Because the absolute value of asymmetry has a characteristic "half-normal" distribution (Van Valen 1962), a Box-Cox transformation, which normalized the distributions, was used before performing parametric analyses: (1) for leaves,

$$\text{asymmetry} = (\sqrt{|L - R|} + 1)^{0.3}$$

(L and R represent surfaces), and (2) for flowers,

$$\text{asymmetry} = (|L - R| + 0.01)^{0.3}$$

(L and R represent lengths of major axes).

We generated a fluctuating asymmetry (FA) index (absolute difference between size of left and right sides, corrected for trait size) independent of variation in trait size, as follows. Because there is often a relationship between individual trait size and asymmetry, we performed a regression analysis of the absolute value of asymmetry, after Box-Cox transformation, on trait size, calculated as the mean of the right and the left trait values, and used the residuals from this regression as the FA index controlled for trait size (Palmer and Strobeck 1986).

The regression employed the data from all genotypes and all treatments because the slope of the regression did not vary among the different treatments or genotypes (ANCOVA analyses: interactions between the covariate and the main effects were never significant). Furthermore, we used the measures of each leaf or flower and not the plant means in order to have multiple measures of individual asymmetry for both organs of each plant. All subsequent analyses used the plant individual as a hierarchical factor, so this procedure did not represent pseudoreplication. We used these residual measures to test whether plants with asymmetrical leaves also had asymmetrical flowers, using Pearson product moment correlation. We calculated intraclass correlations for the three leaves and flowers measured per plant individual to test whether trait size and FA (corrected for size) were consistent within plants.

We tested the effects of genotype, CO_2 treatment, nitrogen treatment, plants (where multiple measures per plant were available, nested within the three previous factors), and all interactions on our FA index, the residuals from the above regression, using a mixed-model ANOVA. Treatments were fixed factors, and genotype and plant individual were random factors. Expected mean squares were calculated by the SAS procedure (SAS Institute 1990) as linear combinations of plant individual nested within the three-way interaction and appropriate interaction effects by Satterthwaite approximation (Sokal and Rohlf 1995). To determine which treatment combinations differed, we considered the factorial nitrogen and CO_2 treatment combinations as a single treatment with four levels, performed a one-way ANOVA, and tested using an *a posteriori* Duncan's test.

To estimate change in asymmetry, we chose the two environments that could be considered most and least perturbing to the developmental process, measured by the expression of leaf and flower asymmetry. We calculated the mean FA of each genotype in the least perturbing environment. Then, for flowers and leaves developed by each plant grown in the most perturbing environment, we calculated the difference between its FA and the mean FA for the same genotype in the least perturbing environment. A random-model ANOVA was used to test genotype and plant individual effect on this change in asymmetry.

We investigated the relationship between FA in flowers and factors associated with fitness: total vegetative dry weight, reproductive effort, nectar production, and pollen quality, using Kendall rank order correlation (Sokal and Rohlf 1995). Individual plants were considered statistically independent observations, so all analyses were performed on plant means where several observations per plant were available. These correlations were performed using data from all treatments together and individually within each treatment. At the genotypic level, we also performed Kendall rank order correlations between change in leaf asymmetry between the most and least perturbing environment and fitness components measured in each of them. Since multiple correlations with the same variables (flower asymmetry or change in leaf asymmetry) were performed, critical values were corrected by sequential Bonferroni procedure (Rice 1989).

All statistical analyses were performed using the SAS software (SAS Institute 1990), and correlations between FA and

Table 1

Mean Size (mm) of Leaves and Flowers of *Lotus corniculatus*, Measured as the Length of the Major Axis, and Asymmetry Characteristics of Signed Left-Minus-Right Trait Values for Each of the Four Experimental Environments

	Trait size		Trait asymmetry					
	Mean (SE)	n	Mean (SE)	t	Skewness	t	Kurtosis	t
$CO_2+/N+$:								
Leaves	10.24 (0.193)	153	1.65 (25.54)	0.06 ns	0.38	1.94 ns	1.28	3.28**
Flowers	8.8 (0.065)	153	−0.21 (0.24)	−0.88 ns	−1.05	−5.35***	5.88	15.08***
$CO_2+/N−$:								
Leaves	10.95 (0.182)	144	22.67 (16.28)	1.39 ns	0.02	0.09 ns	1.30	3.24**
Flowers	9.04 (0.058)	144	0.77 (0.25)	3.13**	−0.19	−0.94 ns	2.84	7.07***
$CO_2−/N+$:								
Leaves	9.24 (0.204)	102	21.69 (19.85)	1.09 ns	1.47	6.15***	6.96	14.69***
Flowers	8.78 (0.09)	102	−0.14 (0.19)	−0.71 ns	0.66	2.76**	1.12	2.36*
$CO_2−/N−$:								
Leaves	10.13 (0.212)	138	−17.23 (20.86)	−0.83 ns	−0.16	−0.78 ns	3.52	8.59***
Flowers	8.44 (0.062)	138	0.33 (0.21)	1.57 ns	1.35	6.54***	7.21	17.59***

Note. Leaf asymmetry was calculated on the surface area and given in pixels^2, while that of flowers, also in pixels, represents the length of the major axis. ns = not significant.

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

fitness components were nonparametric and were performed on ranks.

Results

Measurements Error and Fluctuating Asymmetry

Our observed distributions of signed left-minus-right differences were not completely consistent with fluctuating asymmetry for leaf and flower measures. Mean values did not differ significantly from 0, using one-sample t-tests, except for one case. All distributions of asymmetry were significantly leptokurtic (table 1), with too many values of asymmetry near 0 and too few large positive or negative values. This shows that there was no antisymmetry. Four out of eight distributions of leaf or flower asymmetry were slightly, but significantly, skewed either to the right ($g > 0$) or to the left ($g < 0$) (Sokal and Rohlf 1995).

For both traits, the digitization errors were very small, with repeatabilities for the left and right leaflets and petals all greater than 0.99 ($P < 0.0001$). The repeatabilities for leaf and petal asymmetries were both significantly larger than measurement errors (leaves: $F_{50,300} = 918.61$, $P < 0.0001$; petals: $F_{50,300} = 23.04$, $P < 0.0001$). Digitized measurements corresponded well to the hand measures performed with callipers for leaves. The correlation between calliper and digitized measures for the left leaflet was significant ($r = 0.84$, $n = 51$, $P < 0.0001$). Similarly, leaf asymmetry measured by these two methods corresponded well ($r = 0.80$, $n = 51$, $P < 0.0001$). Unfortunately, flowers were less repeatedly measured by hand, since the petals are very flimsy. Petal measurements taken by the two methods showed no correspondence, nor were the symmetry measures for petals by these two methods correlated ($r = 0.07$, $P > 0.5$). For the manual measurements of asymmetry, measurement errors were small relative to the magnitude of asymmetry for leaves

($F_{50,100} = 199.09$, $P < 0.0001$) but not for the petals ($F_{50,100} = 0.15$, ns).

Large leaves of *Lotus corniculatus* were more asymmetrical than small leaves (correlation between trait size and asymmetry performed for all leaves from all plants: $r = 0.35$, $n = 537$, $P < 0.0001$), and similarly, plants with, on average, larger leaves bore, on average, leaves that were more asymmetrical (correlation between mean trait size and mean asymmetry per plant: $r = 0.48$, $n = 179$, $P < 0.0001$). No such relation between flower size and symmetry was found, either at the level of the individual flowers or at the plant level ($r = −0.01$, $n = 537$, $P = 0.796$ and $r = −0.02$, $n = 179$, $P = 0.747$, respectively). The relative asymmetry (asymmetry divided by trait size) was significantly smaller in flowers ($\bar{x} = 0.028$, SE = 0.0012) than in leaves ($\bar{x} = 0.052$, SE = 0.0022; paired t-test: $t = 8.61$, df = 178, $P < 0.0001$). Similarly, the variance of the signed asymmetry of the major axis of flowers ($s^2 = 7.21$) was significantly smaller than that of the major axis of the leaves ($s^2 = 29.32$, $F_{536,536} = 4.06$, $P < 0.0001$). In addition, we found no correlation between leaf and flower FAs at the level of the individual ($r = −0.05$, $n = 179$, $P = 0.45$). Here, and in all subsequent analyses of asymmetry unless otherwise stated, we used the residual variation from a regression analysis between unsigned Box-Cox-transformed asymmetry and trait size for both leaves and flowers to control for the relationship between size and symmetry found for the leaves. This regression was highly significant for leaves ($F_{1,535} = 73.1$, $P < 0.0001$) but not for flowers ($F_{1,535} = 0.07$, $P = 0.79$), as shown by the correlation analyses above.

The within-plant intraclass correlation for flower size was $r_I = 0.82$, $F_{178,358} = 10.16$, $P < 0.0001$; for leaf size it was $r_I = 0.42$, $F_{178,358} = 2.47$, $P < 0.0001$. This indicates that flower and, to a lesser extent, leaf size may be viewed as repeatable traits of the individual and not the organ. The within-plant repeatability value for petal asymmetry controlled for trait size was

Table 2

Mixed Model ANOVA of the Effect of CO_2 and NO_3^- Addition Treatments, Genotype, and Plant Individual on Flower
and Leaf FA, Calculated as the Residuals from the Regression of the Absolute Value of Asymmetry,
after Box-Cox Transformation, on Trait Size

| | | Flower asymmetry | | | | Leaf asymmetry | | | |
| | | | Denominator | | | | Denominator | | |
Source of variation	Numerator df	Numerator MS	df	MS	P	Numerator MS	df	MS	P
CO_2 ………………………	1	0.454	18	0.05	**	0.21	15.9	0.083	ns
N ………………………	1	0.231	17.9	0.118	ns	0.359	17.6	0.07	*
Genotype …………………	15	0.145	0.4	−0.049	ns	0.131	1.79	0.059	ns
Plant (CO_2 × N × genotype) …..	119	0.129	358	0.101	*	0.07	358	0.078	ns
CO_2 × N …………………	1	0.43	11.35	0.194	ns	0.455	11.4	0.091	*
CO_2 × genotype ………………	15	0.047	10.5	0.198	ns	0.084	10.4	0.092	ns
N × genotype ……………………	15	0.117	10.9	0.196	ns	0.069	10.9	0.091	ns
CO_2 × N × genotype …………	11	0.195	119	0.129	ns	0.091	119	0.07	ns
Residual …………………	358	0.101	…	…	…	0.078	…	…	…

Note. Denominator mean squares (MS) represent linear combinations of the error MS and interaction effects. Degrees of freedom (df)
are calculated by Satterthwaite approximation (Sokal and Rohlf 1995). ns = not significant.
* $P < 0.05$.
** $P < 0.01$.

also significant ($r_I = 0.12$, $F_{178, 358} = 1.27$, $P = 0.03$), though that
for leaves was not ($r_I = 0.053$, $F_{178, 358} = 1.11$, $P = 0.2$).

Influence of Treatment and Genotype on Asymmetry

The genotypes used in this study did not differ significantly
in leaf or flower fluctuating asymmetry (table 2), nor was there
a significant effect of genotype for the increase in flower asym-
metry from the least to the most perturbing environment; how-
ever, for leaves, we found a significant difference among gen-
otypes in their increase in asymmetry (table 3). All of the
interactions using genotype effect were also not significant (ta-
ble 2).

Leaf asymmetry. Nitrate addition significantly increased
leaf FA. A significant interaction effect between nitrogen and
CO_2 treatments was also found (table 2, fig. 1). Leaf FA was
significantly higher under the elevated CO_2 and high nitrogen
treatment than in all the other treatment combinations, which
did not differ significantly from each other.

Flower asymmetry. Elevated CO_2 significantly increased
petal FA (table 2, fig. 1). The two ambient CO_2 treatments did
not differ in asymmetry, and the two elevated CO_2 treatments,

Table 3

Random Model ANOVA of the Effect of Genotype and Plant
Individual on Flower and Leaf Change in Asymmetry

| | | Flower change in asymmetry | | Leaf change in asymmetry | |
Source of variation	df	MS	P	MS	P
Genotype …………………	11	0.173	ns	0.196	*
Plant (CO_2 × N × genotype) …..	29	0.15	ns	0.07	ns
Residual …………………	82	0.129	…	0.101	…

Note. ns = not significant.
* $P < 0.05$.

together with the ambient CO_2 without nitrogen addition,
formed another indistinguishable group (fig. 1). For petal FA,
we also found significant differences among plants of the same
genotype within the same treatment (table 2).

Asymmetry and Fitness at the Individual and Genotype Level

As previously mentioned, there were no significant differ-
ences among genotypes in their mean FA for both flowers and
leaves or for the change in flower asymmetry from least to
most perturbing environment (tables 2, 3). Similarly, individual
plants did not show significant differences for leaf asymmetry.
Therefore, we explored the relationship between asymmetry
and fitness for the flowers at the individual level and between
the change in asymmetry and fitness for leaves at the genotype
level. Using all of the data from all treatments combined, we
found no significant correlations between mean flower FA per
plant and different fitness components (table 4). Looking at
the treatments individually, we found no significant correla-
tions after correction for multiple testing. For the 16 corre-
lations calculated with petal asymmetry, only six out of 16
showed a decrease in the value of the fitness component with
increasing asymmetry. The maximum correlation coefficient
was also with pollen quality ($r = 0.32$, $n = 46$, $P = 0.029$).
Thus, in one treatment, plants with more asymmetrical flowers
produced higher-quality pollen. Similarly, there was no rela-
tionship between the increase in leaf asymmetry between the
least and most perturbing environment and our fitness estimate
for each genotype measured in either environment. Six of the
eight correlation coefficients were negative, with greater in-
crease in asymmetry between environments associated with
lower values for fitness components. The maximum correlation
found was with total vegetative dry weight ($r = 0.45$, $n = 12$,
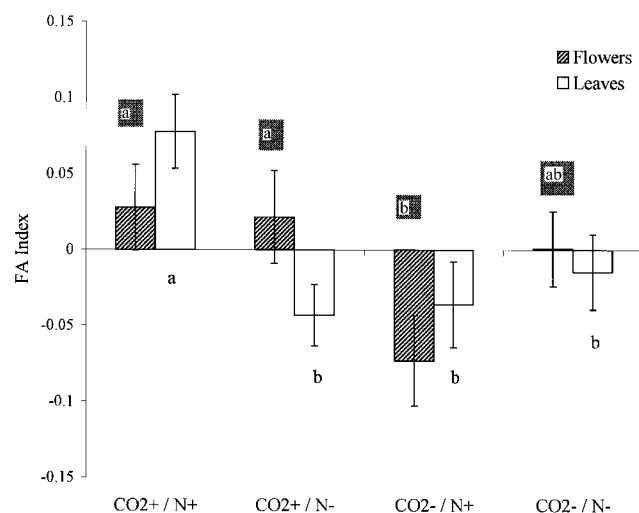$P = 0.14$) measured in the least perturbing environment.

Fig. 1 Mean ($\pm$ SE) FA of flowers and leaves in the four different treatments. FA was adjusted for organ size by calculating the residuals from the regression of the absolute value of asymmetry (Box-Cox transformed) on trait size. Negative values indicate lower asymmetries than expected from the relationship between asymmetry and trait size; positive values indicate greater asymmetry values.

Discussion

Individual asymmetry for traits with an underlying distribution of FA is often used as a measure of developmental stability, the ability of a genotype to produce the ideal phenotype, i.e., identical for both sides of a paired trait in a given set of conditions (Ludwig 1932; Van Valen 1962; Palmer and Strobeck 1986; Zakharov 1989; Parsons 1990; Møller and Swaddle 1997). Individuals that are poorer at controlling their development for genetic or environmental reasons will be more asymmetrical than those with better buffering capacity. If developmental stability is a characteristic of an individual, one might expect that different traits on the same individuals would show positive correlations for their levels of asymmetry. However, if high developmental stability is costly, we might expect high symmetry only for those traits that contribute strongly to fitness. We found that, at the plant level, the degree of asymmetry in flowers did not correlate significantly with the degree of asymmetry in leaves in *Lotus corniculatus*, as has also been shown in others' studies of plants (Paxman 1956; Møller and Eriksson 1994; Evans and Marshall 1996), and a similar lack of correlation for degree of asymmetry between characters is known for animals (review: Møller and Swaddle 1997). However, flowers were similar in their degree of asymmetry within individuals, though leaves were not. Møller and Eriksson (1994) had previously found poor intra-individual consistency for leaf and flower asymmetry in this species. Similarly, Cowart and Graham (1999) showed in *Ficus carica* that the outer leaves most exposed to environmental stress were more asymmetrical than inner leaves. Several possible explanations exist for the lack of a correlation between different

traits, i.e., among leaves and flowers. Foliar and floral traits experience different selection pressures. In particular, floral traits are selected by pollinators and have been shown to have less asymmetry than foliar traits (Evans and Marshall 1996; Sherry and Lord 1996). We also found significantly lower FA and significantly lower variance of the distribution of signed asymmetry in flowers than in leaves, although flowers are considered secondary sexual traits of plants. Insect pollinators prefer symmetrical flowers (Møller 1995a), so consistent directional selection against individuals with asymmetrical flowers could have reduced the variation in this trait in natural populations, thereby reducing mean asymmetry. If the buffering mechanisms for the development of symmetrical traits are costly, there may be a trade-off between allocation of resources to the symmetry of different traits (Møller and Eriksson 1994). Alternatively, the leaves collected from these plants had developed over several weeks, whereas the flowers developed only over a few days. Therefore, leaves and flowers may have developed under somewhat different environmental conditions, considering both the external and the internal environment of the plants.

Several plants show a negative relationship between trait size and asymmetry of flowers but show a positive relationship for leaves, though *L. corniculatus* is an exception to this rule (Møller and Eriksson 1994). We also found no significant correlation between flower asymmetry and size, but larger leaves were more asymmetrical, and plants that bore larger leaves also had more asymmetrical leaves, on average. Since larger structures contain more or larger cells, it is possible that fine control of the developmental process may be more difficult in larger organs.

Asymmetry for flowers and leaves varied over the four environments in our experiment, with highest FA found for both traits under elevated CO_2 and high nitrogen. Asymmetry is known to respond to environmental variation, usually increasing with stress (review: Møller and Swaddle 1997). It is not clear whether the environment that induced the greatest asymmetry in this experiment is stressful for these plants. It is well known that high CO_2 concentration increases photosynthetic assimilation, and high N availability increases growth. Indeed, in this experiment, highest vegetative biomass was found under the elevated CO_2 and high nitrogen treatment (C. Andalo, unpublished data). The high CO_2 and high nitrogen environment could have led to increased asymmetry because of a loss of developmental control with faster growth rate, as Martel et al. (1999) showed in *Betula pubescens*, where higher leaf growth increased FA. The developmental process would be most disturbed under these conditions of accelerated and less controlled growth, but not because of stress. Thomas and Bazzaz (1996) observed an effect of elevated CO_2 on leaf shape in *Taraxacum officinale*. They proposed that elevated CO_2 may change hormone balance or increase leaf turgor pressure during development by reducing stomatal opening. Both of these could influence ultimate leaf shape. Alternatively, an increase in leaf carbohydrate levels often associated with elevated CO_2 may influence patterns of cell proliferation and expansion. We found the greatest asymmetry under elevated CO_2 with high N, under which condition we also found higher carbohydrate levels in leaves (A. Bazin, unpublished data). If these carbohydrates were also accessible to developing flower buds, this

Table 4

Correlations between FA Measured on Flowers and Fitness Components, Using Mean Values from Each Plant Individual

	Total vegetative dry weight	Reproductive effort	Proportion of fertile pollen	Nectar quantity
Flower asymmetry	0.05	0.19	0.06	0.07
n	175	165	164	175
P	0.513	0.015	0.421	0.346

Note. FA represents the residuals from the regression of Box-Cox transformed unsigned asymmetry against flower size, reproductive effort, the residuals from the regression of fruit and flower production against plant vegetative dry mass.

could modify cell proliferation and expansion, thereby increasing asymmetry of flowers as well as leaves.

Interestingly, genotype effect was not significant for leaf or flower asymmetry. Our experimental design did not allow for a real estimate of heritability, but we failed to find evidence for a genetic basis for asymmetry of either flowers or leaves or for the change in asymmetry between nonperturbing and perturbing environments for flowers, as shown by the nonsignificant effect of genotype in our ANOVA models. This was in agreement with recent studies of leaf FA on birch species (Wilsey et al. 1998; Wilsey and Saloniemi 1999), where no genetic variation was found. Heritability for FA in *L. corniculatus* is probably very low, even for sexually selected traits such as flowers, where flowers are signals to pollinators, although sexually selected secondary sexual traits generally show high genetic variation (Pomiankowski and Møller 1995). Further, we found little evidence that fitness was related to asymmetry either at the individual or the genotypic level. The quality of a particular genotype or phenotype could not be predicted in our experiment from leaf or flower asymmetry. This contrasts with previous reports where asymmetry and fitness components were negatively correlated (Eriksson 1996; Møller 1996, 1999). However, other studies of plants found no significant relationship (Wilsey et al. 1998; Roy and Stanton 1999; Wilsey and Saloniemi 1999) or equivocal results for the relationship between fitness components and fluctuating asymmetry, with no relationship in one population but a significant

negative genetic correlation between some fitness components and FA in another (Evans and Marshall 1996).

In conclusion, we found no correlation for asymmetry between traits on the same individual, no significant differences among genotypes for their mean asymmetry, and no significant relationship between asymmetry and fitness. For this species, then, fluctuating asymmetry appears to be of limited utility for understanding fitness differences among individual plants or genotypes. Further, the change in asymmetry from nonperturbing to perturbing environments was not a good indicator of genetic quality for the range of genotypes tested in this experiment. A broader range of genotypes should be tested to determine whether this finding is general or if fluctuating asymmetry could indeed provide information on genotypic quality in this plant species.

Acknowledgments

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