Asymmetry of wild mustard, *Sinapis arvensis* (Brassicaceae), in response to severe physiological stresses

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Keywords:

Brassicaceae;
developmental stability;
fluctuating asymmetry;
Sinapis arvensis;
stress tolerance;
plasticity.

Abstract

It has often been assumed that when a severe stress is applied to a growing organism asymmetry in a number of different traits will occur due to abnormal development. To test whether asymmetry is correlated with fitness in plants, and whether different environmental stresses cause distinct or similar forms of asymmetry, we measured fluctuating asymmetry in *Sinapis arvensis* (Brassicaceae) grown in several environments: five characterized by a distinctive environmental stress (high boron, high salt, low water, low light, low nutrients), and a 'control' environment that was as stress-free as possible with ample water, nutrients and light. Relative to the controls, all of the stress environments increased asymmetry and decreased fitness. Asymmetry can be used to gauge environmental stress in *S. arvensis*, but the organ affected depends on the stress. For example, petal asymmetry was greatest in the high salt treatment, whereas fruit asymmetry was greatest in the low light treatment. Asymmetry also varied among traits within individuals; an individual's asymmetry rank depended on which organ was being examined. Finally, individual fitness was not strongly correlated with asymmetry, indicating that asymmetry cannot be used to cull stress-intolerant individuals from a population during selection. Our results suggest that asymmetry may often be specific, and not general. Under a specific asymmetry model, a particular stress affects the development of a particular organ or set of organs, but not necessarily the whole plant.

Introduction

Although organ asymmetry due to improper development under environmental stress has been well studied in animals, relatively few studies of asymmetry have been done with plants (Evans & Marshall, 1996; Palmer, 1996). There are at least two reasons why asymmetry measurements may be useful in plants. First, if asymmetry is correlated with fitness, then it should be possible to determine the degree of physiological stress a plant is experiencing without waiting for seed set. This could enhance our ability to predict population- and community-level changes as a result of environmental stress, even in long-lived perennials where measuring lifetime fitness is difficult. Second, asymmetry itself may be an ecologically important attribute that is under selection (Fenster & Galloway, 1997). For example, it was recently shown that some pollinators discriminate against asymmetrical flowers (Møller, 1995; Møller & Sorci, 1998). However, not all pollinators are sensitive to floral asymmetry (Jennions, 1996; Midgley & Johnson, 1998).

We define stress as any environmental factor, present in shortage or excess, that reduces fitness when first applied. Fluctuating asymmetry (FA) refers to random deviations in a bilateral trait such as the right and left sides of a leaf. It is assumed that these deviations from symmetry result from developmental abnormalities induced by stresses (Van Valen, 1961; Palmer & Strobeck, 1986). If the deviations between the left and right sides of

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a bilateral trait are showing fluctuating asymmetry, the difference between right and left side measurements fluctuates between negative and positive across individuals, with a normal distribution and a mean of zero (Palmer & Strobeck, 1986).

Palmer (1996) argued that the developmental plasticity of plants makes it very difficult to interpret deviations in asymmetry because 'the remarkable developmental plasticity of plants renders deviations from an ideal form exceedingly difficult to interpret' (Palmer, 1996, pp. 526–527). We suggest that carefully controlled studies will allow us to use FA to gain insights into plant physiology and population biology. Clean interpretation of variation in FA will be facilitated by comparing plants and plant organs that are all of the same age and developmental stage among different test and control environments, by controlling for genetic background, and by minimizing and documenting measurement error. Plants are indeed phenotypically plastic, but their developmental responses to different test environments can be measured by the use of controlled, comparative studies.

The goal of this study was to determine whether or not fluctuating asymmetry can be used to gauge physiological stress in wild mustard, *Sinapis arvensis* L. (Brassicaceae), a commercially important weed (Rollins, 1981; Bing *et al.*, 1996). We grew *S. arvensis* plants under five different stressful environments as well as in a nonstressful control environment, and measured the symmetry of different traits. Because we used several stresses in the experiment we could determine whether the developmental response, in terms of asymmetry, was different or similar across stresses. We asked the following questions:
1 Do the treatments we used cause physiological stress, as defined by reduced fitness relative to the controls?
2a What is the overall relationship between average fitness and average asymmetry across treatments?
2b On average, do environmental stresses increase asymmetry relative to a benign environment?
3 Do the traits we measured for asymmetry respond in similar ways to each stress, or do the responses in asymmetry vary among traits, individuals and treatments?
4 Is there a relationship between fitness and asymmetry in individual *Sinapis arvensis* plants?

Methods

Experimental design and study organism

Because fluctuating asymmetry is often associated with homozygosity (Palmer & Strobeck, 1986; Clarke, 1993; Sherry & Lord, 1996b) as well as with physiological stress, we worked with a species that is known to be highly heterozygous and obligately outcrossing. *Sinapis arvensis* is a self-incompatible, annual mustard species (Ford & Kay, 1985; Lefol *et al.*, 1996) that is native to Europe, but which also exists in large populations as an introduced and invasive weed in N. America (Rollins, 1981). All seeds used in this study were collected in 1993 from at least 1000 individuals within a large ($\gg$1 million individuals) population of *S. arvensis* 1.5 km north of the University of California Davis campus (Yolo County, USA). Seeds were stored with desiccant at 4 °C until used. From the original population sample, we took a random sample of seeds in 1995 and grew 24 plants in the greenhouse under each of six different environmental regimes. Plants grown under control conditions were provided with ample fertilizer, water and light. Each of five environmental stresses differed from the greenhouse control by only a single factor. The six growth regimes were: (1) low nutrient stress – plants received deionized water, but no fertilizer; (2) low water stress – in successive cycles throughout their lifetime, plants received fertilized water for 24 h, followed by drought until severe wilting; (3) low light stress – plants received fertilized water, but were grown under 60% shade cloth canopies; (4) high salt stress – plants received fertilized water, but with 110 mM NaCl in the solution; (5) high boron stress – plants received fertilized water, but with 9 mg L^{-1} boron in solution (boron toxicity is a significant factor for irrigated crops in many arid regions [Manyowa & Miller, 1991]); (6) control plants received natural levels of sunlight (high in Davis) and all the fertilizer and water they could use (fertilizer and water was provided continuously in the subirrigation solution).

For planting, seeds were induced to germinate on filter paper using 1000 p.p.m. gibberellic acid (GA). This procedure results in nearly 100% germination within 24 h, and makes it impossible for experimental plants to escape stress by remaining dormant. Seedlings were transplanted into the greenhouse 24 h after germination (i.e. 48 h after the GA treatment) whereupon environmental treatments were initiated under 16-h days. To ensure evenness of treatment application, all plants were subirrigated with the experimental solutions. Plants were grown individually in 200-cm^3 'Conetainers' (Stuewe & Sons, D-40 cells), filled with a 3 : 1 mixture of Yolo clay-loam and sand, so that the availability of nutrients, toxins and water could be controlled. As the plants flowered they were mass-pollinated every 3 days with feather dusters. The entire experiment was arrayed along one bench in the greenhouse. To minimize environmental effects other than those due to our treatments, the racks of conetainers were rotated and re-randomized 3 weeks after planting.

Measurements

In addition to measuring phenotypic variation in organ size, we measured symmetry traits on four different plant organs from four different stages of plant development: cotyledons, leaves, petals and seed pods. *Sinapis* has two cotyledons and each cotyledon is two-lobed. For the bilateral cotyledon measurements we measured the

length of the lobe nearest to us as we faced the plant, and the length of the companion lobe on the same side of the second cotyledon using digital calipers. To measure the difference in leaf area between the right and left side of leaves (as viewed by placing the leaf top side up with the tip away from the observer) we removed the third leaf during the fifth week of growth and pressed each leaf in a plant press. If the third leaf was missing (rarely), the fourth leaf was collected instead. The leaves were then dried for 48 h at 40 °C. We then sliced each leaf down the middle of the mid vein. The area of each side was measured by photographing the half leaf with a video camera on a DIAS image analysis system which then calculated the area based on the photograph. Crucifer flowers have four petals, two short stamens and four long stamens. We divided the flowers bilaterally between the two short stamens such that each half of the flower had one short stamen. We then measured the difference in length of the petals adjacent to the two short stamens in the flower by removing the petals from the flower and attaching them to a piece of double-sided tape on paper. By taping the petals down, we were able to measure them from the base of the claw to the tip of the petal while flat and fully extended, and we reduced measurement error compared with *in situ* measurements. Because flowering times varied among the treatments, we standardized our flower measurements by developmental stage: all measurements were done during the first week that an individual plant flowered. We were unable to devise a good bilateral character for fruit symmetry (the elongated seed pod found on plants in the Brassicaceae are called siliques, but to increase clarity we are calling them fruits). Instead, we estimated fruit symmetry by subtracting the difference between the tip-to-base length of the fruit along its longest contour and the shortest linear measurement from tip-to-tip. Although this fruit character is not strictly bilateral, it captured the most obvious variation in developmental symmetry: fruits in some treatments were curled due to uneven growth between sides, whereas in others they were straight. To increase measurement precision, fruit measurements were made on photocopies. We photocopied all the fruits a plant produced, and randomly selected one fruit image per individual plant for measurement.

An important consideration in symmetry studies is measurement error (Palmer, 1996). Because measurement error will look like FA, it is essential that the symmetry differences measured are larger than the measurement error. We estimated measurement error by measuring a sample of 10–20 individuals twice for each trait. We used digital calipers to remeasure cotyledons, petals and fruits to the nearest 0.01 mm. For petals, the range in absolute bilateral differences across all treatments was 0–3.1 mm, whereas the average difference between pairs of measurements on a given petal was 0.21 mm with 98% repeatability (Fig. 1A). For cotyledons, the range of absolute differences across all treatments was 0.01–4.94 mm and the average difference between pairs of measurements was 0.27 mm (Fig. 1B), with 97% repeatability. For fruits, the range of absolute differences across treatments was 0–10.4 mm and the average difference between pairs of measurements was 0.1 mm with 99% repeatability. For the fruits, an error estimate of 0.1 mm is only 12.7% of the mean absolute differences. In addition, we verified that our cotyledon, petal and leaf measurements were indeed indicative of fluctuating asymmetry: the frequency distributions of bilateral differences were normally, or nearly normally, distributed, with a mean not significantly different from zero.

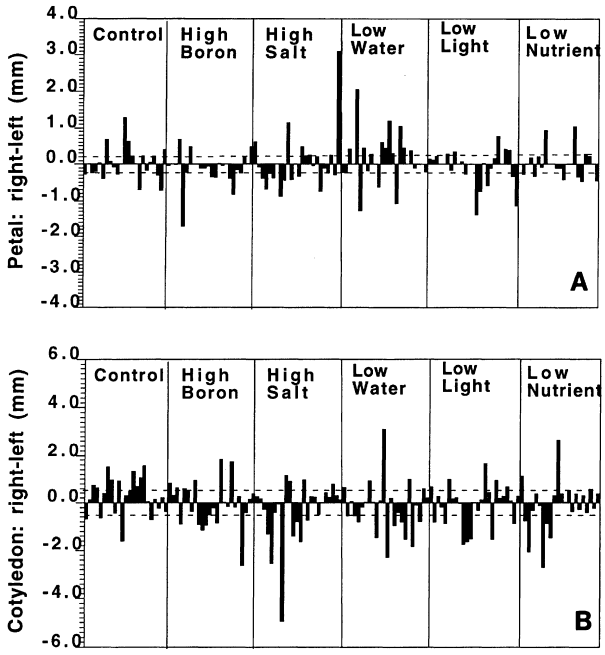

Fig. 1 Fluctuating asymmetry and measurement error. Deviations in symmetry are shown by bars, along with a dotted line showing the measurement error for that trait. (A) Petal left to right differences for each individual plant across all treatments. (B) Cotyledon left to right differences for each individual across all treatments.

Statistical analysis of asymmetry

Palmer & Strobeck (1986) and Palmer (1994) list the common indices used for the dependent variable in analysing FA, and outline the discriminatory ability of each index under particular circumstances. Because each index has different sensitivity to size dependence in the traits, outliers, and other characteristics of the data, Palmer (1994) suggests that values for at least two FA indices be shown. We used three of these indices:

$$\text{Index 1} \quad \frac{\sum |R_i - L_i|}{N},$$

summed over i measurements of a given trait. For each trait, R_i is the value for the right side, and L_i is the value for the left side, and N is the number of measurements made. Index 1 is the most intuitive asymmetry measure, and the simplest. However, it is not very good at detecting differences in asymmetry even when they are known to exist, particularly when there is size dependence (tested in a simulation study by Palmer & Strobeck, 1986). We report values for index 1 for comparative purposes only because some of the older studies used this measure. All analyses using index 1 were performed on log-transformed data which yielded normally distributed residuals.

$$\text{Index 2} \quad \frac{\sum \left[\frac{|R_i - L_i|}{(R_i + L_i)/2} \right]}{N}.$$

For one of our characters, leaf size, variance in FA increased dramatically with size. To correct for size-dependence, we ran a second analysis where we scaled out size by using a size-scaled index, index 2. For MANOVA of all characters we used a log transform of index 2, so the data would meet the standard requirements for parametric statistics.

$$\text{Index 5} \quad \frac{\sum (R_i - L_i)^2}{N}.$$

When FA is independent of character size, index 5 has the highest discriminatory ability (Palmer & Strobeck, 1986). We used this index because for two of our asymmetry measures, cotyledon length and petal length, there was no significant increase in asymmetry with size. To improve the distribution of index 5 for analysis, we applied the log transform.

To determine whether asymmetry varied among stress treatments and between stressful and benign (control) conditions, we used multivariate analysis of variance (MANOVA). MANOVA was appropriate for two primary reasons. First, owing to the number of treatments and characters measured, there were going to be a large number of univariate tests (6 treatments × 4 characters × 3 indices = 72 tests), thus inflating the probability of Type I error (Scheiner, 1993). Second, MANOVA allowed us to test for correlated responses in asymmetry among the four characters measured.

To estimate fitness, we used the combined weight of all the seeds each plant produced; a reasonable estimate of female fitness given that *S. arvensis* is an annual. Because seed weight is treatment-dependent (Fig. 2A), and the treatments were independent of each other, individual correlations of fitness on asymmetry were done for each treatment. We chose correlations over regression because although it is possible that asymmetry causes fitness variation, it is just as likely that FA and fitness could covary because they share a common causation. To test the hypothesis that the sum of FA over the whole plant is correlated with seed weight, we summed the individual scores for FA (log of index 2) for each trait in each individual for a summed FA score, which we then correlated with seed weight.

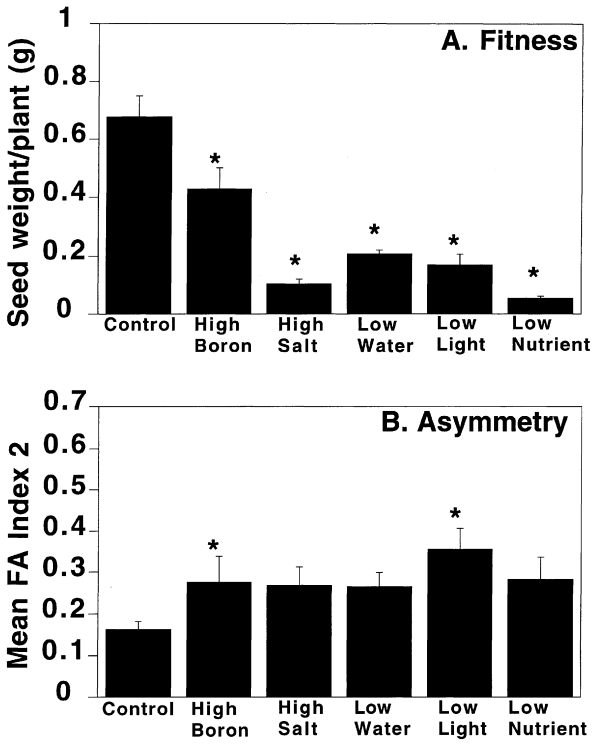

Fig. 2 Mean fitness and mean asymmetry in six growth environments. (A) Fitness is estimated as mean seed weight ± SE within each treatment. Treatments that are significantly different from the controls in a one-way ANOVA at ≤0.05 are marked with an asterisk. (B) Asymmetry is expressed here as the average of Index 2 ± SE, summed across all four plant traits, for each treatment. Raw data are shown; the analysis was performed on log-transformed data. Treatments significantly different from the controls are marked with an asterisk. (Significant contrasts for asymmetry are as follows: high boron versus control, Pillai's Trace $F = 2.35$, $P = 0.05$; low light versus control, $F = 6.30$, $P = 0.0002$.)

Results

Each of the five experimental treatments we used caused stress, which we defined as any physical environmental factor, present in shortage or excess, that reduced fitness when first applied. Total seed weight was considerably reduced in all of the stress treatments, compared with the controls (Fig. 2A). Indeed, plants in four of the five stress environments set less than half as much seed as the controls.

On average, plants in the five stress treatments were less symmetrical than the control plants, and asymmetry was significantly greater in two of the treatments, high boron and low light (Fig. 2B; Index 2, treatment effect, Pillai's Trace test, $F = 1.79$, d.f. = 20,380, $P < 0.0201$). The values for all the traits under all treatments and for

Treatment & Trait	Index 1	Index 2*	Index 5
A. COTYLEDONS			
Control	0.69 ± 0.09	0.07 ± 0.012	0.68 ± 0.16
High boron	0.74 ± 0.13	0.06 ± 0.010	0.89 ± 0.32
High salt	0.84 ± 0.22	0.08 ± 0.026	1.79 ± 1.02
Low water	0.79 ± 0.16	0.07 ± 0.016	1.20 ± 0.44
Low light	0.70 ± 0.12	0.06 ± 0.011	0.80 ± 0.21
Low nutrient	0.73 ± 0.16	0.08 ± 0.017	1.09 ± 0.44
B. PETALS			
Control	0.33 ± 0.07	0.03 ± 0.005	0.20 ± 0.08
High boron	0.34 ± 0.09	0.03 ± 0.008	0.27 ± 0.15
High salt	0.50 ± 0.13	0.05 ± 0.014	0.63 ± 0.41
Low water	0.55 ± 0.11	0.05 ± 0.010	0.55 ± 0.21
Low light	0.34 ± 0.08	0.03 ± 0.007	0.21 ± 0.10
Low nutrient	0.39 ± 0.08	0.03 ± 0.007	0.26 ± 0.10
C. LEAVES			
Control	4.73 ± 1.30	0.07 ± 0.015	54.33 ± 33.33
High boron	8.08 ± 2.21	0.12 ± 0.022	143.25 ± 79.6
High boron + outlier†	12.09 ± 4.50	0.32 ± 0.190	492.63 ± 357.35
High salt	3.75 ± 0.87	0.11 ± 0.017	28.56 ± 14.08
Low water	2.68 ± 0.71	0.15 ± 0.032	18.86 ± 11.64
Low light	13.93 ± 3.17	0.15 ± 0.033	344.92 ± 146.56
Low nutrient	0.74 ± 0.24	0.15 ± 0.032	1.53 ± 0.99
D. FRUITS			
Control	0.18 ± 0.08	0.006 ± 0.003	0.17 ± 0.079
High boron	1.60 ± 0.53	0.067 ± 0.021	8.06 ± 5.575
High salt	0.42 ± 0.13	0.023 ± 0.007	0.52 ± 0.226
Low water	0.48 ± 0.13	0.017 ± 0.005	0.61 ± 0.192
Low light	2.20 ± 0.65	0.083 ± 0.024	11.15 ± 5.14
Low nutrient	0.36 ± 0.18	0.012 ± 0.006	0.73 ± 0.48

Table 1 Mean fluctuating asymmetry values for each treatment and trait for FA indices 1, 2 and 5 (mean ± SE). See text for index descriptions. Raw values are reported here, but the analyses were performed with log-transformed data.

*The Index 2 shown here is the same as Index 2 of Palmer & Strobeck (1986). It differs from the 'index two' of Table 2 of Sherry & Lord (1996a), as the quantity (Right + Left sides) is divided by 2. †There was one serious outlier in the high boron data for leaves (see Fig. 5A); here the values are shown with and without the outlier. This datum was deleted from all the analyses as it eliminated all potential for normal residuals and biased the indices.

three FA indices are shown in Table 1. All MANOVAS analysed with the three indices as dependent variables yielded qualitatively similar results. However, because variance in leaf area increases with size (significant regression of right-left area on (right + left)/2; $r^2 = 0.05$, $F = 5.79$, d.f. = 1,114, $P = 0.0177$) leaf area has a disproportionate influence on some analyses (see Table 1, column for Index 5). When Index 5 is used the low nutrient treatment is also significantly different from the controls, but Index 2, which is the index of choice when there is size dependence, did not identify significantly greater asymmetry in the low nutrient treatment, relative to the controls. Because of the evidence for size dependence, all of the figures were prepared with data from Index 2 and the rest of the discussion focuses on analyses with Index 2.

Although the stresses we used do affect asymmetry, it is important to note that the pattern of asymmetry is both trait- and environment-specific (Figs 2 and 3). We tested the degree of trait- and environment-dependence in a two-way ANOVA, using the scale-independent Index 2 as the outcome variable (Table 2). Fluctuating asymmetry varied among traits and environmental treatments, and there was a nearly significant trait by environment interaction (0.0569). Environmental specificity can be

Table 2 Asymmetry is trait- and environment-dependent. Two-way ANOVA on treatment and trait with Index 2 (log transformed) as the dependent variable. One very asymmetrical outlier (≫ 2 standard deviations from the mean, see Table 1) was removed from the data set as its presence made fitting the assumptions for ANOVA impossible. Model $r^2 = 0.24$, $F = 6.40$, d.f. = 23,478, $P < 0.0001$.

Source	d.f.	SS	*F*	*P*
Treatment	5	0.0098	2.42	0.0353
Trait	3	0.0881	35.85	0.0000
Treatment*Trait	15	0.0203	1.65	0.0569

observed in Fig. 2 where there are large differences in the severity of asymmetry according to treatment. For example, the high boron and low light treatments caused greater asymmetry across all traits compared with the controls. In addition, some traits show more asymmetry than others. For example, the growth environment had little influence on the asymmetry of cotyledons or petals, but strongly influenced leaf and fruit symmetry (Table 1). Pooled or average asymmetry fails to show the full interaction between environmental stresses and organ development. For example, the high salt treatment primarily enhanced cotyledon and petal asymmetry, whereas the low light treatment tended to influence leaf and fruit asymmetry (Fig. 3).

Within individual plants there was little relationship between asymmetry in one trait and asymmetry in other traits. To determine whether high asymmetry for one character predicted relatively high asymmetry for another we produced a 'reaction norm' diagram by plotting individuals according to their rank FA score within a given environment for each trait. Asymmetry was not generally consistent within individuals (example for the low light treatment: Fig. 4). These visual results are corroborated by the MANOVA partial correlation matrix (Table 3), showing little or no correlation among asymmetry values for different traits within individual plants.

An important final question is whether there is a relationship between fitness and asymmetry among individual *Sinapis arvensis*. In Fig. 2(A) we show mean seed weight for all of the stresses and in Fig. 2(B) we show the mean asymmetry value obtained for each treatment. When the data from all environments are combined, more asymmetrical individuals were also less fit (Fig. 5A,B). However, within each environmental treatment, summed asymmetry was not at all correlated with an individual's fitness (Fig. 5C–H).

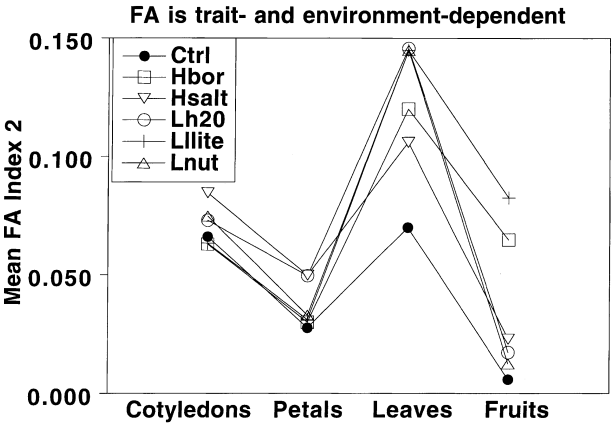

Fig. 3 Asymmetry is trait- and environment-specific. Mean FA (Index 2) by trait and environment. Raw data are shown; the analysis was performed on log-transformed data.

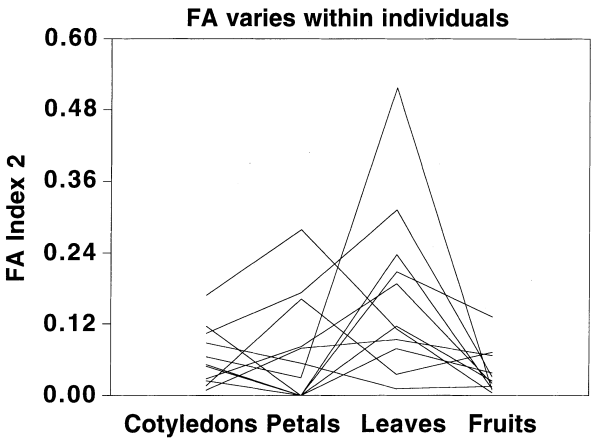

Fig. 4 Fluctuating asymmetry varies among traits within individuals. Here we have plotted individual FA scores (Index 2) on the *y* axis and the four FA traits on the *x* axis. In this example, the individuals were all grown in the low light environment. Only individuals that were scored for all four traits are shown.

Table 3 Partial correlation matrix from a MANOVA on log-transformed Index 2 (n = 142).

	Cotyledon	Petal	Leaf	Fruit
Cotyledon	1.00	−0.08	−0.02	−0.04
Petal		1.00	−0.14	0.03
Leaf			1.00	0.02
Fruit				1.00

Discussion

Asymmetry of organs does indicate population-level stress in *Sinapis arvensis*; individuals in the stressed treatments were more asymmetrical and less fit, on average, than those in the control treatment (Figs 2 and 5A,B). However, because asymmetry for any given character depended on the environment in which it was measured, varied within individuals, and was not strongly linked to fitness, it is difficult to use any single measure of asymmetry to infer the degree of stress an individual plant is experiencing.

Asymmetry varies among traits, even when size dependence of bilateral differences is taken into account by the use of a scale-independent asymmetry index (Table 1, Index 2). Petals were the least asymmetric, and leaves were the most asymmetric. It has been suggested that flowers ought to have greater developmental stability, and thus be more symmetrical, as they are more developmentally canalized than other organs (Bradshaw, 1965; Sherry & Lord, 1996a; Fenster & Galloway, 1997). We tested whether petals were more symmetrical than other organs by using contrasts in the two-way ANOVA reported in Table 2. We found that petals were more symmetrical than cotyledons and leaves (F = 14.66,

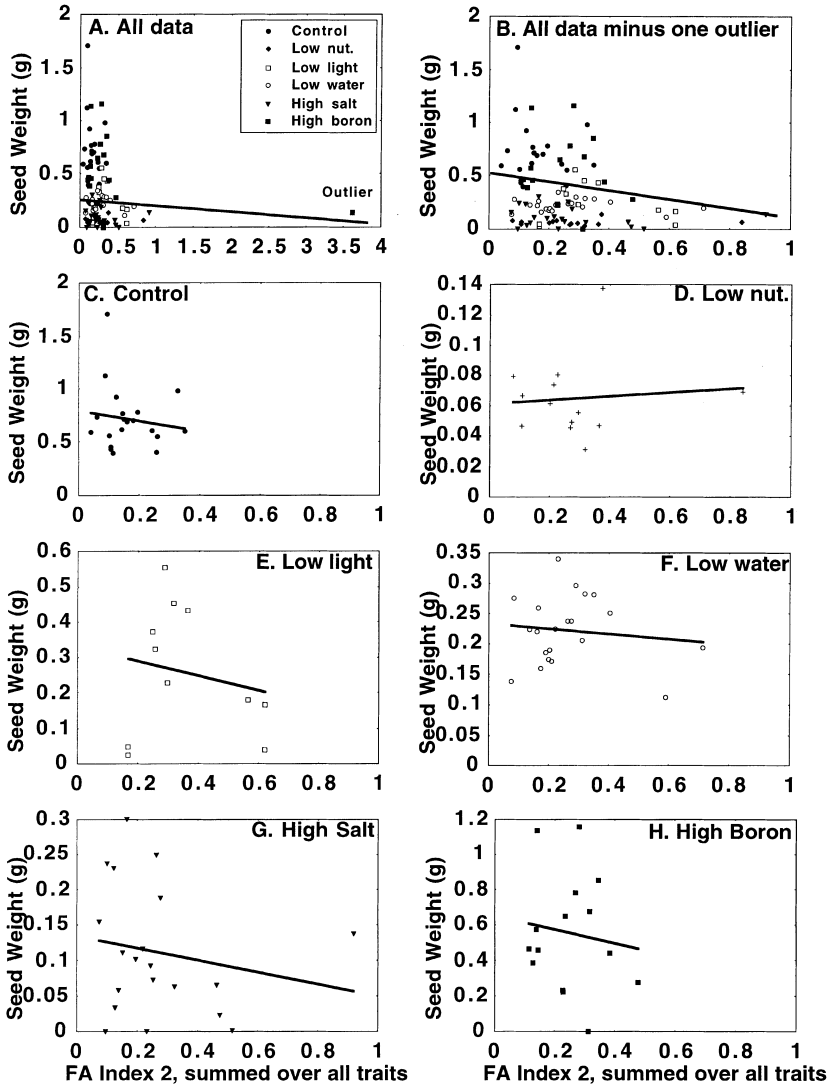

Fig. 5 Correlation between FA and fitness. FA is significantly negatively correlated with summed seed weight when the data are pooled across environmental treatments (A,B), but not within individual environmental treatments (C–H). For each individual, the asymmetry index (log of index 2) was summed for all four traits, then this sum was correlated with individual seed weight. Back-transformed data are shown; the analyses were run on log-transformed data. (A) All data ($r = -0.23$, $P = 0.02$, $n = 101$). (B) All data except for one outlier ($r = -0.27$, $P = 0.01$, $n = 100$). (C) Control environment ($r = -0.15$, $P = 0.53$, $n = 20$). (D) Low nutrient environment ($r = 0.10$, $P = 0.74$, $n = 13$). (E) Low light environment ($r = -0.17$, $P = 0.61$, $n = 11$). (F) Low water environment ($r = -0.08$, $P = 0.72$, $n = 21$). (G) High salt environment ($r = -0.21$, $P = 0.37$, $n = 20$). (H) High boron environment minus one outlier ($r = -0.13$, $P = 0.65$, $n = 15$).

$P = 0.0001$ and $F = 77.15$, $P \ll 0.0000$, respectively) but that there was no significant difference between petals and fruits ($F = 0.1206$, $P = 0.7285$). That petals have higher developmental stability than leaves and cotyledons is in agreement with the findings of others (Møller & Eriksson, 1994; Evans & Marshall, 1996; Sherry & Lord, 1996a).

What are the appropriate organs (traits) for the measurement of asymmetry in plants? The problem lies in the definition of organs. For example, we and others (Møller & Eriksson, 1994; Evans & Marshall, 1996; Møller, 1996; Sherry & Lord, 1996a) have measured asymmetry on flower petals from two sides of a bilaterally symmetrical flower. By so doing, we have made the assumption that a flower is a single organ. However, is the measurement of two separate petals on a flower directly comparable to the measurement of two sides of the same leaf? That is, should we be measuring the same petal instead? We think the measurement of separate petals from the same flower is appropriate for three reasons: (1) petals often have no obvious centre (no midvein from tip to base) making it impossible to measure deviation, (2) the development of flower petals is developmentally synchronized (Coen & Meyerowitz, 1991) and (3) pollinators, which are the major selective force on flowers, focus on the whole flower and respond to differences in petal size (Galen & Newport, 1987; Stanton & Preston, 1988; Møller, 1996). Another character that has been used in plant asymmetry studies are the lengths or widths of the two cotyledons (e.g. Evans & Marshall, 1996). Is measurement of the difference between two cotyledons a measure of a single bilaterally symmetrical organ or of two similar organs? We feel more ambivalent about this character as it could be

argued that the shape of the two cotyledons will vary simply because inside the seed one cotyledon is folded inside the other. However, our study clearly shows that there is more variation in cotyledon length in the stress treatments compared to the controls (Fig. 1B), and thus that even if there was an initial position-based asymmetry, it was subsequently exacerbated by the stress treatments.

Although there was a rough correspondence between average fitness and average asymmetry across the six treatments (Fig. 2), and there was a negative correlation between fitness and asymmetry across all treatments (Fig. 5A,B), we could not detect a significant correlation between an individual's summed asymmetry and its fertility within any given environmental regime (Fig. 5C–H). It is possible that our measurements were not sensitive enough to correlate fitness and asymmetry at the individual level because of small sample size. Sample sizes decreased with increasing stress because more plants died. This meant that fewer individuals were measured at each successive developmental stage, so that by the time the leaf and fruit traits were measured, several individuals had dropped out. For example, the low light treatment started with a sample size of 20 for the cotyledon measurements, but decreased to 10 for flower measurements. The fitness correlations based on the sum of asymmetry across all traits were particularly vulnerable to this loss of individuals, because the sum was scored as missing unless all traits were measured (see Fig. 5C–H). Power analyses suggested by Zar (1984, pp. 309–312) for correlations indicate that the sample size needed was 89, four times larger than what we had. However, a visual inspection of Fig. 5 suggests that the scatter in the data is likely to remain large, and that a tight linear relationship between asymmetry and fitness is unlikely, even if more data points increase the *P* values. A weak relationship between asymmetry and fitness is further indicated by ANOVAS (not shown) in which the class factor was seed set (yes or no) and the *y* variables were asymmetry at earlier stages of development than reproduction. In these tests there were no significant differences in asymmetry for individuals that set seed versus those that did not, again suggesting that asymmetry is not strongly associated with fitness in this species.

Asymmetry varies among organs within individuals under a given environmental stress (Fig. 4, Table 2); high asymmetry in one trait could not be used to predict asymmetry rank in another. Lack of concordance among FA measurements within an individual is common in FA studies of animals (Leamy, 1993), and the same pattern is emerging for plants. For example, individual variation in asymmetry has also been found between flowers and leaves in tobacco (Sakai & Shimamoto, 1965), *Clarkia tembloriensis* (Sherry & Lord, 1996a,b), and between traits of cotyledons and flowers in *Brassica campestris* (Evans & Marshall, 1996). The presence of variation in FA among traits within individuals suggests that a model assuming a single, general relationship between asymmetry and environmental stress is too simple. Specific environmental stresses apparently affect different developmental processes in individualistic ways, suggesting that there is likely to be genetic variation among individuals in their developmental response to stress.

Instead of being general, the association between fitness and FA, when it does occur, appears to be character-specific (Markow & Clarke, 1997). Two previous studies of plants measured both character asymmetry and individual fitness, and our results partially corroborate and generalize these studies. Møller (1996) found that seed abortion rate increased with petal asymmetry in *Epilobium angustifolium* and Brault & Oliveira (1995) found that in apples, as the index of asymmetry increased, the number of seeds decreased. A major difference between our study and the others mentioned is that our study was done in the greenhouse in the absence of pollinators. If the major selective agent on FA in plants, or on flowers in particular, are insects, then our study should have been expected to find no correlation between fitness and FA. However, if developmental instabilities are generally bad because development is less regular, then our results should have shown a decrease in fitness with increasing FA, which they did when pooled across treatments.

It has often been assumed that if a stress is applied to a growing organism, asymmetry in all or many different

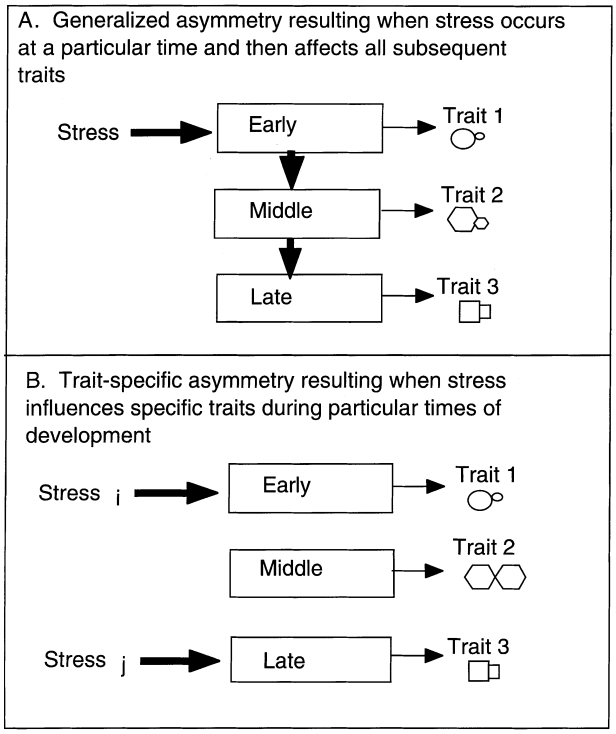

Fig. 6 Asymmetry models. (A) Generalized development of asymmetry. (B) Trait-specific development of asymmetry.

traits will occur due to abnormal development (Fig. 6A). If this model of 'generalized asymmetry' is correct, then FA measures of any single character are likely to inform us about the stresses that an individual is experiencing. Our results and others (e.g. Tarasjev, 1995; Brakefield & Breuker, 1996; Jennions, 1996) suggest that a different model, emphasizing the specific developmental actions of stressors, may sometimes be more accurate. Under our specific asymmetry model (Fig. 6B), a particular stress affects the development of a particular organ or set of organs at a specific stage in development, as appears to be the case for all of the stresses we used. The specific model would also explain why individual plants showed varying asymmetry in response to stresses (Fig. 3). Different genotypes vary with respect to both the magnitude and the pattern of stress response. Leamy (1997) has also suggested that it is reasonable to assume that the genetic basis for FA will be different for different characters. It is also possible that the specific and general models may apply to different circumstances. For example, the general model may apply when the stress affects very early development, or in cases such as inbreeding where many loci are affected (Fig. 6A). In contrast, specific effects of environmental stress may predominate when the stress affects development at later stages, after many tissues have differentiated (Fig. 6B).

It is possible that developmental instability, as expressed by asymmetry, may be very different in plants versus animals. Plants have two characteristics that animals do not have or have to a much lesser degree: more indeterminate growth and modular construction (Schmid, 1992; Fenster & Galloway, 1997). Because of these characteristics, plants can often ameliorate environmental stresses by changing the way in which they grow (i.e. they are more morphologically plastic than animals) (Bradshaw, 1965; Grime, 1986; Chapin, 1991; Schmid, 1992; Sultan, 1992). For example, plants can adjust to shading by changing the orientation of leaves and chloroplasts and by producing larger leaves that are more capable of catching light (Bradshaw & Hardwick, 1989). It would be very interesting to measure whether asymmetry decreases as a plant accommodates to a stress. For example, when the stress remains constant, do later leaves show less asymmetry than early leaves? If asymmetry decreases over a plant's lifetime as a result of a plastic accommodation to stress, then one would not expect for there to be a strong association between asymmetry and fitness in plants.

Acknowledgments

We gratefully acknowledge the help of David Boose in the greenhouse, the members of the Stanton lab for discussion, and anonymous reviewers. This research was supported by NSF grant IBN 94-19800 to M.L.S. and B.A.R.

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Received 12 February 1998; accepted 4 March 1998