TRANSLATIONAL AND FLUCTUATING ASYMMETRY AS TOOLS TO DETECT STRESS IN STRESS-ADAPTED AND NONADAPTED PLANTS

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Plants having experienced previous exposure to a stress are expected to be more resistant to further stress than those not having been exposed. While the assessment of stress in plants is a difficult task, particularly for stress-adapted plants, developmental instability has proven a useful tool for assessing stress in organisms. We examined the effect of water availability on developmental instability (translational asymmetry and fluctuating asymmetry) and growth of *Anthyllis cytisoides* L. under a precipitation gradient. We compared *A. cytisoides* in very xeric (Almería, 256 mm of average rainfall) and subhumid (Málaga, 613 mm of average rainfall) areas, from north- and south-facing slopes, after both a period of extreme drought (1995) and a humid period (1997). Translational symmetry varied between north- and south-exposed plants but differently for the Almería and Málaga populations. We observed that developmental stability was enhanced in south-exposed plants in the population from the more xeric habitat (Almería) after both dry and humid periods. In contrast, *A. cytisoides* living in a subhumid habitat did not alter their developmental stability in response to exposure after a humid period but exhibited a decline in stability in south-exposed slopes after a dry period. That is interpreted as a consequence of the adaptation of *A. cytisoides* to aridity. Growth patterns were also investigated. By reducing growth, plants can mitigate stress through a reduction of water and nutrient demands, allowing the maintenance of a steady supply of nutrients for developmental stability. This strategy was followed by plants acclimated to drought. But in mild weather, such as that of Montes de Málaga, a high growth rate cannot be supported when water is scarce. We also observed that floral fluctuating asymmetry was greatest on north-facing slopes at both the Almería and Málaga sites. That is, southern exposure enhanced floral homeostasis during development. Additionally, comparisons between translational and fluctuating asymmetry showed that translational asymmetry is more sensitive to environmental change than fluctuating asymmetry.

Keywords: developmental instability, translational asymmetry, fluctuating asymmetry, water deficit, *Anthyllis cytisoides* L.

Introduction

Previous studies have demonstrated that the disruption of homeostasis during development (developmental instability) can be used to detect stress in organisms (Soulé 1987; Zakharov 1987; Parsons 1990). Developmental instability is the degree of developmental error (intraindividual variability in an otherwise nonvariable set of traits) induced by environmental disturbances (Graham et al. 1993). Traditionally, it has been assessed by measuring fluctuating asymmetry, random deviations from bilateral symmetry (Ludwig 1932; Van Valen 1962; Palmer and Strobeck 1986). Levels of fluctuating asymmetry have been shown to be related to environmental conditions (Parsons 1990; Alados et al. 1993; Clarke 1993; Freeman et al. 1993, 1999; Graham et al. 1993; Møller and Swaddle 1997) and individual quality (Møller and Pomiankowski 1993; Watson and Thornhill 1994; Markow 1995;

Møller and Thornhill 1998). Because their modular nature presents repeated structures developed under the same pathway, plants are good subjects for estimating the capacity of the genotype to control developmental error. Most studies on plant developmental instability have utilized fluctuating asymmetry of leaves and flowers (see Møller and Shykoff 1999 for review). In addition to bilateral asymmetry, statistical noise in other allometric relations can also be used to detect random intraindividual variability during development (Freeman et al. 1993; Graham et al. 1993; Alados et al. 1994, 1998a, 1998b, 1999; Escós et al. 1995, 1997, 2000; Sherry and Lord 1996; Anne et al. 1998). Additionally, and because previous studies have revealed that vegetative and reproductive structures might vary differently in response to environmental conditions (Alados et al. 1998b), we performed developmental instability analyses on both vegetative and reproductive structures.

Plant physiological and evolutionary responses to water stress depend on the range of resource availabilities to which the plants are adapted (Chapin 1988). Plants having experienced previous exposure to a stress are expected to be more able to resist further stress than organisms that previously have not faced such situations. Plants facing frequent drought pe-

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riods evolve a conservative strategy that consists of the ability to reduce growth under severe drought (Grime 1979; Chapin 1980, 1991; Levitt 1980) while still maintaining homeostasis of fundamental structures for fitness. In contrast, plants living in more mesic habitats may present a more competitive strategy (Bertness and Callaway 1994), allocating more energy to production, while still being able to maintain developmental stability (homeostasis) in growth structures, except in the face of occasional drought periods.

In this study, we analyze trends in developmental stability and growth rhythm of *Anthyllis cytisoides* L. under different water-deficit conditions. *Anthyllis cytisoides* is intimately associated with grazing regimens, being a preferred food species for domestic animals (Barroso 1991). Past studies on the effect of grazing on *A. cytisoides* showed that intermediate grazing pressure favors both adult survival and homeostatic maintenance of morphological allometries (Alados et al. 1994; Escós et al. 1997, 2000). Varying slope and aspect provide a variation in solar energy and its effects on evapotranspiration that control the soil moisture regimen (Ayyad and Dix 1964; Coxson and Looney 1986; Nevo 1997, 1998), providing a gradient to study plant tolerance to water deficit. We used plants living on north- and south-facing slopes from two populations, which differ in average rainfall (256 mm in Almería, 613 mm in Málaga) after both a dry (1995) and a humid period (1997). This experiment permitted us to investigate adaptive plant responses to environmental change. We can use the relation between growth and developmental stability to explore strategic responses to environmental stress and to determine whether stress resistance mechanisms have evolved.

Material and Methods

Study Area

We selected two areas ca. 250 km apart. One was the Rambla Honda Valley, located near Tabernas in Los Filabres Range (Almería, Spain), lat. 37°08'N, long. 2°22'W, and 600–900 m altitude. The climate is semiarid; annual mean temperature is 15.8°C and annual mean rainfall is 256 mm (Puigdefábregas et al. 1996). Rainfall in the spring of 1995 was 14.5 mm, i.e., 21% of average spring rainfall (R. Lázaro, personal communication). During 1996, rainfall increased to 333.29 mm, 104.72 mm falling in the spring of 1997.

The other study area was located in the Montes de Málaga Mountain Range (Málaga, Spain), lat. 36°47'N, long. 4°33'W, and 200–850 m altitude. The climate is subhumid. Annual mean temperature is 15.6°C and annual mean rainfall is 613 mm. During 1995, rainfall was 64% of average, and most precipitation (203 mm) fell in December. During the spring of 1995, there was only 48.2 mm of rainfall. In 1996, rainfall was 1179.2 mm, while in the spring of 1997 (March, April, and May), there was 106.2 mm of rainfall.

The natural vegetation at Rambla Honda (Almería) includes shrubs and thorny shrubs, with monospecific patches of *Anthyllis cytisoides* and *Stipa tenacissima* L. grass. *Artemisia barrelieri* Besser is scattered throughout the area. The vegetation at Montes de Málaga is a shrub land of *Genista umbellata* (L'Hér.) Poir. and *A. cytisoides*.

Anthyllis cytisoides (Leguminosae) is a western Mediter-

anean, endemic leguminous species that lives in nearly monospecific patches. It is a winter-deciduous shrub, 50–125 cm high, much branched with an apical inflorescence. The inflorescence is a simple spikelike raceme of fasciculated (two to three) flowers in the axils of bracts. The corolla is zygomorphic, Papilionaceae type, and yellow. Flowers are mellitophilous, stamotribic (chest rubbing), and bee pollinated (Arroyo 1981). Previous experiments consisting of isolating plants from pollination by transparent net bags yielded no seeds, demonstrating that *A. cytisoides* has obligate insect pollination. We have studied the vexilla and alae, which are essential floral pieces of the pollination mechanism.

Data Collection and Analyses

We performed a 2 × 2 factorial design, comparing the effect of slope exposure on the plant morphological response of two populations with different levels of water deficit: Almería (xeric) and Málaga (subhumid). Later, we compared observations after a dry period (1995) and after a humid period (1997). Differences between slope exposures were due only to sun irradiance and its related effects on water evaporation and enhanced UV-B radiation. Nitrogen is the major nutrient limiting productivity in most terrestrial habitats (Tilman and Downing 1994). Because *A. cytisoides* is a symbiotic N-fixing legume, it is not dependent on soil nitrogen availability. Additionally, symbiotic nitrogen fixation is very sensitive to water deficit (Sprent 1972; Gallacher and Sprent 1978). Thus, despite possible soil characteristic difference at the Málaga and Almería study sites, we assume that water deficit is the most important factor affecting performance of this leguminous plant.

We selected 40 *A. cytisoides* plants per treatment in two parallel transects (20 per transect), with a total of 320 plants collected for this experiment. The two areas were ungrazed. We chose adult plants of similar size in order to reduce variability in the analysis. Each plant was divided into three parts with respect to height. From each individual plant, we selected from the middle strata one 3-yr-old branch. Additionally, we selected a well-developed annual shoot from each branch.

Plant growth was estimated as the total length of a well-developed annual shoot. Floral asymmetries and internode lengths were all examined to detect consistency in developmental stability.

Fluctuating asymmetry. The asymmetry of flowers was examined by measuring the width on each side of the midaxis at the midpoint of the vexillum at the level of the maximum width, and by measuring the largest width of each alae (fig. 1). Floral asymmetry was measured only in plants collected in 1997. We selected 15 plants per transect and two flowers per plant, making a total of 352 flowers analyzed, 118 from Málaga and 234 from Almería. Each flower was measured two times in order to calculate measurement error. Measurements were taken with a Visilog5.1 from Noesis image processing over a SPARC STATION 20 sun and with a camera CCD B/N (Sony) with macro objective. The image obtained is 512 × 512 pixels with 256 gray scale.

Absolute fluctuating asymmetry (AFA) was calculated as the unsigned left (*L*) minus right (*R*) difference. Relative fluctuating asymmetry (RFA) was calculated as the absolute value

Fig. 1 Illustration of 2-yr-old branch of *Anthyllis cytisoides*. Inset, magnification of corolla parts: vexillum and alae, showing left and right width measurements.

of left (L) minus right (R) divided by the average $(L + R)/2$, to correct for possible associations between asymmetry and size (index F2 of Palmer and Strobeck 1986). We also used the variance of left and right traits (index F4 of Palmer and Strobeck 1986). The validity of fluctuating asymmetry interpretations depends on, according to Palmer and Strobeck (1986, 1992), an absence of directional asymmetry, antisymmetry (bimodality or platykurtosis), and a normal distribution for $(L - R)$ with mean zero. However, antisymmetry and directional symmetry are many times present, are difficult to distinguish from fluctuating asymmetry (Rowe et al. 1997), and may not be related to developmental error. Graham et al. (1998) proposed alternative approaches to estimate fluctuating asymmetry in situations where directional asymmetry or antisymmetry are present. In this study, we calculated the residual variance obtained from a principal component ANCOVA matrix on the two sides (left and right) of each floral trait. Given no measurement error, factor scores on the second principal component are estimates of fluctuating asymmetry.

Translational symmetry. We selected a developed new

shoot per branch located in the second or third position from the previous year's stem. Later in the laboratory, we measured vegetative length (from shoot base to the inflorescence), inflorescence length, and internode length (distance between leaf insertions) from bottom to the top using an electronic caliper, recording to the nearest 0.01 mm (fig. 1).

In *A. cytisoides*, the relation between internode length and node order follows a self-similar sequence, where internode order declines regularly as we proceed up the stem (Alados et al. 1994; Escós et al. 1997, 2000). The relation between internode length (L) and node order (N), starting from the shoot base to the uppermost, fits the general equation

$$L = kN^b e^{aN}, \quad (1)$$

where e is the natural base and k , a , and b are fitted constants. This equation has two components. The first component (kN^b) corresponds to the allometric relationship between internode length and node order (Escós et al. 1997). The second component (e^{aN}) represents the inhibition mechanism patterns of flower formation (Meinhardt 1984). The use of this equation, however, does not imply biological meaning for parameter values; it is simply a convenient function that is able to express the differences between individuals.

The constants of the equation were obtained from regression analyses. Under stress, the impact of random perturbations increases, leading to enhanced developmental instability, that is, to a decrease in the accuracy of the curve fitting. Regressions were performed separately for each plant and the resulting R^2 , S_{yx} , and S_b were then analyzed. Because the number of internodes varies among shoots, we calculated R^2 adjusted to the degrees of freedom. The standard error of the parameter b , S_b , is positively correlated with b (Pearson's correlation coefficient, $r = 0.37$, $P < 0.001$). In consequence, we used S_b/b to avoid bias due to the relation between treatment effects and size. Finally, because S_{yx} is related to average internode length, it was divided by $\bar{y}$ mean, $S_{yx}/\bar{y}$, following Zar's (1984) recommendations.

Statistical Analysis

To find out whether we have to correct for directional symmetry or antisymmetry, we performed two different kinds of analyses: (1) We first did normality tests on the distribution of signed $(L - R)$ differences to test the presence of systematic nondirectional (antisymmetry) or directional deviations from bilateral symmetry. Correlations between the magnitude of AFA, $|L - R|$, and character size, $(L + R)$, were also calculated to investigate the possible need for normalizing $|L - R|$ (Palmer 1994). (2) We did a mixed-model two-way ANOVA with side and treatment as fixed-effect factors. Side effects represent directional asymmetry, treatment effects represent size and shape variations, and the "side-by-treatment interaction" term represents antisymmetry (Palmer and Strobeck 1986). Individual variance, nested within the side-by-treatment interaction as a random effect factor, represents the nondirectional asymmetry. The error term refers to the measurement variation and represents the measurement error.

In order to investigate the variation in fluctuating asymmetry due to treatment, we performed two different analyses. (1) We

did a mixed-model ANOVA, with two fixed-effect factors, and four levels nested analysis (transect nested in treatment, individual nested in transects, flower nested within individual, and measurement nested in flower as error term). We compared floral trait size in the same way. We used parametric statistics on raw data because they are robust to violations of normality assumptions and because transformations or the use of non-parametric tests result in loss of power (Gangestad and Thornhill 1998). (2) Within-trait variance was calculated from the side within flower term of the four nested ANOVAs, one per treatment. To determine robust probability values, we used a bootstrap procedure (Manly 1991), consisting of sampling with replacement from the observed distribution of untransformed data. An empirical distribution was created by repeatedly randomizing and reanalyzing. After 1000 random reanalyses, the actual MS (mean square of the side within flower term) value was compared to the distribution generated by the bootstrap procedure to determine the probability of obtaining a value of MS that large or larger. A *t*-test was used to compare the MS from the bootstrap distribution with the MS obtained from the different treatment.

Finally, in order to see the effect of treatments on curve-fitting estimators R^2 , $S_{yx}/\bar{y}$, S_b/b and on the regression parameters a , b , and $\ln k$, a three-level nested ANOVA was performed, with transects nested within treatment, individuals nested within transects, and shoots nested within individuals as the error term.

Data were analyzed by Model III ANOVA (GLM routine [SAS Institute 1995]). Nested effects were pooled following recommendations of Sokal and Rohlf (1981). Comparisons of means between treatments were made using Tukey's Studentized range test.

Results

Preliminary Floral Asymmetry Tests

To detect factors confounding analyses of fluctuating asymmetry, a series of preliminary tests was performed on the distribution of signed asymmetry. Signed ($L - R$), averaged for 704 alae pairs, gave a mean $\pm$ SE of 0.37 ± 0.14 , which differs significantly from zero ($t = 2.66$, $P < 0.05$). The Wilks-Shapiro test value was 0.95 ($P < 0.001$). The signed difference between left and right of vexillum, averaged for the 704 flowers (-0.55 ± 0.17), also differed significantly from zero ($t = 3.31$, $P < 0.01$). The Wilks-Shapiro statistic was 0.98 ($P < 0.05$). Separate analyses were performed for the Almería and Málaga populations. Directional asymmetry calculated from the side factor of two-way ANOVA (side $\times$ treatment) indicated no directional asymmetry for alae ($F_{1,460} = 0.90$) or vexillum ($F_{1,460} = 2.31$) in the Almería population, at the 0.05 significance level. The side-by-treatment interaction, representing antisymmetry (Palmer and Strobeck 1986), was not significant for either alae ($F_{3,460} = 1.24$, ns) or vexillum ($F_{3,460} = 0.99$, ns). Treatment effects significantly affected floral morphometry (half vexillum and alae widths), which represents size and shape variation in floral traits. Similar analyses were performed for the Málaga population and showed a lack of alae ($F_{1,232} = 0.87$, ns) and vexillum ($F_{1,232} = 2.42$, ns) directional asymmetry. Antisymmetry (side-by-treatment inter-

action) was not significant, in any case, either for alae ($F_{1,232} = 0.07$, ns) or vexillum ($F_{1,232} = 0.05$, ns). That is, neither Almería nor Málaga populations presented directional asymmetry or antisymmetry.

The vexillum presented low trait size variability with a coefficient of variation equal to 10.84. The frequency distribution of vexillum size was platykurtic at the 0.05 significance level ($g_2 = -0.36$, $tg_2 = 1.96$, $P = 0.05$) and skewed to the right ($g_1 = 0.23$, $tg_1 = 2.46$, $P < 0.05$), indicating disruptive selection. Left and right alae size distributions also presented low coefficients of variation (CV = 16.24, for left alae; CV = 15.30, for right alae) with leptokurtosis ($g_2 = 2.97$, $tg_2 = 16.09$, $P < 0.001$, for left alae; $g_2 = 1.37$, $tg_2 = 7.42$, $P < 0.001$, for right alae) and right skewed ($g_1 = 0.88$, $tg_1 = 9.53$, $g_1 = 0.56$, $tg_1 = 6.07$, $P < 0.001$, for left and right alae, respectively), indicating stabilizing selection.

The ratio of measurement MS (mean square) to the among-individual MS (MS due to treatment effects) represents the relative trait size variation due to measurement error and ranged between 0.86% for alae width to 1.00% of half vexillum width. The proportion of measurement MS to the individual within-trait MS (MS of individual within side-by-treatment interaction) represents the fraction of developmental instability variation due to measurement error. It was close to 4% (3.93% for half alae width and 4.33% for half vexillum width).

Size scaling appeared unnecessary for investigating vexilla, as the correlation between the magnitude of absolute fluctuating asymmetry and character size was not significant ($r = -0.04$, $n = 704$, ns). Alae, on the contrary, presented a significant Pearson's correlation coefficient between absolute fluctuating asymmetry and the sum of left plus right alae ($r = 0.31$, $n = 704$, $P < 0.01$). In consequence, we used relative fluctuating asymmetry in further analyses.

In order to control the possible effects of the observed directional asymmetry and antisymmetry, we performed a principal component ANCOVA matrix on the two sides of each floral trait analyzed for both populations clumped together. Because previous exploration of residuals showed an increase with size, we assumed a multiplicative error model and used the logarithm of left and right sides in the analysis (see Graham et al. 1998). The relationship between left and right alae fits the equation

$$L_{\text{alae}} = 1.12R_{\text{alae}}^{1.04},$$

while vexilla fit

$$L_{\text{vex}} = 2.27R_{\text{vex}}^{0.75},$$

where L_{alae} and R_{alae} represent left and right alae width, and L_{vex} and R_{vex} represent left and right vexillum width. Because the slope (1.04) does not differ significantly from 1.0 and 95% confidence limits are 0.94 and 1.15, we presume that alae present isometric growth. Confidence limits for vexillum slope are 0.57 and 0.97, exhibiting anisometric growth. However, the low slope value compensates for the high intercept value, as demonstrated by the low vexillum skewness (-0.17). The absolute value of factor scores on the second principal component, λ_2 , represents the individual fluctuating asymmetry

Table 1

Partitioning ANOVA with Two Fixed-Effect Factors (Location and Exposure) and Four-Level Nested Analysis

Source	RFA _{alae}		AFA _{vex}		λ_2 Alae		λ_2 Vex	
	df	F	df	F	df	F	df	F
Exposure (E)	(1, 472)	3.89*	(1, 472)	1.47	(1, 472)	3.88*	(1, 1)	0.98
Location (L)	(1, 472)	0.07	(1, 472)	0.05	(1, 472)	0.12	(1, 1)	0.00
Interaction	(1, 472)	0.006	(1, 472)	0.58	(1, 472)	0.73	(1, 472)	3.54**
$T \subset L \times E$	(4, 468)	1.42	(4, 111)	0.49	(4, 468)	1.54	(4, 111)	1.27
$I \subset T$	(111, 119)	0.94	(111, 119)	1.78*	(111, 119)	0.88	(111, 119)	1.83**
$F \subset I$	(119, 238)	14.6**	(119, 238)	3.55**	(119, 238)	15.85**	(119, 237)	3.4**

Note. Transect (T) is nested within location-exposure interaction ($T \subset L \times E$), individuals are nested with transects ($I \subset T$), and flower is nested within individuals ($F \subset I$). Measurement error was the error term. Dependent variable is relative fluctuating asymmetry of alae (RFA_{alae}), absolute fluctuating asymmetry of vexillum (AFA_{vex}), and residual variance of alae and vexillum (λ_2 Alae, λ_2 Vex). Absence of asterisks indicates $P > 0.05$.

* $P < 0.05$.

** $P < 0.01$.

(Graham et al. 1998) and was used as the dependent variable in the mixed-model ANOVA.

Fluctuating Asymmetry

The results shown in table 1 reveal that alae asymmetry was significantly related with slope aspect, whatever the index used. South-exposed plants presented lower asymmetry than north-exposed plants. No significant differences between locations existed. Vexillum asymmetry did not vary significantly with either exposure or location when ANOVA was performed. Mean fluctuating asymmetry values for the different indices used are presented in table 2. The larger differences observed correspond to the F4 index (within side MS).

MS_{alae} (F4 index) for south-exposed plants presented significantly lower fluctuating asymmetry than expected based on the bootstrap distributions for Almería ($t = 8.96$, $P < 0.001$) and Málaga ($t = 4.66$, $P < 0.001$). However, no significant differences were observed when plants were north exposed ($t = 1.10$, ns, for Almería; $t = 1.79$, ns, for Málaga). Comparisons between treatment MS_{alae} revealed that Almería south-exposed plants are more symmetrical than Almería north-exposed plants. The same results were observed for Málaga

plants, i.e., alae being more symmetrical when south exposed (table 2).

MS_{vex} presents a different tendency, exhibiting increasing asymmetry for south-exposed plants (table 2). Málaga and Almería south-exposed vexilla are significantly more asymmetrical than that expected from the bootstrap distribution ($t = 6.40$, $P < 0.001$; $t = 2.14$, $P < 0.05$, respectively), while north-exposed Málaga plants presented the most symmetrical vexilla ($t = 3.41$, $P < 0.01$).

Average $\pm$ SE values of floral trait sizes for north- and south-exposed slopes at Almería and Málaga are presented in table 1. The results show that floral trait size was significantly affected by location ($F_{1,472} = 28.6$, for vexillum; $F_{1,472} = 26.6$, for left alae; $F_{1,472} = 22.8$, for right alae, all significant at the 0.001 level), with Málaga flowers larger than Almería flowers for all traits.

Translational Symmetry

The curve-fitting accuracy of the relationships between internode length and node order is presented in table 4 and figure 2, for each treatment separately. The parameter b represents the rate of internode enlargement with node order, which

Table 2

Means $\pm$ SE of *Anthyllis cytisoides* Floral Traits Inhabiting Almería and Málaga under North and South Slope Exposures

Trait	Almería		Málaga	
	North ($n = 120$)	South ($n = 120$)	North ($n = 116$)	South ($n = 120$)
RFA _{alae}	0.114 $\pm$ 0.01 ^A	0.095 $\pm$ 0.007 ^A	0.109 $\pm$ 0.01 ^A	0.095 $\pm$ 0.008 ^A
AFA _{vex}	3.13 $\pm$ 0.24 ^A	3.24 $\pm$ 0.28 ^A	2.99 $\pm$ 0.18 ^A	3.51 $\pm$ 0.30 ^A
λ_2 alae	0.084 $\pm$ 0.007 ^A	0.066 $\pm$ 0.005 ^B	0.076 $\pm$ 0.01 ^{AB}	0.069 $\pm$ 0.005 ^{AB}
λ_2 vex	0.084 $\pm$ 0.007 ^A	0.077 $\pm$ 0.007 ^A	0.070 $\pm$ 0.004 ^A	0.090 $\pm$ 0.007 ^A
Left alae	24.02 $\pm$ 0.34 ^A	25.37 $\pm$ 0.34 ^B	26.77 $\pm$ 0.49 ^C	26.54 $\pm$ 0.35 ^{BC}
Right alae	24.65 $\pm$ 0.40 ^A	24.27 $\pm$ 0.31 ^A	26.34 $\pm$ 0.35 ^B	25.95 $\pm$ 0.35 ^B
Vexillum	52.39 $\pm$ 0.48 ^A	55.38 $\pm$ 0.48 ^B	59.42 $\pm$ 0.50 ^C	59.36 $\pm$ 0.50 ^C
MS _{alae}	17.22 ^A	10.48 ^B	18.77 ^A	12.18 ^B
MS _{vex}	14.95 ^A	17.34 ^{AB}	10.15 ^C	21.57 ^B

Note. Means squares (MS) are tested with t -test. Means with different letters are significant at the 0.05 level, Tukey's Studentized range test.

Fig. 2 Scatterplots of nonlinear best-fit equation $L = kN^b e^{-aN}$ for internode length versus node order for Almería and Málaga populations, north and south exposed.

changes significantly with treatment ($F_{3,314} = 9.70, P < 0.001$), increasing in the south-exposed plants. The parameter a , representing the inhibition mechanism of the growth pattern, describes internode shrinking along the stem. In consequence, the larger the absolute value of a , the more pronounced is the shortening. The parameter a varied significantly between treatments ($F_{3,314} = 57.90, P < 0.001$), reaching its largest absolute value in Almería south-exposed plants and the lowest in Málaga north-exposed plants (table 4). A clear picture of the morphological aspect that produces the variations in the parameter values averaged per treatment effect is presented in figure 2. This figure shows that shoots produced by plants living in Málaga grew more slowly during the first internodes, and as node order progressed, the decline in internode length with order was less steep. In contrast, in Almería internode length dropped drastically after a few internodes. The total length (mm) reached at the end of the seasonal growth period was larger in north-exposed Málaga plants ($374.6 \pm 7.7; 80$) than south-exposed Almería ($208.2 \pm 9.0; 80$).

To determine how exposure affects the translational symmetry of internode length, we performed nested ANOVA with the adjusted coefficient of determination, R^2 , standard error of the regression, $S_{yx}/\bar{y}$, and standard error of the parameter b , S_b/b , as dependent variables (table 3). The results presented

in table 4 show a lower value for R^2 and a higher $S_{yx}/\bar{y}$, indicating lower developmental instability in the Almería than in the Málaga population. The worst fit was observed in the south-exposed Málaga plants. The worst fit for S_b/b also was found in the south-exposed Málaga plants. No difference among transect within treatment occurred, and variation among shoots within individuals was lower (i.e., MS is 0.015 for the R^2) than variation between individuals within transects (MS is 0.036 for the R^2). We can ascribe differences among the treatments to main effects. Comparisons between means were assessed by Tukey's Studentized range test (table 4).

Because only vegetative growth pattern was measured in plants collected in 1995, we assessed curve-fitting accuracy of the reduced equation ($L = kN^b$), which corresponds to vegetative growth. To do that, we calculated the equation parameters from the vegetative part of the shoot, dismissing the inflorescence. Previous analysis revealed that the adjusted coefficients of determination (R^2) obtained from the extended equation (1) and the reduced equation were highly correlated (Pearson's correlation coefficient $r = 0.70, P < 0.001, n = 160$). Similar results were obtained for the parameter b ($r = 0.76, P < 0.001, n = 160$).

Comparisons of plant response after dry versus mesic years revealed that in Almería, south-exposed plants were developmentally more stable than north-exposed plants, with that tendency being more pronounced after a dry 1995 year. On the contrary, Málaga north-exposed plants demonstrated less stress than south-exposed plants after a dry year, with this trend disappearing after a humid period (1997) (fig. 3). That is, a significant interaction exists between location, year, and exposure ($F_{1,308} = 8.24, P < 0.01$). Separate analyses for Almería and Málaga populations reveal a significant effect of average annual rainfall in Almería, with plants less stressed after a humid year (means $\pm$ SE are 0.78 ± 0.01 for 1997, 0.70 ± 0.03 for 1995; $F_{1,154} = 4.77, P < 0.05$). On the contrary, no significant difference between years occurred in the Málaga population ($F_{1,154} = 1.21, ns$), while there was a significant interaction between exposure and year ($F_{1,154} = 6.76, P < 0.05$).

Total shoot length was significantly lower in the drier 1995 than in 1997 ($F_{1,309} = 220.52, P < 0.001$) for both the Málaga and Almería populations and for both the north- and south-exposed populations.

Table 3

Partitioning ANCOVA with One Fixed-Effect Factor (Location $\times$ Exposure) and Three-Level Nested Analysis

Source	R^2		$S_{yx}/\bar{y}$		S_b/b	
	df	F	df	F	df	F
$L \times E$	(3, 314)	13.73**	(1, 314)	124.84**	(1, 314)	5.00**
$(T \subset L \times E)$	(4, 151)	0.27	(4, 151)	0.60	(4, 151)	0.98
$(I \subset T)$	(151, 159)	2.38**	(151, 159)	2.68**	(151, 159)	3.18**

Note. L = location, E = exposure. Transect (T) is nested within location-exposure interaction ($T \subset L \times E$), individual is nested within transect ($I \subset T$), and shoot nested within individuals as error term. Dependent variables are R^2 , $S_{yx}/\bar{y}$, and S_b/b for Almería and Málaga population under north and south exposures. Absence of asterisks indicates $P > 0.05$.

** $P < 0.01$.

Table 4

Means $\pm$ SE Values of Dependent Variables and Equation Parameters Are Present for Each Treatment Effect

	Málaga		Almería	
	North ($n = 80$)	South ($n = 78$)	North ($n = 80$)	South ($n = 80$)
R^2	0.65 ± 0.01^A	0.56 ± 0.01^B	0.67 ± 0.02^A	0.71 ± 0.02^A
$S_{yx}/\bar{y}$	0.26 ± 0.01^A	0.41 ± 0.01^B	0.19 ± 0.01^C	0.20 ± 0.01^C
S_d/b	0.14 ± 0.001^A	0.19 ± 0.01^B	0.18 ± 0.02^B	0.17 ± 0.01^{AB}
b	1.95 ± 0.05^A	2.18 ± 0.04^B	1.92 ± 0.07^A	2.32 ± 0.07^B
a	-0.16 ± 0.01^A	-0.18 ± 0.01^A	-0.27 ± 0.01^B	-0.39 ± 0.02^C
$\ln k$	-0.25 ± 0.11^A	-0.86 ± 0.04^B	0.86 ± 0.07^C	0.85 ± 0.06^C

Note. Means with different letters are significant at the 0.05 level, Tukey's Studentized range test.

Discussion

Knowledge of the capacity of long-lived species such as *Anthyllis cytisoides* to adjust to environmental changes is critical because of the importance of such species in preventing soil erosion. In this study, we observed that translational symmetry varied in north- and south-exposed plants but differently between the Almería and Málaga populations. We observed that developmental stability was enhanced in south-exposed plants in the population inhabiting the more xeric habitat (Almería) after both dry and humid periods. In contrast, *A. cytisoides* living in a subhumid habitat did not alter their developmental stability in response to exposure after a humid period but exhibited a decline in stability on south-exposed slopes after a dry period. That is interpreted as a consequence of the response of *A. cytisoides* to aridity. Plants that are habituated to long periods of aridity present a conservative, slow-growth strategy and modify their allocation patterns in response to water deficit; i.e., saving energy in production (low growth rate) allows the plant to transfer energy to homeostasis (developmental stability). This conservative strategy has been called "stress resistance syndrome" (Grime and Hunt 1975; Grime 1979; Chapin et al. 1993), and it is frequently found among plants in dry areas (Edelin 1977; Thomasson 1977). When the same species grows in more benign habitats (Málaga), extreme drought conditions, as seen in 1995, make the plants on south-facing slopes developmentally less stable because the reduction in growth rate is not enough to compensate for the energy required for homeostasis during development. That is, the Málaga population presents a competitive strategy that makes *A. cytisoides* less resistant to dry periods. An association of high developmental instability and increased growth also has been observed in nutrient fertilization experiments (Møller 1995; Andalo et al. 2000; Lappalainen et al. 2000).

Corroborating former results, we observed that some phenomorphological features of *A. cytisoides* also varied along a precipitation gradient. Plants inhabiting xeric areas (Almería) started growing sooner (Navarro et al. 1993) than plants living in a subhumid climate (Málaga) (Hidalgo and Cabezero 1994), with earlier apex inhibition and a lower number of internodes. As a result, Málaga shoots reached larger size than Almería shoots. Additionally, north-exposed plants, growing for a longer period and with lower inhibition, reached larger size than south-exposed plants. That is, *A. cytisoides* reduced shoot enlargement in response to water deficit. This effect was

more evident under mild weather conditions (Málaga) than under the extreme xeric conditions of Almería. Microgeographical experiments in Israel revealed that microclimatic variation in opposite slopes appeared as an evolutionary driving force of genotypic and phenotypic evolution Nevo (1997, 1998). Thus, under extreme environmental conditions the potential for evolutionary change was low because of the high cost associated with the accommodation to environmental stress. In other words, plants from less stressful environments had a broader reaction norm (Stearns 1989; Parsons 1994).

In summary, *A. cytisoides* accustomed to xeric stress are less likely to exhaust water resources, while plants with high growth rates have high intrinsic respiration requirements that cannot be supported when water is scarce. Prolonged drought

Fig. 3 Comparative effects of water deficit on developmental stability (adjusted coefficient of determination R^2) and growth (shoot length) of *Anthyllis cytisoides*.

exposure enables the plant to make physiological and morphological adjustments that allow for normal functioning even while the stresses persist. By reducing shoot elongation and energy/nutrient allocation to reproduction the plant can ameliorate stress by reducing nutrient demand, allowing it to maintain a steady supply of nutrients for restoring and maintaining homeostasis under environmental stress.

Alae floral asymmetry also increased in north-exposed plants in both the Almería and Málaga populations, both with respect to traditional relative fluctuating asymmetry and in terms of the directional component of asymmetry, as measured with residual variance. That is, south exposure enhanced floral homeostasis. However, these indexes of stress were less sensitive to environmental change than translational asymmetry of vegetative structures.

Fitness depends on the phenotypic expression of traits that are functionally important to the organism. Consequently, natural selection should act to minimize the developmental error of such traits (Fowler and Whitlock 1994). Previous studies have reported adult survival to be more important than reproduction to the total inclusive fitness of *A. cytisoides* (Escós et al. 1997). Consequently, we may expect higher canalization of traits that enhance adult survival in comparison with floral asymmetries. Given that allometric relations between plant parts are important in order to sustain plant mechanical stability (Niklas 1992; Niklas and Buchman 1994), they must be maintained during growth. It is, therefore, not surprising that translational symmetry presented a lower variation coefficient ($CV = 24.66$ for R^2 and 27.23 for the scaling parameter b) than floral asymmetries. Similar results were observed in *Cistus ladanifer* L. ($CV = 34$ for the standard error of the regression; Alados et al. 1999). In contrast, we observed high variability of floral asymmetries in this study (variation coefficient of vexillum AFA_{vex} is 87.61 and 90.85 for RFA_{alae}) in comparison with the values observed for floral asymmetry of *C. ladanifer* ($CV = 55$) and close to the one observed in the leaves of the same species ($CV = 84$). This makes sense because *C. ladanifer* and *A. cytisoides* have different types of corollas according to their mode of pollination and their functional structure. Zygomorphy has evolved from actinomorphy by establishment of a reflectional symmetry and has been suggested to promote

specialist pollinators such as bees, which are more efficient than generalist pollinators (Cronk and Möller 1997). Recently, evolved genotypes may be responsible for the larger developmental instability observed in zygomorphic corollas, as occurred in the gynodioecious *Teucrium lusitanicum* Schreb. where recently evolved male sterile individuals are more asymmetric than hermaphrodites (Alados et al. 1998b).

Leptokurtosis is more pronounced in bilaterally symmetrical organs where selection acts to minimize asymmetry (Leung and Forbes 1997). We observed leptokurtosis in floral asymmetries of *A. cytisoides*, indicating the existence of selection mechanisms for symmetrical flowers. Leptokurtosis also was observed in the petals of 22 plant species (Møller and Shykoff 1999). The lack of relationship between vexillum size and asymmetry and the fact that vexillum size is platykurtic and skewed to the right demonstrates the existence of disruptive selection in vexillum size. That, together with the larger developmental stability of vexilla in north-exposed areas, is in accordance with the functional value of the vexillum both in the protection of flower buds (Tucker 1987) and as an attractant to pollinators. Indeed, the size of floral parts in Papilionaceae flower types is genetically correlated with other floral features, such as production and size of pollen (Primak 1987). Nectar production, the principal floral reward in *Anthyllis*, is also correlated with petal size (Herrera 1985; Petanidou and Smets 1995). Alae size is leptokurtic and positively correlated with asymmetry, indicating a stabilizing and directional selection for symmetrical and small alae that is enhanced by the favorable effect of light on flower formation on south-facing slopes.

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