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DEVELOPMENTAL INSTABILITY AND FITNESS IN *PERIPLOCA LAEVIGATA* EXPERIENCING GRAZING DISTURBANCE

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We investigated the sensitivity of developmental instability measurements (leaf fluctuating asymmetry, floral radial asymmetry, and shoot translational asymmetry) to a long-standing natural stress (grazing) in a palatable tannin-producing shrub (*Periploca laevigata* Aiton). We also assessed the relationship between these measures of developmental instability and fitness components (growth and floral production). Developmental instability, measured by translational asymmetry, was the most accurate estimator of a plant's condition and, consequently, environmental stress. Plants with less translational asymmetry grew more and produced more flowers. Plants from the medium-grazed population were developmentally more stable, as estimated by translational and floral asymmetry, than either more heavily or more lightly grazed populations. Leaf fluctuating asymmetry was positively correlated with tannin concentration. The pattern of internode growth also responded to grazing impact. Plants under medium to heavy grazing pressure accelerated early growth and consequently escaped herbivory later in the season, i.e., at the beginning of the spring, when grazing activity was concentrated in herbaceous plants. *Periploca laevigata* accelerated growth and finished growing sooner than in the other grazing treatment. Thus, its annual growth was more mature and less palatable later in the season when grazers typically concentrate on shrubs. The reduction of developmental instability under medium grazing is interpreted as a direct effect of grazing and not as the release from competition.

Keywords: developmental instability, growth rhythm, grazing disturbance.

Introduction

Developmental stability (DS) is the ability of an individual to produce consistently a given phenotype under particular environmental conditions (Mather 1953; Thoday 1955). The expression of DS requires the suppression of genetic and environmental disturbances (Leary et al. 1992). When an individual cannot buffer genetic and/or environmental perturbations during development, the phenotype is altered and developmental instability results (Waddington 1957; Graham et al. 1993b). Disruption of the normal allometry of repeated structures produced by the same developmental process can be used to assess the capacity of organisms to control environmental and genetic perturbation (Emlen et al. 1993; Graham et al. 1993b; Alados et al. 1998b). Under stress, the effect of perturbations will increase because of energy dissipation reducing the efficiency (Parsons 1997; Emlen et al. 1998; Møller and Pagel 1998) of the nonlinear feedback mechanisms that control morphological development (Emlen et al. 1993).

As the “optimal” phenotype of bilaterally symmetrical organs is known—i.e., zero differences between left and right

side of the particular trait—random deviations from bilateral symmetry, i.e., fluctuating asymmetry (FA), have been used to detect disruption of homeostasis during development (Ludwig 1932; Van Valen 1962; Palmer and Strobeck 1986). Nowadays, it is accepted that increased FA is related to genetic and environmental stress (Zakharov 1987; Parsons 1992; Clarke 1993; Graham et al. 1993a; Markow 1994; Møller and Swaddle 1997) and poor individual quality (Møller and Pomiankowski 1993; Watson and Thornhill 1994; Markow 1995; Møller and Thornhill 1998). Several studies reported positive correlations between FA and insect herbivory (Møller 1995; Zvereva et al. 1997; Martel et al. 1999; Lempa et al. 2000). Møller (1999b) presented evidence that FA of elm leaves (*Ulmus glabra*) reflects the susceptibility to disease as well as the negative effect of the disease on phenotypic development.

Given that developmental instability depends on developmental noise, a suite of random processes that disrupt development by increasing random differences in homologous structures, and stability, which arises from genetic components as a consequence of stabilizing selection (Watson and Thornhill 1994), different traits may respond to stress differently (Palmer 1994). This can influence the outcome of studies because investigators usually only study a few traits (Freeman et al. 1999; Hoffmann and Woods 2001), and choosing a trait that did not show increased fluctuating asymmetry may result in a false negative outcome; i.e., a particular environmental perturbation was stressful but affected traits other than those examined by

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the investigator. This is one of the limitations of fluctuating asymmetry. Moreover, the relationship between fluctuating asymmetry and trait size may produce spurious effects (Nachman and Heller 1999; but Møller 1999c).

The reduced fluctuating asymmetries observed in stressed individuals have led several authors to use composite measures in studies of fluctuating asymmetry (Leung et al. 2000) or to look for other developmental instability measurements. Emlen et al. (1993) and Watson and Thornhill (1994) argued that symmetries of complex traits may be more sensitive indicators of stress because such traits are under the control of higher organized processes; i.e., allometric growth implies the coordination of size and shape relationships. Thus, in addition to FA, other methods have been used in order to estimate developmental instability by measuring the within-plant variation, such as frequency of phenodeviants and deviations from radial, spiral, translational, and fractal dimensions of plant structures (Freeman et al. 1993; Graham et al. 1993b; Alados et al. 1994, 1998a; Escós et al. 1995, 1997; Sherry and Lord 1996). In addition, the relationship between different indices of developmental instability and fitness is largely unknown. Although some studies indicate that developmental stability is an indirect estimate of the fitness of individuals and that changes in developmental stability may predict changes in fitness, few studies have assessed the magnitude of the relationship between these parameters (Leung and Forbes 1996; Møller 1999a).

Previous studies have reported a positive effect of moderate grazing on plant fitness (McNaughton 1977, 1979, 1983; Collins 1987), although excess or a lack of grazing may constitute important agents of perturbation (Escós et al. 1997). In addition, grazing influences developmental instability in some species, with grazing-tolerant plants having the least sensitivity under moderate grazing (Alados et al. 1994, 1998a; Escós et al. 2000). Here we compare the sensitivity to grazing of different developmental instability measurements (leaf fluctuating asymmetry, floral radial asymmetry, and translational asymmetry) to assess grazing impact and fitness components (growth and floral production) in *Periploca laevigata* Aiton, a palatable, tannin-producing shrub of semiarid Mediterranean ecosystems.

Methods

Study Area

The study area is located at Cabo de Gata Natural Park (Almería, Spain), 36°46'N, 2°09'W. This preserve was established in 1987 with 37,570 ha. Altitude ranges from sea level to 493 m at El Fraile peak. The climate is semiarid Mediterranean. The average annual rainfall between 1973 and 1996 at 43 m is 193.9 mm, and the mean annual temperature is 19.4°C (Passera 1999). The southeast part of the park, where this research was conducted, is occupied by a volcanic mountain range, with the stony soil predominant by a Litico Lepotosol (Oyonarte et al. 1999).

Grazing pressure was calculated from direct observations of livestock (sheep and goats) landscape use. Animal movements were located with a geographical position system and transferred to the map in a geographical information system format.

Effective stocking rate was calculated from the mean stocking rate multiplied by the correction factor resulting from livestock landscape use (percentage of time using each grazing site). Additional information about these sites is available in Boza et al. (1998). We selected four sites along a grazing disturbance gradient; Sabinar as control site is not grazed. The low grazing site, located at 1 km from the Romeral farm, has a stocking rate of 0.46 individual ha⁻¹. The medium grazing site, located at 2 km from the main shed (Presillas), has a stocking rate of 0.53 individual ha⁻¹. The heavy grazing site is closer (100 m) to the main animal shelters of the Romeral farm and presents a stocking rate of 0.65 individuals ha⁻¹.

The vegetation is composed mainly of *Chamaerops humilis* L., *Thymus hyemalis* Lange, *Stipa tenacissima* L., *Brachypodium retusum* (Pers.) Beauv., *Teucrium* ssp., *Lavandula* ssp., *Rhamnus lycioides* L., and *Periploca laevigata* Aiton.

Periploca laevigata, the subject of this study, is a highly palatable woody shrub and is readily consumed by domestic animals (7.2% of dry matter; Barroso and Martínez 1999) despite producing tannins, which increase in response to grazing (Barroso et al. 2002).

Data Collection and Analyses

We selected randomly 40 adult plants per treatment from two different transects (20 per transect) at the valley side from midslope position. Elevation, slope angle, and aspect were similar to avoid variability caused by habitat characteristic. The *P. laevigata* population was continuously distributed along the study area, with not genetic isolation among the investigated sites, being the main causes of stress grazing pressure. A 2-m-diameter circle was located around the plant to estimate the percentage of ground covered by the target individual and by the other plant species in the circle. The number of flowers was counted on each of the 40 target plants per site along two separated transects. Four short shoots per plant, two leaves per shoot of two of those shoots, and two flowers per plant were collected in April 2000 and taken to the laboratory for measurement. Short shoots are produced during the first flush in spring. To reduce variability in the analysis, we selected well-developed and undamaged short shoots from the middle stratum and at the same orientation of adult plants of similar size. Two leaves of the same age were collected per short shoot from the fourth or fifth internode to avoid variability caused by leaf position on the shoot (Coward and Graham 1998). A total of 569 shoots, 636 leaves, and 368 flowers were analyzed.

Corollas were cut from the calyx base and placed between two microscope slides embedded in glycerin. Shoots with their leaves were pressed for later analyses.

Plant growth was estimated as the total length of a well-developed new shoot. Leaf fluctuating asymmetry, floral radial asymmetry, and translational asymmetry (internode lengths as a function of node position) were all examined to determine the consistency of the different developmental stability measurements.

Floral and leaf measurement were taken using Optimas 6.2 image processing. Images were taken with a Sony CV-M90 color camera (512 × 512 pixels), with a macro lens and a tripod from a fixed focal length. Leaves and flowers were flattened to keep the same focal length. Measurements were per-

formed twice to allow for an assessment of measurement error, which was then removed from the analyses.

Chemical Analyses

Tannin content was investigated in 45 *P. laevigata* individuals, 15 from the ungrazed control, 15 from low grazed, and 15 from medium grazed. The individuals were tagged to compare tannin production with developmental instability. Tannin contents of plant extracts were determined according to the procedure described by Hagerman (1987) with some minor modifications; 200 mg of wilted, ground plants were added to 1.5 mL methanol-distilled water (1 : 1). After 1 h, the mixture was centrifuged (15,000 g, 10 min), and the supernatant was recovered. Extracts were then stored at -20°C until used. Extracts were then loaded in 4-mm-diameter wells excavated in petri dishes filled with a mixture of agarose (1% w/v) and BSA (0.1% w/v) in 50 mM acetic acid, 60 mM ascorbic acid, pH 5.0. The amount of material loaded into the wells from some of the extracts was doubled because of their small tannin content. Petri dishes were incubated at 37°C for 96 h, and the precipitation rings were measured and compared with a tannic acid standard previously prepared. Two perpendicular diameters were measured for each and averaged. Results were expressed as percentage of tannic acid in dry matter (tannic acid equivalents). All samples were assayed at least in triplicate.

Measurements

Translational symmetry. We measured basal shoot diameter and internode length (distance between leaf insertions) from bottom to the top of the shoot using a digital caliper recording to the nearest 0.01 mm. Shoot length was measured from the shoot base to the top.

In *P. laevigata*, the relation between internode length (L) and node order (N) fits the equation

$$L = kN^a e^{-bN}, \quad (1)$$

where internode order declines regularly as we proceed up the stem; e is the natural base, and k , a , and b are fitted constants. The parameter k represents the starting conditions of shoot enlargement (length of the first internode), a represents the exponential growth, and b represents the inhibition process of growth. This relation has been used to calculate developmental instability in several plant species (Alados et al. 1994, 1998a, 1998b, 1999; Escós et al. 1995, 1997; Sherry and Lord 1996; Anne et al. 1998). This equation has two parts. The first component (kN^a) corresponds to allometric growth (Escós et al. 1997), and the second component (e^{-bN}) represents the inhibition process of competition between the growth of the main shoot and the development of axillary branches as well as floral bud formation (Borchert 1976; Meinhardt 1984; Remphrey and Powell 1985). The constants of the equation were obtained from regression analyses.

Under stress, the effects of random perturbations increase, 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 was then analyzed. Because the number of internodes varies among

shoots, we used R^2 adjusted to the degrees of freedom (Sokal and Rohlf 1995).

To see the effect of treatments on curve-fitting estimators (R^2) and on the regression parameters (a , b , and $\ln k$), a three-level nested ANOVA was performed, with transects nested within grazing treatment, individuals nested within transects, shoots nested within individuals, and the natural logarithm of basal diameter treated as a covariate (Model III GLM routine in SAS; SAS Institute 1996). Grazing treatment was the fixed-effect factor. Comparisons of means between treatments were made using Tukey's studentized range test.

Radial asymmetry. The flowers of *P. laevigata* are actinomorphic, with five petals. We measured the distance from the flower center to the top of each petal through the petal's midaxis. We also measured the angles between midaxes of each pair of consecutive petals. Floral asymmetry was estimated as the standard deviation of petal angle.

Data were analyzed by a four-level nested ANOVA, with transects nested within grazing pressure, individuals nested within transects, flowers nested within individuals, and repeated measures nested within flower (Model III GLM routine in SAS). Grazing treatment was the fixed-effect factor. Comparisons of means between treatments were made using Tukey's multiple range test.

Fluctuating asymmetry. We measured the width of the right (R) and left (L) halves of the blade at the midpoint of the leaf between petiole insertion and tip. Each leaf was measured twice in order to calculate measurement error.

Absolute fluctuating asymmetry (AFA) was calculated as the unsigned left (L) minus right (R) difference. The validity of fluctuating asymmetry interpretations depends, according to Palmer and Strobeck (1986, 1992), on an absence of directional asymmetry, antisymmetry (bimodality or platykurtosis), and a normal distribution for $L - R$ (with a mean of zero).

Before the analysis of asymmetry, we determined whether a correction for directional symmetry or antisymmetry was necessary. We performed normality tests on the distribution of signed $L - R$ differences to test the presence of systematic non-directional (antisymmetry) or directional deviations from bilateral symmetry. We calculated skewness and kurtosis statistics for the signed differences between sides and tested to determine whether there were significant deviations from the expected value of zero (Sokal and Rohlf 1995). We also tested whether signed $L - R$ differences had means not deviating significantly from zero using a two-tailed t -test. We then used a mixed-model two-way ANOVA to assess the significance of FA. In this model, "side" was the fixed-effect factor representing directional asymmetry; "individual" was a random factor that assessed variation among individuals, representing size and shape variations; and the "side-by-individual" interaction term represented FA (Palmer and Strobeck 1986). The error term refers to the measurement variation and represents the measurement error. To determine whether it was necessary to correct for size dependence of FA, a linear regression between FA and mean $(L + R)/2$ was performed for each grazing pressure, according to Palmer (1994). In order to examine possible effects of treatment on leaf size, univariate ANOVAs were performed for the means of the left and right sides, $(L + R)/2$. According to Sokal and Rohlf (1995), absolute asymmetries have half-normal distributions. We then used the Box-Cox

Table 1

Means \pm SE (n) and F Values of the Nested ANCOVA, with Transect Nested with Grazing, Individual Nested within Transects, Shoot Nested in Individuals, and Natural Logarithm of the Basal Stem Diameter as Covariate

Grazing pressure	n	a	b	$\ln k$	Nodes
Control	136	2.814 \pm 0.059 ^A	-0.432 \pm 0.014 ^A	0.685 \pm 0.053 ^A	14.21 \pm 0.24 ^A
Low	145	3.048 \pm 0.074 ^A	-0.528 \pm 0.021 ^A	0.770 \pm 0.046 ^A	12.98 \pm 0.21 ^B
Medium	145	3.627 \pm 0.088 ^B	-0.721 \pm 0.027 ^B	0.862 \pm 0.058 ^A	11.49 \pm 0.22 ^C
Heavy	143	3.453 \pm 0.142 ^B	-0.779 \pm 0.049 ^B	0.928 \pm 0.066 ^A	9.34 \pm 0.18 ^D
$F_{3, 458}$		3.46*	2.88*	1.37	23.10***

Note. Grazing treatment is the fixed-effect factor. Dependent variables equation parameters a , b , and $\ln k$, from the leaf arrangement equation $L = kN^a e^{-bN}$; a = exponential growth; b = inhibition process of growth; k = starting conditions of shoot enlargement (length of the first internode). Means with the same letters show no significant differences at the 0.05 level, Tukey's studentized range test.

* $P < 0.05$.

*** $P < 0.001$.

transformation $[(\ln L - \ln R) + 0.00005]^{0.33}$ that successfully achieved normality and removed any size scaling (Swaddle et al. 1994; Graham et al. 1998).

To investigate the variation in fluctuating asymmetry from treatment, we performed a nested ANOVA (Model III GLM routine in SAS), with grazing pressure as the fixed-effect factor. We had four levels of nesting (transect nested within grazing pressure, individual nested in transect, shoot nested in individual, and leaf nested in shoot). We compared leaf size in the same way.

To remove measurement error from the data, we performed, following Palmer (1994), a two-way ANOVA (side \times individuals) partitioned by location. The mean squares of the interaction minus error mean squares divided by the number of replicate measures give the index FA10 of Palmer (1994). Comparisons among variances were performed following Bartlett's test (Snedecor and Cochran 1980).

Relationship between DI plant interactions and fitness.

Plant fitness components measured were number of flowers and growth (shoot length and crown cover). The number of flowers, F , was related to the crown size, C (percentage of the 2-m-diameter circle occupied by the target), according to the equation

$$\ln F = r + s \ln C. \quad (2)$$

To remove the effect of crown size, we used the regression residuals. Plant size was estimated by the percentage of crown cover and by the average shoot length obtained measuring four complete developed shoots per plant.

We performed a reduced major axis analysis to compare the intensity of the association between developmental instability estimated by translational asymmetry (adjusted coefficient of determination R^2 from the curve fitting of eq. [1]) and fitness components estimated by the crown cover and the regression residuals of equation (2). This method of principal axis provides one way to estimate the slope of the relationship between two variables in Model II regression when both variables are subject to error. The variables have been standardized to mean zero and 1 SD (Sokal Rohlf 1995).

To determine how other plants in the community affect the fitness of target plants, we performed a multiple regression of the cover of the target species, after logarithmic transformation, on the cover of the different perennial species living

within the quadrat (arcsine transformed), as explanatory variables. Plants were categorized by life-form group. Annual herbs and annual grasses were combined. We also regressed the natural logarithm of R^2 from the regression of internode length on internode order as a measure of developmental stability on the plant cover of the co-occurring plant species (arcsine transformed) as explanatory variables. To control for the target plant-size effect on the remaining 2-m-diameter circle available, we multiplied the cover of the interacting species by the percentage of quadrat not occupied by the target (100%/100% *P. laevigata* cover). Subsequently, we performed a stepwise regression with a backward elimination procedure with an exclusion criterion of 0.1. Later, we performed a multiple regression analysis with all variables left in the model at the 0.1 levels. A negative value of the parameter indicates that either the presence of the interacting species or the conditions favoring the interacting species negatively affect *P. laevigata*.

Results

Grazing Effects on Developmental Instability

Translational symmetry. Grazing significantly affected all the model parameters (table 1). The scaling parameter, a , increased significantly for the medium and heavy grazing. The absolute value of parameter b also increased significantly for the medium and heavy grazed; i.e., plants in the medium and heavy grazed regimens started and also finished growth sooner. As a result, the total shoot length at the end of the growing period was smaller than for the control and low grazing treatments ($F_{3, 458} = 25.09$, $P < 0.0001$; fig. 1). The control and low grazed plants grew slower (lower a parameter) for a longer period (lower absolute b parameter) (fig. 1).

The coefficient of determination, R^2 , was significantly affected by grazing disturbance ($F_{3, 458} = 12.80$, $P < 0.0001$) and showed that *Periploca laevigata* was developmentally more stable at medium grazing (fig. 1). Variation among shoots within individuals was not significant ($F_{63, 458} = 0.65$). Similarly, variation among individuals within the transects was not significant ($F_{19, 458} = 1.06$), nor did transects within grazing treatment differ ($F_{3, 458} = 1.82$). The coefficient of variation of the translational asymmetry parameter (R^2) was 26.6%.

Radial asymmetry. Floral trait size was significantly affected by grazing treatment. Flowers from the medium grazed

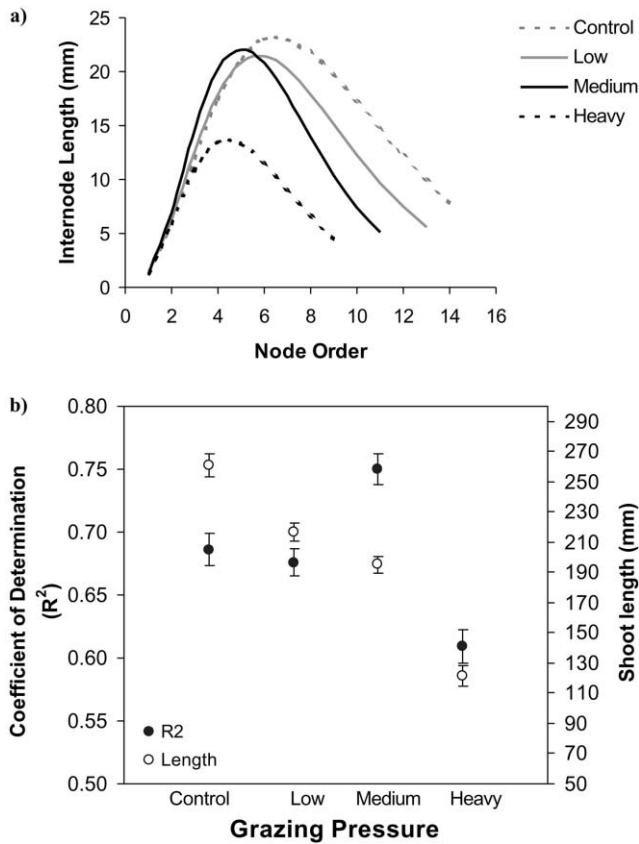


Fig. 1 a, Graphical representation of the estimated nonlinear equation $L = kN^a e^{-bN}$ for internode length (L) versus node order (N) for the different grazing treatment. b, Comparative effects of grazing pressure on adjusted coefficient of determination R^2 and shoot length (means \pm SE) for *Periploca laevigata*. Significant differences between grazing treatments were obtained from the three-level nested ANCOVA, with transect nested with grazing, individual nested within transects, shoot nested in individuals, and natural logarithm of the basal stem diameter as covariate. Grazing treatment is the fixed-effect factor.

treatment were the largest ($F_{3,567} = 7.45$, $P < 0.001$; fig. 2). Developmental instability of floral traits was calculated as the standard deviation of petal angle. This index allows stress detection without spurious scaling size effects. In this study, we observed a significant relationship with treatment (table 2). In concordance with translational asymmetry, developmental error declined in medium-grazed plants (fig. 2); i.e., plants in the medium grazed regimen had both larger and more symmetrical flowers. The coefficient of variation for standard deviation of angle was 54%. The ratio of measurement mean square (MS) error to the MS caused by treatment effects, representing the relative trait size variation caused by measurement error, is 1.02%.

Fluctuating asymmetry. The signed difference between left and right leaf width, averaged for the 636 leaves (0.008 ± 0.006), did not differ significantly from zero (t -test = 1.34, ns). The distribution did not differ significantly from normality; the Wilks-Shapiro statistic was 0.99 ($P < 0.9$). The frequency distribution was leptokurtic ($g_2 = 1.20$, $tg_2 = 6.30$,

$P < 0.001$). Skewness did not differ from zero ($g_1 = -0.09$, $tg_1 = 0.91$, ns). No directional asymmetry as calculated from the side factor of two-way ANOVA (side \times individual) was observed ($F_{1,635} = 1.74$, ns). The side-by-individual interaction, representing fluctuating asymmetry (Palmer and Strobeck 1986), was significant ($F_{635,1271} = 10.09$, $P < 0.001$), indicating that fluctuating asymmetry was larger than measurement error. Individual effects significantly affected leaf morphometry (half leaf), which represents size and shape variation ($F_{636,1271} = 222.69$, $P < 0.001$).

The ratio of measurement mean square (MS) to the among-individual MS (MS caused by treatment effects), representing the relative trait size variation caused by measurement error, was 0.45%. The proportion of measurement MS to the individual within-trait MS (MS of individual within side-by-individual interaction) was 9.9%. This represents the fraction of developmental instability variation (FA) from measurement error.

Size scaling was significant only for low grazing plants (table 3). A significant effect of treatments was observed for mean trait size. To avoid spurious differences among samples, relative fluctuating asymmetry (RFA) was calculated from $\log(L/R)$.

The results show that only absolute fluctuating asymmetry (AFA) was significantly related to grazing disturbance (table 3). Control and medium-grazed plants had the largest asymmetry values. The significant differences disappeared when we controlled for measurement error (FA10) and when relative fluctuating asymmetry was considered. Mean leaf size also increased in the control and medium grazing populations.

The coefficient of variation of AFA was very high, 81.3%, though it is reduced to 30.8% after the Box-Cox transformation of RFA.

Leaf fluctuating asymmetry was positively correlated with tannin content (Pearson's correlation coefficient, $r = 0.354$, $P < 0.02$, $n = 42$) (fig. 3). Plants with high leaf fluctuating

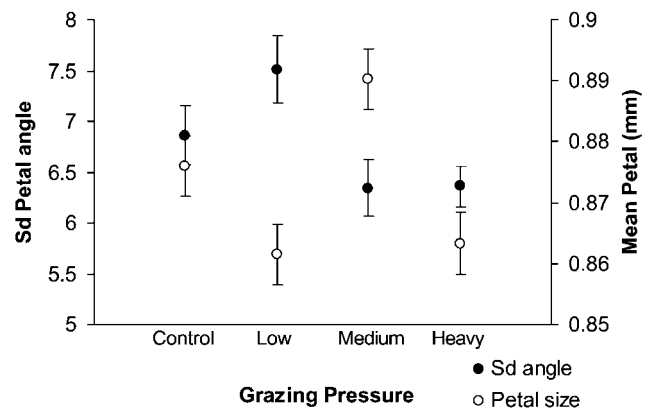


Fig. 2 Comparative effects of grazing pressure on standard deviation of petal angle and average petal length (means \pm SE) for *Periploca laevigata*. Significant differences between grazing treatments were obtained from the four-level nested ANOVA, with repeated measures nested within flower, flower nested within individual, individual nested with transects, and transect nested within grazing pressure. Grazing intensity is the fixed-effect factor.

Table 2
Partitioning ANOVA of the Three-Level Nested Analysis

Source	Standard deviation of petal angle		
	df	MS	F
Grazing	3	46.22	3.94**
Transect C grazing	3	2.64	0.23
Individual C transect	19	36.69	3.13***
Flower C individual	19	23.50	2.0**
Repetition C flower	2	1.41	0.12
Error	567	11.73	

Note. With grazing treatment as fixed effect, transect nested in grazing ($T \subset S$), individuals nested within transect ($I \subset T$), flowers nested within individuals, and repeated measures nested within flower. Dependent variable is standard deviation of petal angle. No asterisk indicates $P > 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

asymmetry had enhanced tannin concentrations. Other estimates of developmental instability were not significantly correlated with tannin concentration content (data not shown).

Grazing Effects on Fitness Components

The relationship between flower number (F) and crown cover (C) fit the equation $F = e^{0.87C^{1.01}}$ ($R^2 = 0.20$, $P < 0.0001$). Residuals of the regression between $\ln F$ and $\ln C$ differed significantly among the sites ($F_{3,115} = 13.21$, $P < 0.0001$) and was significantly greatest at medium grazed (fig 4). Variation among individuals within transect was not significant ($F_{38,115} = 1.15$, ns), nor was variation among transect within grazing treatment significant ($F_{3,115} = 2.22$, ns). Crown cover also varied significantly among the sites ($F_{3,115} = 38.94$, $P < 0.0001$), as did average shoot length (table 1). Variation among individuals within transect was not significant ($F_{3,115} = 0.66$, ns), nor was variation among transect within grazing treatment significant ($F_{38,115} = 1.41$, ns). The smallest shoot length and crown cover were in heavy grazed plants, while the largest plants were at control site (fig. 4). In general,

plant cover declined with grazing pressure as expected for such a highly palatable species.

Relationship of DI to Plant Interactions and Fitness Components

To determine whether competitive interactions between *P. laevigata* and the other species influenced developmental instability, we performed a multiple linear regression of developmental stability measured by translational asymmetry of the target species (log transformed) against the cover of the co-occurring species (arcsine transformed; table 4). No significant increase in developmental instability was observed with the presence of any species, indicating the lack of competitors of this dominant shrub in this particular ecosystem; only *Fumana laevipes* showed a light competitive influence on *P. laevigata*. Interaction strength between *P. laevigata* cover and the cover of the co-occurring species showed a positive association with the annual species *Brachypodium retusum*, *Stipa tenacissima*, and *Chamaerops humilis*, while *F. laevipes* was negatively related to *P. laevigata* in concordance with previous results.

The relationship between developmental instability and fitness components (growth and floral production) was also investigated. The results of the reduced major axis analyses showed that flower production estimated from the standardized residuals of $\ln F$ on $\ln C$ significantly increased with translational symmetry (R^2) (floral production = $-0.0035 + 4.30$ translational symmetry; 95% confidence limits for the slope was $2.12 < 4.30 < 63.36$). Similarly, crown cover increased significantly with translational symmetry (crown cover = $-0.0028 + 1.01$ translational symmetry; 95% confidence limits for the slope of the principal axis was $0.39 < 1.01 < 2.66$). That is, the more developmentally stable plants produce more flowers and grow larger than plants less able to buffer random perturbations during development.

Discussion

Periploca laevigata, a palatable tannin-producing shrub traditionally grazed by sheep and goats, was developmentally

Table 3
Means \pm SE (n) of Average Leaf Size ($L + R$)/2, Slope \pm SE of the Regression between Fluctuating Asymmetry (FA) and Average Leaf Size, and Fluctuating Asymmetry Indices

Sample (n)	$(L + R)/2$		$(L - R)$			AFA	RFA	FA10 ^a		
	$x \pm$ SE	Slope \pm SE	$x \pm$ SE	Skewness \pm SE	Kurtosis \pm SE	$x \pm$ SE	$x \pm$ SE	Msm	σ^2	df
Control (160)	1.786 \pm 0.04	0.037 \pm 0.019	-0.021 \pm 0.014	-0.386 \pm 0.19*	1.542 \pm 0.39***	0.479 \pm 0.01 ^A	0.400 \pm 0.009 ^A	0.004	0.0137	118
Low (160)	1.602 \pm 0.02	0.048 \pm 0.02*	-0.011 \pm 0.01	0.109 \pm 0.19	1.111 \pm 0.39**	0.443 \pm 0.01 ^B	0.381 \pm 0.01 ^A	0.001	0.0096	135
Medium (156)	1.668 \pm 0.02	0.021 \pm 0.02	0.016 \pm 0.01	0.006 \pm 0.20	0.178 \pm 0.39	0.479 \pm 0.01 ^A	0.400 \pm 0.009 ^A	0.003	0.0113	122
Heavy (160)	1.625 \pm 0.02	0.022 \pm 0.022	-0.017 \pm 0.011	0.198 \pm 0.19	1.204 \pm 0.39**	0.442 \pm 0.01 ^B	0.379 \pm 0.009 ^A	0.001	0.009	139
$F_{3,570}$	10.16***					2.80*	1.26			

Note. L = left; R = right. Means \pm SE, skewness and kurtosis of the signed ($L - R$) difference. Absolute fluctuating asymmetry (AFA) and relative fluctuating asymmetry (RFA) are Box-Cox transformed of $|L - R|$ and $|\log(L/R)|$, respectively. F values are from the four-level nested ANOVA for the grazing effect factor. FA10 values from Palmer (1994) (Msm is measurement error; σ^2 is nondirectional asymmetry) for *Periploca laevigata* along a grazing pressure gradient. Means with the same letters indicate no significant differences at the 0.05 level, Tukey's studentized range test.

^a $\chi^2 = 6.66 < \chi^2_{0.05,3} = 7.86$ (from Bartlett's test).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 4
Linear Regression Analysis

Variable	Parameter	F
<i>Periploca laevigata</i> cover ($F_{3,155} = 6.08$):**		
Intercept	3.51	1422.46***
Annuals	0.72	36.66***
<i>Brachypodium retusum</i>	0.33	6.33**
<i>Stipa tenacissima</i>	0.21	4.85*
<i>Chamaerops humilis</i>	0.17	3.10
<i>Fumana laevipes</i>	-3.56	8.49**
R^2 ($F_{1,156} = 4.37$):*		
Intercept	-0.37	218.38***
<i>F. laevipes</i>	-0.94	3.53

Note. Analysis between natural logarithm of *Periploca laevigata* cover or of developmental stability (R^2 from the regression between internode length on internode order) as independent variables and presence of different co-occurring species as explanatory variables (arcsine transformed).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

more stable under medium-grazing pressure. Developmental instability of internode translatory asymmetry and the standard deviation of petal angle both declined under medium grazing. In contrast, heavily grazed plants suffered a large reduction in biomass and could not maintain stability of developmental structures. Previous studies have examined the effect of grazing on the developmental instability of *Anthyllis cytisoides* or *Seriphidium novum* (Escós et al. 1997; Alados et al. 1998a). Both studies also report lower developmental instability under medium grazing and higher levels of developmental instability in light or heavy grazing as we observed in this study. These three species are all palatable species to domestic livestock, and all have an evolutionary history of grazing. While moderate grazing may directly enhance the fitness of the grazed plants (McNaughton 1977, 1979, 1983; Collins 1987; Escós et al. 1997), reduced developmental instability could also arise indirectly from a release from competition (Belsky 1986, 1987). In this study, we did not observe any competitive effects on the developmental instability of *P. laevigata*. Thus, our work tends to support the former hypothesis.

While the majority of plant studies on developmental instability have examined leaf fluctuating asymmetry, previous studies and the results reported here demonstrate that translatory asymmetry is a more sensitive and powerful measurement of developmental instability (Alados et al. 1999; Freeman et al., in press). Natural selection should favor processes that minimize developmental error of traits that are functionally important to maintain fitness in organism (Fowler and Whitlock 1994). Allometric relationships among plant parts are important in maintaining mechanical stability (Niklas 1992, 1996; Niklas and Buchman 1994) and in minimizing the mutual shading of leaves on the same plant (Freeman et al., in press). Allometry, however, must also be responsive to changes in the local environment (e.g., internode lengths typically respond to changes in the light regime). Thus, translational asymmetry can be expected to be a more reliable measure (lower coefficient

of variation) than, for example, leaf asymmetry (larger coefficient of variation). Consequently, leaves may not accurately reflect stress effects (Anne et al. 1998 for an example under controlled conditions). However, floral morphology is important in pollination mechanisms. Petals are a major visual attractant for animal pollinators, and petal size and symmetry are important in pollination processes (Bell 1985; Willson 1990; Møller and Eriksson 1994; Møller 1995; Cronk and Möller 1997). Floral traits are more highly canalized than leaves (Bradshaw 1965; Evans and Marshall 1996; Sherry and Lord 1996; Fenster and Galloway 1997; Alados et al. 1999; Roy and Stanton 1999), leading to lower sensitivity to stress; i.e., leaves were more asymmetric than petals.

Like several prior studies, we too found that floral and leaf estimates of developmental instability are often uncorrelated (Paxman 1956; Bagchi et al. 1989; Evans and Marshall 1996; Leung and Forbes 1996; Alados et al. 1998b; Møller and Shykoff 1999; Andalo et al. 2000). These different traits are under different selection pressures, develop at different times, and involve different developmental pathways (Freeman et al., in press); thus, they should not be expected to respond similarly. Indeed, Roy and Stanton (1999) observed, in an experimental study in *Sinapsis arvensis*, that the organ affected depended on the type of stress. Salinity, for example, did not influence leaf fluctuating asymmetry (Anne et al. 1998).

In this study, leaf fluctuating asymmetry was positively related to tannin concentration. Tannins occur in the vacuoles of intact plant cells. The exact function of tannin is not clear; nevertheless, most researchers consider tannins to be involved in protecting vulnerable parts of the plant from herbivore attacks (McLeod 1974) and to contribute to tough and durable cell walls (Kause et al. 1999). These authors observed that high tannins concentrations were associated with reduced physiological performance in birch leaves. *Periploca* produces more tannin in grazed treatments than in ungrazed treatments (Barroso et al. 2002). This defensive mechanism may impose a cost that reduces leaf developmental homeostasis, leading to the formation of asymmetrical leaves as we observed (also Lempa et al. 2000). Further study is needed to see if this is a general pattern.

When we compared the growth patterns among the sites,

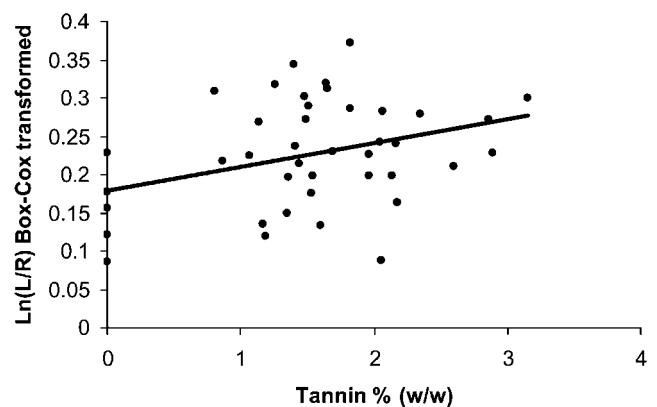


Fig. 3 Scatter plot of the relation between leaf fluctuating asymmetry ($\ln [L/R]$ Box-Cox transformed) and tannins contents.

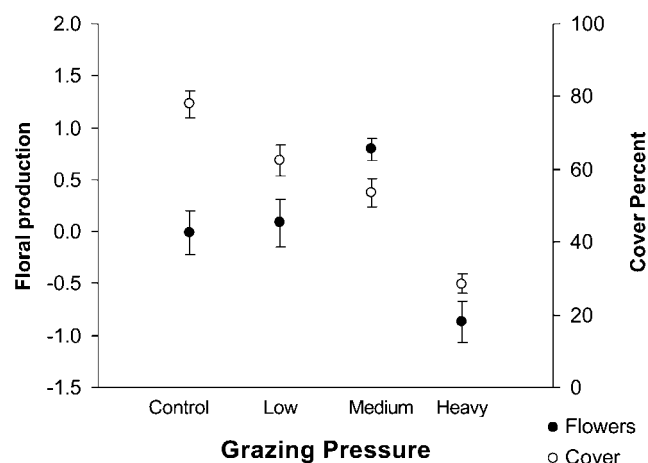


Fig. 4 Means \pm SE of the regression residuals of $\ln F$ versus $\ln C$ and cover percent of *Periploca laevigata* along a grazing perturbation gradient. Significant differences between grazing treatments were obtained from two-way nested ANOVA, with individual nested within transects and transect nested within grazing pressure. Grazing intensity is the fixed-effect factor.

the medium-grazed plants had more rapid shoot elongation during the first internodes, as did the very heavily grazed plants. The heavily grazed population does not, however, maintain this high growth rate, and it ends its growth sooner. In contrast, plants in the low grazed and control areas reach larger shoot size and have slower internode elongation and node production but do so over a more protracted period, producing a larger number of internodes. The rapid development response of *P. laevigata* under heavy grazing may be related to the strategy of accelerated early growth, such that plants can escape herbivores that feed on that plant later in the season. This change of strategy is advantageous for the plant given that during the beginning of the spring, when the availability of herbaceous plants is high, grazers are concentrated in herbaceous consumption; it is during this period that *P. laevigata* invests its energy in shoot enlargement. This has been confirmed by the photosynthesis measurements performed in that species under grazing treatment by C. Nabais, H. Castro, and H. Freitas (personal communication). These authors reported larger photosynthetic activity in heavy grazed plants than in low grazed plants in April.

Stress is generally considered to be any environmental factor that reduces the efficient use of energy (Emlen et al. 1993; Parsons 1997), leading to a reduction in developmental ho-

meostasis (Zakharov 1987; Parsons 1992; Clarke 1993; Graham et al. 1993a; Alados et al. 1994, 2001; Markow 1994; Sommer 1996; Møller and Swaddle 1997) and ultimately long-term total inclusive fitness (Koehn and Bayne 1989; Hoffmann and Parsons 1991). Theory predicts that plants with limited resources will show trade-offs in biomass allocation among maintenance, growth, reproduction, and herbivore defense (Coley et al. 1985; Sibley and Calow 1986; Bazzaz et al. 1987; Fageström 1989), which is alleviated when nutrient availability increases (Sugiyama and Bazzaz 1998). Thus, we expect a positive correlation between components of fitness and measures of developmental homeostasis under extreme stress, although under moderate stress, trade-off between different fitness components was expected (Parsons 1997; Roy and Stanton 1999). In this study, we observed a positive association between fecundity and developmental stability under medium grazing but not with growth, although under extreme stress, plants had high developmental instability, low growth, and reduced fecundity.

In summary, our results support the position that *P. laevigata* fitness was enhanced by medium-grazing pressure, showing both greater flower production and more translational symmetry. Plants with high translational symmetry were developmentally more stable plants, grow larger, and produce more flowers per unit cover than plants with reduced translational symmetry. Moreover, stem growth integrates developmental instability and plant investment in growth and reproduction, providing a more reliable indicator of plant adaptation to environmental conditions (Escós et al. 1997, 2000) than fluctuating asymmetry.

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