Instability of development and fractal architecture in dryland plants as an index of grazing pressure

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Developmental instability has been used to monitor the well-being of natural populations exposed to physical, chemical and biological stressors. Here, we use developmental instability to assess the impact of grazing on *Chrysothamnus greenii* and *Seriphidium novum* shrubs, and *Oryzopsis hymenoides* grass, common in the arid intermountain west of the U.S.A. Statistical noise in allometric relations was used as an indicator of developmental instability arising from grazing-induced stress. Unpalatable species that are not grazed (*Chrysothamnus greenii*) or species that are dormant during the winter–spring grazing period (*Oryzopsis hymenoides*) show lower allometric variability under high grazing pressure. Palatable species (*Seriphidium novum*) exhibit high developmental instability under low and high grazing pressure. Grazing pressure imposed by presumably co-adapted wild herbivores enhances developmental stability in species habituated to moderate grazing, like *Oryzopsis hymenoides*, but stresses plants such as *Chrysothamnus greenii* that prefer disturbed environments. These grazing effects are probably due to the impact grazing has on competitive relationships and not to the direct action of the herbivore on the plants.

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Introduction

Developmental stability, the ability of a genotype to consistently produce a given phenotype in a given environment, has been shown to decline as a result of genetic stresses such as inbreeding or hybridization between disparate taxa, and as a result of exposure to both physical and chemical stressors (see Markow (1994) for a review).

Developmental instability is manifested as developmental error reflected in exaggerated intra-individual variation in repeated traits and patterns.

Traditionally, developmental instability has been measured by random deviations away from bilateral symmetry, fluctuating asymmetry (Sumner & Huestis, 1921; Ludwig, 1932; cited in Van Valen, 1962; see Palmer & Strobeck, 1986; and Graham *et al.*, 1993a), and increases under stress. For example, audiogenic stress increased left-right differences in rats (Siegel & Smookler, 1973; Siegel & Doyle, 1975a). Temperature stress also caused increased fluctuating asymmetry in rats and mice (Beardmore, 1969; Siegel & Doyle 1975b, c; Siegel *et al.*, 1977) and in the salmon *Oncorhynchus keta* Walbaum (Beacham, 1990). Fluctuating asymmetry increased in grunion (*Leuresthes tenuis*) exposed to DDT (Valentine & Soulé, 1973). Similar results were observed by McKenzie & Clarke (1988) in the blowfly, *Musca vetustissima* Walker, exposed to Avermectin. However, bilateral symmetry is only one of many measures of developmental instability. Other kinds of symmetry in development—radial symmetry, translational symmetry, and symmetry of spatial scale (Freeman *et al.*, 1993; Graham *et al.*, 1993a; Alados *et al.*, 1994, in press; Escós *et al.*, 1995b, 1997)—have also proven useful in detecting and assessing the biological effects of stress.

Until recently most studies concerned animals with bilateral symmetry, though studies have now revealed that plants also are suitable subjects to detect developmental instability caused by environmental disturbance (Freeman *et al.*, 1993, 1995; Alados *et al.*, 1994, in press; Møller & Eriksson, 1994, 1995; Escós *et al.*, 1995b, 1997; Møller, 1995a; Tracy *et al.*, 1995; Sherry & Lord, 1996).

The extent to which developmental instability reflects the ability of an organism to increase fitness will depend on the character analysed. Traits that directly and strongly affecting fitness and are under intense stabilizing selection may not be appropriate for developmental instability analysis (Soulé & Cuzin-Roudy, 1982). On the other hand, high plastic characters may present high developmental instability even under good conditions. In consequence, in choosing measures of developmental instability it will be useful to consider alternate developmental instability measurements. Plants, because of their modular nature and taxon-specific consistent architecture, present repeated allometries (i.e. branch diameter, branch length, and dry weight; Kimura, 1960; Ogawa & Kira, 1977; Kohyama, 1987; Niklas, 1992) that can be used to assess developmental instability. In allometric relations, variables are related by a power law. Power law scaling results from self-similarity, which means that each part of an object resembles the whole object. For example, the branch system of a plant consists of self-similar branches. Self-similar structures can be analysed via their fractal dimension, which is a measure of how thoroughly they fill space, and thus how efficiently they interact with their environment; complex, space-filling structures are characterized by high fractal dimension (Zeide & Pfeifer, 1991; Bassingthwaite *et al.*, 1994; Bunde & Havlin, 1995). There are several types of fractal dimension, including capacity dimension, information dimension, correlation dimension, and the Lyapunov dimension, and there are several different algorithms to compute fractal dimension; but until methods of fractal analysis are standardized it will be difficult to compare fractal dimension values taken out of the context of a given study. In spite of this, application of fractal analyses to biology is rapidly increasing. Here we not only use statistical noise in allometric relations, but also complexity of fractal structures as a way to detect disturbances in natural ecosystems.

Few studies have examined the influence of natural biotic stressors on developmental stability. Zakharov *et al.* (1991) have shown that in some populations of the common shrew, *Sorex*, developmental stability is lowest when the local population density is the highest. Several authors (Polak, 1994; Escós *et al.*, 1995a, b; Møller 1995b; Alados *et al.*, 1996; Mara & Freeman, unpublished data) have shown that developmental stability declines as parasite loads or disease increases, provided that

the parasitism occurs before the cessation of development. But in general, developmental stability has not been used to assess the impact of one species upon another. Yet, developmental stability has much to offer as a means of assessing the impact of interspecific interactions, and specifically to the understanding of grazing systems.

The 'beneficial' effects of grazing have been the subject of considerable debate (McNaughton, 1977, 1979, 1983, 1986; Belsky, 1986, 1987; Crawley, 1987, 1992). Grazing has been shown to change plant community structure (Cargill & Jefferies, 1984; Ruess & McNaughton, 1987; Jefferies, 1988; Brown & Gange, 1989; Ruess *et al.*, 1989; McInnes *et al.*, 1992; Montalvo *et al.*, 1993), and to affect life history feature of both grazed and non-grazed species. The effects of grazing depend upon the intensity and frequency of damage (Harper, 1977; Crawley, 1983; Marquis, 1984, 1992; Doak, 1991; Edenius *et al.*, 1993), the plant phenological stage at the time of herbivory (Maschinski & Whitham, 1989; Marquis, 1992), which plant tissues are removed (Brown & Gange, 1989; Oesterheld, 1992), competition with non-grazed species (Fowler & Rausher, 1985; Maschinski & Whitham, 1989), and the biological characteristics of the plant species in question (Brown & Gange, 1989). The methodology traditionally used to detect grazing effects is thoroughly discussed by Belsky (1986, 1987), and Trlica & Rittenhouse (1993); it is time-intensive. Developmental instability, on the other hand, is relatively inexpensive and quickly provides an assessment of stress.

Here, we analyse the effects of plant competition in response to different herbivore pressure on developmental stability of one non-grazed shrub species (*Chrysothamnus greenii* A. Gray), a palatable shrub species (*Seriphidium novum* (Nelson) W. H. Weber (syn. *Artemisia nova* Nelson)), and one grass species (*Oryzopsis hymenoides* (Roem. & Schultes) Ricker) subjected to long-term grazing experiments. In addition, we propose several new measures of developmental stability.

Methods

Study areas and species

The Desert Experimental Range (DER) of the USDA Forest Service is located in the cold desert shrub-steppe of western Utah. It has been the subject of a continuous grazing experiment since its inception in 1933 (see Clary & Holmgren (1982) for a description of the DER and long-term grazing experiment). Plants exposed to three levels of winter grazing by sheep (light, medium and heavy with 25, 35 and 42 sheep days ha⁻¹, respectively) (Hutchings, 1966) and a control area that is naturally grazed by pronghorn, jackrabbits and cottontail rabbits (the most important herbivore species inhabiting the DER) were examined.

Populations of *Oryzopsis hymenoides*, a perennial winter dormant grass, and a preferred food species for domestic animals, and two shrubs *Chrysothamnus greenii* (a rabbit brush inhabiting disturbed habitats and not preferred by sheep) and *Seriphidium novum* (black sagebrush, a long-lived woody plant highly preferred by domestic animals and antelope) were examined.

Healthy plants of similar size were chosen in order to reduce variability in the analysis. A randomized branch sampling was performed, where the crown was divided into strata of similar size based on relative height and orientation, and one well developed branch was randomly selected from the specific strata. Additionally, in order that the loss of material as a direct effect of grazing did not interfere with plant architecture, only undamaged stems were selected for the analyses. In consequence, the effect of grazing pressure was studied on the undamaged plant part.

Data collection

Individuals of *C. greenii* and *O. hymenoides* were collected from the Desert Experimental Range during July 1992. Samples were collected from 89 *C. greenii* plants, 39 of them in areas heavily grazed by sheep, 12 in moderately grazed areas and 38 in the control area. Two undamaged stems per plant were measured. A total of 160 individuals of *O. hymenoides* were collected, 40 from each of the four grazing treatment areas, in two separate replicates. *Seriphidium novum* was collected between the end of May and the beginning of June 1993. Seventeen plants were collected from four small fenced grazing exclosures and 20 from the heavily grazed area outside the exclosures. To control for the effects of plant size and age, height, base perimeter, and two orthogonal crown diameters was measured for each plant.

Data analysis

Relationship between internode length and node order

The relation between internode length and node number from bottom to top fits a simple general equation with different species having different coefficients. *Chrysothamnus greenii* and *S. novum* fit the following expression:

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

where N is node number, and k , a and b are fitted constants unique to each plant. Taking natural logarithms of both sides of the equation:

$$\ln(L) = \ln(k) + a \ln(N) - bN \quad (\text{Eqn 2})$$

Stress is predicted to decrease the fit of these equations, so the standard error of the regression, S_{yx} , and the standard error of the parameter a , S_a , are good estimators of developmental instability (Freeman *et al.*, 1993; Graham *et al.*, 1993a). Low R^2 also indicates developmental instability. Regressions were performed separately for each plant and the resulting data were then analysed using mixed model analysis of variance, with herbivory treatments as a fixed effect factor and stem nested with treatments as a random effect factor, since two stems per individual plant were measured. In order to avoid side effects, all the plants were of similar size.

Cumulative frequency exponent

Chrysothamnus greenii has numerous leaves per stem, arranged at increasing distance from bottom to top. The increment of stem length ($\Delta L_{(N)}$) with increasing order from bottom to top fits to a hyperbolic function, where stem length L , with respect to node order N , is given by:

$$\Delta L_{(N)} = kN^D \quad (\text{Eqn 3})$$

This relation has the form of a cumulative frequency equation; using node order, N , as a measure of scale, D therefore fits the definition of a fractal dimension (Hasting & Sugihara, 1993). This equation is similar to the mass-radius relation in lattice models (Avnir, 1990). D can be fitted with a regression analysis of the log transformed data. The error in the curve fitting is also a measure of developmental instability. Consequently, we used the standard error of the regression, S_{yx} , standard error of the slope D , S_D , and one minus the coefficient of determination R^2 , as estimators of developmental instability. To test differences among treatments a mixed model analyses of variance was performed for the scaling parameter D and curve fitting indexes, S_{yx} , S_D and R^2 .

Information dimension (Evenness index)

The Shannon index is a general expression for statistical entropy, and is a measure of the complexity of a system. Seed-to-base pathways from the first order inflorescence branches of *Oryzopsis hymenoides* to the seed base were measured (mm):

$$H = - \sum_{i=1}^N p_i \ln(p_i) \quad (\text{Eqn 4})$$

where, $p_i = x_i / \sum_i x_i$, x_i is the seed-to-base pathway length for the i th seed, and N is the number of seeds. The maximum value of H is obtained when all seeds have equal seed-to-base pathway lengths, $H_{\max} = \ln(N)$. The ratio $H/H_{\max} = J$ is called the evenness (Frontier, 1987; Brooks & Wiley, 1988). It has the form of a fractal dimension, and has been called the information dimension (Schroeder, 1991, p. 221). This dimension measures the loss of information in the dynamic development of a chaotic system. Thus J is an index of order, and is expected to decline under stress. Comparisons among treatments were accomplished as before by mixed model analysis of covariance.

Korcak exponent

The inflorescence of *O. hymenoides* trifurcates into three branches. Each branch point represents a new order or generation. Segment length and diameter decrease, on average, with respect to the preceding order (Durian theory). We calculated the hyperbolic distribution of the scalar relation between branch size, 1, and the number of branches F_1 of length at least 1. By ordering the internode length of the inflorescence in order of magnitude and calculating the cumulative frequency ($F_{(1 \geq L)}$) for categories of branches ranking 5 mm in decreasing order, 5, 10, 15. . . etc, we obtained a hyperbolic distribution for the scaling equation. The relation between number of occurrences (F) and length (1) follows a power law:

$$F_{(1 \geq L)} = k l^{-\beta} \quad (\text{Eqn 5})$$

where β is the Korcak-Mandelbrot exponent (Hastings & Sugihara, 1993; Kaye, 1993). The exponent β represent the heterogeneity of inflorescence branch length. The larger the absolute value of β , the more heterogeneous is branch size. The standard error of the log-log regression (S_{yx}), the standard error of the slope (S_β), and one minus R^2 were used in this study as indices of developmental instability.

Box counting fractal dimension

We examined the fractal dimension of the branching system of *S. novum*. Photographs (slides) were taken of *S. novum* branches during the spring, when the year's new biomass was fully grown. The branches were randomly chosen from among branches that had basal diameters of about 10 mm, allowing us to standardize branch size. The fractal dimension of branch architecture was measured using a box counting method, i.e. by enclosing the projection of the plant in a grid of side A , divided into $(A/\lambda)^2$ squares of size λ . Square size ranged from 0.5 to 32 mm, scaling in geometric progression with ratio equal 2. Let N_λ be the number of squares of side λ needed to cover the object. Then:

$$N_\lambda = b \cdot \lambda^{-D} \quad (\text{Eqn 6})$$

where D is the fractal dimension of the projection of the plant onto a plane. By regressing N_λ against the size λ , after logarithmic transformation, we obtain the slope of the regression (D) (Mandelbrot, 1982). We used an analysis of covariance to determine the significance of grazing treatment on the fractal morphology of the plant, with the volume of the cylinder occupied by the aerial plant part as the covariate.

Results

Chrysothamnus greenii

Internode length

The relation between internode length and node order fits Eqn (1). The significance value of the associated regression was $P < 0.001$ for 93% of the cases (184 vs. 13 where the significance was lower). Averaged over all branches, $L = k \cdot N^{0.7} \cdot e^{-0.002N}$. Figure 1 present scatterplots of one representative case. In this figure we observe a good fit of the curve to the data point. The standard error of the regression (S_{yx}), and the standard error of the slope a (S_a), are estimates of the curve's inaccuracy. Since *C. greenii* is not grazed by sheep, increased grazing should adversely affect competitors, alleviating stress on *C. greenii*. Therefore, the poorest curve fits should characterize low-grazing sites, the best fits the heavily grazed plants. As an inhabitant of disturbed areas, *C. greenii* should experience greatest stress at control sites where grazing is minimum.

Our results show that grazing significantly influenced both the standard error of the regression and the standard error of the slope a ($F_{2,180} = 6.24$, $p < 0.002$ and $F_{2,180} = 7.47$, $p < 0.001$ respectively; see Table 1). They indicate that as grazing increases the standard error of the regression and the standard error of the slope drops, as expected. Differences in R^2 were almost significant ($F_{2,180} = 2.31$, $p < 0.1$).

In *C. greenii* the internode distance increases from the bottom to the top, except at the very top where this pattern is reversed. Thus (Eqn 1), the parameter a is positive and the parameter b is negative, but small, because the effect is not highly apparent.

To determine if the grazing treatments affect internode length, we performed a mixed model analysis of variance on both parameter a and parameter b , with grazing pressure as treatment, and stem nested in treatment. The results indicate that parameter a decreases significantly with increased grazing ($F_{2,180} = 19.26$, $p < 0.0001$), making the internode length smaller (Table 1). The relationship between the parameter b and grazing pressure is also significant ($F_{2,180} = 23.95$, $p < 0.0001$).

Figure 1. Scatterplots of the non-linear, best fit equation $L = 1.10 N^{0.52} e^{-0.03N}$ for internode length (L) against order for one representative case in *Chrysothamnus greenii*. Equation parameters were obtained by least square regression analyses of the linearized equation ($\ln L = \ln k + a \ln N + bN$).

Higher grazing also leads to lower absolute values of the parameter b and thus slower decrease in internode length, particularly at the top of the stem (Table 1). In summary, grazing decreases the rate at which internode distance increase with node order, i.e. decreasing the distance between leaves.

Cummulative frequency

The cummulative frequency exponent represents the intensity of leaf formation; the larger the slope the larger is the increase in stem length with order and the fewer leaves are produced per stem length. In this case fractal dimension indicates the unfilled space between leaves. In consequence the scaling exponent should increase with stress, as observed (Table 2). The error in the curve fitting also varied significantly among the grazing treatments. Plants in heavily-grazed areas had significantly lower standard error of the slope, standard error of the regression and higher regression coefficient than plants inhabiting medium-grazed and control treatments (Table 2).

Oryzopsis hymenoides

Information dimension

Oryzopsis hymenoides is not eaten by sheep during the winter because the above-ground part of the plant is dead. Therefore, *O. hymenoides* should behave like *C. greenii*; heavy grazing alleviates stress from competitors. On the other hand, *O. hymenoides* is adapted to undisturbed conditions, suggesting low stress also in the control area. Light and

Table 1. Mean $\pm$ SE (N) and F values of the mixed model analysis of variance, comparing scale asymmetry parameters (S_{yx} , S_a , and R^2), equation parameters a and b from the leaf arrangement equation $\ln L = \ln k + a \cdot \ln N - b \cdot N$, under three different grazing pressures

	Control	Medium	High	$F_{(2, 180)}$
S_{yx}	0.34 $\pm$ 0.02 (78) ^a	0.29 $\pm$ 0.03 (29) ^{ab}	0.25 $\pm$ 0.01 (90) ^b	6.24**
S_a	0.20 $\pm$ 0.01 (78) ^a	0.18 $\pm$ 0.02 (29) ^{ab}	0.14 $\pm$ 0.01 (90) ^b	7.47***
R^2	0.69 $\pm$ 0.02 (78) ^a	0.62 $\pm$ 0.03 (29) ^a	0.68 $\pm$ 0.02 (90) ^a	2.31
a	0.98 $\pm$ 0.06 (78) ^a	0.65 $\pm$ 0.09 (29) ^b	0.47 $\pm$ 0.05 (90) ^b	19.26***
b	0.05 $\pm$ 0.005 (78) ^a	0.03 $\pm$ 0.008 (29) ^b	0.0008 $\pm$ 0.004 (90) ^c	23.95***

*** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$; no asterisks indicates $p > 0.05$.

^{a-c}Means with the same letters show no significant differences at the 0.05 level, Tukey test.

Table 2. Mean $\pm$ SE (N) and F values of the mixed model analysis of variance, comparing scale asymmetry parameters (S_{yx} , S_D , and R^2), and slope (D) in the cummulative frequency equation ($\Delta L_{(N)} = kN^D$) of *Chroothamnus greenii*

	Control	Medium	High	$F_{(2, 172)}$
D	1.51 $\pm$ 0.02 (70) ^a	1.36 $\pm$ 0.04 (28) ^b	1.37 $\pm$ 0.02 (80) ^b	10.05***
S_{yx}	0.16 $\pm$ 0.01 (70) ^a	0.12 $\pm$ 0.02 (28) ^a	0.09 $\pm$ 0.01 (80) ^b	12.39***
S_D	0.036 $\pm$ 0.003 (70) ^a	0.036 $\pm$ 0.005 (28) ^a	0.02 $\pm$ 0.003 (80) ^b	8.89***
R^2	0.982 $\pm$ 0.002 (70) ^a	0.988 $\pm$ 0.003 (28) ^{ab}	0.993 $\pm$ 0.002 (80) ^b	9.84***

*** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$; no asterisks indicates $p > 0.05$.

^{a-c}Means with the same letters show no significant differences at the 0.05 level, Tukey test.

intermediate grazing might stimulate growth of foraging species which compete with *O. hymenoides* causing stress.

Information dimension is a measure of complexity. Using a mixed model analysis of covariance, we compared the relationship between the J index and grazing intensity. Plant crown diameter was a covariant. The results indicate that grazing significantly impacts J ($F_{3,154} = 3.11$, $p < 0.05$). Significant differences also occurred between the replicates, but since the interaction between grazing and replicate is not significant ($F_{3,151} = 0.556$, NS), we conclude that grazing treatment significantly influences the information dimension. As expected, J increases under high (0.988 ± 0.001 , $N = 40$) and natural (control) (0.987 ± 0.001 , $N = 39$) grazing, and declines under light (0.985 ± 0.001 , $N = 40$) and medium (0.985 ± 0.001 , $N = 41$) grazing.

Korcak exponent

The Korcak exponent is related to the number of ramifications of the inflorescence. A linear regression between the seed number and the β exponent is significant ($R^2 = 0.09$, df. = 155, slope = 0.007, $t = 4.01$, $p = 0.0001$). The larger the number of ramifications, the higher is the β exponent. The β exponent is not significantly related to grazing intensity ($F_{3,149} = 0.273$, NS). Because developmental instability is the degree to which disturbances lead to exaggerated intra-individual variance, the accuracy in fits of the scalar equation may be a measure of developmental stability; under stress conditions S_{yx} and standard error of the slope, S_β , should increase. Results of the two way analysis of variance (Table 3) indicate that grazing significantly affects the standard error of the regression in *O. hymenoides* when size is controlled ($F_{3,149} = 2.584$, $p = 0.05$). The standard error of the slope (S_β) also differs significantly among the grazing treatments ($F_{3,149} = 4.433$, $p = 0.005$), i.e. S_β is lowest in the control and heavy grazing treatments. Differences in R^2 are not significant.

Seriphidium novum

Internode length

The multiple regression of log internode length on node number and log node number (Eqn 2) was performed, and for 78% of the cases the significance value of the associated analysis of variance was $p < 0.05$ (in 13 out of 59 cases the significance was lower). The equation obtained with the mean values over all treatments is $L = k \cdot N^{0.93} \cdot e^{-0.12N}$. A scatterplot of one representative case is presented in Fig. 2. In this figure we also observe a good fit of the curve to the data point.

To determine if grazed and ungrazed plants exhibit different levels of developmental instability, we compared S_{yx} , S_a and R^2 for grazed and ungrazed treatments using a nested analysis of covariance with two stems measured per plant, and mean crown diameter as covariant. The results do not indicate any significant difference for S_a

Table 3. Mean $\pm$ SE (N) and F values of the mixed model analysis of variance, comparing scale asymmetry parameters (S_{yx} , S_p , and R^2), and slope (β) in the cumulative frequency equation ($F_{(l \geq L)} = k l^{-\beta}$) of *Oryzopsis hymenoides*

	Control	Low	Medium	High	$F_{(3, 149)}$
β	1.34 $\pm$ 0.07 (40) ^a	1.42 $\pm$ 0.07 (39) ^a	1.42 $\pm$ 0.07 (40) ^a	1.38 $\pm$ 0.07 (38) ^a	0.27
S_{yx}	0.66 $\pm$ 0.03 (40) ^a	0.72 $\pm$ 0.03 (39) ^{ab}	0.79 $\pm$ 0.03 (40) ^b	0.73 $\pm$ 0.03 (38) ^{ab}	2.58*
S_β	0.035 $\pm$ 0.03 (40) ^a	0.037 $\pm$ 0.02 (39) ^a	0.44 $\pm$ 0.02 (40) ^b	0.36 $\pm$ 0.02 (38) ^a	4.43**

*** $p < 0.0001$; ** $p < 0.001$; * $p < 0.05$; no asterisks indicates $p > 0.05$.

^aMeans with the same letters show no significant differences at the 0.05 level, Tukey test.

($F_{1,54} = 0.25$, NS), nor for R^2 ($F_{1,54} = 0.08$, NS), although a tendency towards larger standard error of the regression (S_{yx}) is observed for ungrazed plants ($F_{1,54} = 2.21$, $p < 0.1$, $Sy_{x_{ungrazed}} = 0.35$, $N = 30$, $SE = 0.02$, $Sy_{x_{grazed}} = 0.31$, $N = 29$, $SE = 0.02$).

To determine if grazing affects the parameter values in the equation, we performed a nested analysis of covariance with mean crown diameter as covariant. The parameter a declines under heavy grazing from 1 ($N = 30$, $SE = 0.08$) in the non-grazed enclosure, to 0.85 ($N = 29$, $SE = 0.08$) with grazing ($F_{1,54} = 3.75$, $p < 0.06$). As in *C. greenii*, the parameter a increases under higher stress conditions, increasing the distance between leaves. No trend is observed for the parameter b ($F_{1,54} = 0.10$, NS).

Fractal dimension

Fractal dimension is a measure of the efficiency with which plants fill space. Plants with many terminal branches should have higher fractal dimension than plants with poorly developed branching. Prior analyses demonstrated that internode length increases with stress. That is, stress produces plants with fewer leaves. Therefore, fractal dimension should decline under stress conditions.

Although the measures of developmental instability reported above did not differ significantly between the grazed and ungrazed treatments, there was a tendency toward higher instability under the ungrazed treatment. Consequently, we do not necessarily expect significant differences in fractal dimension, but if a tendency is observed it must be towards a reduction in dimension under ungrazed treatment. A one-way analysis of covariance produced a $p < 0.1$ level of significance ($F_{1,34} = 2.56$), with mean dimension = 1.54 ($N = 17$, $SE = 0.02$) for ungrazed plants, mean dimension = 1.58 ($N = 20$, $SE = 0.01$) for grazed plants, in keeping with our expectations.

Figure 2. Scatterplots of the non-linear, best fit equation $L = 0.91 N^{1.55} e^{-0.16N}$ for internode length (L) against order for one representative case in *Seriphidium novum*. Equation parameters were obtained by least square regression analyses of the linearized equation ($\ln L = \ln k + a \ln N + bN$).

Discussion

Not all developmental instability measurements are equally sensitive to stress (Soulé & Cuzin-Roudy, 1982; Freeman *et al.*, 1993; Graham *et al.*, 1993a). Sensitivity depends on the degree of canalization (Waddington, 1942), on the non-linear dynamics of development at the molecular level (Emlen *et al.*, 1993), and on non-linear feedback processes regulating allometric growth (Graham *et al.*, 1993b). It would be useful to define the more sensitive indices of stress for use as stress detection tools. Here, we examine the usefulness of several such indices *vis-à-vis* stress from plant competition under grazing pressure.

When studying developmental instability in plants, we should take into account that plants, in contrast to animals, are very plastic organisms. Plasticity, accordingly to Bradshaw (1965), is the ability of an organism to alter its physiology or morphology in response to environmental changes. Its phenotypic differences are consistent in sign among generations and have a genetic origin. In contrast, developmental instability is the random intra-individual variation of a character which is not genetic in origin. It is the result of error during development (Mather, 1953). Developmental stability, on the other hand, is the ability of an organism to buffer environmental and genetic disturbances during development, it has a genetic component but is controlled by a different genetic system, as has been demonstrated in *Arabidopsis thaliana* (L.) Heynh. by Bagchi & Iyama (1983). It is based on the assumption that stabilizing selection maintains a well integrated combination of genes acting to reduce intra-individual variation in repeated structures with identical genetic and environmental influences. In consequence, one can use any phenotypic trait to measure developmental instability provided that one *a priori* knows what phenotype should be produced in the absence of stress. This seemingly impossible task is actually rather simply achieved by examining traits that do not normally vary during development, i.e. developmental invariants (Graham *et al.*, 1993a).

Studies in animals have demonstrated that it is preferable to avoid traits with strong stabilizing selection, for example traits that directly and strongly affect fitness (Soulé & Cuzin-Roudy, 1982; Parsons, 1990). On the contrary, in choosing measures of developmental instability in plants, it is more useful to consider structural details and to select highly canalized characters in order to avoid masking developmental error by high variability.

In this study we observe that grazing clearly affected the intra-individual variability of *C. greenii* and *O. hymenoides*. However, for *S. novum*, there was no difference in the levels of instability between the control and heavily grazed treatments. Species that are either dormant at the time of grazing (*O. hymenoides*) or are not grazed (*C. greenii*) show the lowest level of developmental instability in the most heavily grazed treatments. These results are consistent with most studies of grazing (see Belsky (1986, 1987) for reviews), which show that grazing reduces the abundance of competitors. Thus, unpalatable species are expected to have the highest level of developmental instability in the highly competitive (i.e. ungrazed) treatments and the lowest level of developmental instability in the least competitive (most heavily grazed) environments, as observed. Conversely, palatable species that are dormant during the winter-spring grazing period are expected to, and do exhibit elevated levels of developmental instability under moderate grazing. Natural grazing pressure enhances developmental stability in species habituated to moderate grazing, such as *O. hymenoides*. Seen in this light, the response of *S. novum* is not surprising. We examined this species in only two treatments, an ungrazed control (highly competitive) and a heavily grazed treatment. The failure to find elevated levels of developmental instability in the control *vs.* the heavily grazed treatment does not invalidate the technique, because in fact both habitats are stressful. What differs is the nature of the stress. This points out one of the unique advantages of developmental instability as a measure of stress; it can be used

to resolve the controversy over whether light to moderate grazing enhances the fitness of grazed plants directly (see McNaughton, 1977, 1979, 1983; Collins, 1987; Escós *et al.*, 1997) or, alternately, arises indirectly from a release from competition (see Belsky, 1986, 1987). Our results support the contention that grazing reduces competition.

Efficiency of transport systems in animals can be enhanced by increasing the surface area available for absorption through the intestine, or by proliferation in the branching of blood vessels, bile ducts and bronchial trees (Frontier, 1987; West & Goldberger, 1987; Goldberger *et al.*, 1990). In similar vein, it is expected that increased complexity of branch structure, which can be measured by fractal geometry, enhances energy flow and nutrient cycling in plants. For example, the branching structure of trees may allow sufficient contact between plant and atmosphere for efficient photosynthetic and metabolic gas exchange (Frontier, 1987; Zeide & Pfeifer, 1991). Fractal structure also is a form of symmetry, in this case invariance with scale. The ultimate importance of fractal analysis for developmental instability analysis is not well known (Glenny *et al.*, 1991; Emlen *et al.*, 1993). In this study we observe that the fractal dimension of branch architecture declines as stress increases. In the same way, fractal dimension of the root system in *Zea mays* L. declines under nitrogen stress (Eghball *et al.*, 1993). This result is consistent with changes in the parameter values of the leaf and branch arrangement equations, which lead to increased foliage (shorter internode length) and larger branches under unstressed situations. Similar results are observed in another grazed shrub, *Anthyllis cytisoides* L. (Alados *et al.*, 1994; Escós *et al.*, 1997). The cumulative frequency exponent represents the intensity of leaf formation; the larger the exponent, the longer the internode enhancement and the lower the density of the leaves. In consequence, the cumulative frequency scaling exponent declines with increased disturbance.

The results shown here are important in other ways. Developmental instability is usually assessed as a departure from symmetry. Fluctuating asymmetry, for example, measures departures from bilateral symmetry. There are other forms of symmetry. For example, where internode length or diameter scales with node number, one can translate any point on the regression line to any other simply by using the scaling relationship. Therefore, dispersion about the regression line becomes a measure of the departure from perfect translational symmetry. As noted above, grazing does indeed increase the dispersion about the regression line as predicted. Grazing also changes the allometric relationship between node number and internode length, i.e. the parameters of the regression lines. These changes may occur in conjunction with changes in the dispersion about the line (*C. greenii*), and therefore may be attributed to poor curve fitting. Our results suggest that the allometric relationships themselves also contain information about stress. Thus, it would appear that a combination of both allometric measures and symmetry measures can be used to assess the impact of grazing on the architecture of plants.

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