Fractal structures and fractal functions as disease indicators

J. M. Escós, C. L. Alados and J. M. Emlen

Developmental instability is an early indicator of stress, and has been used to monitor the impacts of human disturbance on natural ecosystems. Here we investigate the use of different measures of developmental instability on two species, green peppers (Capsicum annuum), a plant, and Spanish ibex (Capra pyrenaica), an animal. For green peppers we compared the variance in allometric relationship between control plants, and a treatment group infected with the tomato spotted wilt virus. The results show that infected plants have a greater variance about the allometric regression line than the control plants. We also observed a reduction in complexity of branch structure in green pepper with a viral infection. Box-counting fractal dimension of branch architecture declined under stress infection. We also tested the reduction in complexity of behavioral patterns under stress situations in Spanish ibex (Capra pyrenaica). Fractal dimension of head-lift frequency distribution measures predator detection efficiency. This dimension decreased under stressful conditions, such as advanced pregnancy and parasitic infection. Feeding distribution activities reflect food searching efficiency. Power spectral analysis proves to be the most powerful tool for characterizing fractal behavior, revealing a reduction in complexity of time distribution activity under parasitic infection.

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Stress is an energy dissipative process (Parsons 1993) that consumes energy otherwise available for growth and reproduction. Under stress conditions, environmental fluctuations, together with the lack of enough energy available for homeostatic buffering, lead to increased variation in the development of structures under identical genetic and environmental influence (e.g., left and right sides of bilaterally symmetric organisms). This increased variance is known as developmental instability (Waddington 1957). Many empirical studies have demonstrated that stress increases random variation in normally bilaterally symmetric traits i.e. fluctuating asymmetry (Zakharov 1987, Parsons 1992, Graham et al. 1993, for a review). But bilateral symmetry is only one within-individual developmental invariant. Deviations in radial symmetry or symmetry of scale (Graham et al. 1993, Freeman et al. 1993) are also useful in detecting stress. The arrangement of leaves on a stem often follows a consistent Fibonacci sequence, that optimizes the acquisition of light and resource distribution. Internode length often follows a simple self-similar (or self-affine) sequence where internode order (counted from the stem base) is interpreted as a scaling factor. The error of the curve fitting is a good estimator of the scale asymmetry, defined as error in the repetition of an object at different spatial scales, and thus, of developmental instability (Paxman 1956, Freeman et al. 1993, Alados et al. 1994). A decline in accuracy of the curve fit is expected under stress situations.

But increased variability is not the only consequence of stress. In some structures energy flow as well as environment nutrient exchange is enhanced by fine structure (e.g. of blood vessels, bronchial tree, neurites) (Frontier 1987, West and Goldberger 1987, Goldberger et
Table 1. One way analyses of variance, with curve fitting indexes (Sa, Syx, R²) and regression parameters (k, a and b) as dependent variables and treatment as fixed factor, in the leaf arrangement equations of green pepper.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa</td>
<td>1.37</td>
<td>1.54</td>
<td>31.48</td>
<td>0.000</td>
</tr>
<tr>
<td>Syx</td>
<td>1.36</td>
<td>1.20</td>
<td>152.20</td>
<td>0.000</td>
</tr>
<tr>
<td>R²</td>
<td>1.37</td>
<td>2.92</td>
<td>97.43</td>
<td>0.000</td>
</tr>
<tr>
<td>k</td>
<td>1.37</td>
<td>20.38</td>
<td>19.36</td>
<td>0.000</td>
</tr>
<tr>
<td>a</td>
<td>1.37</td>
<td>35.87</td>
<td>11.74</td>
<td>0.002</td>
</tr>
<tr>
<td>b</td>
<td>1.37</td>
<td>7.77</td>
<td>7.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Means

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa</td>
<td>0.34±0.05 (19)</td>
<td>0.74±0.05 (20)</td>
</tr>
<tr>
<td>Syx</td>
<td>0.18±0.02 (19)</td>
<td>0.52±0.02 (19)</td>
</tr>
<tr>
<td>R²</td>
<td>0.89±0.04 (19)</td>
<td>0.35±0.04 (20)</td>
</tr>
<tr>
<td>k</td>
<td>4.69±0.23 (19)</td>
<td>3.25±0.23 (20)</td>
</tr>
<tr>
<td>a</td>
<td>1.71±0.40 (19)</td>
<td>-0.21±0.39 (20)</td>
</tr>
<tr>
<td>b</td>
<td>-0.61±0.21 (19)</td>
<td>0.16±0.20 (20)</td>
</tr>
</tbody>
</table>

In trees, branch structure allows sufficient contact between the atmosphere and chlorophyll to perform photosynthesis efficiently. The self-similar nature of branches provides a useful way to measure complexity by its fractal dimension. In these structures stress can be expected to reduce complexity. Decreased fractal dimension under stress has been found in previous studies (Alados et al. 1994).

Physiological and behavioral responses, as well as morphological irregularities, can be used to detect stress. A number of diseases are characterized by a loss of the variability associated with fractal structures (West and Golberger 1987, Weinstein et al. 1992, Cross et al. 1993, Boxt et al. 1994) and functions (Waddington et al. 1979, West and Golberger 1987, Golberger et al. 1990, Butler et al. 1993). A loss of physiological variability in a variety of systems appears to be characteristic of the aging process (Waddington et al. 1979, West and Golberger 1987). West (1990) argues that fractal processes are more adaptive to internal and environmental changes than non-fractal ones since they are error tolerant.

Methods

To analyze the effect of viral infection (tomato spotted wilt virus, TSWV) on the scale invariance of leaf arrangement in green pepper, we collected samples from two different populations under plastic protected facilities, in the Mojonera Experimental Station (INIA), Spain. Twenty plants were infected with TSWV virus and 19 plants served as controls. Internode length was measured from bottom to top. Curve fitting accuracy was calculated for each individual plant. The plants fit the equation \( L = k \cdot N^a \cdot e^{-bN} \), where \( L \) is internode length, \( N \) is node order, and \( k, a \) and \( b \) are fitted parameters. After linearizing the equation by log-log regression analyses, the uncertainty of the curve fitting, measured as the standard error of the regression \( S_e \), standard error of the slope \( S_b \) and the coefficient of determination \( R^2 \) are good estimators of the scale asymmetry. Fractal dimension of green pepper was calculated, using the box-counting method (Sugihara and May 1990), from slides taken of well developed plants at the same distance from the side, focusing on the center of the plant.

To study the effect of stress on animals, we analyzed the fractal structure of the time distribution activity while feeding for female Spanish ibex under natural conditions, and under two stressful situations: advanced pregnancy and parasitic infection by Sarcopset scabiei. We expect that behavioral feeding patterns vary in relation to energy available for maintenance. Feeding occurs in temporal bouts of different length, separated by gaps of different duration. When the animal interrupts its feeding to lift its head for just a moment, a head lift pattern is recorded. Number of head lifts per observation minute, on one hand, and feeding bout length and interfeding time gap duration in seconds, on the other, were collected from focal females of Spanish ibex from the south of Spain during spring. A total of 22 female Spanish ibex were observed at Cazorla and Segura Mountains during May 1984. Each female was observed for at least 30 min, and the number of head-lifts per min was noted. Twelve females showed advanced pregnancy and 10 manifested no sign of pregnancy.

During May 1994 a total of 32 females, 22 healthy and 10 sick, parasitized by Sarcopset scabiei, were observed at Sierra Nevada. The infection started because of domestic goat transmission to the infected population, but no ecological differences exist between the healthy and sick females. This allowed us to compare sick vs healthy females under identical ecological and physiological circumstances. Focal sampling for continuous periods of time during feeding activity was recorded by the researcher on a Psion Organizer computer with automatic time recording. Each time the animal activity changed was registered in the computer. Subsequently the data was converted into samples at one-second intervals.

The regression between cumulative frequency of head-lifts per minute \( F (\Delta t) \) and time interval \( (\Delta t) \) after log-log transformation, yields the dimension, \( D \) (Hasting and Sugihara 1993) from the equation \( F (\Delta t) = k \cdot (\Delta t)^D \). The Mandelbrot-Weierstrass fractal exponent (Barry and Lewis 1980, Mandelbrot 1982) was calculated by applying the Fast Fourier Transform algorithm to the time series of the binary processes (feed = 1, lag = 0) over discrete intervals of one-second length. Time distribution activity was recorded for at least 1024 s, and no longer than 4096 s. The power spectrum of the FFT function is the square of the amplitude and is inversely related with the frequency \( E (f) = k \cdot f^D \), where \( E (f) = power \)
Table 2. One way analysis of covariance with the fractal dimension \( D \) from the head lift frequency equation \( F(\Delta t) = k \cdot (\Delta t)^\beta \), as dependent variable and phase of the reproductive cycle as fixed factor. Observation time is included as a covariant. Means, standard error and number are also included.

<table>
<thead>
<tr>
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<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repr. cycle minutes</td>
<td>1</td>
<td>0.415</td>
<td>8.231</td>
<td>0.01</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>0.951</td>
<td>5.007</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Means

Pregnant 0.896(12)±0.07
Not pregnant 1.184(10)±0.07

The spectrum at frequency \( f \), \( k \) is a constant and \( \beta \) is the Mandelbrot-Weierstrass exponent.

Results and discussion

We analyzed the effect of viral infection on the scale invariance of the leaf arrangement equation in green pepper, by comparing an infected and an uninfected population in plastic protected facilities. The results indicate that the infected population had higher developmental instability than the non-infected population. That is, the standard error of the regression \( S_{by} \) and the standard error of the parameter \( a \), \( S_a \), are significantly higher in the infected population, while the regression coefficient \( R^2 \) declines (Table 1).

So, in plants, because of their iterate modular nature, stress can be measured by the inconsistency of the geometrical relations between the plant’s parts (Alados et al. 1994). Moreover, since stresses cause the plant to mobilize its resources and to reduce the amount of energy available for growth, we expect a reduction in the internode length with order. In consequence, the slope \( a \) from the equation \( L = k \cdot N^a \cdot e^{-58} \) significantly decreased in the diseased plants.

Plants develop fractal structures. The self-similar nature of branches provides a useful way to measure complexity by its fractal dimension. Branch structure of plants allows sufficient contact between the atmosphere and the chlorophyll to perform photosynthesis efficiently. We observed that healthy plants have larger fractal dimensions \( (D = 1.65, se = 0.01, n = 20) \) than infected plants \( (D = 1.59, se = 0.01, n = 19) \). The difference was significant when tested by one-way analyses of variance \( (F_{1,38} = 8.42, P < 0.01) \).

To detect stress in wild animals, we analyzed the fractal structure of the time distribution activity, while feeding, for female Spanish ibex. Animals need to compromise in their use of time (or energy), between vigilance and feeding activities. If we consider the amount of energy intake of an organism to be fixed, the allocation of energy consumption into one component is necessarily at the expense of others.

We computed the fractal dimension, \( D \), of the cumulative frequency of head lift over time intervals of duration \( \Delta t \). The head-lift frequency \( F(\Delta t) \) scales as: \( F(\Delta t) = k \cdot (\Delta t)^\beta \), where the scaling exponent measures the rate of head-lift frequency with the time interval. Comparisons between females with advanced pregnancy against non-pregnant females show a significant reduction in the fractal dimension (Table 2). When we compare females parasitized by Sarcoptes scabiei with apparently healthy females, we also observed a significant reduction in the fractal dimension with illness \( (F_{1.39} = 5.468, P = 0.026) \), mean value is \( 1.146 \pm 0.05, n = 22 \) for healthy females, \( 0.936 \pm 0.074, n = 10 \) for sick females).

Food is consumed over the course of walking, alternating searching time with feeding bouts. The distribution of time between feeding bouts and the time gap represents the searching efficiency, especially during feeding hours in spring, when animals are mostly feeding in the same

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**Fig. 1.** Natural logarithm of the power spectrum against the natural logarithm of the frequency of time distribution feeding activity in healthy and unhealthy female Spanish ibex.
such or looking for adequate patches. In consequence we expect the complexity in the time distribution of feeding activity to be higher when animals are in better condition, giving them higher success in searching for food.

Fig. 1 shows the log-log relation between the power spectrum of the time distribution of feeding activity and frequency for healthy and unhealthy females. Our data show a highly significant effect of poor health on the exponent, 0 = 15.544, P < 0.0001, with ß lower in females (mean = 3.57 ± 0.024, n = 20) than in males with scabiosis (mean = 1.52 ± 0.034, n = 10).

Differences in the ß exponent in relation to the observation time were not significant (Wilcoxon test z = 0.169, P = 0.866). Approximately the same mean values were observed when only sequences of 2048 s were included, ß = 1.329 ± 0.036, n = 12, for healthy females; mean ß = 1.2 ± 0.05, n = 6, for unhealthy females; 0 = 0.1, P = 0.01).

The relation between ß and the fractal dimension D is discussed (Anguiano et al. 1994), but in all cases it is closely related, that is, the fractal dimension decreases under stress.

Previous authors have used fractal dimension of walking paths to measure searching efficiency in terms of utilization rather than in terms of encounter rate (Novak et al. 1992, Bundy et al. 1993). The fact that the fractal dimension of particular structures is intrinsically linked (J. and Iannaccone 1992 in liver tissue, Smith et al. 1993 in neurons), is not altered after its formation in the renal arterial tree (Cross et al. 1994), and not vary between taxonomic groups (Smith et al. 1993 in cerebellar Purkinje cells, Garcia-Ruiz et al. 1990 in monite suture) makes fractal dimension a relevant indicator. Crist et al. (1992) observed that fractal analysis of pathway structure in different beetle species does not change over a broad range of microlandscape diversity.

Currently, literature is giving enough evidence of a role in the fractal dimension of structures. For example, fractal dimension of the pulmonary arteries is from 1.62 to 1.6 in hypoxic lungs and to 1.44 in hypoxic lungs (Boxt et al. 1994). Renal arterial trees reduced fractal dimension in congenitally abnormal kidneys (Cross et al. 1993). Fractal dimension of botic sutureless in comparison with normal (Feinstein et al. 1992). In horned ungulates, where some has evolved structures to absorb the shock of encounters, the complexity of the sagittal suture bone separation. The greater the fractal dimension the frontal suture the more pronounced is its effect (Long 1985). Under stress situations due to changes, fractal dimension of the skull sagittal suture Dama gazelle (Gazella dama) suture (Alados and Frias). In plants, fractal dimension of branch architecture with grazing stress (Alados et al. 1994).

In conclusion, we observe, on one hand, an increase in a error reducing stress where homeostasis is maintained because consistent relations between organ-isms parts are adaptive. This was observed in the case of plant leaf arrangement. On the other hand, where complexity is an advantage because it promotes increased efficiency (West and Goldberger 1987) in interchange with the environment, or because complexity buffers environmental variability (West 1990, Shinbrot et al. 1993), a reduction in complexity should accompany stress. That expectation was borne out in the fractal dimension of branch architecture and in head-lift and feeding bout distributions.

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