Effects of generalist and specialist parasitic plants (Loranthaceae) on the fluctuating asymmetry patterns of ruprestrian host plants

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Abstract

Leaf fluctuating asymmetry (FA) is an important indicator of plant responses to various sources of stress, which range from poor nutritional and harsh environmental conditions to herbivory and parasitism. Mistletoes are important hemiparasites, which represent a source of stress for their host plants but, in spite of being common in all major biogeographical regions of the world, no study has measured the response of their hosts in terms of leaf FA. We addressed the effect of mistletoes on host–plant foliar FA by comparing parasitized and non-parasitized individuals of four host species in two sites in southeastern Brazil, Serra do Cipó and Serra do Rola Moça. In the four host species, parasitized individuals presented significantly higher levels of leaf FA than unattacked plants. The specialist mistletoe \textit{Phoradendron cf. amplexicaule} induced higher levels of FA on its host \textit{Erythroxylum suberosum} (Erythroxylaceae) than the generalist mistletoe \textit{Struthanthus flexicaulis} did on either \textit{Mimosa calodendron} (Fabaceae), \textit{Lychnophora pinaster} (Asteraceae) or \textit{Stachytarpheta glabra} (Verbenaceae). We hypothesized that specialized parasites could be more damaging to their hosts than generalist mistletoes, which could explain the higher FA levels observed. Significant differences in FA levels among parasitized individuals within species were found, which were significantly correlated with intensity of infection (number of mistletoes per host individual). The results indicate that mistletoes represent an important factor of stress for their host plants, and that FA can be an excellent instrument for evaluating the effects of infections by parasitic plants.

Zusammenfassung

Die fluktuierende Asymmetrie (FA) von Blättern ist ein wichtiger Indikator für pflanzliche Reaktionen auf verschiedene Stressfaktoren, die von schlechter Nährstoffversorgung und extremen abiotischen Umweltbedingungen bis zu Herbivorie und Parasitierung reichen. Mistelkraut sind wichtige Hemiparasiten, die einen potentiellen Stressfaktor für ihre Wirtspflanzen darstellen, aber, obwohl Misteln in allen größeren bedeutenden biogeographischen Regionen der Welt angetroffen werden, hat bisher keine Untersuchung die Reaktion der Wirt in Form von FA gemessen. Wir behandelten den Effekt von Misteln auf die FA bei Blättern von Wirtswurzeln, indem wir parasitierte und nicht parasitierte Individuen von vier Wirtsarten an zwei Standorten im Südosten Brasiliens (Serra do Cipó and Serra do Rola Moça) untersuchten.

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Bei den vier Wirtsarten zeigten parasitierte Pflanzen signifikant höhere Blatt-FA als nicht parasitierte Pflanzen. Die spezialisierte Mistel Phoradendron cf. Amplexicaule induzierte stärkere FA an ihrem Wirt Erythroxylum suberosum (Erythroxylaceae) als die generalistische Mistel Struthanthus flexicaulis an ihren Wirten Mimosa calodendron (Fabaceae), Lycnophora pinaster (Asteraceae) und Stachytarpheta glabra (Verbenaceae). Wir vermuten, dass spezialisierte Parasiten schädlicher für ihre Wirte sein könnten als generalistische Misteln, was die beobachteten höheren FA-Werte erklären würde.


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Introduction

Most organisms exhibit developmental instability during ontogenetic stages as a result of environmental disturbances caused by both abiotic (i.e. soil fertility, extreme climatic conditions, pollution, urbanization, salinity, temperature, irradiation) (Kozlov, Wilsey, Koricheva, & Haukojoa 1996; Freeman, Graham, Tracy, Emlen, & Alados 1999; Hohen, Blanckenhorn, & Ward 2000; Valkama & Kozlov 2001; Cornelissen, Stiling, & Drake 2003; Jentsch, Kohler, & Schumacher 2003; Weller & Ganzhorn 2004) and biotic factors (i.e. competition, herbivory, parasitism) (Reimchen 1997; Rettig, Fuller, & Getty 1997; Cárcamo, Floate, Lee, Beres, & Clarke 2008). One of the best measures of developmental instability is fluctuating asymmetry (FA) that is defined as random morphological differences in size or shape between the two sides of bilateral characters (Møller & Shykoff 1999). Since both sides of morphological traits are controlled by the same set of genes, the degree of FA reflects the inability of individuals to maintain homeostasis during development under stress conditions (Palmer 1996; Møller & Swaddle 1997). Therefore, FA is considered as a reliable indicator of environmentally induced stress in organisms and has been evaluated in different groups such birds (Hansen, Amundsen & Forsgren 1999; Anciles & Marini 2000), mammals (Wauters, Dhondt, Knothe, & Parkin 1996; Wauters, Dhondt, & Dhondt 1997; Allenbach, Sullivan, & Lydy 1999), amphibians (Söderman, van Dongen, P亢kesmaa, & Merila 2007), insects (Bronies & Guinéz 2005), humans (Rikowski & Grammer 1999) and plants (Møller & Shykoff 1999; Leamy & Klingenberg 2005).

Parasites impose a multitude of stresses to their hosts and while a few studies have examined how parasitism influences FA levels in animals (e.g., Fernandes, Mattos, Franco, Lütte, & Ziegler 1998; Møller & Shykoff 1999), the effects of parasitic plants on FA levels of their host plants practically have not been analyzed. Lorantaceous mistletoes photosynthesize all or most of their carbohydrates and obtain water and nutrients from the host xylem (i.e. hemiparasitism) (Calder 1983; Marshall & Ehleringer 1990). These plants represent a main group of parasitic species that colonize branches and roots of tropical and temperate host plants (Thorne 1992; Calvin & Wilson 2006). Compared with holoparasitic plants, lorantaceous mistletoes are in general not considered pests in natural and agricultural systems. Some studies have indicated that they have negative and sublethal effects on their host plants (Cameron et al. 2005; Press & Phoenix 2005) but only in few cases may cause the death of the host plant (Reid, Yan, & Fittler 1994).

Mistletoes have a broad variation in the degree of specificity on their host plants (Pennings & Callaway 2002; Westbury 2004). Spatial distribution and architecture of host plants, environmental conditions and feeding behaviour by dispersing animals have been proposed as explaining factors of the differences in mistletoe host specificity (García-Franco & Rico-Gray 1996; Martínez del Río, Silva, Medel, & Hourdequin 1996; Arruda, Carvalho & Del Claro 2006).

Parasitism by mistletoes has been documented to reduce host growth, photosynthesis and respiration rates, and also to affect host architecture, and water and nutrient balance (Wanner & Tinnin 1986; Tennakoon & Pate 1996; Meinzé, Woodruff & Shaw 2004). Also, in some cases, crown deformation and tree death affect plant composition and biotic interactions, modifying the community structure (Pennings & Callaway 2002; Press & Phoenix 2005; Reblin, Logan, & Tissue 2006). Therefore, mistletoe parasitism probably represents an important factor of stress for host plants (Lütte et al. 1998; Schwartz et al. 2003) and consequently may produce instability during the development of organs such as leaves (Press & Phoenix 2005). In this study, we evaluated the effects of both specialist and generalist parasitic mistletoes on the fluctuating asymmetry patterns of four rupestrian host plant species. We expected foliar FA to be higher in infected hosts and to increase with the intensity of the infection. This is a first empirical study evaluating the patterns of foliar FA in relation to ecological interactions between plants in rupestrian fields. We addressed the following questions: (i) what is the degree of FA in parasitized and nonparasitized rupestrian host plant species? (ii) Is there variation in the levels of FA within each host plant species and is it related to the intensity of the infection?
Materials and methods

This study was conducted in two different ruprestrian field regions in the Espinhaço mountain chain in southeastern Brazil. Ruprestrian fields are the only exclusively Brazilian biome where highly sclerophyllous species predominate and exhibit a rich biological diversity and endemism. This vegetation is under a severe habitat destruction caused primarily by mining activities (Jacobi & Carmo 2008). The first study area was in Serra do Cipó (19º10’–20º’S, 43º30’–40º’W). This region is above 1000 m in elevation and supports ruprestrian fields characterized by the presence of sclerophyllous shrubs (Madeira & Fernandes 1999). The climate is characterized by dry winters and rainy summers, with an annual mean temperature of 21 ºC and average annual precipitation of 1500 mm (Menezes & Giulietti 2002). This site can be considered relatively well conserved. The second area of study was in Serra do Rola Moça State Park, located in the southern portion of Espinhaço Mountains (20º03’60”S, 44º02’00”W). The elevation of this site ranges between 800 and 1200 m, the average annual temperature is 25 ºC and the mean annual precipitation is 1300 mm (Vincent 2004). The predominant vegetation is sclerophyllous shrubs and the most representative plant families are Poaceae, Cyperaceae, Velloziaceae, Fabaceae, Asteraceae and Rubiaceae (Vincent 2004). This site is characterized by a higher degree of human-induced perturbation.

Study system

Erythroxylum suberosum (Erythroxylaceae) occurs in Serra do Cipó and is a perennial species that varies from shrubs to treelets, characteristic of cerrado and ruprestrian fields. This plant species is parasitized by Phoradendron cf. amplexicaule Eichl. (Loranthaceae), which is a specialist hemiparasitic shrub typical of cerrado (Kuijt 1994). Mimosa calodendron Mart. (Fabaceae), Stachytarpheta glabra (Verbenaceae) and Lychnopora pinaster (Asteraceae) are perennial shrubs typical of ruprestrian fields and occur in Serra do Rola Moça State Park. These host plant species are frequently attacked by the mistletoe Struthanthus flexicaulis (Loranthaceae), a generalist parasitic plant in Brazil (Arruda et al. 2006).

Sampling design and measurement of FA

In order to estimate the effects of parasitic plant species on the patterns of leaf FA in each of the four host plant species (E. suberosum, M. calodendron, S. glabra, and L. pinaster), we first determined the proportion of parasitized hosts of each species at the two sites and then randomly selected 15 host plants parasitized by mistletoes and 15 unparasitized. From each individual host plant we collected 50 fully expanded leaves.

A digital image was taken of each leaf and FA was calculated as the absolute value of the difference between the distances from the midvein to the right and left-side leaf margin (Ai–Bi), divided by the average distance (Ai + Bi/2), to correct for the fact that asymmetry may be size-dependent (Cornelissen & Stiling 2005). A mean FA value was obtained for each individual from the values of the 50 leaves measured. Measurement error was assessed by re-measuring a random subsample of a total of 25 leaves from various individuals, without reference to previous measurements. Then, the significance of FA relative to measurement error was estimated using a two-way mixed-model ANOVA considering as factors individual, leaf (random) and side (left or right). Both measurements were considered as replicates (Palmer & Strobeck 2003). The significance of the interaction (individual × leaf × side) indicated that variation in FA was greater than expected by measurements error (F24, 50 = 17.2; P < 0.0001).

Statistical analysis

A generalized linear model applying the GENMOD procedure (SAS 2000) was conducted to analyze foliar FA patterns between host plants in each condition (with and without parasitic plants). The model used host plant species and condition as the independent variables. Mean foliar FA per individual was used as the dependent variable. An LSMeans test was used for a posteriori comparisons. Because the scale and distribution of these variables did not follow a normal distribution, a Poisson distribution was used for the analysis of foliar FA.

To test if foliar FA caused by parasitic plants was different between individuals of each host plant species, we used a generalized linear model applying the GENMOD procedure (SAS 2000). Individuals were considered as the independent variable and FA values of the 50 leaves measured per individual were used as the dependent variable. LSMeans test were used for a posteriori comparisons between plant species (P < 0.05). Finally, linear regression analyses were used to determine the relationship between the number of mistletoes per host plant and foliar FA, in each host plant species.

Results

The results of the analysis using the GENMOD procedure indicated a significant effect of plant species, condition (with or without mistletoes), and the interaction between species and condition, on foliar FA values. In the four species studied, individuals parasitized by mistletoes presented higher leaf FA values (E. suberosum: 0.13 ± 0.005; M. calodendron: 0.1 ± 0.003; L. pinaster: 0.09 ± 0.005 and S. glabra: 0.11 ± 0.002) than non-parasitized individuals (E. suberosum: 0.05 ± 0.006; M. calodendron: 0.04 ± 0.003; L. pinaster: 0.04 ± 0.001; and S. glabra: 0.04 ± 0.002)
Fig. 1. Comparison of foliar fluctuating asymmetry between the four host plant species and between parasitized and non-parasitized individuals. Host species infected by the specialist mistletoe (*Phoradendron cf. amplexicaule*) and by the generalist mistletoe (*Struthanthus flexicaulis*) are indicated. Values with the same letter did not differ significantly after a LSMeans multiple comparison test (*P* < 0.05).

(conditions: $\chi^2 = 231.7$; d.f. = 1; *P* < 0.0001) (Fig. 1). Also, while non-parasitized individuals of the four species displayed similar leaf FA values, highly significant differences were found among species for parasitized individuals (species: $\chi^2 = 20.76$; d.f. = 3; *P* < 0.001, and interaction: $\chi^2 = 48.36$; d.f. = 3; *P* < 0.0001) (Fig. 1). The highest FA values were found for *E. suberosum* individuals parasitized by *P. cf. amplexicaule*, the specialist mistletoe.

In addition, we found significant variation in leaf FA among individuals of *E. suberosum* ($\chi^2 = 28.2$; d.f. = 27; *P* < 0.0001), *M. calodendron* ($\chi^2 = 40.2$; d.f. = 29; *P* < 0.0001), *L. pinaster* ($\chi^2 = 19.4$; d.f. = 29; *P* < 0.0001), and *S. glabra* ($\chi^2 = 25.8$; d.f. = 29; *P* < 0.0001) (Fig. 1). Finally, we found a positive relationship between the number of mistletoes per host and mean FA values (*E. suberosum* $R^2 = 0.87$; $F = 68.1$; *P* < 0.0001; *M. calodendron* $R^2 = 0.82$; $F = 60.3$; *P* < 0.0001; *L. pinaster* $R^2 = 0.72$; $F = 39.3$; *P* < 0.0001 and *S. glabra* $R^2 = 0.87$; $F = 88.3$; *P* < 0.0001) (Fig. 3).

Discussion

Plant species experiencing environmentally induced stress such as poor nutritional conditions, extreme temperatures and radiation, high CO2 concentration, pollution, herbivory and parasitism usually show increased FA levels (Hardersen...
In conclusion, the results of this study indicate that mistletoes represent an important factor of stress for their host plants, and that FA can be an excellent instrument for evaluating the effects of infections by parasitic plants in a wide range of habitats and conditions.
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