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# Fluctuating asymmetry of and herbivory on *Poincianella pyramidalis* (Tul.) L.P. Queiroz (Fabaceae) in pasture and secondary tropical dry forest

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## ABSTRACT

Plants subjected to stressful environments tend to be more asymmetric with reduced defenses and are therefore more vulnerable to herbivory. This study investigates the relationship between herbivory and fluctuating asymmetry (FA) in *Poincianella pyramidalis* in two contrasting habitat types in the Brazilian *caatinga* (shrublands). We tested the following hypotheses: that FA occurs in *P. pyramidalis*; that FA of *P. pyramidalis* leaves is greater in individuals located in pasture than in those located in secondary tropical dry forest; that herbivory by insects (leaf chewers and leaf miners) increases in parallel with increases in the level of FA; and that herbivory is more common in pasture than in secondary tropical dry forest. In each of the two environments, we sampled 20 plants and evaluated 400 leaflets. We submitted FA data to the Shapiro-Wilk test of normality, and we investigated the other variables using generalized linear models. We found that FA was present in all *P. pyramidalis* individuals evaluated but was greater in those located in the more degraded habitat (pasture). In addition, although herbivory was similar between the two habitats, there was positive relationship between FA and herbivory. This indicates that herbivores select plants that are more asymmetric, regardless of the type of habitat involved, which might be attributable to the mechanisms posited in the plant stress hypothesis.

**Key words**: *caatinga*, forest regeneration, leaf miners, plant-insect interactions, tropical dry forests

## Introduction

Some conditions of stress (e.g., drought, pollution, and nutritional deficiency) can alter the characteristics of plants and thus their chances of survival (Cornelissen & Stiling 2005; Larcher 2006; Torrez-Terzo & Pagliosa 2007). When such conditions reach the adjustment threshold of organisms, certain disturbances, including small random variations in bilateral symmetry in symmetrical characters, collectively known as fluctuating asymmetry (FA), are manifested (Palmer & Strobeck 1986; Leung *et al*. 2000; Cornelissen & Stiling 2005). Therefore, FA reflects environmental instability caused by various stressors and has consequently been considered a reliable indicator of environmental stress in different plant species (Hagen *et al*. 2008; Cornelissen & Stiling 2011).

In some systems, it has been found that FA is greatest in plants under stress (Leung *et al*. 2000; Torrez-Terzo & Pagliosa 2007), although there have been inconsistent findings across studies (Graham *et al*. 2010, Costa *et al*. 2012). That inconsistency is primarily attributable to two different factors. First, plants that suffer stress (and are therefore more likely to be asymmetric) appear to be a better food source because they provide greater availability of soluble nitrogen and have lower concentrations of defense compounds (Cornelissen & Stiling 2005), making them more susceptible to herbivory (White 1969). In contrast, vigorous plants should be the best source of resources due to higher food quality and a lack of induced defensive compounds, as stated by Price (1991). That author postulated that, in vigorous plants, herbivore fitness should be higher than in less vigorous plants, as well as that insect herbivores whose larval development is associated with host-plant growth processes should prefer to attack the most vigorous plants or plant modules, where the subsequent larval performance would be the highest.

The positive correlation between FA and herbivory indicates that plants with FA are more prone to herbivory

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than are symmetric plants but also indicates that herbivory itself acts as a stressor and directly increases the level of FA (Zvereva *et al.* 1997; Moller & Shykoff 1999; Cornelissen & Stiling 2005). However, this correlation is not always causal, because the chemical and nutritional differences between symmetric and asymmetric leaves probably influence leaf selection by herbivores (Lempa *et al*. 2000).

The various aspects of FA in plants have been studied in different environments, such as coastal areas, estuaries, tropical dry forests, rain forests, neotropical savannas, and rupicolous habitats (Torrez-Terzo & Pagliosa 2007; Constantino *et al*. 2009; Graham *et al*. 2010; De Sibio & Rossi 2012). Most such studies have shown that plant asymmetry is greater in disturbed areas. However, studies involving the effects of habitat type on FA in semi-arid environments are scarce and relatively recent (Cuevas-Reyes *et al*. 2011; Costa *et al*. 2012) and there have as yet been no studies involving the plant life of the Brazilian *caatinga* (shrublands). In *caatinga* environments, stressors such as long periods of drought affect the vegetation as a whole and can thus increase FA. These effects may be more severe on plants in areas with a recent history of human disturbances, such as abandoned pastures, than in areas with advanced regeneration, such as secondary forests.

The aim of the present study was to investigate the relationship between herbivory and FA in *Poincianella pyramidalis* (Tul.) L. P. Queiroz (Fabaceae) in two contrasting habitats: an abandoned pasture and a fragment of secondary tropical dry forest. We tested the following hypotheses: that FA occurs in *P. pyramidalis*; that the level of FA in *P. pyramidalis* varies under distinct abiotic conditions, being higher in plants located in abandoned pastures than in those located in secondary tropical dry forest; that herbivory of *P. pyramidalis* by insects (leaf chewers and leaf miners) increases in parallel with increases in the level of FA; that the number of *P. pyramidalis* leaves damaged by insect herbivores is greater in pasture than in secondary tropical dry forest.

## Material and methods

### *Study area*

This study was conducted in the *Monumento Natural Grota do Angico* (MNGA), one of many specially protected areas in Brazil, where such areas are referred to as "conservation units". The MNGA is located in northeastern Brazil (09°39'S; 37°40'W), in the state of Sergipe, between the municipalities of Poço Redondo and Canindé de São Francisco. The study site comprised lands situated over sedimentary basins, encompassing the mountains, plateau, and ravines that surround the São Francisco River in this region (IBGE 2010), featuring fragments of secondary tropical dry forest in various stages of succession, surrounded by a matrix of active and abandoned pastures (Souza *et al.* 2003). The vegetation types found in the MNGA match the physiognomic forms of the *caatinga*, which range from seasonally tropical dry forest vegetation (sensu Andrade-Lima 1981; Pennington *et al.* 2000) to scrub vegetation (Sampaio 1995). Throughout the *caatinga* domain (736,000 km$^2$), the climate is classified as tropical semi-arid (Bsh in the Köppen climate classification), with annual rainfall of 250-900 mm and a mean temperature of 24-26°C (Andrade-Lima 1981).

### *Sampling*

The arboreal species *P. pyramidalis* (Fabaceae), also known by its common name in Portuguese, *catingueira*, is endemic to and widely distributed throughout the *caatinga* biome, occurring in the states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, and Sergipe, as well as in the northern part of the state of Minas Gerais (Andrade-Lima 1981; Silva *et al*. 2009). The species is widely used by local communities in folk medicine (Albuquerque *et al.* 2007), for firewood, and for the production of charcoal (Silva *et al.* 2009). Its leaves are compound, with 5-11 leaflets, and the adult plants flower for four months during the rainy season.

We sampled leaves of *P. pyramidalis* within two areas (of 2 ha each) that differed in terms of abiotic conditions (such as the richness and abundance of tree species, as well as the type of land use) and states of regeneration. The first area was pasture that had been abandoned two years prior, and the second area was a secondary tropical dry forest that had been in a state of regeneration for approximately 10 years. In each area, we established a linear transect of 100 m, along which we sampled 20 trees, all with a diameter at breast height > 6 cm. The average distance between trees in each transect was approximately 5 m. The two sites were within approximately 1 km of each other.

From each of the trees samples, we collected ten compound leaves (the first two compound leaves at the base of five branches per tree). We then randomly selected ten leaflets from each of the leaves collected, which gave us 400 leaflets to be analyzed. Leaflets were identified, dried at 48°C, and then photographed with a digital camera (Kodak EasyShare C182; Eastman Kodak, Rochester, NY, USA), at a resolution of 10 megapixels. The area of each leaflet was measured by analysis of digital images with the ImageJ program, version 1.44 (National Institutes of Health, Bethesda, MD, USA).

### *Data analysis*

***FA*** - Leaf FA was calculated as the absolute value of right-side distance from the midvein to the leaf margin minus the left-side distance from the midvein to the leaf margin, following two formulas (Graham *et al.* 2010; Cornelissen & Stiling 2011):

$$\text{Index 1} = [\Sigma|R_i - L_i|]/N$$

$$\text{Index 2} = [\Sigma|R_i - L_i|/(\breve{Y})]/N$$

where $R_i$ and $L_i$ are the right- and left-side distances, respectively, $N$ is the total number of samples, and $\breve{Y}$ is the mean value ($[R_i + L_i] / 2$) for each leaflet. The first index measures the absolute asymmetry, whereas the second corrects for the fact that larger leaves have greater asymmetry (Cornelissen & Stiling 2011). Therefore, Index 1 could be used if the leaflet area were similar between the sites studied; otherwise Index 2 would be more appropriate. All leaflets with damage that could affect FA analysis were excluded from the calculations.

Leaf characters are considered to exhibit FA if signed right-minus-left values are normally distributed with a mean value of zero, reflecting randomly directed deviations from the optimal symmetric phenotype. We determined the normality of the FA data using the Shapiro-Wilk test, testing the null hypothesis that the values of FA did not differ from a normal distribution, or p>0.05 (Palmer & Strobeck 1986; Cornelissen & Stiling 2011).

***Herbivory levels*** - To determine the herbivory levels, we use the same 400 leaflets used to calculate FA (n = 200 habitat$^{-1}$). The insect herbivores of *P. pyramidalis* were leaf chewers and leaf miners. To estimate the leaf area lost to herbivory, we analyzed the digital images using the ImageJ software. Leaflets in which the damage (i.e., small bite marks or signs of insect herbivory) could not be identified were discarded and another leaflet was randomly selected from the same plant.

We used generalized linear models to compare FA (Index 1 or 2, explanatory variable) between trees in abandoned pasture and those in secondary *caatinga* (response variable) as well as to evaluate the effect of FA (explanatory variable) on the loss of leaf area (%), the number of mines, and the number of injuries (response variables). We also used generalized linear models to compare leaf damage between tree individuals in pasture and those in secondary *caatinga*. In addition, leaflet size was included as a explanatory variable (covariate) in the model. All statistical analyses were performed with the program R, version 2.13.1 (R Development Core Team, 2010).

# Results

We identified FA in leaves of *P. pyramidalis*, as demonstrated by the normal distribution of the signed right-minus-left values (Shapiro-Wilk test; W=0.9958, p=0.374), which did not deviate significantly from a mean of zero.

In general, the type of habitat had an influence on FA, as well as on the number of mines, number of injuries, and percentage of leaf loss (Tab. 1). However, only FA values differed significantly between pasture and secondary tropical dry forest. Therefore, the second hypothesis was confirmed, because plants in abandoned pasture were more asymmetric than were those in secondary tropical dry forest (F=17.06; p<0.01; Tab. 1). However, there was no difference in the size of leaflets between plants of the two habitat types (F=3.7; p>0.05), indicating that Index 1 can be used as a proxy for FA.

The hypothesis that herbivory of *P. pyramidalis* by insects (leaf chewers and leaf miners) increases in parallel with increases in the level of FA was confirmed by our finding that FA correlated positively with the number of mines (F=6.8; p=0.013), as shown in Figure 1a, as well as with the number of injuries caused by leaf chewers (F=10.39; p=0.02), as shown in Figure 1b. The number of mines, number of injuries, and the proportion of leaf lost, however, did not differ between plants in abandoned pasture and those in secondary tropical dry forest (Tab. 1).

# Discussion

In this study, we have demonstrated that *P. pyramidalis* shows a pattern of FA in its leaflets and that this pattern differs between habitats differing in their structural complexity, such as abandoned pasture and secondary tropical dry forest. In addition, in these habitat types, plants that present higher levels of FA are more prone to suffer leaf damage from insect herbivores such as leaf chewers and leaf miners.

Previous studies have demonstrated the occurrence of FA in other woody species in various environments, such as coastal areas (Cornelissen & Stiling 2011), tropical dry forests (Cuevas-Reyes *et al*. 2011) and mangroves (Torrez-

**Table 1.** Comparative values of FA, number of mines, number of injuries from leaf chewers, and leaf area lost in 20 plants in the Brazilian *caatinga* (shrublands), in secondary tropical dry forest or abandoned pasture.

| Parameter | Abandoned pasture | Secondary tropical dry forest | F | p |
|---|---|---|---|---|
| Fluctuating asymmetry (Index 1) | 1.4 ± 0.08 | 0.99 ± 0.06 | 17.06 | 0.001 |
| Number of mines | 5.10 ± 0.5 | 3.95 ± 0.47 | 2.26 | ns |
| Number of injuries | 6.9 ± 0.46 | 6.6 ± 0.42 | 0.23 | ns |
| Leaf loss (%) | 3.9 ± 0.6 | 3.5 ± 1.0 | 0.03 | ns |

ns = not significant

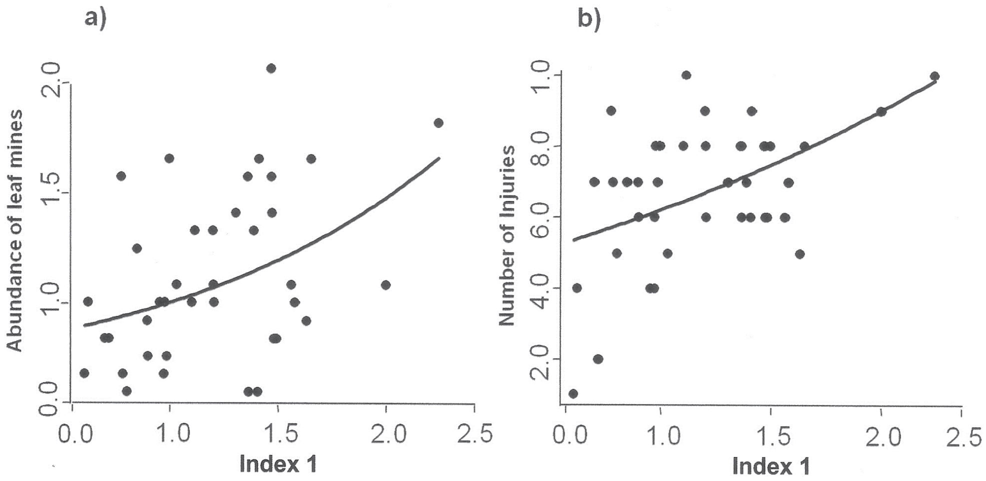

**Figure 1.** Relationship that absolute fluctuating asymmetry (Index 1) was found to have with a) abundance of leaf mines, equation: [Y = exp (-1.46 + 0.77x)] and with b) number of injuries from leaf chewers, equation: [Y = exp (-1.46 + 0.37x)].

-Terzo & Pagliosa 2007). Such studies have shown that plants generally respond to the stressful effects of certain biotic or abiotic conditions. In coastal areas, for example, FA has been positively correlated with the concentration of salts in leaves (indicating environmental stress), and the rates of herbivory (mainly by leaf miners) in such areas were found to be higher in asymmetric plants than in symmetric plants (Cornelissen & Stiling 2011). This provides a valuable cue to evaluating the early stages of an environmental disturbance (Dongen 2006; Torrez-Terzo & Pagliosa 2007; Hagen *et al.* 2008).

In semi-arid environments such as the *caatinga*, periods of drought may be the main limiting factor for the plant community. In these environments, stressors act in a more intense way than in other ecosystems, such as tropical rain forest, and ecological processes such as edge effects do not seem to accentuate the stress on plants (Santos & Santos 2008). Therefore, compounding the effects of the already stressful environment, the variation in FA between the habitat types indicates that *P. pyramidalis* is highly sensitive to small environmental changes, such as microclimatic differences in air temperature and relative humidity. This pattern, however, varies among species in semi-arid habitats, as evidenced by the fact that FA does not differ between individuals of *Chamaecrista semaphora* (H. S. Irwin & Barneby, Fabaceae) sampled in restored and natural environments within a tropical dry forest (Costa *et al.* 2012).

Although we found the rate of FA to be higher among plants in abandoned pasture than among those in secondary tropical dry forest, the proportion of leaflets showing herbivory damage did not differ between the two habitats, indicating that asymmetric plants are more susceptible to herbivory, regardless of the habitat type. This preference for asymmetric plants can be explained by the plant stress hypothesis (White 1984; Mattson & Haack 1987), which postulates that herbivore abundance is higher on hosts under stressful conditions. For some plant species, for example, a lower nutritional status leads to decreased production of secondary compounds or inefficiency of other defense mechanisms (Cornelissen *et al.* 1997). Because asymmetric leaves are associated with a higher nutrient content value than are symmetric leaves (Torrez-Terzo & Pagliosa 2007), they are more attractive to herbivores (Cornelissen & Stiling 2005). Some studies have demonstrated a significant and positive relationship between herbivory and FA, which is indicative of high quality plant tissues for insect consumption (Lempa *et al.* 2000; Cornelissen & Stiling 2005; Telhado *et al.* 2010; Cornelissen & Stiling 2011). This hypothesis could explain why the levels of herbivory were high for asymmetric plants at both of our study sites.

The plant vigor hypothesis proposes that there is an effect opposite of that posited in the plant stress hypothesis: that plants with better nutritional status are also more susceptible to herbivory because they contain higher levels of essential nutrients such as nitrogen (Cornelissen *et al.* 2008). To illustrate the relationship between insect herbivores and plant vigor, Cornelissen *et al.* (2008) conducted a meta-analysis and found that the herbivores were almost 65% more abundant on healthy plants than on stressed plants. In addition, Cornelissen & Stiling (2006) found that the duration of the larval stage was shorter among leaf miners on fertilized host plants than among those on control plants. This explains why some studies found no significant relationship between FA and herbivore abundance or herbivory rates (Cornelissen *et al.* 1997; Costa *et al.* 2012), suggesting that the use of FA as an indicator of plant susceptibility to herbivores requires more in-depth investigation.

Further studies, associating the occurrence of FA in different strata and different plant species (woody and herbaceous) in relation to environmental variables such as soil and foliar nutrient content, as well as herbivory rates, could help establish a reliable index of environmental stress in the *caatinga*, using FA as an indicator.

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