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Plasticidade foliar em resposta à luz de cinco espécies de Passiflora em ecótonos campo-floresta no sul do Brasil

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*Plasticidade foliar em resposta à luz de cinco espécies de Passiflora em ecótonos campo-
floresta no sul do Brasil*

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RESUMO

A heterogeneidade ambiental, seja ela espacial ou temporal, traz desafios aos organismos e a forma como eles respondem a ela é através de mudanças no fenótipo para a sobrevivência e *fitness* (i.e. plasticidade fenotípica). Investigamos se atributos foliares de cinco espécies de *Passiflora* respondiam de forma plástica à variações na disponibilidade de luz (Radiação Fotossinteticamente Ativa) em ecótonos de floresta-campo. O estudo abrangeu três regiões geomorfológicas do estado do Rio Grande do Sul e os atributos foliares escolhidos foram: área total e específica, espessura, dureza e forma. Utilizamos informações genéticas e filogenéticas dos indivíduos bem como medidas de luz como preditores da expressão fenotípica. Observamos plasticidade (maior influência do ambiente de luz) em três atributos: SLA, espessura e dureza, apenas observada em três espécies, de clados distintos, indicando que a plasticidade neste grupo não é conservada. Ainda, nestes ecótonos, a dinâmica de expansão e retração florestal pode estar levando à plasticidade de atributos foliares e, possivelmente, de uma radiação adaptativa no recurso de luz, através da criação de oportunidades para as plantas de colonizar novos ambientes.

Palavras-chave: Atributos foliares, filogenia, evolução, trepadeiras

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INTRODUÇÃO GERAL

Atributos e plasticidade fenotípica

A captura de energia fotossintética garante às plantas a obtenção de quase toda sua energia química e tem papel central no seu fitness. A fotossíntese, por sua vez, é diretamente influenciada pela quantidade de luz que chega até as plantas, que varia, entre outros fatores, conforme características do dossel. Sendo assim, as plantas não dependem apenas de características intrínsecas ao organismo (e.g. taxa fotossintética) para o crescimento, defesa e competição, mas também da dinâmica e geometria do dossel, tornando esta variável ambiental muito estudada sob o aspecto de atributos foliares de importância ecológica (Givnish, 1988).

O fenótipo de um organismo inclui todos os aspectos do indivíduo além do genótipo, desde enzimas até características físicas e comportamentos aprendidos (West-Eberhard, 1989). Ao alternar de ambientes, ele pode permanecer o mesmo ou ser alterado. O quanto a expressão do fenótipo é modificada é uma medida da plasticidade dos caracteres. Portanto, plasticidade fenotípica é demonstrada por um genótipo quando sua expressão é capaz de se alterar de acordo com influências do meio (Bradshaw, 1965).

Em 1881, Charles Darwin escreveu, em carta a Karl Semper: “Especulei se uma espécie muito sujeita a repetidas e grandes mudanças de condições não poderia assumir uma condição flutuante pronta para ser adaptada a qualquer condição.” Esta ideia é o princípio básico da plasticidade fenotípica e ressalta o seu papel importante na evolução biológica. Devido a sua capacidade de gerar respostas fenotípicas imediatas ao estímulo ambiental, a plasticidade fenotípica ajuda a gerar fenótipos

divergentes entre populações, podendo, assim, levar à especiação (West-Eberhard, 2005, Pfennig et al., 2010).

Um dos nomes mais proeminentes neste ramo é Mary Jane West-Eberhard, cuja maior contribuição tem sido tratar plasticidade fenotípica como um fator que contribui à evolução, não sendo apenas um resultado dela, como já era visto. Dessa relação com a diversidade de espécies, somado ao quadro atual de mudanças climáticas, surge a importância de se estudar as causas e implicações da plasticidade fenotípica. Ainda, como os efeitos da variação intraespecífica na dinâmica de populações e comunidades são de grande importância, estudos ecológicos necessitam considerar plasticidade de atributos (Bolnick et al., 2011).

Ecótonos

Ecótonos são zonas de tensão que limitam habitats distintos, formando assim um sistema ecológico único, com características de ambos ambientes e com uma determinada permeabilidade dos fluxos ecológicos entre eles (Fonseca & Joner 2007). A vegetação no Rio Grande do Sul (latitudes 27° a 33° S) é marcada por ecótonos entre campos nativos e florestas, formando mosaicos naturais de vegetação diversificada (Rambo, 1958). Estes ambientes estão em uma constante dinâmica em que a vegetação florestal tende a se expandir sobre o campo em processos de expansão da borda ou nucleação (Oliveira & Pillar, 2004; Duarte et al., 2007).

Florestas são dinâmicas, com o dossel em contínuo estado de fluxo, sendo alterado devido a várias mudanças estruturais ao longo do tempo e do espaço (Whitmore, 1989). A radiação fotossinteticamente ativa (PAR) varia de 1 mol/m²/dia em interiores de florestas fechadas a 35 mol/m²/dia em clareiras (latitudes

equatoriais) (Whitmore 1998). Assim, devido ao contraste entre esses ambientes distintos e o gradiente luz que é gerado, os ecótonos entre campo e floresta tornam-se ambientes ideais para a observação de respostas plásticas de plantas em relação à disponibilidade de luz solar.

Família Passifloraceae

A família Passifloraceae é constituída por cerca de 600 espécies, compreendidas em 20 gêneros, com distribuição em regiões tropicais a temperadas (Cervi, 2005). O gênero *Passiflora* possui o maior número de espécies da família e do seu total de ca. 520 espécies (MacDougal & Feuillet, 2004), 140 ocorrem no Brasil (Cervi, 2005). Segundo levantamento mais recente, Mader et al. (2009) cita 18 espécies do gênero para o Rio Grande do Sul. São características do gênero o hábito escandente com gavinhas axilares, corona de filamentos, além de androceu e gineceu dispostos em um pedúnculo, formando um androginóforo (Judd et al., 2009). Podem ser herbáceas ou lenhosas, com algumas espécies arbustivas de pequeno porte (Cervi, 1997).

O gênero *Passiflora* é um grupo de plantas de importância econômica por produzirem frutos comestíveis e de propriedades medicinais, caso de *P. edulis* e *P. alata*, respectivamente (Gosmann et al., 2011, Crochemore et al., 2003). Também despertam interesse sobre estudos de interação inseto-planta, os quais têm sido focados na herbivoria, como o caso das larvas de Heliconíneos (Lepidoptera: Nymphalidae) (Moreira et al., 2011). A polinização é feita principalmente por abelhas, mas também por outros insetos e vertebrados, como morcegos e beija-flores. Estes últimos também são responsáveis pela dispersão de sementes, atraídos pela

coloração e cheiro dos frutos (Semir & Brown, 1975). O subgênero *Passiflora* é o maior da família, com 236 espécies conhecidas e distribuição da metade sul dos EUA até o sul da América do Sul. O subgênero *Decaloba* contém 214 espécies de distribuição nas Américas, no sudeste da Ásia e na Austrália.

Apesar de sua grande diversidade, pouco se sabe sobre plasticidade fenotípica no gênero *Passiflora*. Apenas dois estudos foram desenvolvidos até o presente: o de Cutri et al (2013), com meristemas axilares em *P. edulis*, e o de Barp et al (2006) com folhas de *P. suberosa*.

Capítulo 1. Light-driven leaf plasticity in five *Passiflora* species in south Brazilian forest-grassland ecotones*

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Abstract

Environmental heterogeneity, spatial or temporal, poses serious challenges to organisms and the way they respond to it is by changing their phenotype in order to improve their survival and fitness. This ability is called phenotypic plasticity. Studies of phenotypic plasticity do not usually address both genetic and environmental effects in phenotypes. We aimed to investigate whether leaf traits of five *Passiflora* species were plastic in response to light availability in forest-grassland ecotones. Study sites comprised three geomorphological regions of the state of Rio Grande do Sul, Brazil, and leaf traits were: total and specific leaf area (SLA), thickness, hardness and shape. We used phylogenetic and genetic information of individuals as well as measures of light intensity as predictors of trait expression. We observed phenotypic plasticity (influence of the environment instead of or stronger than the other predictors) in three traits: SLA, thickness and hardness, which were only observed in three species, from distinct clades, indicating that plasticity in this group is not a phylogenetically conserved trait. Further, in these ecotones, the dynamics of forest expansion/retraction might be the drivers of phenotypic plasticity in leaf traits and, possibly, of an adaptive radiation on the light resource, by creating opportunities for plants to shift to novel environments.

Key-words: leaf traits, phylogeny, evolution, vines

Introduction

An organism may face distinct environments throughout its life, often different from those its parents experienced. Evolution by constructive changes is not able to provide the changes necessary for its fitness to be maintained in this case, thus arising the importance of an adaptive phenotypic response within the individual phenotype, i.e. phenotypic plasticity (Bradshaw 1965, Bradshaw and Hardwick 1989)

Phenotypic plasticity (PP) is the capacity of a genotype to express different phenotypes in the presence of environmental stimuli (Pigliucci 2001). It has a strong, frequently adaptive, influence on differential survival and reproduction, by increasing the chances of success on new environmental conditions. By promoting intraspecific variation, PP may also facilitate interspecific diversification and population divergence, thus providing conditions for speciation to occur (Pfennig et al. 2010). The developmental pathways underlying environmentally induced phenotypes consist of many genetic components and it is in this variation that selection acts upon (Pfennig et al 2010). The process of understanding the evolution of phenotypic plasticity should then consider the molecular and cellular mechanisms by which individual organisms express flexibility in the face of environmental change (Schlichting and Smith 2002).

Although the influence of plasticity on evolution and speciation has a widespread acceptance among developmental ecologists, its specific function and significance is still controversial for many evolutionary ecologists (West-Eberhard 2005b, Pfennig et al 2010). The role of PP in speciation can follow two main pathways: (1) through phenotypic differences that eventually lead to reproductive isolation, or (2) facilitating colonization of new environments (West-Eberhard 2005b,

West-Eberhard et al. 1989, de Jong 2005, Pigliucci et al. 2006, Nosil et al. 2009). Although it is clear that phenotype expression is influenced by the external conditions, the relationship between species traits and the environment can be masked by the phylogeny of that taxon, i.e. the history of trait - environment interactions over the evolution of that group. A consideration of phylogenetic relationships becomes, therefore, relevant in ecological studies of traits (Westoby et al. 1995, Desdevises et al. 2003, Staggemeier et al. 2010).

Light is one of the most important resources for plants and many studies have been addressing trait responses to light heterogeneity for decades (Bjorkman 1981; Valladares et al. 2000, Avramov et al. 2007). Light harvesting is thus of great significance for plants in shaded environments, with genetic and mechanical limitations to its efficiency, such as leaf structure and biomass requirements (Niinemets 2010). Leaf morphology is highly plastic according to light spectral quality and intensity (Crawley 1986, Pigliucci 2001). That is because plants, as sessile organisms, developed many means to cope with shading (e. g. leaf arrangement, reduced respiration rate and increased leaf area per unit weight), which often reflects in their morphology (Avramov 2007, Valladares and Niinemets 2008). Although this relationship is well established in the literature, only recently a connection between the causes and consequences of plasticity of characters in an evolutionary context has been addressed (see West-Eberhard 2005).

In forest – grassland ecotones, where light spectra and availability changes abruptly, natural selection favors phenotypic plasticity, whereas genetic variation instead would be expected in more stable environments (Svanbäck et al. 2009). Plants in those varying habitats are an ideal group with which to work when studying these relationships, especially for the ease of trait measurements. Climbers are an

important component of tropical forests, although often neglected by studies (Putz and Mooney 1991). In forest – grassland ecotones, forest edges form an ideal habitat for those plants, with structures called “trellises” (i.e. plants that offer physical support on which other plants climb). Vines are thin-stemmed climber plants and include the widely distributed passion vines (*Passiflora* genus), with 140 Brazilian species (Cervi 1997), of which 18 can be found in the southernmost state, Rio Grande do Sul (Mäder et al. 2009).

Studies of environmental responses in populations do not usually combine the effects of genetic differentiation and phenotypic plasticity (Svanback et al 2009). This study aimed to answer the following question: Is the leaf phenotypic variability among species being explained by their genetic and phylogenetic background or by the light environment? Should phenotypic plasticity be the main factor driving the evolutionary pathway of the study species, we expected to find a stronger explanation from the environment than from the phylogenetic and genetic factors. Furthermore, there is still little knowledge on the aspects of carbon economy in vines such as costs of maintenance of leaf biomass and carbon balance. We hope this study will also contribute to the understanding of this dynamics by providing some information on environmental responses of vine leaves to variations in light availability.

Material and Methods

Study site

Rio Grande do Sul is the southernmost state in Brazil. The climate fits into Köepen's categories Cfa and Cfb, with mean annual temperatures ranging from 15 to 20°C and lower rainfall in the south half of the state. Study sites comprised three of the six geomorphological provinces in the state (Fig. 1), described below. Each city had a sampling site, a plot of 4 km², which included forest - grassland ecotones, within which we searched for adult individuals of the *Passiflora* species chosen for this study.

Araucaria Plateau: Located in the northeast of the state, with a mean annual temperature of 14.5°C and average altitude of 1000m (Behling 2002), this region is characterized by a mosaic of forest and grasslands. The forest has the often dominant presence of the pine *Araucaria angustifolia*, giving its name "Araucaria forest" (Rambo 1956, Hueck 1972). The grasslands are marked by tall grasses and *Baccharis uncinella* (Asteraceae) shrubs. Study sites were located in the cities of São Francisco de Paula (*Pró-Mata* Research and Conservation Center - 29°29' S, 50°12' W) and Cambará do Sul (*Aparados da Serra* National Park - 29°09' S, 50°04' W).

Serra do Sudeste: This region is situated in the south - central part of the state, with a mean annual temperature of 18.3°C. The vegetation forms a mosaic of semi-deciduous seasonal forest patches in a grassland matrix of *Campos* grasslands and pastures (Pillar and Quadros 1997, Jurinitz and Jarenkow 2003) and is marked by the curious presence of the pine trees *A. angustifolia* and *Podocarpus lambertii*; both species are characteristic of the *Araucaria Plateau* region (Carlucci et al. 2011). Study

sites were located in the cities of Santana da Boa Vista (30°52' S, 53°08' W), Encruzilhada do Sul (30°32' S, 52°31' W) and Herval (32°01' S, 53°24' W), all in private properties.

Campanha: Occupies the western part of the state. Climate normal to dry with a mean annual temperature of 18.9 °C. This region has a high diversity of habitats, dominated by vast, non-uniform grasslands with patches of forests of several kinds (Rambo 1956). Study sites were located in the cities of São Francisco de Assis (29°33' S, 55°08' W) and Santana do Livramento (30°51' S, 55°31' W).

Study species

Six species of the genus *Passiflora* were chosen for this study, from three distinct clades (Fig. 2). Each pair was chosen as to have one species that occupies more shaded habitats and another, occupying those more exposed to sunlight. However, one of the species was not found, thus remaining only five. Sampled species were: *Passiflora caerulea*, *P. organensis*, *P. misera*, *P. actinia* and *P. elegans*.

Phenotypic traits

A synthesis of the chosen vegetative traits with their ecological relationship with light variation and their possible impacts on fitness is shown in Table 1.

During the summer of 2012, we searched inside the forest and along the forest edge for adult plants that were at least 2/3 of its extension in a homogeneous environment (i. e. with no or minimum light exposure variation on the same branch). In each individual, six leaves were collected, avoiding the five newest. They were

fixed in a solution of 90% ethanol 70%, 5% formaldehyde and 5% acetic acid (FAA) for subsequent laboratory analyses. Two extra leaves were collected and stored in paper bags with silica gel to preserve the DNA for molecular analyses.

At the moment of collection the light environment was characterized using a quantum sensor attached to a multimeter to quantify the amount of photosynthetically active radiation (PAR) at the precise place in which the plant was. However, this measure varies according to the time of day and cloud condition. Thus, a measure was taken at full exposure to sunlight (away from any possible shadow) at the same moment of the capture of PAR at each plant, in order to quantify the transmittance ($PAR_{\text{leaf}} : PAR_{\text{full sunlight}}$).

Trait measures were carried out according to Cornelissen et al. (2003) and were the following:

- *Total and specific leaf area (SLA)*: three leaves were scanned and oven-dried. The areas were calculated in the software Image J (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997-2012) and the dried leaves were weighted in a precision scale. SLA was calculated as the sum of the three measures of areas divided by the sum of the three measures of dry mass.
- *Leaf shape*: with the same images used to measure leaf area, on Image J, the shape was calculated as the average square perimeter divided by the area.
- *Leaf thickness*: a longitudinal section of the leaf lamina, parallel to the major vein, was cut in three leaves, which were measured in a stereomicroscope with a micrometric scale.

- *Leaf hardness*: We used a penetrometer as described in (Feeny 1970), which consists of a device that combines shearing and tearing forces. The measure given is the weight required to detach the leaf disc.

Molecular analyses

DNA was extracted from leaf samples following Mäder et al. (2010) and Desfeux and Lejeune (1996). We used the genetic markers called nuclear ribosomal ITS 1 and 2 (Internal transcribed spacers), which were amplified using primers and amplification conditions as described by Desfeux and Lejeune (1996). To exclude the presence of low stability templates, 10% dimethyl sulfoxide (DMSO) was used (Buckler IV et al., 1997; Fuertes-Aguilar et al., 1999). PCR products were checked by electrophoresis in 1% agarose gel stained with GelRed. Polymerase chain reaction products were purified using Exonuclease I and Shrimp Alkaline Phosphatase following the guidelines of the suppliers, and sent for sequencing at Macrogen Inc. (Geumcheon-gu, Seoul, Korea), using the forward primer. Sequence electropherograms were visually inspected using Chromas 2.31 (http://www.technelysium.com.au/chromas_lite.html) and the fragments obtained were aligned using Clustal W, implemented in MEGA 5.0 (Tamura et al. 2011). Alignments were checked and edited by hand. The mean number of pairwise differences (Nei, 1987) was calculated in the MEGA 5.0 program as well. The result was a genetic distance matrix, which we used in the statistical analyses as an independent variable. The genetic distance tree can be seen in Figure 1 (Appendix).

Statistical analyses

Initially, in order to evaluate the influence of the light environment on leaf trait distribution, we performed linear regressions between each trait and transmittance. Traits that related significantly to the environmental factor were submitted to Analyses of Covariance (ANCOVAS) to quantify the relative contribution of environment and phylogeny to trait values, considering the phylogenetic nodes connecting the five *Passiflora* species. Additionally, we computed a Coefficient of Variation (CV) for each trait, considering individual variation within each species, as a measure of the amount of phenotypic plasticity (higher CVs indicate greater plasticity of response). Because CV is a very common estimator of plasticity, but weak for statistical comparisons, we also used RDPI (Valladares et al. 2006), which calculates distances between all pairs of individuals of a species that are in distinct environments, giving a relative distance plasticity index for that species, ranging from 0 (no plasticity) to 1 (maximum plasticity).

Finally, aiming to assess the influence of the genotype and the environment on the plant traits within each species, we followed the method of variation partitioning proposed by Desdevises et al. (2003), with the eigenvectors we extracted from a PCoA with the genetic distance matrix (Diniz-Filho et al. 1998) as our genetic component and the measures of transmittance as our environmental component. A stronger influence of environment would be a more accurate indicator of phenotypic plasticity. We also tested correlations between all pairs of traits, in order to infer possible developmental constrains to plasticity.

Results

We sampled a total of 149 individuals: *P. actinia* (n=18), *P. elegans* (23), *P. caerulea* (59), *P. misera* (19) and *P. organensis* (30). *P. caerulea* was better represented because we collected individuals in two of the three sampled regions (*Araucaria* Plateau and Serra do Sudeste). Thirty-three samples were excluded from our molecular analyses for being unable to amplify or for posterior distance calculation errors, generating a final matrix with 116 individuals.

Mean transmittance values (ranging from 0 to 1) of species were 0.73 for *P. actinia*, 0.80 for *P. elegans*, 0.78 for *P. caerulea*, 0.85 for *P. misera* and 0.75 for *P. organensis*. *P. misera* had the highest mean SLA, while leaf area were the largest in *P. actinia*. Leaf shape was the highest in *P. caerulea* and *P. elegans* showed the highest means of leaf thickness and hardness (Fig. 4). The correlations between all pairs of traits and revealed at least one correlation in all but one species: *P. elegans* (Tab. 1, Appendix 1).

There was a significant influence of the light environment on leaf thickness and hardness, as well as on SLA. This relationship was only observed in the three species with the highest environmental variation: *P. actinia*, *P. organensis*, and *P. caerulea* (Tab. 3). Leaf area and shape did not vary significantly according to the environmental variable; therefore they were removed from ANCOVAs.

Phylogeny also had an influence on trait expression, although less important (Tab. 2). At some phylogenetic nodes, this factor had an influence stronger than the environment in all traits: for SLA, in the node *P. actinia* x *P. elegans*; for hardness and thickness, in the most basal node (see Fig. 2).

The CVs showed a higher variation in *P. elegans*, *P. caerulea* and *P. organensis* for the traits SLA, thickness and hardness, respectively. On the other hand, *P. elegans* had the least variation in the other four traits. *P. misera* had the highest variation in

total leaf area and *P. caerulea* also had the highest CV for leaf shape. RDPIs were mostly low, varying from 0.081 (thickness in *P. elegans*) to 0.409 (hardness in *P. actinia*). For *P. caerulea* e *P. organensis*, SLA was the most plastic trait when considering the R^2 of the variation partitioning. However, the CVs and RDPI indices showed the highest values for leaf hardness (Tab. 3 and 4), which was the trait with the highest RDPI values for the other species as well (Tab. 4).

Variation partitioning between the genetic and environmental components showed a much stronger influence of the environment on trait expression (Tab. 3). The genetic component did not account for a significant amount of trait variation in any case, except for a low relationship with leaf area in *P. elegans*. In general, individuals of the same species were not as genetically different as they were phenotypically.

Discussion

Light was an important factor determining the expression of leaf traits in some of the studied species. However, species had different responses to the light environment. By comparing the influence of genotype and environment on trait expression, we observed phenotypic plasticity in three species; by analyzing the phylogenetic relationships among them, we noticed that plastic species belong to distinct clades, indicating that the evolution of plasticity in leaf traits for light heterogeneity might have occurred before the divergence of these clades. Moreover, in this scale, we can observe a lack of phylogenetic niche conservatism, which is when species maintain their niches throughout time, thus retaining similar ecological responses with the closely related species (Harvey and Pagel 1991).

Plasticity can diverge substantially among phylogenetically related species (Schlichting and Levin 1986, Pigliucci 2001) and we have found so in our study, where species of the same genus showed distinct plastic responses. This difference in the presence or in the amount of plasticity between closely related species might be due to differences in their niches, as evidenced by a decrease in the CVs of the environment with decreasing plasticity. Moreover, if we consider PP as a trait within the phylogenetic relationships among our study species, we can infer a pattern of phylogenetic trait lability in a larger scale.

Studies have shown that shade-tolerant species are more plastic than intolerant ones; however this plasticity is mostly expressed in their physiology (Ellsworth and Reich 1996, Portsmouth and Niinemets 2007, Valladares and Niinemets 2008). Correspondingly, in our study we found that *P. misera* and *P. elegans*, which had the highest mean exposure to light (thus possibly the lowest shade tolerance), were not plastic in their leaf morphology. Nonetheless, they might be responding in a different level of biological organization (Valladares and Niinemets 2008), such as whole-plant chemical and structural traits (Niinemets 2010).

Both species from the Campanha region, *P. misera* and *P. elegans* did not show significant responses to light in the five leaf traits we measured and had the least variation in light environments. During our sampling period there was a strong draught, which is common for this region (Buriol et al. 1979), that probably maintained only the plants that were in shaded and more humid habitats, such as those inside the forest patches. That might explain the shortest light gradient in those species and thus our inability to observe light-driven plasticity. Light variation

according to photoperiod and seasonality changes across geographical scales, unlike the one caused by small-scale patchiness inside tropical forests (Pigliucci 2001).

Similar to the study by Valladares *et al* (2006), we found no relationship between the coefficient of variation and the estimators of plasticity, which is understandable, since phenotypic plasticity is only a part of the whole phenotypic variability. Further, these authors argue that the difference between these two – the unexplained variance – still cannot be 100% explained by most ecological studies, although methods to control confounding variables, experimentally or statistically, help to understand variation in ecological variables. According to them, a dissection of character variation into as many components as possible would generate a better understanding of plant responses to environmental variation, which we aimed to do by adding phylogenetic and genetic information to our data.

The plastic development may be constrained by strong genetic correlations between traits (Schmitt 1997). In only one species (*P. elegans*) there were not such correlations. Moreover, an interaction of multiple stresses can affect the tolerance to light variation (Valladares and Niinemets 2008). For example, shade tolerance was proven to be inversely associated to tolerance to draught and flooding in temperate trees and shrubs (Niinemets and Valladares 2006).

Leaf shape responds to different environmental stimuli, but one of the most common is water availability (Pigliucci 2001). Even though water availability is expected to change according to irradiance intensity we found no significant relationship between this trait and the light variable. The high plasticity in leaf hardness for *P. actinia* is probably helping in the defense against predation by insects, confirmed by the fact that there was almost no noticeable herbivory in the plants we collected closer to forest edges. The reduced leaf thickness in individuals less exposed

to light might be due to decreased development of the palisade (Witkowski and Lamont 1991), which, for being responsible for photosynthesis, might cause a decrease in photosynthetic rate. However, the plastic plants are compensating with an increase in SLA, as evidenced by an even stronger plasticity in this trait.

An increase in leaf area per unit leaf biomass is a common response of plants to low irradiance, because this way they maximize carbon gain, making SLA an important trait in plants that experience variation in the light environment (Niinemets 1999, Valladares and Niinemets 2008). Steinger et al. (2003) found an association between high SLA and increase in fitness for the weed *Sinapis arvensis* in light-limiting conditions, supporting the idea of a role of plasticity in SLA in adaptation to light environments. The carbon gain hypothesis, according to which shaded species achieve better survival and fitness by minimizing CO₂ losses rather than by enhancing carbon gain, would explain why leaf thickness in the plastic species increased significantly with increasing light. That is, the most shaded species compensated the lack of PAR by investing less in mass.

Beside the genetic and phyletic constraints that limit plasticity, there are those simply related to physiological limitations and/or costs. For example, in a recent experimental study, Svanback et al (2009) observed that, in a predator-prey model, high levels of phenotypic plasticity evolved in the population when physiology-related costs of plasticity were removed. In order to identify possible costs of plasticity in our studied species, a more thorough study, with the understanding of the genetics behind it and fitness implications, would be necessary. However we can infer that, for the three plastic species, either the cost was low or it was absent. Some of the possible costs for those species with no plasticity might be involved with mortality rate, such as being more palatable to herbivores.

A recent study by Lorenz-Lemke et al. (2005), with the geographical distribution of *P. actinia* e *P. elegans* found that the genetic variability in *P. actinia* is structured through a north – south gradient that ends in the northeast part of Rio Grande do Sul (where it was collected in our study). This low genetic variability (Fig. 3) could be a reason why the genetic factor did not explain trait variation. This is the region where *P. elegans* begins to occur towards the southwest of the state and, although this species shares a strong genetic similarity with *P. actinia*, only the latter was plastic.

When the phenotypic response needs time to take place, plasticity will have a better chance of occurring if the environment varies relatively slowly (Stomp et al. 2008). So it is a matter of adequacy of the pace at which trait expression occur to that of the environmental variation. In our study system, ecotones, light variation is not only present during the course of the day, but – and maybe most importantly – throughout the dynamics of forest expansion/retraction, which are relatively slow events. Therefore, this may be a cause that supports our hypothesis (that plasticity would be driven in these transition environments).

The presence of leaf plasticity in *Passiflora* reinforces the importance of these structures to climbers (Putz and Mooney 1991): by helping them survive in early stages, when they are only a few leaves easily shaded by other plants, until when they are adults, with several branches exposed to distinct levels of light. Furthermore, these forest-grasslands ecotones might be the drivers of not only leaf plasticity itself, but also of an adaptive radiation on the light resource, because they create the opportunities for plants to shift to novel environments and thus open up new adaptive zones – an ‘escape and radiate’ scenario (see Ehrlich and Raven 1964).

The interplay between ecological and evolutionary processes is essential to the understanding of population dynamics and to analyze and predict conservation status of species. The knowledge of how heritable phenotypic traits determine population fluctuations starts with studies of how these interact with the environment and the genotype. Although we know that a more detailed study with this subject (e. g. fitness - testing experiments) is necessary to fully comprehend these relationships, we aimed to contribute with the first step: testing for environmentally driven phenotypic plasticity.

Observing phenotypic plasticity among distinct clades can give us an idea of how plasticity in traits can be heritable and thus help us predict future consequences of anthropogenic threats in biodiversity. So far, we can agree with Pigliucci (2001), that development, plasticity and genetic variation for a trait are connected in a complex, yet understandable, causal web.

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Table 1. Summary of the leaf traits chosen for this study.

Trait	Effect on fitness	Expected response	References
Total leaf area	Helps photosynthesis due to better light absorption in shade	Inversely related to increasing light	Niinemets (2010), Ishi and Asano (2010)
Specific leaf area (SLA)	Trade-off between light absorption and cost of developing protective tissues	Inversely related to increasing light	Bjorkman (1981), Niinemets (2009), Carlson et al. (2010)
Shape	Affects temperature, transpiration, photosynthesis and defense against herbivores	Any alteration according to light variation	Pigliucci (2001), Gottschlich and Smith (1982), Gilbert (1975) Rausher (1978)
Thickness	Affects temperature, transpiration and protection against herbivores and radiation	Increase with increasing light availability	Witkowski and Lamont (1991), Garnier et al. (1999), Pigliucci 2001, Barp et al 2006
Hardness	Affects transpiration, protection against herbivores	Increase with increasing light availability	Wright et al. (1989), Groom and Lamont (1997)

Table 2. ANCOVAs between the environmental (transmittance) and phylogenetic (contrasts between clades) components. Asterisks indicate significance levels (* p<0.05, **p=0.01 and *p<0.01). Act: *P. actinia*; Ele: *P. elegans*; Cae: *P. caerulea*; Mis: *P. misera* and Org: *P. organensis*.**

Trait	Factor	R ²			
		Act x Ele	Act + Ele x Cae	Act + Ele + Cae x Mis + Org	Mis x Org
SLA	Phylogeny	0.277***	0.107***	0.192***	0.005
	Phylogeny + environment	0.08**	0.001	0.018*	0.02
	Environment	0.213***	0.322***	0.212***	0.221***
Hardness	Phylogeny	0.185***	0.017	0.387***	0.005
	Phylogeny + environment	0.120***	0.020	0.004	0.061
	Environment	0.176***	0.148***	0.062***	0.064
Thickness	Phylogeny	0.196***	0.019	0.258***	0.094*
	Phylogeny + environment	0.042	0.009	0.011	0.001
	Environment	0.264***	0.283***	0.146***	0.173**

Table 3. Variation partitioning of the traits between the ecological (transmittance) and genetic (genetic distances) components. Values shown are adjusted R². Values too close to 0 are not shown. CV_{env}: Coefficient of variation of the environmental variable; CV_{trait}: Coefficient of variation of the trait. Asterisks indicate significance levels (* p<0.05, **p=0.01 and *p<0.01).**

Species	CV _{env}	Traits	CV _{trait}	Ecological component		Unexplained	
				Genetic component			
<i>P. actinia</i>	24.3	SLA	54.5	0.645***	-	-	0.361
		Thickness	22.7	0.521***	-	-	0.510
		Hardness	59.6	0.722***	-	-	0.303
		Area	36.5	-	-	0.11	0.93
		Shape	17.1	-	-	-	1
<i>P. elegans</i>	15.7	SLA	62.4	-	-	-	1
		Thickness	14.2	0.602	-	0.055	0.479
		Hardness	28.3	0.056	-	-	1
		Area	30.9	-	0.228	0.166*	0.624
		Shape	5.8	-	-	-	1
<i>P. caerulea</i>	16.0	SLA	38.3	0.421***	0.016	-0.012	0.574
		Thickness	28.5	0.179***	-	-	0.836
		Hardness	60.9	0.014	-	0.04	0.936
		Area	58.8	-	-	-	1
		Shape	38.5	0.019	-	0.061	0.958
<i>P. misera</i>	13.6	SLA	36.4	-	0.020	-	0.980
		Thickness	21.9	-	0.077	-	0.922
		Hardness	64.3	-	-	-	1
		Area	73.6	-	-	-	1
		Shape	22.2	0.119	0.119	0.010	
<i>P. organensis</i>	17.2	SLA	35.0	0.474***	-	-	0.561
		Thickness	24.2	0.183**	-	-	0.859
		Hardness	69.6	0.248*	-	-	0.794
		Area	56.7	-	-	0.07	0.935
		Shape	10.5	-	-	-	1

Table 4. Relative Distance Plasticity Index (RDPI) values of leaf traits with a significant relationship with the environmental variable.

Species	RDPI		
	SLA	Thickness	Hardness
<i>P. actinia</i>	0.306	0.135	0.409
<i>P. elegans</i>	0.17	0.081	0.152
<i>P. caerulea</i>	0.203	0.159	0.301
<i>P. misera</i>	0.199	0.143	0.397
<i>P. organensis</i>	0.208	0.132	0.332

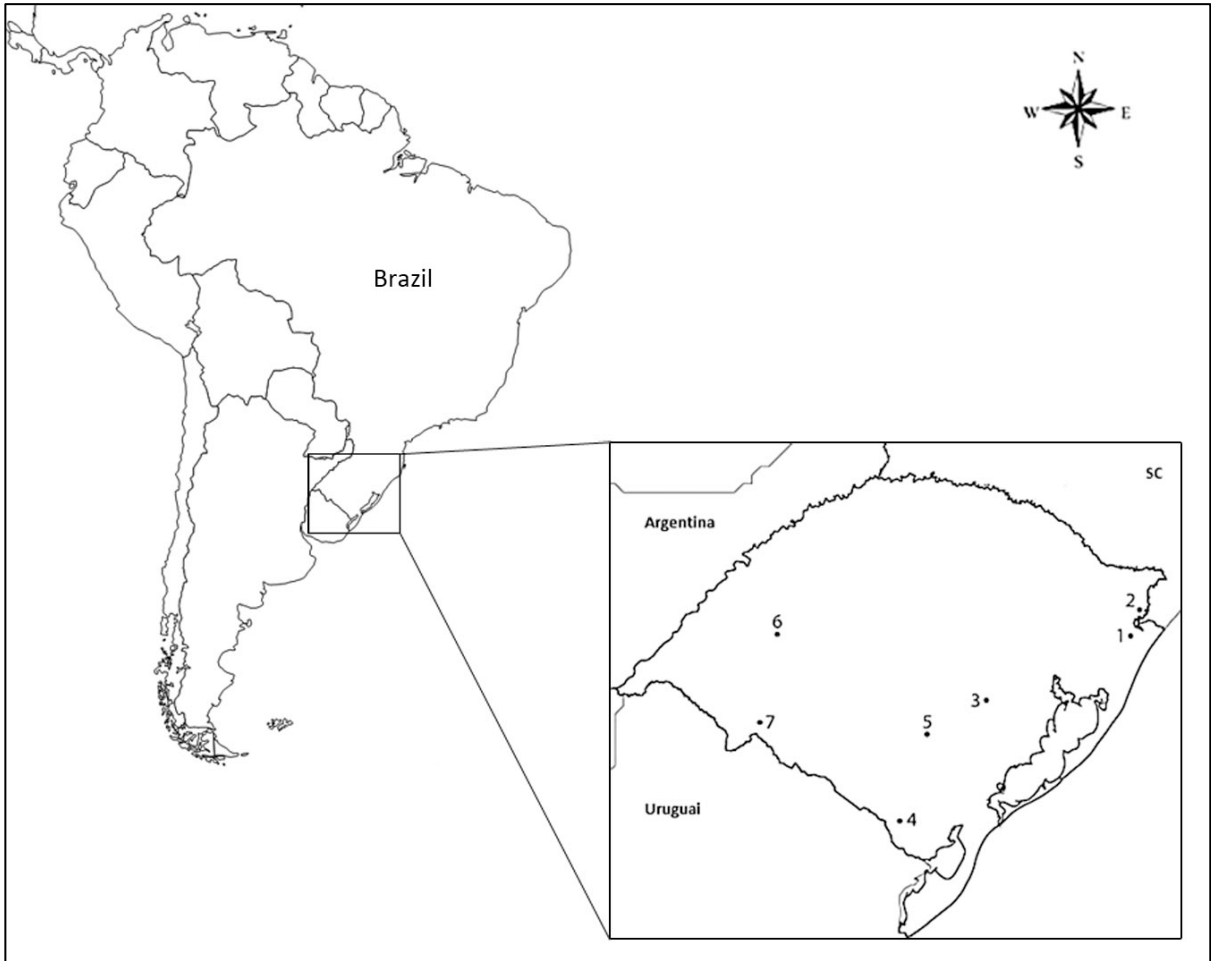


Figure 1. Map indicating sampling sites, which were located in seven cities in the State of Rio Grande do Sul. 1- São Francisco de Paula; 2 - Cambará do Sul; 3 - Encruzilhada do Sul; 4 - Herval; 5 - Santana da Boa Vista; 6 - São Francisco de Assis; 7 - Santana do Livramento

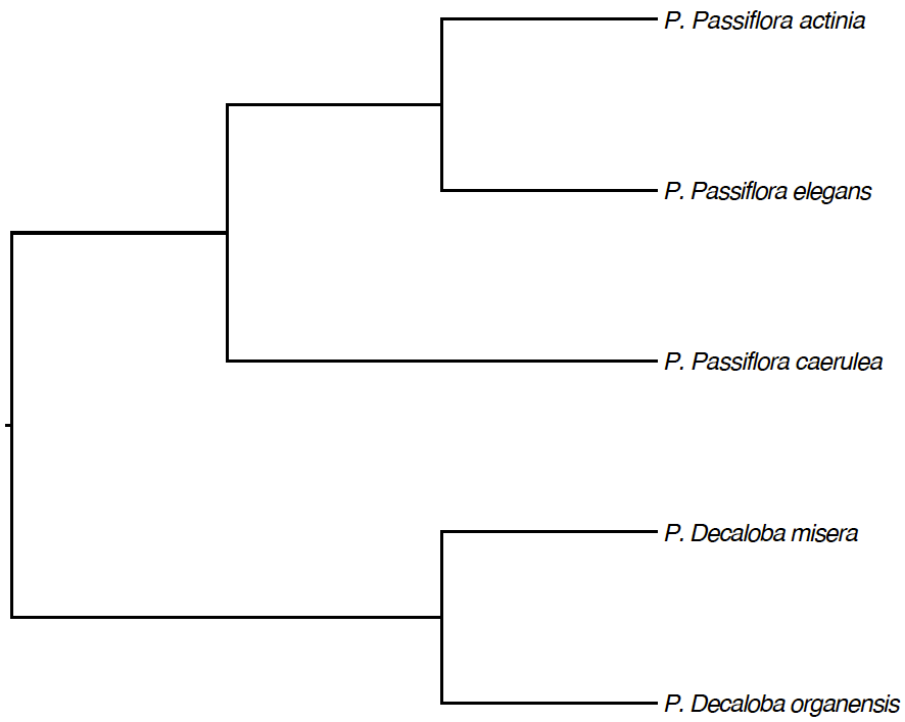


Figure 2. Schematic phylogenetic relationship among the five studied species based on Muschner et al. (2003).

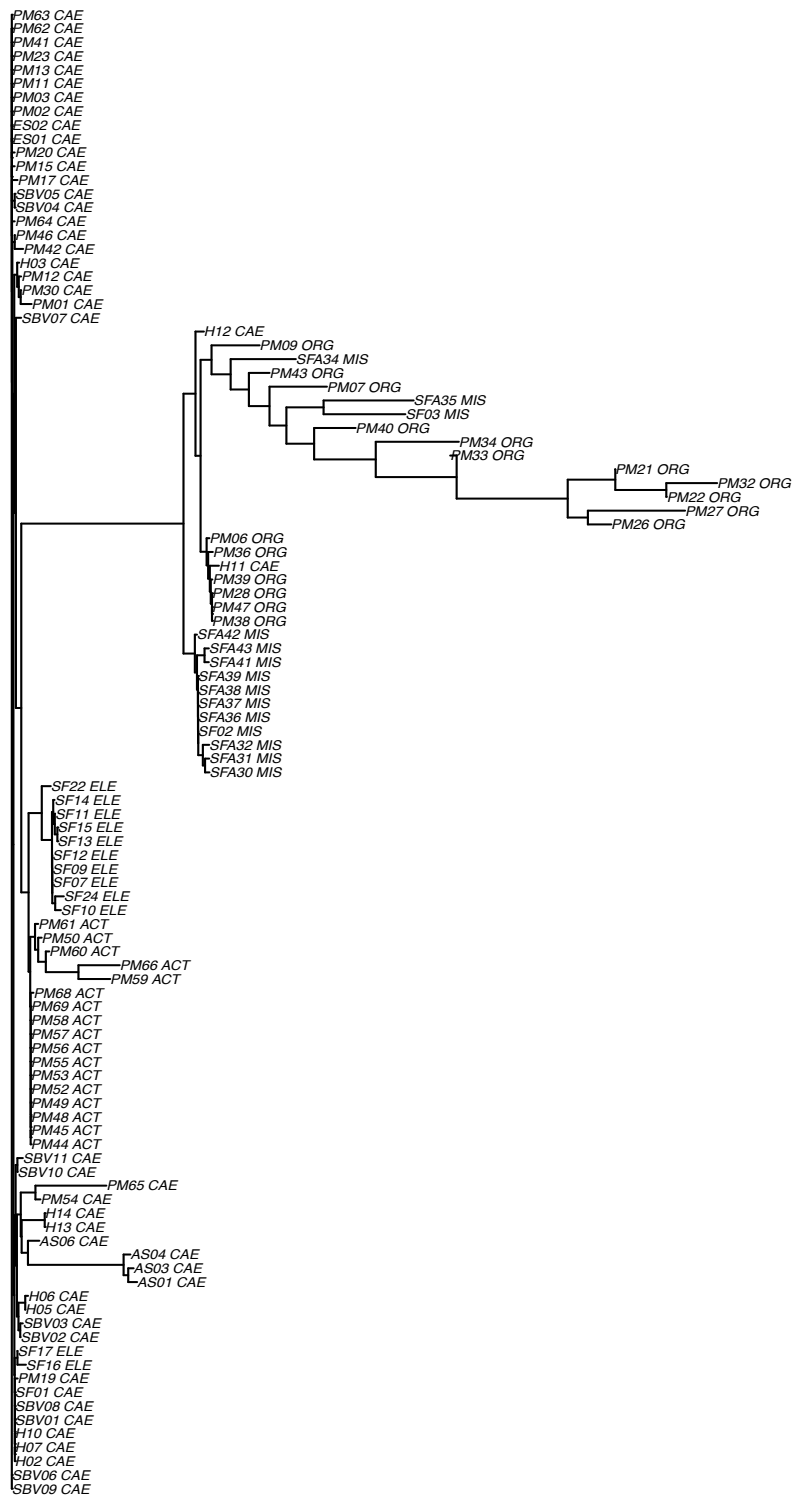


Figure 3. Genetic distance tree among 120 individuals of *Passiflora* using the method Neighbor-Joining. Initial codes indicate site where it was collected (AS: Cambará do Sul; PM: São Francisco de Paula; H: Herval; SF: São Francisco de Assis; ES: Encruzilhada do Sul; SBV: Santana da Boa Vista). End codes indicate species (ACT: *P. actinia*; ELE: *P. elegans*; CAE: *P. caerulea*; MIS: *P. misera*; ORG: *P. organensis*).

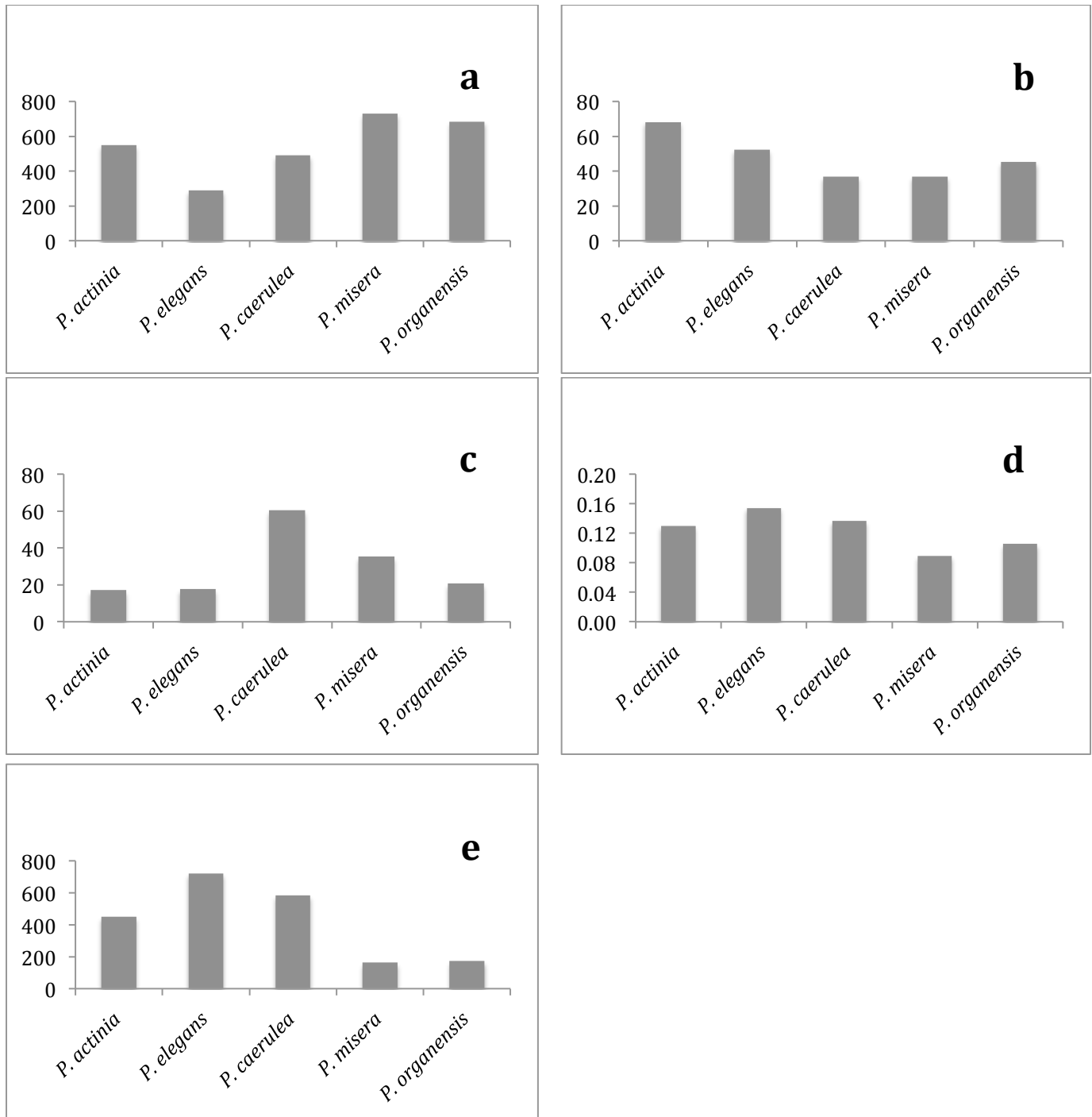


Figure 4. Mean traits for study species. a. SLA (index); b. leaf area (mm²); c. leaf shape (index); d. leaf thickness (mm) and e. leaf hardness (weight, in grams, necessary to perforate the leaf lamina).

Appendix

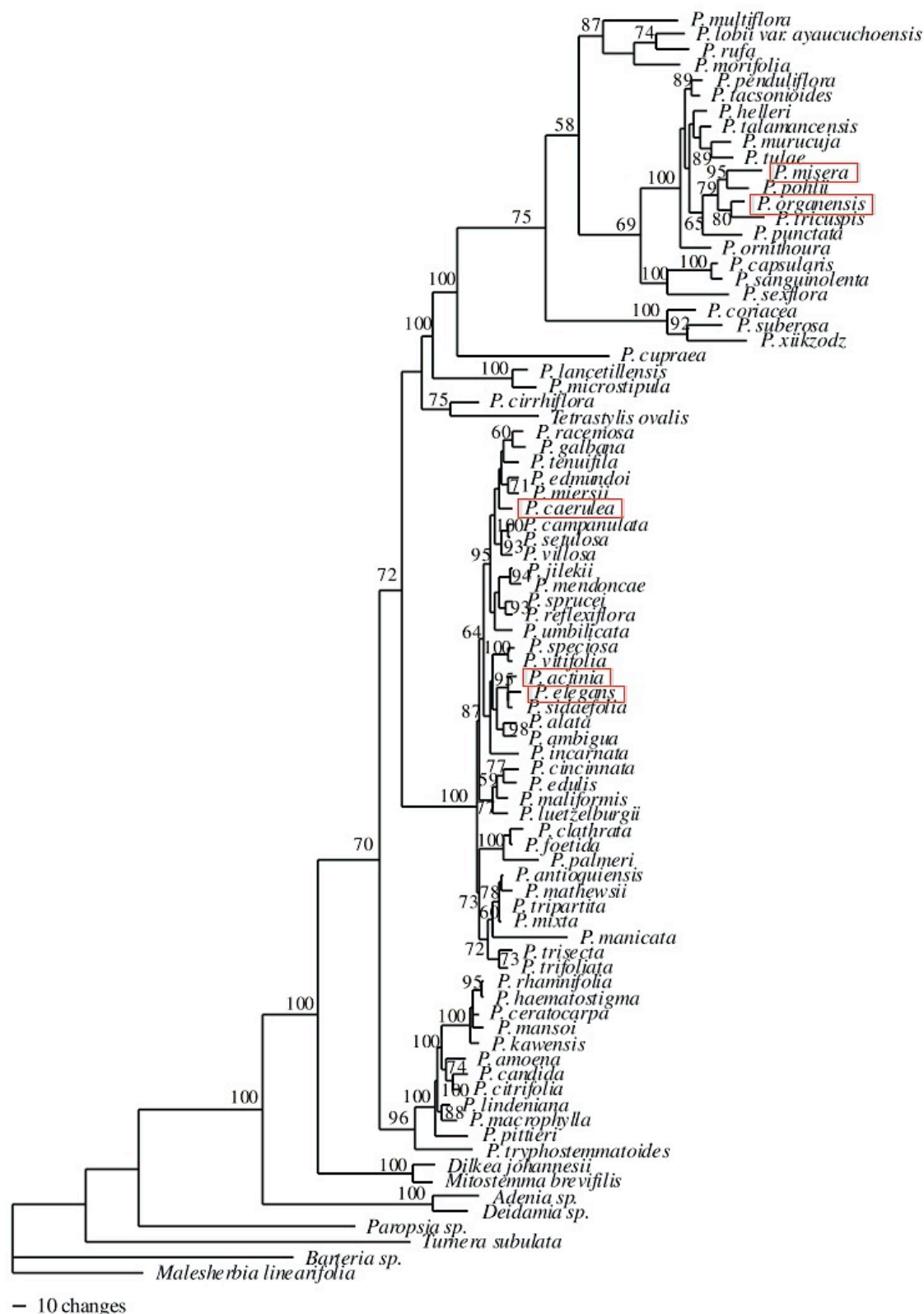


Figure 1. Maximum likelihood phylogenetic tree considering all seven DNA regions, for the genus *Passiflora*, elaborated by Mushner (2005). Numbers above the branches refer to bootstrap (BS) values. Study species are highlighted in red.

Table 1. Correlation between all pairs of traits in the studied species, which had a significant relationship with the environmental variable. Asterisks indicate significance levels (* p<0.05, **p=0.01 and *p<0.01).**

Species	Trait	SLA	Thickness
<i>P. actinia</i>	Thickness	-0.729***	
	Hardness	-0.894***	0.853***
<i>P. elegans</i>	Thickness	-0.331	
	Hardness	-0.054	0.277
<i>P. caerulea</i>	Thickness	-0.716***	
	Hardness	-0.378*	0.221
<i>P. misera</i>	Thickness	-0.882***	
	Hardness	-0.430	0.458*
<i>P. organensis</i>	Thickness	-0.721***	
	Hardness	-0.577***	0.386

CONSIDERAÇÕES FINAIS

Em florestas tropicais, a disponibilidade de recursos varia temporal e espacialmente. Nestes ambientes, a luz é um dos recursos mais importantes – e limitantes – para plantas, que evoluíram uma variedade de estratégias fisiológicas e morfológicas. Trepadeiras, comparadas às árvores, possuem adaptações de crescimento e alocação de biomassa diferentes (Putz & Mooney, 1991), tendo, em geral, taxas de crescimento superiores (Schnitzer et al., 2005). Por dependerem do apoio em outras plantas, trepadeiras podem investir menos em mecanismos de sustentação e mais em folhas. Portanto, atributos foliares são de grande importância a essas plantas, podendo responder ao ambiente de diversas formas.

Como os atributos das espécies são também resultado da história evolutiva de seus ancestrais, ao avaliarmos o quanto da expressão fenotípica é independente desse histórico, é possível observar a presença da capacidade de resposta ao ambiente atual. A partir da comparação entre espécies aparentadas, podemos inferir padrões em uma escala filogenética, isto é, se espécies mais próximas tem respostas mais semelhantes. Este padrão é resultado da Conservação Filogenética de Nicho, que é a tendência das espécies de manter seu nicho ao longo do tempo evolutivo, assim mantendo também os atributos correspondentes, gerando fenótipos mais similares entre espécies mais próximas filogeneticamente (Harvey & Pagel, 1991).

A partir da comparação da influência do genótipo e do ambiente na expressão dos atributos nos indivíduos, neste estudo observamos a presença de plasticidade fenotípica em três espécies; analisando as relações filogenéticas entre elas, observamos que as espécies plásticas pertencem a clados distintos, indicando que não há conservação filogenética de nicho na escala estudada. Características que

ocorrem em algumas espécies e não em outras, embora filogeneticamente próximas, são evidência de que essas respostas foram geradas por processos evolutivos em relação ao ambiente em que elas ocorrem (Bradshaw & Hardwick, 1989). É importante ressaltar que as espécies que não demonstraram respostas plásticas pertencem a uma região fitoecológica distinta das outras espécies mais próximas filogeneticamente e mais plásticas. Ou seja, é possível que aquele ambiente esteja influenciando de outra maneira e/ou em outra escala evolutiva.

A plasticidade fenotípica garante à planta a vantagem de não precisar gastar energia na produção de um atributo até que seja necessário. Por exemplo, o caso conhecido das fitoalexinas em algumas espécies de plantas, que são produzidas apenas quando ocorre o ataque por patógenos (Bradshaw & Hardwick, 1989). Diferentemente da produção de compostos químicos, no entanto, no caso do nosso estudo, os atributos são irreversíveis, de forma que são produzidos (e passíveis ou não de alterações conforme o meio) durante o desenvolvimento da folha. Isso pode ser considerado uma limitação à plasticidade fenotípica, uma vez que ela não pode ocorrer em qualquer momento, na planta. Contudo o crescimento rápido das trepadeiras pode ser uma forma de compensação a este limite.

Alterações fenotípicas promovidas pelo ambiente gera alvos para a seleção dentro de uma geração, gerando o que é chamado de evolução rápida. Assim, o ambiente assume um novo papel, além daquele de selecionar entre variações fenotípicas geneticamente fixadas: ele ajuda originar a variação de atributos que serão selecionados. Portanto, a plasticidade pode estar auxiliando as espécies de *Passiflora* não apenas na sua sobrevivência em habitats variados, mas também – em uma escala maior – 1) no processo de especiação, através da facilitação da

divergência populacional com a colonização de ambientes e 2) através do processo de assimilação genética, que é quando a variação fenotípica se torna constitutivamente produzida (West-Eberhard, 2005; Pigliucci et al., 2006; Pfennig et al., 2010). Finalmente, a plasticidade fenotípica pode ser vista como um atributo, sujeito à seleção como qualquer outro caractere (Scheiner, 1993; Schlichting & Smith, 2002; Svanbäck et al., 2009; Pfennig et al., 2010).

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