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REVIEW

The importance of evolutionary history in studies of plant physiological ecology: examples from cerrados and forests of central Brazil

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Studies that compare physiology, anatomy, and ecology across species have offered some of the best insight into adaptation and evolutionary constraints in plants. As a result, the comparative approach has become increasingly used in studies of plant physiological ecology. The high species diversity of Brazilian biomes provides many excellent opportunities for comparative plant ecophysiology, and could be better exploited for understanding the evolution of plant form and function. In this paper we emphasize the importance of considering phylogenetic information for the design and analysis of comparative studies, relying on examples from comparisons of woody plants from cerrado and gallery (riparian) forests. The use of congeneric species pairs, each containing one cerrado species and one forest species of the same genus, greatly improves statistical power while assuring phylogenetic independence, an essential condition for inference in comparative studies. For example, in a study of seedlings of nine cerrado species and nine forest species, when we ignore phylogenetic relationships, it is not be possible to conclude that cerrado and forest species differ with regard to leaf area ratio (LAR), specific leaf area (SLA), or seedling height. If, however, we use the generic groupings to account for phylogenetic effects, then we find that seedlings of savanna species have lower LAR, SLA, and seedling height. Much of the phylogenetic inertia observed for these traits appears related to the fact that these traits are all strongly correlated with seed mass, which has been previously shown to be strongly conserved phylogenetically. These examples emphasize the importance of phylogenetic information in comparative studies.
Key words: Cerrado, forest, specific leaf area, leaf area ratio, phylogeny

A importância da história evolutiva em estudos de ecofisiologia vegetal: exemplos dos cerrados e florestas do Brasil Central: Estudos que comparam a fisiologia, anatomia e ecologia das espécies têm oferecido algumas das melhores apreciações sobre as limitações adaptativas e evolucionárias em plantas. Como resultado, a abordagem comparativa tem sido cada vez mais utilizada em estudos de ecofisiologia vegetal. A alta diversidade de espécies dos biomas brasileiros fornece excelentes oportunidades para estudos de ecofisiologia vegetal comparada, uma abordagem que poderia ser melhor explorada em pesquisas que visam a compreensão da evolução da forma e função em plantas. Neste artigo nós enfatizarmos a importância de considerar a informação filogenética no desenho e analises de estudos comparativos, utilizando como exemplos espécies lenhosas do cerrado e de mata de galeria (mata ripária). O uso de pares congenéricos, cada par contendo uma espécie do cerrado e uma espécie de floresta do mesmo gênero, aumenta o poder estatístico das análises, enquanto assegura concomitantemente a independência filogenética, uma condição essencial para inferência em estudos comparativos. Por exemplo, em um estudo comparativo de nove espécies do cerrado e nove de mata, não seria possível concluir que as espécies de cerrado e de mata diferem na razão de área foliar (LAR), área foliar especifica (SLA) e altura da plântula, se as relações filogenéticas forem ignoradas. Entretanto, se utilizarmos agrupamentos genéricos para contabilizar os efeitos filogenéticos, nós vamos encontrar que as plântulas de espécies de cerrado tem menores valores de LAR, SLA e altura da plântula. Muito da inércia filogenética observada para estes atributos em plântulas parece estar relacionada ao fato de que estas características se correlacionam fortemente com a massa da semente, que estudos anteriores demonstraram ser um atributo fortemente conservado em termos filogenéticos. Estes exemplos ressaltam a importância da informação filogenética em estudos comparativos.

Palavras-chave: Cerrado, floresta, área foliar especifica, razão da área foliar, filogenia

INTRODUCTION

Plant adaptation to the environment is a universal theme that underlies the study of plant physiological ecology. Even when a study does not explicitly focus on evolutionary processes, adaptation usually provides a logical basis for considering optimal patterns of phenology, resource uptake and use, allocation, morphology and defense (Ackerly et al., 2000). By comparing these traits across species adapted to different environmental conditions, we can gain insight into the selective pressures and constraints that explain the diversity in plant form and function observable in nature. As a result, the comparative approach has become an increasingly important tool for plant physiological ecology.

Species diversity is the fundamental basis for comparative studies, therefore the vast species richness of Brazilian biomes provides unparalleled opportunities for studying plant adaptation to the environment. However, when designing a comparative study, careful consideration should be given to the choice of species for the question of interest (Harvey, 1996). In diverse ecosystems, this can be best done with a knowledge of the phylogenetic relationships among the potential study species, but in tropical ecosystems, this information is often unavailable. Nevertheless, even with incomplete phylogenetic information we can greatly enhance our ability to perform robust statistical tests of ecological and evolutionary hypotheses.

In this paper, we examine the importance of phylogenetic information for studies of evolution of plant form and function, relying on examples from our own comparative studies of savanna and forest woody plants of the Brazilian Cerrado. Three fundamental questions motivated this work: 1) How do plant adaptations to the environment differ between savanna and forest environments? 2) What environmental factors limit the success of forest tree species within the cerrado and *vice versa?* 3) How do cerrado and forest species differ in their effect on ecosystem structure and function? In this paper, we focus on the first of these three questions, but the comparative approach is a valuable tool in addressing all of the above questions. The last two questions are not explicitly evolutionary in nature, yet here we hope to illustrate the importance of considering evolutionary history even when addressing questions of a purely ecological nature.

SAVANA AND FOREST WOODY PLANTS AS DISTINCT FUNCTIONAL TYPES

The Cerrado region of central Brazil is characterized by a mosaic of savanna and forest vegetation. The savanna vegetation ranges in tree density from grassland (campo limpo), open shrub savanna (campo sujo), open tree-shrub savanna (campo cerrado), and tree-dominated savanna (cerrado *sensu stricto* or cerrado sentido restrito; Coutinho, 1978; Ribeiro and Walter, 2001). A diversity of forest types occur in the region, including riparian forests and a diversity of upland forests ranging from deciduous to evergreen. The region also contains close-canopy woodlands (cerradão), which have been commonly been grouped with forest formations (Coutinho, 1978; Ribeiro and Walter, 2001).

There tends to be little overlap in the tree communities of these savanna and forest formations, with the exception that cerradão woodlands commonly contain both savanna and forest species. While it is straightforward to classify most species into savanna or forest functional types, however a few species appear intermediate in their distributions and are not so easily classified. Other species occur predominantly in cerrado vegetation in some regions, while persisting within forest environments elsewhere (or visa versa). In some cases, taxonomic problems may contribute to these confusions. Fire suppression, a common policy in many cerrado reserves, adds to this problem by allowing forest species to invade cerrado (Durigan and Ratter, 2006), obscuring natural patterns of species distribution.

These complications in classifying species into savanna or forest functional types do not present serious problems to comparative studies. In most cases it is sufficient to infer the relative tendency to occur in savanna versus forest habitats. In this respect, classification of woody plants into savanna and forest species approach is similar to studies focusing on other continua of plant strategies such as pioneer/climax, r/K selected species, which have proved to be a valuable tool for ecosystem studies and studies of plant adaptations to the environment (Bazzaz, 1991).

The low similarity in species compostion between savanna and forest vegetation suggests that contrasting environmental conditions impose strong constraints on species distributions in these habitats. Light tends to be more abundant in savanna, though seedlings in savanna may experience very low light levels (Nardoto et al., 1998, Kanegae et al., 2000) and forest canopy trees experience very high light intensities. Relative to savanna, forest soils tend to have higher nutrient availability (Furley, 1992; Ruggiero et al., 2002, Silva et al., 2008), though this is not always the case for cerradão (Haridasan, 1992). Similarly, forests are associated with greater water availability in the case of riparian forests and, at regional scales, in the Amazon and Atlantic forest regions that border the Cerrado. These differences in resource availability, combined with the higher fire frequencies typical of savanna (Hennenberg et al., 2006), should exert very different selective pressures for species inhabiting these environments. One could expect that this would be manifested in savanna species exhibiting reduced investment in light interception (for instance, low ratio of leaf area to whole-plant mass, shorter seedlings, and lower specific leaf area) and greater investment for acquisition of soil resources (higher root:shoot ratio).

THE COMPARATIVE APPROACH

Perhaps the simplest conceivable approach to testing the above hypotheses would involve choosing one representative savanna tree species and one representative forest tree species. Seeds of these two species would then be germinated and the resulting seedlings grown in a common environment to ensure that any observed differences are not caused by differences in growing conditions. Then, at some pre-determined point in time, multiple seedlings of each species would be harvested and the various plant traits are measured. In such study, a two-sample comparison test (for instance a t-test) would be applied to test whether each of the traits differ between the two species.

Figure 1 shows data for such a comparison. The data were obtained from seedlings of one savanna tree species (*Enterolobium gummiferum* (Mart.) MacBride) and one forest tree species (*Ouratea castaneaefolia* (St. Hil.) Engl.) grown under full sun for 150 days. Contrary to the hypotheses, the savanna species had higher LAR, SLA, and seedling height than did the forest species. However, by relying on only a single species from each environment, we have no ability to make inferences about savanna and forest species in general, and therefore are unable to test our hypotheses. To understand this point, it is important to consider that any two species, even if they both come from a single habitat, are almost certain to exhibit differences in any quantitative trait we measure, provided that sufficient sample sizes are used. In our example, the observed differences in the species traits may result from their differences in phenology (*E. gummiferum* is deciduous but *O. castaneaefolia* is evergreen), seed size (*E. gummiferum* has larger seeds than *O. castaneaefolia*), nitrogen metabolism (*E. gummiferum* is N-fixing, but *O. castaneaefolia* is not), or any of the other traits that these species acquired from their ancestors.

The suite of traits exhibited by any species is the product of its particular evolutionary history, and only partly reflects specialization to the particular environment that it currently inhabits (Ackerly, 2004). For example, in *E. gummiferum*, deciduousness, nitrogen fixation, and large seeds should not be considered as adaptations to the cerrado environment, since these traits are shared by closely-related species that principally inhabit forest environments. Only novel traits provide useful information for inferring natural selection (Harvey, 1996; Wanntorp et al., 1990).

However, if novel traits arise independently within multiple evolutionary lineages, this indicates convergent evolution, which provides strong evidence for adaptation (Wanntorp et al., 1990). Therefore it becomes

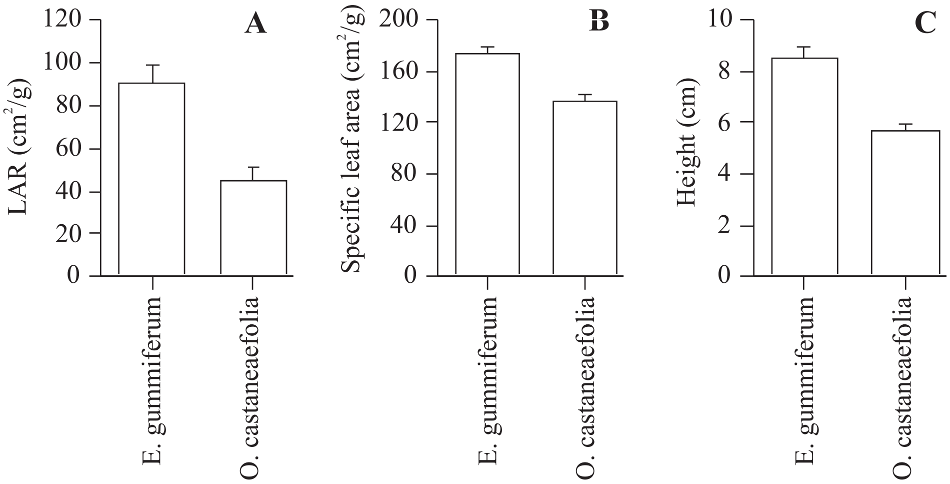

Figure 1. A comparison of three seedling traits for one savanna tree species (*Enterolobium gummiferum*) and one forest tree species (*Ouratea castaneaefolia*). All three variables differed significantly (t-test, P<0.05) between the two species, but none of the comparisons support the initial hypotheses. However, since only one species of each functional type is compared, it is not possible to determine whether the observed differences are due to specialization to their respective environments or due to unrelated historical evolutionary events. Data are adapted from Hoffmann and Franco (2003).

important to determine if species are similar because of convergent evolution or because of descent from a common ancestor. Phylogenetic information is essential for distinguishing these two cases.

Figure 2 shows the hypothetical phylogenetic relationships for two sets of species that could potentially be chosen for a comparative study. In the first phylogeny, all savanna species share a common ancestor, while all forest species share another common ancestor. If, as hypothesized, the forest species have consistently taller seedlings than savanna species, this may simply reflect the fact that they share a common ancestor, which happened to have tall seedlings. In contrast, the second scenario reflects a situation in which divergence into savanna and forest species has occurred independently in multiple lineages. In this case, if we find that seedlings of savanna species are consistently shorter than those of closely-related forest species, this reflects convergence, and consequently, adaptation.

In Brazilian ecosystems, it may initially seem to be a difficult task to obtain the phylogenetic information necessary to determine whether traits are shared due to convergence or to common ancestry. However, the phylogenetic relationships among the principal plant families have been published (Davies et al., 2004), and online tools can be used to extract these relationships for a particular subset of families (Webb and Donoghue, 2005). A practical consequence of this is that it is generally possible to determine the phylogenetic relationships among a set of potential study species, and then use this information to choose appropriate species for a comparative study.

For comparing savanna and forest species, we have relied on congeneric species pairs, each of which contain one savanna species and one forest species. By avoiding pairs from closely-related genera, we can safely assume that each pair represents an independent divergence into savanna and forest functional types. Therefore, differences between savanna and forest species that occur consistently in many pairs of species can be interpreted as evidence of natural selection (Wanntorp et al., 1990).

If we now examine the original hypotheses using multiple congeneric species pairs, the value of utilizing phylogenetic information becomes evident (Figure 3). For each of the three variables, if we choose not to use the phylogenetic information and disregard species pairings, we find no significant difference between savanna and forest species due to the large amount of interspecific variation within each of the groups. However, if we utilize the phylogenetic information by pairing species, it become evident that, within genera,

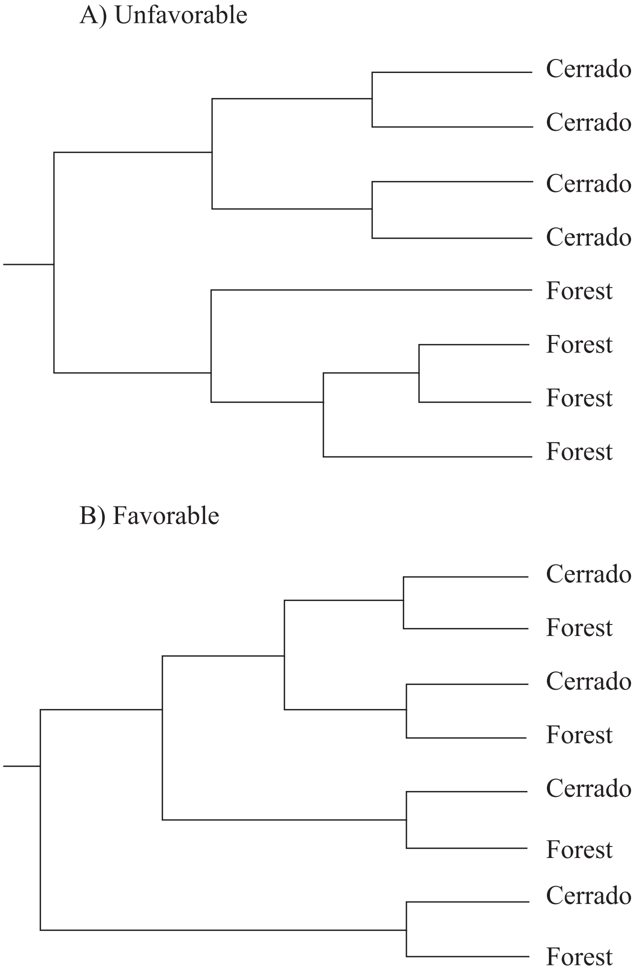

Figure 2. Two hypothetical phylogenies showing two extremes of potential relationships among a set of study species. Phylogeny A is poorly suited for statistical comparisons between cerrado and forest species because of the high degree of phylogenetic dependence. The divergence between the savanna and forest species occurred only once, and it is therefore not possible to determine whether any differences between them are caused by phylogenetic effects or by adaptation to the two environments. In contrast, phylogeny B is much more suitable for testing for differences between savanna and forest species.

forest species tend to have higher LAR, SLA, and seedling height as was originally hypothesized (Figure 3). Here, an improvement in statistical power was attained, making it possible to discern adaptation to the particular environment even though much of the interspecific variation in species traits can be attributed to phylogeny.

In this example, LAR, SLA and height of seedlings were strongly conserved within genera, as indicated by the tendency for both species within a genus to have similar values of a variable. For example, if a forest species had high values of LAR, the savanna species of the same genus also had high values of LAR. By grouping the species in congeneric pairs, we can eliminate the effects of genus and strengthen our ability to observe convergence within each functional type.

In these examples, analysis of variance can be used to quantify the contributions of phylogeny (i.e. genus) and functional type to the total variation among species (Hoffmann and Franco, 2003). For the data presented in Figure 3, phylogeny accounted for 81%, 74%, and 86% of the interspecific variation in LAR, SLA, and height, respectively. In contrast, functional type accounted for only 12%, 15%, and 10% of the variation among species. The fact that so little of the total variation can be attributed to functional type should not suggest that selective pressure for these traits has been weak, but instead emphasizes the dominant role of phylogenetic history in determining species characteristics.

It is important to note that the species is the statistical sampling unit in these analyses, while individuals are considered subsamples. It is critical to emphasize this point, because many researchers are accustomed to considering individual organisms as the sampling unit, as is typically the case in experimental settings. But when testing an evolutionary hypothesis based on differences among species, two individuals of a species do not provide independent support for this hypothesis because this is merely an extreme case where traits are shared because of a common ancestor. A practical implication is that statistical power will be more strongly enhanced by increasing the number of study species than by increasing the number of individuals per species. Nevertheless, measurements on multiple individuals per species will generally be needed to attain reliable mean trait values for each study species.

CORRELATIONS AMONG TRAITS

Within a species, evolutionary change in one trait almost always occurs concurrently with change in other traits. This non-independent evolution of species traits can result from pleiotropy, which occurs when one gene influences multiple traits, or because of tradeoffs and

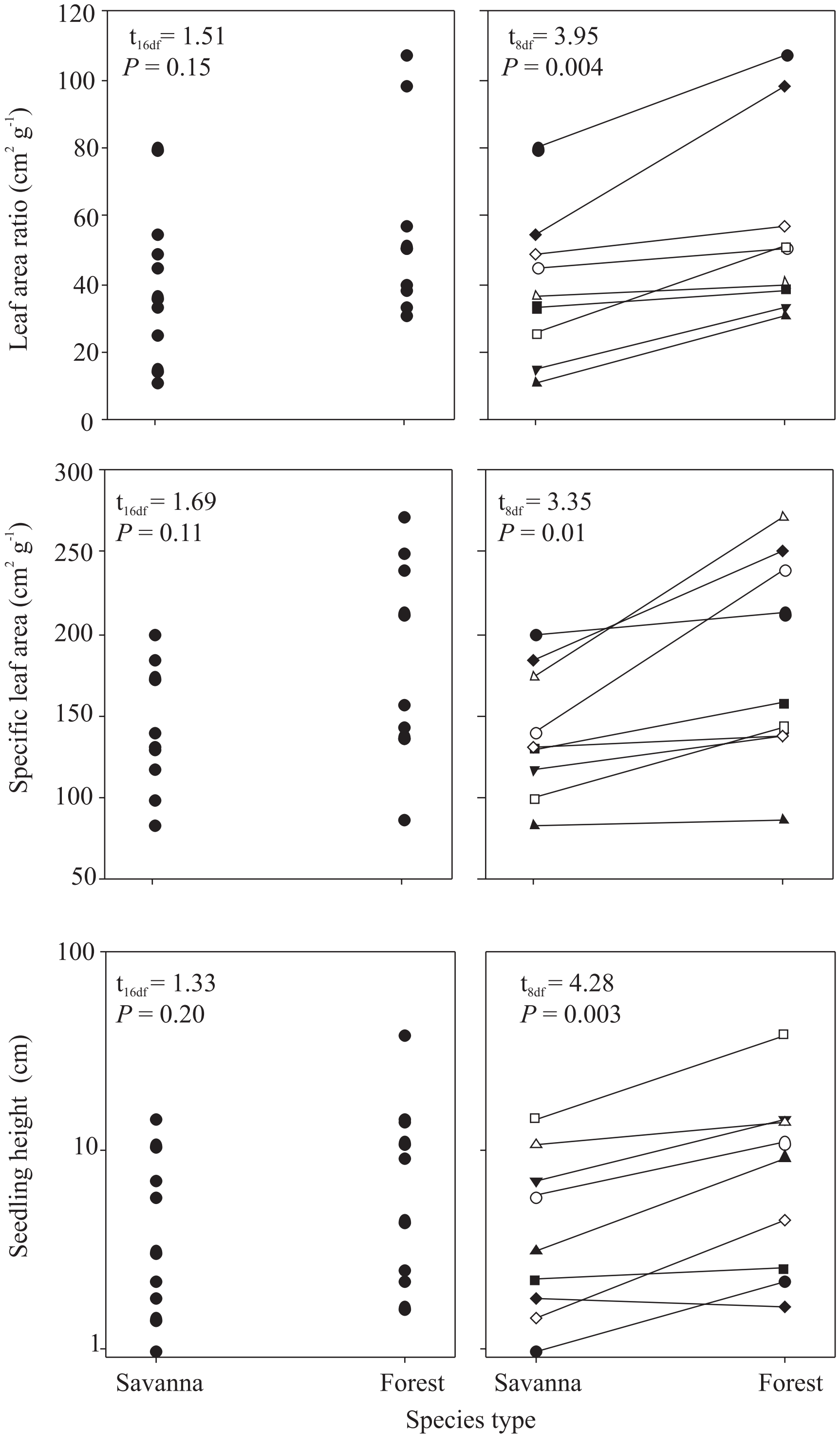

Figure 3. Demonstration of the increase in statistical power that can be attained with the use of phylogenetic information. If the phylogenetic relationships are ignored and a simple t-test is used to compare savanna and forest species, there is no significant difference for leaf area ratio (leaf area/seedling mass), specific leaf area (leaf area/leaf mass), or seedling height because of the large amount of overlap in species means. However, when phylogenetic information is used to pair species of the same genus, a paired t-test results in significant differences for all traits. Symbols used for genera: ● *Alibertia*, ○*Aspidosperma*, ▼*Brosimum*, △*Enterolobium*, ■*Guapira*, □*Hymenaea*, ◆*Jacaranda*, ◇*Ouratea*, ▲*Salacia*.

constraints that limit the combination of traits that are physically possible or that are beneficial to the plant. Much insight into such constraints can be gained by examining correlations among traits across species. Consider, for example, the relationship between seedling height and seed mass. Wherever there is competition for light, a selective pressure should exist for greater investment in seedling height, which increases the amount of light interception (Figure 4). However, biophysical constraints limit the maximum height that can be attained with a given mass of seed reserve (Figure 4). The combined effect of these two constraints should determine the strength of the relationship, as well as its slope and intercept.

Such constraints are important to consider when inferring natural selection and conservatism of the traits. If two traits are strongly correlated, when natural selection acts on one of them, the other is likely to evolve as well. This correlated evolution can be inferred if the phylogenetic relationships among the species is known (Felsenstein, 1985).

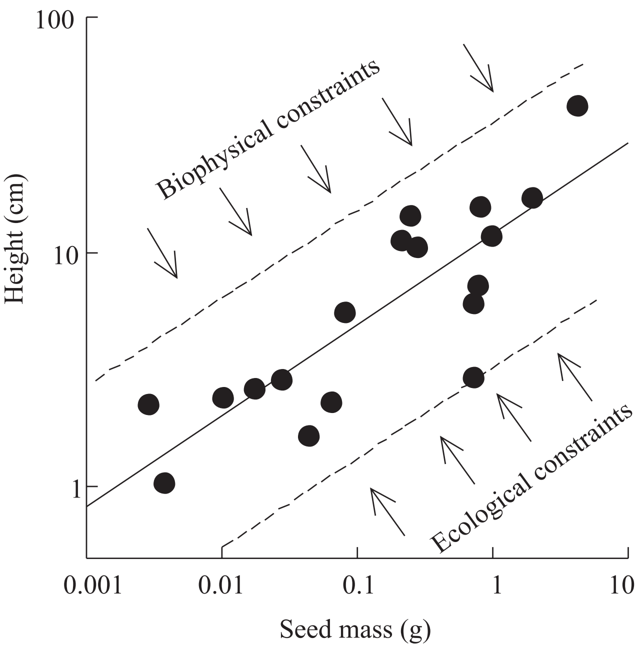

Figure 4. Representation of the hypothesized constraints that result in correlations between seed mass and seedling height. Species with small seeds and tall seedlings are not observed because it is energetically impossible, while competition for light selects against seedlings that invest too little of their seed reserves for growth in height.

Many seedling traits, for example, are strongly correlated with seedling mass. LAR has been shown to be negatively correlated with seed mass, while seedling height is positively correlated with seed mass (Figure 5). The correlation between seed mass and seedling height may result simply because large seeds have more resources for quickly producing large seedlings. The negative relationships between seed mass and LAR may largely arise from an ecological constraint in which seedlings of small-seeded species need greater leaf area to ensure rapid growth, as small-seeded species are more sensitive to stresses such as drought (Baker, 1972; Leishman and Westoby, 1994) and herbivory (Armstrong and Westoby, 1993).

Regardless of the mechanisms that give rise to these correlations between seed mass and seedling traits, the fact that these same relationships have been observed repeatedly in other systems (Marañón and Grubb, 1993) suggests that they arise from strong constraints on the evolution of seedling traits. This is of particular importance for the evolution of seedling traits because seed mass is a trait that is very strongly conserved phylogenetically (Peat and Fitter, 1994). For the species included in this example, genus accounted for 94% of the total interspecific variation in seed mass (Hoffmann and Franco, 2003). Seed mass, in turn, explains 52% of the variation in LAR across species and 92% of the variation in seedling height (Figure 5). Because of the strong phylogenetic conservatism of seed mass, combined with the strong relationship between seed mass of other seedling traits, it follows that these seedling traits should also be conserved within genera.

When comparing species from different habitats, it is useful to compare how these relationships among traits are influenced by the contrasting environments under which they evolved. For the relationship between LAR and seed mass, the relationship was similar for cerrado and forest species (Figure 5A). For the relationship between seedling height and seed mass, there was a shift in the intercept of the relationship (Figure 5B), indicating that the evolution of reduced seedling height among cerrado species occurred independently of any change in seed mass. Alternatively, evolution may proceed as a shift along the main axis of variation, as is seen in the relationship between specific leaf area and leaf P concentration (Figure 5C, Hoffmann et al., 2005b).

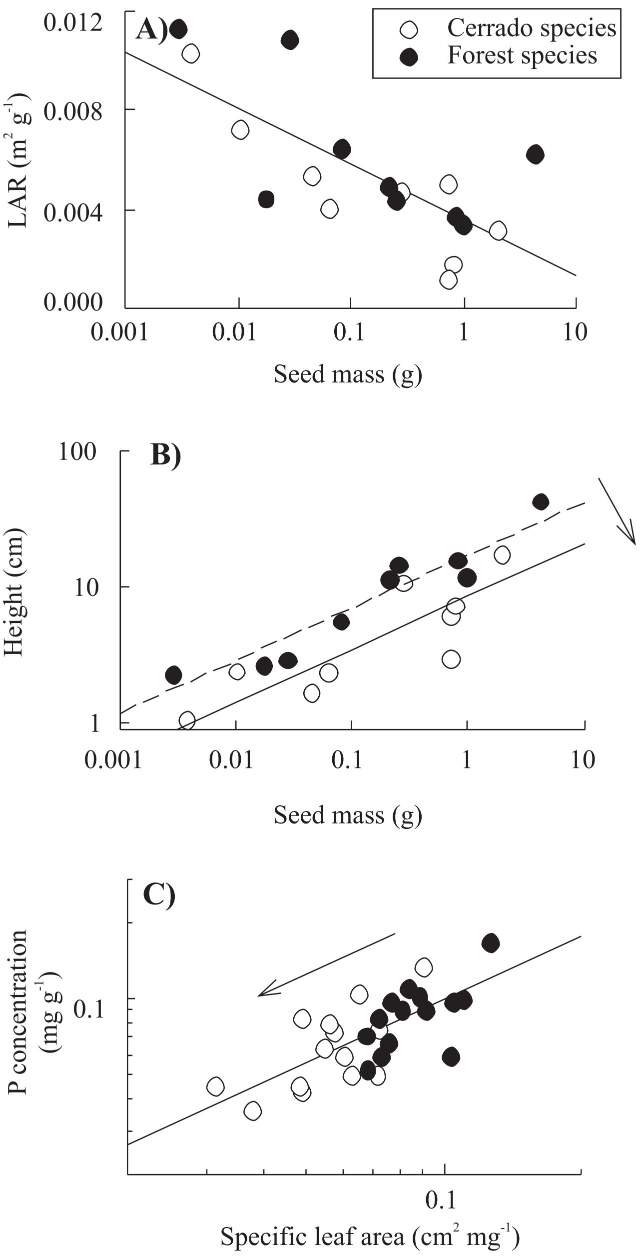

Figure 5. Examples of patterns of trait evolution when strong correlations exist between traits. Leaf area ratio (A) and seedling height at 150 days (B) are both strongly correlated with seed mass. In the case of LAR, there was no shift in position of species means along the overall relationship between savanna and forest species. However, for height, savanna species exhibited a downward shift in the intercept of the relationship. In other cases, such as the relationship between SLA and leaf nutrient concentration for adult plants, there was a shift along the main axis, with no shift in intercept. Data are adapted from Hoffmann and Franco (2003) and Hoffmann et al. (2005b).

Strong relationships between SLA and nutrient concentrations have been well demonstrated in ecosystems throughout the world (Reich et al., 1999; Wright et al., 2004), suggesting that these relationships arise from universal constraints on leaf physiology and function. Savanna species tend to have lower SLA and lower nutrient concentrations than forest species (Figure 5C, Hoffmann et al., 2005b). In this case it is not clear whether this has arisen primarily from the action of natural selection on SLA or on nutrient concentration, though it is likely that both traits are adaptive in the cerrado.

Examining relationships between only two variables can be quite informative, but in reality, tradeoffs commonly involve many traits simultaneously (Reich et al., 1999). This is particularly true when we consider patterns of carbon allocation. Cerrado species tend to have greater investment in bark (Hoffmann et al., 2009), roots (Hoffmann et al., 2004), and nonstructural carbohydrates (Hoffmann et al., 2004), at the expense of lower allocation in stem biomass (Hoffmann and Franco, 2003) and leaf area (Hoffmann and Franco, 2003, Hoffmann et al., 2005a). A result of these differences is that cerrado species have lower growth rates and greater hydraulic conductance per unit leaf area (unpublished data).

CONCLUSIONS

Boundaries between cerrado and forest are dynamic systems, having undergone past shifts (Silva et al., 2008) and will probably respond to future climate change (Salazar, 2007). Humans contribute to these dynamics through deforestation and increased fire frequency in some cases (Cochrane et al., 1999) and by suppressing fire in others (Durigan and Ratter, 2006). The response of these ecotones to changing climate and disturbance regimes depends largely on the physiological and ecological characteristics of cerrado and forest trees species. Comparative studies will aid in understanding these characteristics. The objective of this paper was to emphasize the importance of considering the evolutionary history of plants in any comparative studies. This has value not only for robust inference of the role of natural selection but also for increasing the statistical power of comparisons.

The large number of genera containing both cerrado and forest species offers a unique opportunity that is not available in any other tropical savanna region of the world. Similar approaches can be applied to many other interesting comparisons, such as differences between trees versus shrubs, deciduous versus evergreen species or terrestrial versus epiphytic habits. While congeneric species pairs may often not be available, the growing understanding of phylogenetic relationships among plant taxa is increasing the opportunities for designing comparative studies.

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