

Comparative growth analysis of tropical forest and savanna woody plants using phylogenetically independent contrasts

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Summary

1 The savanna–forest boundary in the tropics is marked by a discontinuity not only in tree density, but also in species composition, with few tree species regularly occurring in both savanna and forest environments.

2 We performed comparative growth analysis for nine congeneric pairs, each containing one savanna species and one forest species, grown in a factorial design involving two light and two nutrient levels.

3 Contrary to predictions, there was no difference in relative growth rates (RGR) between savanna and forest species. However, there were clear differences in allocation patterns and in phenotypic responses to light intensity. Savanna species allocated more biomass to roots and maintained lower leaf area per unit plant mass (LAR), and lower leaf area per unit leaf mass (SLA).

4 Savanna species also exhibited greater phenotypic plasticity in specific leaf area, leaf area ratio and net assimilation rate in response to light intensity. An increase in LAR in response to shading of savanna species offset a decrease in net assimilation rate per unit leaf area (NAR), such that RGR was largely unaffected.

5 For most plant traits measured, more of the interspecific variation could be attributed to differences among genera than to differences between the two functional types, indicating that these seedling traits are highly conserved within the congeneric species pairs. Many of these traits were correlated to seed mass, which is itself highly conserved within genera.

Key-words: allocation, functional type, light, plasticity, relative growth rate

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Introduction

Understanding the factors that determine the current location of an ecotone is necessary to predict vegetation distribution following altered climate or disturbance regimes. In the tropics, this is particularly true of the savanna–forest boundary, which has undergone widespread shifts in the past (Desjardins *et al.* 1996; Pessendra *et al.* 1998; Delegue *et al.* 2001), and is expected to shift under future climate scenarios (Neilson *et al.* 1998; Bergengren *et al.* 2001). This ecotone not only reflects a discontinuity in tree density, but also a discontinuity in species composition, with most species effectively limited to either the savanna or forest (Adejuwon & Adesina 1992; Felfili & Junior 1992). Ecological and physiological differences will determine the distribution of these two groups of species, thereby playing a

dominant role in the dynamics of the savanna–forest boundary, but there have been few comparative studies (Longman & Jenik 1992). This contrasts with the large number of comparative studies of pioneer and climax species in tropical forests, which have greatly enhanced our understanding of gap dynamics.

The savanna–forest boundary represents a balance between gradual forest advance and occasional forest retreat, caused primarily by fire (Hopkins 1992). Forest species are more sensitive to fire than savanna species (Hoffmann & Moreira 2002), so savanna fires kill forest species establishing in the savanna environment (Hoffmann 2000) and degrade the forest edge. The forest understorey tends to be much less flammable than savanna (Biddulph & Kellman 1998; Cochrane *et al.* 1999), but occasional fires do cause widespread mortality of forest species. The increase in frequency of fires in many savanna areas, due to human activity, has led to increasing amounts of former forest becoming savanna (Cochrane *et al.* 1999).

Table 1 Species used in this study

Savanna species	Forest species	Family
<i>Alibertia concolor</i> (Cham.) Schum.	<i>A. macrophylla</i> Schum.	Rubiaceae
<i>Aspidosperma macrocarpon</i> Mart.	<i>A. subincanum</i> Mart.	Apocynaceae
<i>Brosimum gaudichaudii</i> Trec.	<i>B. rubescens</i> Taub.	Moraceae
<i>Enterolobium gummiferum</i> (Mart.) MacBride	<i>E. contortisiliquum</i> (Vell.) Morong	Leguminosae (Mimosoideae)
<i>Guapira noxia</i> (Netto) Lundel.	<i>G. graciliflora</i> (Mart. Ex J.A. Schmidt) Lundel.	Nyctaginaceae
<i>Hymenaea stignocarpa</i> Mart. Ex Hayne	<i>H. courbaril</i> (Hayne) Lee et Lang.	Leguminosae (Caesalpinoideae)
<i>Jacaranda ulei</i> Burm. & K. Schum.	<i>J. puberula</i> Cham.	Bignoniaceae
<i>Ouratea hexasperma</i> (St. Hil.) Bail.	<i>O. castaneaefolia</i> (St. Hil.) Engl.	Ochnaceae
<i>Salacia crassifolia</i> (Mart.) G. Don.	<i>S. elliptica</i> (Mart.) G. Don.	Hippocrateaceae

The process of forest advance is much less understood. In some savanna regions, it is relatively rapid when fire is excluded (Hopkins 1992; Swaine *et al.* 1992; King *et al.* 1997), but in others, including the Brazilian Cerrado, there is only scattered establishment of forest trees even after one or two decades of fire exclusion (Bowman & Fensham 1991; San José & Fariñas 1991; Ratter 1992; Bowman & Panton 1995). This establishment may be limited by the high light intensity and temperature characteristic of savanna, as well as the lower nutrient and/or water availability relative to forest. As a result, the establishment and growth of forest species is low in open savanna, and is facilitated by the presence of adult savanna trees that ameliorate water and nutrient stress for establishing seedlings (Kellman 1985; Hoffmann 1996). However, it is not clear that forest species differ fundamentally from savanna species, as the establishment of the latter may also be facilitated by tree cover (Hoffmann 1996).

The present study focuses on the comparative ecology of tree and shrub species of the cerrado, a region of predominantly savanna vegetation occupying approximately 2×10^6 km² in south-central Brazil. Gallery forest is found along streams, and dry forest occurs locally throughout the cerrado region as well as at the transition zones between the cerrado and the Amazonian and Atlantic forests.

Light is a primary limiting resource in forest, whereas nutrients and water are limiting resources in savanna (Frost *et al.* 1986), so we expect forest species to allocate more resources to light capture and savanna species to capture of below-ground resources. Similarly, savanna fire should select for increased investment in coarse roots for storage and reduced investment in permanent aerial structures. Savannas are considered more stressful, unproductive environments than forests, so suites of traits typical of stress-tolerant species are expected (Grime 1977; Chapin *et al.* 1993), including low relative growth rate (RGR), low net assimilation per unit leaf area (net assimilation rate, NAR), and low leaf area per unit plant mass (leaf area ratio, LAR). Finally, species adapted to high resource availability tend to exhibit greater phenotypic plasticity in variables directly related to growth (Lortie

& Aarssen 1996), such as NAR and RGR. For other traits in which plasticity represents a strategy to improve resource uptake, such as LAR and specific leaf area (SLA), species adapted to more heterogeneous environments should exhibit greater phenotypic plasticity (Bazzaz 1996). As savannas exhibit both higher mean values and greater variability in light availability than forests, savanna species are expected to exhibit greater plasticity in both kinds of traits.

In order to better understand the ecological differences that determine the properties and dynamics of the savanna–forest boundary, we compare the morphological and growth responses of savanna and forest species to contrasting light and nutrient levels. We test our predictions of the phenotypic differences within nine congeneric species pairs, each containing one savanna and one forest species. This approach ensures phylogenetic independence, an important condition for inference in comparative studies (Felsenstein 1985), and improves the statistical power of comparisons between the two groups when there is a large amount of variation among genera (Garnier 1992; Ackerly 1999).

Methods

Nine pairs of woody plants for which seed was available were chosen (Table 1), each consisting of one savanna species and one forest species of the same genus. Except for two genera from separate subfamilies within Leguminosae, each pair represented a different family (Table 1). Two of the ‘forest’ species (*Guapira graciliflora* and *Alibertia macrophylla*) might be considered transitional species because they occur in dense savanna woodland (cerradão) as well as in forest. Only *Aspidosperma* spp. and *Enterolobium* spp. are deciduous, and except the subshrub *J. ulei* and the shrubs *A. elliptica* and *O. hexasperma* all are trees.

Seeds from all of the savanna species and most forest species were collected in the Federal District of Brasília, Brazil. Among the forest species, seeds of *B. rubescens* were collected near Nova Xavantina, Mato Grosso, and *E. contortisiliquum* was collected near Alto Paraiso, Goiás. All seeds were collected between September 1998 and January 1999. For each species, the seeds were pooled and 20–30 were sampled,

extracted from the integument, dried at 70° for at least 7 days and weighed.

Growth studies were performed in the nursery of the Forestry Department of the University of Brasília. Seeds were sown in opaque black polyethylene sacks, 10 cm in diameter and 25 cm deep, with perforated bottoms, in a substrate of oxisol subsoil collected under cerrado. Chemical analysis on this soil revealed (mean ± SE, $n = 6$) pH = 4.9 ± 0.1, N (%) = 0.28 ± 0.11, P (p.p.m.) = 0.72, K (p.p.m.) = 6.7 ± 1.8, Ca (p.p.m.) = 19.0 ± 3.2, Mg (p.p.m.) = 2.5 ± 0.8.

The seeded sacks were assigned to high or low light and nutrient treatments in a factorial design. The high nutrient level received 50 mL of 1/4 strength Johnson's solution, weekly; low light levels were provided by three identical shade houses covered with shade cloth. Measurements of photosynthetic photon flux density (PPFD) were made at six locations in each of the three shade houses on a clear day (3 August 2000) with Licor 190s quantum sensors. Measurements were made at hourly intervals for each of these sample points and paired with readings in full sun (high light treatment). The shade houses provided 26% ambient light, approximating conditions in dense cerrado woodland, the densest vegetation type considered to be cerrado, where mean daily PPFD at 10 cm height has been measured at 19% full sun (Kanegae *et al.* 2000)

Plants were organized into 10 blocks within each of the light treatments. For the low light treatment, the blocks were distributed among the three shade houses, with low and high nutrient plants randomly arranged within each block. For each congeneric species pair, both species were seeded on the same date. The plants were watered daily, when necessary, to supplement natural rainfall.

The nutrient treatment was initiated when approximately 50% of the seedlings of both species within a congeneric pair had emerged. Destructive harvests of randomly chosen individuals were performed at 50, 100 and 150 days after this date. Prior to each harvest, the second youngest mature leaf was collected from each plant, scanned on a flatbed scanner, and its area determined. This leaf was then dried and weighed to determine specific leaf area. Leaf area for each plant was estimated by multiplying total leaf mass by the mean specific leaf area for the appropriate treatment. For *Enterolobium* and *Hymenaea*, this procedure was applied to leaflets rather than entire leaves. Similarly, for species with leaf-like cotyledons, specific area was also determined for one cotyledon per plant.

At each harvest, individuals from each treatment were randomly chosen, washed, and dried for at least a week at 70 °C. Due to insufficient seed availability, sample sizes differed among species. Ten individuals were harvested in each treatment at each date, except for both species of *Ouratea*, where only eight individuals were harvested and *E. elliptica*, *E. crassifolia* and *A. macrocarpon*, where the number was reduced to four or five.

Each harvested plant was separated into leaf, stem, cotyledon, coarse roots and fine roots. For *Hymenaea* and *Enterolobium*, leaves were separated into leaflets and rachis. Each fraction was weighed separately.

ANALYSES

Classical growth analysis was used to examine patterns of growth and allocation. Relative growth rate (RGR) was calculated using

$$\text{RGR} = \frac{\overline{\ln(M_2)} - \overline{\ln(M_1)}}{t_2 - t_1}$$

where $\overline{\ln(M_1)}$ and $\overline{\ln(M_2)}$ are the mean ln-transformed plant dry masses at times t_1 and t_2 (Hoffmann & Poorter 2002). Net assimilation rate (NAR) was calculated using the equation

$$\text{NAR} = 2 \frac{M_2 - M_1}{(t_2 - t_1)(A_2 + A_1)}$$

A_1 and A_2 are total leaf area at times t_1 and t_2 . This equation was chosen, following Chiariello *et al.* (1991), after regression analysis revealed a linear relationship between leaf area and plant mass. Leaf area ratio (LAR) was calculated by dividing total leaf area by plant dry mass.

Analysis of variance was used to test for the effects of light, nutrients, harvest date, genus and plant type (savanna/forest) on total plant biomass, root : shoot ratio and leaf area ratio. As we are interested in generalizing about differences between savanna and forest species, genus was treated as a random factor, whereas all others were considered fixed factors. Note that, for comparisons between the savanna and forest types, this mixed-model ANOVA is equivalent to a paired *t*-test applied to species means (Zar 1999). Analysing the data in this manner treats each species as a statistical replicate as is appropriate in comparative studies, and as would be done with a paired *t*-test. In contrast, prescribing genus as a fixed factor would have treated each individual plant as a replicate, greatly inflating the error degrees of freedom.

When testing for an overall effect of a factor, independently of plant type, we substituted genus and plant type for a single random factor, species. For the derived variables RGR and NAR, which are based on intervals, the values were calculated for each interval and each treatment, and then analysed with ANOVA as above.

Congeneric pairs with unusually large differences can dominate the test of significance between savanna and forest species. This can occur systematically because large mean values for a trait can often be associated with large differences within a pair, resulting in non-independence in species differences (Freckleton 2000). To determine whether transformation of a variable was necessary to avoid this, we tested for a significant correlation between the pair mean and the pair difference. A significant correlation was detected for SLA, NAR, seed mass and seedling height, so these

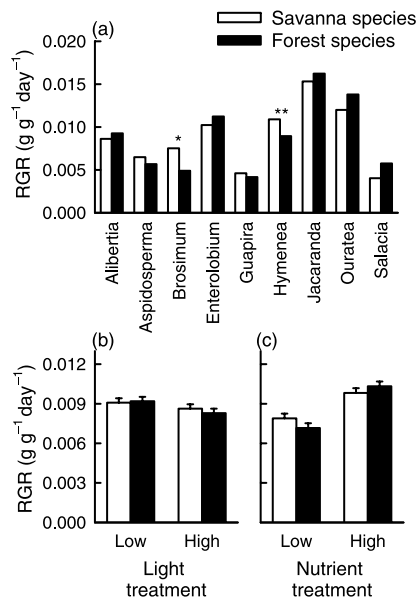


Fig. 1 (a) Relative growth rate of 18 savanna and forest species, averaged over all treatments ($*P < 0.05$, $**P < 0.01$ for differences within genera). (b) Overall effects of light treatments. (c) Overall effects of nutrient treatments. For b and c, each mean and standard error was calculated from the nine species values. All RGR values were calculated for the 50–150 day interval.

were log transformed before performing further analysis. Additional inspection failed to reveal other cases in which outliers dominated the comparison of savanna and forest species.

To compare how much of the overall interspecific variance of a trait can be attributed to differences among genera or to differences between functional types, we calculated the r^2 values for each trait. These were calculated from factorial ANOVAs, following Rosenthal & Rosnow (1985) as

$$r_x^2 = \frac{SS_x}{SS_{total}}$$

where SS_x and SS_{total} are the sum of squares for factor X and total sum of squares, respectively.

Results

RELATIVE GROWTH RATE (RGR)

There was no significant difference in relative growth rate between savanna and forest species when compared over all treatments (Fig. 1a, $F_{1,8} = 0.10$, $P = 0.76$), nor under low or high light (Fig. 1b), nor under low or high nutrient treatments (Fig. 1c). Nevertheless, when individual genera are examined *Hymenaea* and *Brosimum* do exhibit greater RGR for the savanna species (Fig. 1a).

Light had no overall effect on RGR when tested over all species (Fig. 1b, $F_{1,17} = 1.14$, $P = 0.25$), nor when tested separately over forest ($F_{1,8} = 0.0018$, $P = 0.097$)

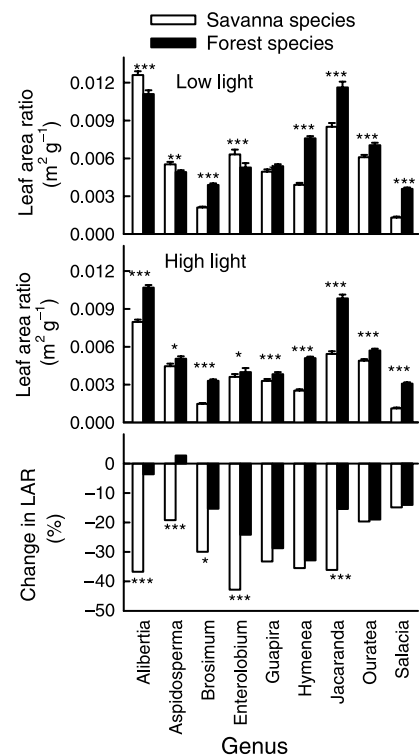


Fig. 2 Leaf area ratio (LAR) of savanna and forest species in response to light: (a) under 26% sun; (b) under 100% sun; (c) proportional decrease between treatments. All values represent means over the entire study period. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

or savanna species ($F_{1,8} = 0.79$, $P = 0.40$). However, when species were analysed individually, growth of many species did respond to light level, with the effect being positive for some species and negative for others (data not shown).

There was an overall positive effect of nutrients on RGR (Fig. 1c, $F_{1,17} = 11.99$, $P = 0.003$). The two functional types did not respond differently to nutrient level, as there was no type–nutrient interaction in RGR ($F_{1,8} = 1.22$, $P = 0.30$).

Mean RGR over all species and all treatments declined from 0.0109 to 0.0067 g g⁻¹ d⁻¹ from the first (50–100 days) to second (100–150 days) period ($F_{1,17} = 16.88$, $P = 0.003$). There was no light–nutrient interaction overall ($F_{1,16} = 2.64$, $P = 0.12$)

LEAF AREA RATIO (LAR)

Overall, leaf area per unit plant mass (leaf area ratio, LAR) was significantly higher for forest species than for savanna species ($F_{1,8} = 7.40$, $P = 0.026$; Fig. 2). This was due to differences under high light, where savanna species had lower LAR ($F = 13.38$, $P = 0.006$, Fig. 2b). Under low light, there was no overall difference in LAR between savanna and forest species ($F = 2.39$, $P = 0.16$), although many species pairs showed differences (Fig. 2a). When tested over all species, LAR was higher under low light than under high light ($F_{1,17} =$

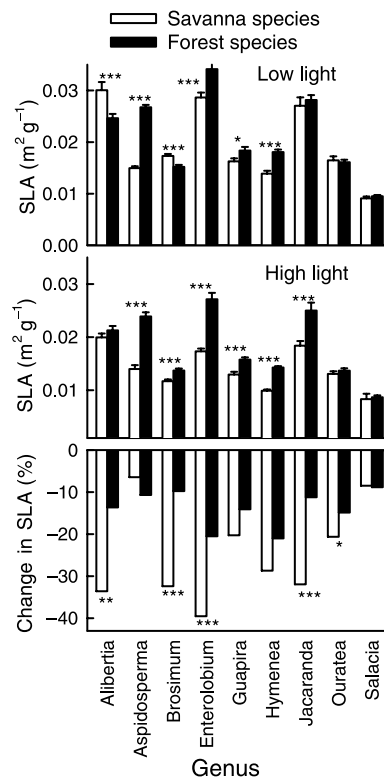


Fig. 3 Specific leaf area (SLA) of savanna and forest species in response to light: (a) under 26% sun; (b) under 100% sun; (c) proportional change between treatments. All values represent means over the entire study period. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

26.57, $P \ll 0.001$), and there was a significant light \times type interaction ($F_{1,8} = 12.76$, $P = 0.007$), with savanna species showing greater response to light than forest species (Fig. 2c).

LAR can be decomposed into leaf mass per unit plant mass (leaf weight ratio, LWR) and leaf area per unit leaf mass (specific leaf area, SLA), such that $LAR = LWR \times SLA$. Savanna species had lower LWR ($F_{1,8} = 6.40$, $P = 0.035$) as well as lower SLA ($F_{1,8} = 18.72$, $P = 0.003$, Fig. 3b) than forest species in full sun. In contrast, under 26% sun, neither LWR ($F_{1,8} = 3.27$, $P = 0.107$) nor SLA ($F_{1,8} = 1.77$, $P = 0.22$, Fig. 3a) differed significantly between savanna and forest species. Savanna species exhibited greater plasticity in SLA in response to increased light than did forest species ($F_{1,8} = 12.93$, $P = 0.007$, Fig. 3c).

LAR was also greater under high nutrients than low ($F_{1,17} = 11.29$, $P = 0.0037$), with no light–nutrient interaction ($F_{1,17} = 0.08$, $P = 0.78$), nor nutrient–functional type interaction ($F_{1,8} = 0.032$, $P = 0.86$). This response to nutrients was due to increased LWR ($F_{1,8} = 10.94$, $P = 0.01$) while SLA was unaffected ($F_{1,8} = 0.44$, $P = 0.52$).

There was an overall decline of 25% in LAR from the first to last harvest ($F_{2,17} = 14.17$, $P < 0.001$). LAR was positively correlated with RGR among species ($r = 0.55$, $P = 0.02$).

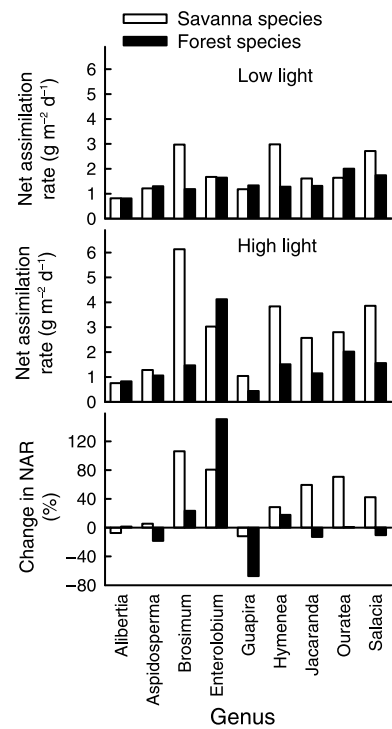


Fig. 4 Net assimilation rate (NAR) of savanna and forest species in response to light: (a) NAR under 26% sun; (b) NAR under 100% sun; (c) proportional change in net assimilation rate under 100% sun relative to 26% sun. All values represent means over the entire study period.

NET ASSIMILATION RATE (NAR)

Over all treatments, NAR did not differ significantly between savanna and forest species ($F_{1,8} = 3.66$, $P = 0.09$). However, there was a light \times type interaction ($F_{1,8} = 15.44$, $P = 0.004$), such that savanna species had higher NAR than forest species under high light (Fig. 4; $F_{1,8} = 5.62$, $P = 0.04$), but not under low light ($F_{1,8} = 0.19$, $P = 0.67$). Over all species, NAR was greater under high light ($F_{1,17} = 4.85$, $P = 0.04$), but when analysed separately for each functional type, light significantly enhanced NAR for savanna species ($F_{1,8} = 5.41$, $P = 0.05$) but not for forest species (Fig. 4; $F_{1,8} = 0.38$, $P = 0.56$). Nutrients did not have an overall effect on NAR ($F_{1,17} = 3.25$, $P = 0.11$) nor was there a light–nutrient interaction ($F_{1,17} = 0.54$, $P = 0.48$). There was a 19% decline in NAR from the first to the second interval, though this was not significant overall ($F_{1,17} = 1.21$, $P = 0.30$). NAR was not correlated with RGR among species ($r = 0.15$, $P = 0.56$).

BIOMASS PARTITIONING

There were clear differences in biomass partitioning between savanna and forest species. Savanna species allocated less to leaves and stems ($F_{1,8} = 16.32$, $P = 0.004$), and more to coarse roots ($F_{1,8} = 5.43$, $P = 0.05$) than did forest species (Fig. 5), but there was no difference in fine roots (Fig. 5, $F_{1,8} = 0.37$, $P = 0.85$). The fraction of biomass in fine and coarse

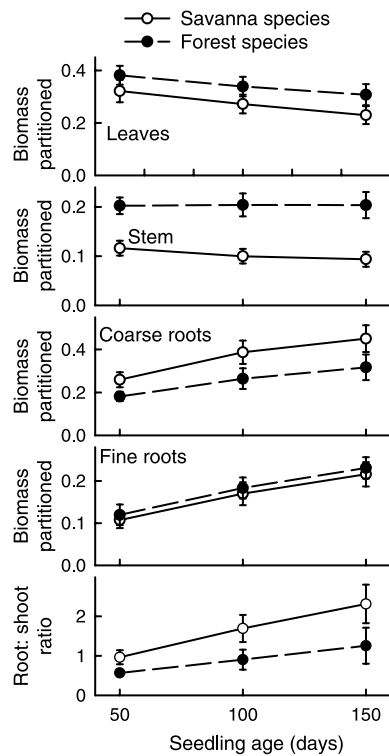


Fig. 5 Biomass partitioning, as the fraction of total plant biomass, of savanna and forest trees into leaf, stem, coarse roots and fine roots. Error bars represent standard errors for the variation between species ($n = 9$ species each).

roots increased steadily over the course of the experiment, whereas that in leaves declined, and stems remained relatively constant (Fig. 5). The mean root:shoot ratio of savanna species therefore increased from 0.96 to 2.31 from the 50th to the 150th day, while that of forest species increased from 0.57 to 1.26 (Fig. 5).

COMPARISONS AMONG GENERA

More of the overall interspecific variance is explained by differences among genera than by differences between savanna and forest species (Table 2), except for the ratio of stem mass to plant mass (stem weight ratio), for which plant type accounted for nearly 50% of the overall variance. In contrast, 94% of the interspecific variation in seed mass is attributed to genus, whereas less than 1% can be attributed to differences between functional types (Table 2). Among genera, mean seed mass spanned more than three orders of magnitude, explaining a large portion of the intergeneric variation in other variables that are correlated to seed mass (Fig. 6). Both SLA and LAR were negatively correlated with seed mass (Figs 6a, b), as was allocation to fine root biomass (not shown, $r^2 = 0.42$, $P = 0.004$). The negative relationship between LAR and seed mass was offset by a positive correlation between seed mass and NAR (Fig. 6c), so there was no significant relationship between seed mass and RGR for 50–150 days (not shown, $r^2 = 0.14$, $P = 0.13$), although there was a

Table 2 Fraction of total interspecific variance (r^2) that is explained by phylogeny (genus) and by functional type. The r^2 values were calculated from ANOVA sums of squares as explained in Methods. Seed mass, LAR and NAR were log-transformed before analysing

Trait	r^2_{genus}	r^2_{type}
Seed mass	0.94	< 0.01
Relative growth rate	0.94	< 0.01
Leaf area ratio	0.87	0.06
Net assimilation rate	0.65	0.14
Specific leaf area	0.85	0.06
Root:shoot ratio	0.57	0.17
Coarse root weight ratio	0.61	0.16
Fine root weight ratio	0.92	< 0.01
Stem weight ratio	0.38	0.49
Plasticity in NAR	0.69	0.10
Plasticity in LAR	0.53	0.27
Plasticity in SLA	0.47	0.30

weak relationship for the high-nutrient treatment ($r^2 = 0.24$, $P = 0.04$). Also when RGR is calculated for the post-emergence stage (0–50 days), using seed mass as initial mass, there was a strong relationship between seed mass and RGR ($r^2 = 0.61$, $P < 0.01$). Seedling height was strongly correlated with seed mass, with forest species averaging more than twice the height of savanna species (Fig. 6d, $F_{1,15} = 11.74$, $P = 0.004$). When tested only within genera by regressing the difference in a trait value against the difference in $\log(\text{seed mass})$, none of these relationships is significant, with the exception of seedling height ($r^2 = 0.52$, $P = 0.03$).

Discussion

There were clear differences between savanna and forest species, despite the fact that the forest species included a diversity of functional types, including pioneers (*E. contortisiliquum*), climax (*H. courbaril* and *B. rubescens*), understory trees (*S. elliptica*) and savanna/forest intermediates (*A. macrophylla* and *G. graciliflora*), which were collected from transitional Amazonian forest (*B. rubescens*), dry forest (*E. contortisiliquum* and *A. subincanum*), closed cerrado woodland (*G. graciliflora*) and gallery forest (remaining species). Similarly, the savanna species included a range of growth forms from subshrubs to trees. The emergence of clear differences between savanna and forest species, despite the high functional diversity within each of these groups, reinforces the generality of these results.

The most striking differences between savanna and forest species arose in biomass partitioning. Savanna species allocated more biomass to coarse roots and less biomass to leaves and stems than did forest species, as predicted based on the physical differences between the savanna and forest environments. Coarse roots enable the carbohydrate and nutrient storage necessary for surviving frequent fire, and provide greater rooting

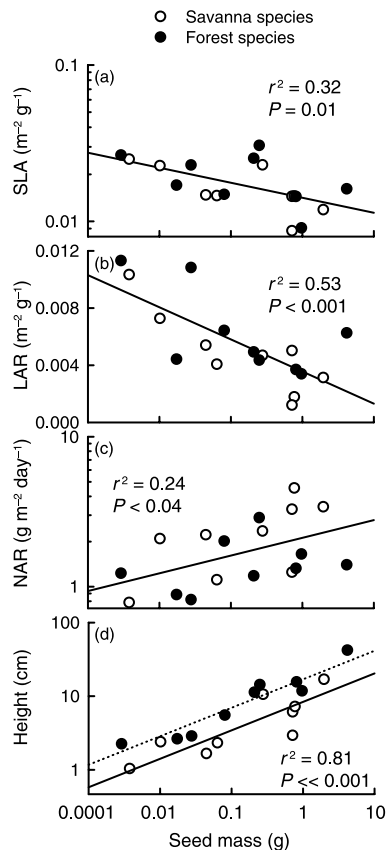


Fig. 6 Relationship between seed mass and (a) specific leaf area, (b) leaf area ratio, (c) net assimilation rate, and (d) seedling height. All values represent means over all treatments and over the study period. For A–C the regression lines were fit using all species, but for D, separate lines were fit for savanna and forest species using ANCOVA as forest species averaged twice the height of savanna species.

depth, which permits access to deeper soil water during the 5-month dry season. Indeed, first-year seedlings of cerrado savanna species exhibit high survival during this dry season, even when burned (Oliveira & Silva 1993; Hoffmann 2000; Kanegae *et al.* 2000). While there is little comparable information for survival of forest seedlings in the cerrado, three forest species were unable to survive burning, and survived the dry season only in shaded sites (Hoffmann 2000).

In contrast to the open savanna environment, competition for light is important in the forest environment, conferring an advantage to taller individuals with greater leaf area. Forest species were on average twice as tall as savanna species and had 51% greater leaf area. This greater leaf area was the result of greater allocation to leaf biomass (leaf mass ratio, LMR) as well as greater leaf area per leaf mass (leaf area ratio, LAR). The difference in leaf area ratio increased, in relative terms, as the seedlings aged. In the 100 days that elapsed between the first and the last harvest date, the fraction of biomass partitioned as leaves declined by 29% among savanna species, but only by 19% among forest species (Fig. 5). If this trend continues through development, it could have important implications for

RGR, which is strongly correlated with LAR (Lambers & Poorter 1992; Kitajima 1994; Wright & Westoby 2000). If biomass partitioned to leaves continues to decline more rapidly in savanna species, these are likely to exhibit lower RGR than forest species later in development. Nevertheless, during the first 150 days of growth studied here, savanna species did not have lower RGR than forest species, contrary to predictions.

RGR of the two functional types was similar, despite differences in LAR, because of a corresponding difference in NAR. Because $\text{RGR} = \text{NAR} \times \text{LAR}$, growth rate is dependant on leaf area as well as net photosynthesis on a leaf area basis. Therefore the higher LAR of forest species, combined with the lower NAR, resulted in no net difference in RGR relative to savanna species.

The greater phenotypic plasticity of savanna species in response to light probably can reflect adaptation to either higher mean light levels (e.g. Lortie & Aarssen 1996; Valladares *et al.* 2000) or to a more variable light environment (e.g. Bazzaz 1996). For NAR, the arguments of Lortie & Aarssen (1996) suggest that higher light levels are responsible, although they suggest that RGR of savanna species should also be more plastic, contrary to our findings that neither the savanna nor forest types exhibited a consistent response to light. The constancy in the mean RGR of savanna species across light levels despite a decline in NAR under shading was due to a corresponding increase in SLA, LWR and consequently LAR. This plasticity in SLA, LWR and LAR stabilizes resource capture across a range of light environments, as expected for the highly variable light environment of the cerrado (e.g. Bazzaz 1996).

Either of these explanations would imply that the low plasticity of forest species is an indication they are not as well adapted to the savanna light environment. This is particularly evident when examining NAR, which is an integrated measure of net photosynthesis. With the exception of the pioneer *E. contortisiliquum*, forest species exhibited a decrease or only a slight increase in NAR in full sun relative to low light, whereas the savanna species tended to exhibit an increase. Such results are consistent with differences in photosynthetic light response curves between sun and shade species, in which sun species typically reach saturation at higher light intensities, and shade species frequently exhibit photoinhibition in high light. Indeed many species from gallery forests and other tropical forests maintain greatest growth rates at 50% full sun or less (Rezende *et al.* 1998; Ageyman *et al.* 1999; Mazzei *et al.* 1999; Poorter 1999). The low values of full-sun NAR of forest species relative to savanna species indicate a lower maximum photosynthetic rate, another indication of adaptation to lower light levels.

These responses of NAR to light level are consistent with differences in shade tolerance between savanna and forest species. Low shade tolerance of savanna species could explain their scarcity in mature forest, which typically present understorey photosynthetic

photon flux densities (PPFD) an order of magnitude lower than the lowest value tested here. In contrast, adaptation of forest species to shaded environments is not sufficient to explain their low success in savanna, as many forest species, namely pioneers, are also adapted to high PPFD. Therefore, other traits seem critical in determining plant success in cerrado. While forest pioneers exhibit high NAR and high phenotypic plasticity, traits shared with savanna species, they also exhibit high SLA and LAR and low root : shoot ratio (Kitajima 1996; Walter & Reich 1999), traits not adaptive to the greater water and nutrient stress, nor the higher fire frequency of savannas. Holmes & Cowling (1993) also noted that savanna species deviated from shade-intolerant forest species, pointing to the combination of high photosynthetic rates and low relative growth rates. The adaptations to the savanna environment, in particular the low LAR and high root : shoot ratio, appear to preclude the possibility of a high maximum RGR, a trait fundamental to forest pioneers. Furthermore, as savanna species invest little in height growth, they would be overtopped quickly in a forest gap. These trade-offs would explain why savanna species do not participate in gap-phase regeneration in forest, and why forest pioneers do not rapidly invade savanna despite both being light-demanding species.

Nevertheless, coexistence of savanna and forest species can occur, as in *cerradão*, a woodland formation that sometimes forms transitional vegetation between savanna and forest, and can occur as patches within landscapes that are otherwise dominated by savanna. While there is little consensus as to the origin of these mixtures, it appears that coexistence of savanna and forest species is made possible by low enough water and/or nutrient availability to prevent rapid succession to forest, and low enough grass biomass to prevent frequent or intense fires causing retrogression to savanna. The high frequency of anthropogenic fire typical of the cerrado today tends to eliminate these fire-sensitive forest species from *cerradão* (Moreira 2000), a process that sharpens the savanna–forest boundary.

While there were clear differences between savanna and forest species within genera, differences among genera explained much more of the interspecific variation of most traits than did differences between the savanna and forest types. The relatively small amount of divergence between savanna and forest species indicates that these traits have been highly conserved within these congeneric species pairs, at least for traits expressed at the seedling stage. Seed mass, RGR and allocation to fine roots were the traits most highly conserved, with genus explaining > 90% of the total interspecific variation (Table 2).

In the cerrado, the radiation of woody plants into savanna and forest species has occurred independently in a large number of genera. For example, at one site where the flora has been particularly well described, 45 of the 82 genera of savanna trees and shrubs also contain species occurring in gallery forests in the same

ecological reserve (Pereira *et al.* 1992), and many of the remaining genera contain forest species native to other regions. Furthermore, in a single family, Chrysobalanaceae, the divergence of savanna and forest species occurred independently in at least 14 lineages (Prance 1992). The large number of independent transitions between savanna and forest habitats suggests that relatively little genetic change is involved. Chapin *et al.* (1993) argue that changes in a small number of genes may be sufficient for the expression of whole suites of traits that confer stress tolerance. This could permit relatively rapid adaptation to the savanna environment.

Due to such pleiotropic effects, and due to constraints imposed by ecological and physiological trade-offs, it is unlikely that individual traits evolve independently of others as evidenced here by correlations between seed size and other variables (Fig. 6). If these are indeed functional relationships, then this may constrain the evolution of such traits. Seed mass in particular is highly conserved within genera (Table 2; Peat & Fitter 1994), while other seedling traits are correlated to seed mass (Fig. 6; Marañón & Grubb 1993), so it follows that these other seedling traits need also be conserved within genera. The negative relationship between seed mass and LAR may be 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 & Westoby 1994) and herbivory (Armstrong & Westoby 1993). The correlations between seed mass and other traits are based on cross-species relationships, but although they could therefore arise from phylogenetic history, such relationships have been observed elsewhere (Marañón & Grubb 1993), suggesting that they are indeed real.

The great amount of variation in plant traits among genera, relative to the difference between savanna and forest functional types, emphasizes the importance of considering phylogeny in comparative studies. Many of the traits shown here to differ between savanna and forest species would not have been statistically significant had the species not been paired into genera. So it is not surprising that other comparative studies have not been able to conclusively show seedling differences in LAR, photosynthesis (Paulilo & Felipe 1998) or root : shoot ratio (Moreira & Klink 2000) between cerrado and woodland or forest species.

Conclusions

Savanna–forest boundaries have always been in a state of change, responding to changes in climate, fire regimes and more recently, anthropogenic disturbance. In future decades, all three of these factors are likely to intensify. Our results demonstrate that forest species invest much more in leaf area and stem biomass (adaptations for a competitive light environment), albeit at the expense of root biomass, in which savanna species

invest heavily. This predisposes the forest species to perform poorly in the savanna environment, which is characterized by water stress, fire and high light intensity. Savanna species are light-demanding species with large investment in coarse roots, improving their capture of soil water during the dry season and permitting resprouting following burning or drought.

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