BIOTROPICA ■■(■■): 1–9 2011 10.1111/j.1744-7429.2011.00760.x

Which Extent is Plasticity to Light Involved in the Ecotypic Differentiation of a Tree Species from Savanna and Forest?

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ABSTRACT

Light intensity and heterogeneity are some of the main environmental factors that differ between forest and savanna habitats, and plant species from these habitats form distinct functional types. In this study, we tested the hypothesis that not only differences in morphological and physiological traits but also phenotypic plasticity in response to light are involved in adaptation to forest and savanna habitats by investigating ecotypic differentiation between populations of *Plathymenia reticulata* (Leguminosae: Mimosoideae), a tree from the Brazilian Atlantic Forest and the Brazilian Cerrado (savanna). Seeds from four natural populations (one from each biome core area and two from ecotonal regions) were grown in a common garden with four light treatments. Fifteen morphological and physiological characteristics were evaluated until individuals reached 6 mo old. Comparisons among populations showed differences for seven traits in at least one light treatment. These differences pointed to local adaptation to different biomes. Populations showed different levels of phenotypic plasticity in response to light in seven traits. Higher plasticity was found either in the forest core population or ecotonal populations; lower values were found in the cerrado core population. Lower plasticity in the cerrado population emphasizes the stress resistant syndrome, as lower plasticity is probably advantageous in a habitat where a conservative resource use is crucial. Higher plasticity in forest individuals suggests higher ability in exploiting the light heterogeneity in this habitat. Also, higher plasticity in ecotonal populations can be important to ensure the maintenance of *P. reticulata* in these temporally and spatially dynamic areas.

Abstract in Portugese is available at http://www.blackwell-synergy.com/loi/btp.

Key words: Brazilian Atlantic Forest; Brazilian Cerrado; common garden; ecotone; phenotypic plasticity; *Plathymenia reticulata*.

TROPICAL ECOSYSTEMS INCLUDE THE MOST DIVERSE PLANT COMMUNITIES ON EARTH DUE TO A complexity of biotic and abiotic factors (Givnish 1999, Wright 2002). Among such factors, the spatial and temporal heterogeneity of light has been noted as an important factor underlying niche partitioning among plants (Poorter & Arets 2003, Balderrama & Chazdon 2005). Although less studied and understood, phenotypic plasticity in response to light could also be important for the maintenance of plant diversity (Valladares *et al.* 2000b). This may seem paradoxical because phenotypic plasticity enables a given genotype to occupy different environments, and thus might be expected to retard evolutionary change (West-Eberhard 1989). Plasticity can, however, be a diversifying factor in evolution because it is itself a trait subject to natural selection (West-Eberhard 1989, Lortie & Aarssen 1996, Valladares *et al.* 2000b, Zou *et al.* 2009).

The tropical Brazilian Cerrado and the Brazilian Atlantic Forest are among the most biodiverse ecoregions in the world, both considered priority hot spots for conservation (Myers *et al.* 2000). The cerrado biome comprises widely varying physiognomic types of vegetation that ranges from treeless grasslands to dense woodlands. The most frequent physiognomy is named cerrado *sensu stricto* and originally occupied more than 65 percent of the biome (Haridasan 1992). It is a savanna characterized by a community of trees and shrubs, usually about 2–8 m in height with contorted trunks, thick corky bark, sclerophyllous leaves and crown cover of 10–60 percent, below which there is a well-developed grassy ground layer (Ratter *et al.* 1997). The Brazilian Atlantic Forest includes both rain forest, which runs along the coastline and semi-deciduous forest, extending across a plateau in the center and southeast interior of Brazil (Morellato & Haddad 2000, Oliveira-Filho & Fontes 2000).

Tree species from the forest and the cerrado form distinct functional types differing in life history attributes (Hoffmann *et al.* 2005) partially due to adaptations to differences in light availability

Received 11 June 2010; revision accepted 15 December 2010.

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between these habitats (Hoffmann & Franco 2003). Although climate, soil characteristics, fire and seasonality may influence plant distribution across forest and savanna boundaries (Furley 1992, Haridasan 1992, Hao *et al.* 2008, Hennenberga *et al.* 2008), light could be considered one of the abiotic factors that differ most between them. In the semi-deciduous forest the leaf area index (LAI) is around five in the wet season (Paula & Lemos Filho 2001) while in the cerrado *senso stricto*, LAI values in the wet season are around one (Miranda *et al.* 1997). Forest light regimes are also highly heterogeneous, ranging from deeply shaded understories to sunlit treefall gaps. The light variation among microsites may be 50-fold (Niiments 2007). Moreover, forest understories are highly dynamic with temporal changes spanning from years (*e.g.*, canopy opening by treefall) to minutes, as when the very low background understory irradiation is occasionally punctuated by short duration bright sunflecks (Pearcy 2007). Cerrado may also show high spatial and seasonal heterogeneity in understory light (Lemos *et al.* 2010), but the very low light regimes observed in the forest understories are probably not found in cerrado savannas. Thus, a forest plant may face, throughout its life, a more variable light regime than a cerrado plant.

Comparative studies that evaluate plasticity among functional plant groups are important in determining the implications of plasticity for the distribution, invasion and persistence of populations, and also to understand evolutionary diversification (Sultan 2000, Zou *et al.* 2009). The accumulated knowledge on plastic responses of plants to the light environment suggests the existence of a general trend of higher plasticity in sun-adapted than in shade-tolerant plant species (Valladares *et al.* 2000a, 2005). The trend may depend, however, on the traits analyzed, as sun plants seem to be more plastic for physiological features while plasticity for morphological and architectural traits seem to be greater in shade plants (Valladares *et al.* 2002, 2007; Niinemets & Valladares 2004).

In this work we investigate the existence of local adaptation in populations of the tree *Plathymenia reticulata* (Leguminosae: Mimosoideae) from the semi-deciduous Atlantic Forest and from the cerrado (*sensu stricto*), hypothesizing that differential response to light is involved in ecotypic differentiation among them. We predicted that habitat-based selection should lead to different light responses in saplings from forest and cerrado grown in a common garden. We also evaluated *P. reticulata* from ecotone areas, which have distinctive traits in comparison with individuals from core populations (Lacerda *et al.* 2002; Goulart *et al.* 2005, 2006; Lemos Filho *et al.* 2008). We also predicted that forest saplings should show morphological and physiological characters that enhance growth and survival in shaded habitats. We also expected that higher phenotypic plasticity in traits related to light capture should be found in forest populations, as an evolutionary response to the higher light heterogeneity experienced by them in relation to cerrado.

METHODS

Studied species and populations.—*Plathymenia reticulata* is found in the cerrado and the Atlantic Forest. Originally, populations from different biomes were considered vicariant species until gene flow between them was determined by a molecular study (Lacerda *et al.* 2002). This was by a taxonomic revision that recognized only *P. reticulata* (Warwick & Lewis 2003). Individuals differ in size with those from the cerrado being shorter (height up to 12 m) with contorted trunk while individuals in the forest reaching up to 30 m. *Plathymenia reticulata* is deciduous, though populations differ in the timing of leaf senescence, which starts earlier in drier areas (cerrado), later where the drought period is less severe (semi-deciduous forest), while individuals in transitional regions exhibit an intermediate pattern (Goulart *et al.* 2005).

We evaluated four populations of *P. reticulata*. Two populations were from the Atlantic Forest semi-deciduous physiognomy, one located in the core area (19°45′ S, 43°31′ W) and another in its periphery, in an ecotone with the Cerrado (19°56′ S 46°56′ W). Two populations were from cerrado *stricto sensu* physiognomy: one located in the biome's core area (18°43′ S, 45°03′ W) and another in its periphery, in an ecotone with the Atlantic Forest (19°49′ S, 43°48′ W).

Cerrado and forest core studied sites differ in the length and severity of the dry season with an average of 1200 and 1480 mm of annual precipitation respectively (Goulart *et al.* 2005). The sites also differ in soil characteristics, especially in organic matter content, which is about 51.2 and 24.8 g/kg for forest and cerrado sites, respectively (M. Goulart, unpubl. data). The studied ecotones are characterized by a mosaic of adjacent patches of cerrado and forest floristically and physiognomically very similar to their respective core habitat and not characterized by intermediate vegetation type.

Experimental design.—Seeds were collected as mixed samples from ten individuals from each population. Seeds were submitted to mechanical scarification and germination was conducted at 28°C in darkness over 6 d. In the first experiment, a total of 120 seedlings from each population were planted (18 cm × 32 cm pots) in soil collected in a Atlantic Forest site (pH = 5.6; N = 1.4 mg/kg; P = 2.2 mg/kg; K = 128 mg/kg; Ca^{2+} = 1.65 cmolc/kg; Mg^{2+} = 0.68 cmolc/kg). Pots were watered daily. Seedlings were randomly arranged on four benches (1 m × 8 m), each of them receiving different light intensities: sun (100% of ambient sunlight) and three levels of shade, achieved through the use of layers of neutral shade cloth. The shade cloths were supported by wood frames and stood 1 m above the benches covering also its sides. Simultaneous measurements of temperature, relative humidity and photosynthetic photon flux density (PPFD) using quantum sensors (Li-Cor, U.S.A.) were performed in three locations on each bench during an entire sunny summer day at 30-min intervals. These measures showed that the cloths resulted in 53 percent, 36 percent and 22 percent of sunlight (Table 1). Three blocks were established to test for homogeneity of irradiance; however, the statistical analysis showed no significant block effects on the evaluated traits.In another experimental, 20 individuals from each population, excluding the Cerrado in ecotone due to low seed number, were planted (25 cm × 40 cm pots) in 3:1 nutrient-enriched peat and sand mixture. Seedlings were equally and randomly arranged on the benches with 100 percent and 22 percent of ambient sunlight. As in the first experiment, pots were watered daily.

Morphological and physiological measurements.—Individuals in the first experiment were evaluated after 6 mo of growth. With

TABLE 1. *Environmental conditions in the four growing houses, representing four different light treatments. Data were recorded every 30 min during a summer sunny day from 0700 h to 1800 h. PPFD stands for photosynthetic photon flux density.*

	Light treatments (percentage of ambient sunlight)			
Environmental variable	100	53	36	22
Daily PPFD (mol m^2/day)	48.96	25.91	17.41	10.90
Mean temperature (°C)	31.5	31.3	31.3	30.3
Mean relative humidity (%)	50.2	50.9	55.3	57.1

the use of a digital calipter (0.01 mm precision) and common ruler, shoot length (cm) and base diameter (mm) were obtained and number of internodes counted. Mean internode length (cm) was estimated as shoot length/number of internodes, considering only individuals with a single bud, and slenderness index (cm/mm) as shoot length/base diameter. *In vivo* chlorophyll fluorescence measures were evaluated in six 6-mo old individuals from each population growing at ambient sunlight and also at 22 percent of ambient sunlight treatments. Measurements were made using a pulse amplitude modulated photosynthesis yield analyzer (Mini-PAM, Walz, Germany). Potential quantum yield of photosystem II was calculated as $F_v/F_m = (F_m - F_0)/F_m$, where F_m and F_0 are the maximum and the minimum fluorescence, respectively, measured in fully developed leaves after 30 min of dark adaptation. Light saturation curves were obtained using the light curve program of the instrument, and used to determinate maximum apparent photosynthetic electron transport rate (ETR$_{max}$) and saturating photosynthetically active photon flux density (PPFD$_{sat}$), following Rascher *et al.* (2000). Leaf pigment content (total chlorophylls, carotenoids:chlorophylls ratio and chlorophyll *a*:*b* ratio) was determined on the same six individuals sampling a fully developed leaves per individual. Leaflets were weighted and ground in 80 percent acetone, and after centrifugation at 3000 rpm for 5 min, the absorbance was measured spectrophotometrically at 470, 646 and 663 nm and pigment content was determined using equations described in Lichtenthaler and Wellburn (1983) and expressed on a fresh weight basis.

In the second experiment, half of the individuals were harvested after 1 mo of growth and the other half after 6 mo of growth. Roots were extracted from the soil by wet-sieving and plants were separated into leaves, stems and roots. Dry mass of each fraction was determined after 3 d at 70°C. Leaf area was measured on a flatbed scanner with computer software (Easy Quantify), before drying. The following measurements were carried out: total dry mass, shoot:root ratio (shoot dry mass/root dry mass), specific leaf area (SLA = leaf area/leaf dry biomass) and leaf area ratio (LAR = leaf area/total dry biomass). We also calculated the relative growth rate (RGR) by the paring method (Hunt 1982) where $RGR = [\ln(M_2) - \ln(M_1)]/t_2 - t_1$, M_1 and M_2 being plant dry masses at times t_1 (1 mo) and t_2 (6 mo) and net assimilation rate (NAR) as $NAR = (M_2 - M_1)/(t_2 - t_1)(\ln A_2 + \ln A_1)$ being M_1 and M_2 plant dry masses and A_1 and A_2 leaf area and at times t_1 and t_2.

ANALYSIS OF DATA.—In order to compare light treatments and also the populations, data were logarithmically transformed and the assumptions of normality and homoscedasticity were met. Analyses of variance (ANOVA) considered as sources of variation: light treatment, population and also population × light treatment interaction. Whenever a factor did not show significance, it was removed from the model and a new analysis was conducted. *Post-hoc* Tukey mean comparison tests were performed for population and for light treatments, for all morphological and physiological traits.

For each population, plasticity was quantified under 22 percent and 100 percent of ambient sunlight treatments, using the relative distance phenotypic index (RDPI) described by Valladares *et al.* (2006). The relative distances (RD) among trait values for all pairs of individuals of a given population grown under different light treatments were determined as $RD_{ij \to i'j'} = d_{ij} \to i'j'/(x_{i'j'} + x_{ij})$, where j and j' are individuals belonging to different light treatment i and i'; and x is the attribute value. The RDPI ranges from 0 (no plasticity) to 1 (maximum plasticity) and is obtained as $RDPI = \sum \left(d_{ij} \to i'j'/(x_{i'j'} + x_{ij})\right)/n$, where n is the total number of RD. Comparisons of RDPIs among populations were made by ANOVAs and *post-hoc* Tukey test, or by Kruskal–Wallis and *post-hoc* Holm test when nonparametrical distributions were found.

RESULTS

A significant population × light treatment interaction was found for shoot length (Table 2). Different light conditions did not affect significantly shoot length of plants from cerrado core and ecotones, but plants from forest core exhibited twice the shoot length in the highest shade treatment than in the ambient sunlight treatment (Fig. 1A). Plants from forest core had a greater shoot length than cerrado core and ecotones at 32 and 22 percent of full sunlight. Differences were greatest in the darkest shade treatment, where plants from forest core grew almost three times taller than plants from the cerrado core. No significant population × light treatment interaction was found for slenderness index and internode length (Table 2). Populations differed in slenderness index and internode length with individuals from forest core showing mean values almost twice as those from cerrado core, while ecotonal populations showed intermediate mean values (Fig. 1B). Plants from all populations responded to light treatments by significantly increasing the slenderness index in shade although not by elongating the internodes (Fig. 1B).

No significant population × light treatment interaction was found for chlorophyll fluorescence traits and leaf pigment content (Table 2). For potential quantum yield of photosystem II (F_v/F_m), photosynthetic electron transport rate (ETR$_{max}$) and saturating photosynthetically active photon flux density (PPFD$_{sat}$), differences among populations and among light treatments were not significant (Table 2 and Fig. 2A). In relation to leaf pigment content, populations were different only for carotenoids:chlorophyll ratio,

TABLE 2. *Analyses of variance for morphological and physiological characters recorded in* Plathymenia reticulata *from four populations and submitted to two or to four light treatments.*

	Sources of variance		
Traits	Population	Light treatment	Population × light
Shoot length (cm)	25.00**	3.12*	2.28*
Slenderness index (cm/mm)	43.96***	7.99***	ns
Internodes length (cm)	16.33***	ns	ns
Dry mass (g)	5.4*	7.89*	ns
Shoot:root ratio	14.06***	ns	5.57*
SLA (cm^2/g)	ns	32.61***	ns
LAR (cm^2/g)	ns	21.18**	ns
RGR (g/g/month)	10.74***	ns	4.79*
NAR (g/cm^2/month)	ns	19.60**	4.91**
F_v/F_m	ns	ns	ns
ETR_{max} (μmol/m^2/s)	ns	ns	ns
$PPFD_{sat}$ (μmol/m^2/s)	ns	ns	ns
Chlorophylls (μm/mg)	ns	57.88***	ns
Carotenoids:chlorophylls ratio	8.53***	10.42**	ns
Chlorophyll *a*:*b* ratio	ns	ns	ns

Variance ratios (*F*-values) are reported with associated level of significance $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

LAR, leaf area ratio; NAR, net assimilation rate; ns, not significant; RGR, relative growth rate; SLA, specific leaf area.

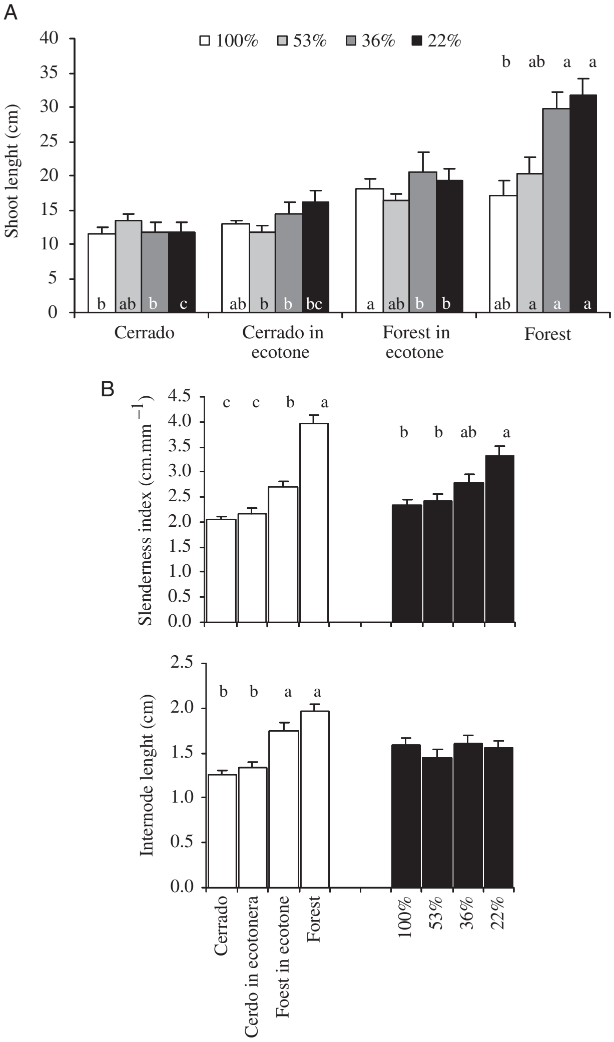

FIGURE 1. Means ± standard error of morphological traits (experiment 1) obtained in 6-mo old individuals of *Plathymenia reticulata* from four populations (cerrado, cerrado in ecotone zone, Atlantic Forest in ecotone zone and Atlantic Forest) grown under four different light levels (100%, 53%, 36% and 22% of ambient sunlight). In (A), there is significant population × light treatment interaction, letters inside bars indicate differences among populations within each light treatment while letters above bars indicate differences among light treatment within each population. In (B), there is no significant population × light treatment interaction, letters above white bars indicate comparisons among population while letters above black bars indicate comparisons among light treatments. Alphabetical order of letters corresponds with ranking mean value and absence of letters indicates no significant differences considering 95% CI.

with plants from cerrado (core and ecotone) showing higher mean values than forest ones. Comparison between light treatments showed significant differences for chlorophyll content and carotenoids:chlorophyll ratio, but not to chlorophyll *a*:*b* ratio. Individuals growing in the shade showed significantly higher chlorophyll and lower carotenoids:chlorophyll ratio than those growing in ambient sunlight (Table 2 and Fig. 2B).

Dry mass, SLA and LAR did not show significant population × light treatment interaction (Table 2). Forest core and ecotone individuals accumulated more than two times the dry biomass accumulated by cerrado (Fig. 3B), but no differences were observed among populations concerning SLA and LAR. Plants from all populations showed higher dry mass in ambient sunlight and higher SLA and LAR under shade (Fig. 3B). Shoot:root ratio, RGR and NAR showed a significant population × light treatment interaction (Table 2). Only forest core individuals showed differences in shoot:root ratio between sun and shade treatments, with plants growing in the shade showing mean values two times larger than plants in ambient sunlight. Under full sunlight, populations showed a similar shoot:root ratio, while under shade, forest core population showed significant higher values than all others (Fig. 3A). Once more, only forest core individuals showed differences in RGR between light treatments, with higher mean values in full sunlight. At both light treatments, forest core and ecotone plants showed higher RGR than cerrado individuals (Fig. 3A). No significant differences among populations were found for NAR, which significantly differed among light treatments for cerrado and forest core populations. In these populations, individuals growing in full sunlight showed higher NAR than those growing in shade (Fig. 3A).

Phenotypic plasticity in response to light (Table 3) was comparatively higher for dry mass, NAR, SLA, LAR and chlorophyll content (final mean RDPI ranging from 0.28 to 0.41). Intermediate plasticity was found for shoot length, slenderness index,

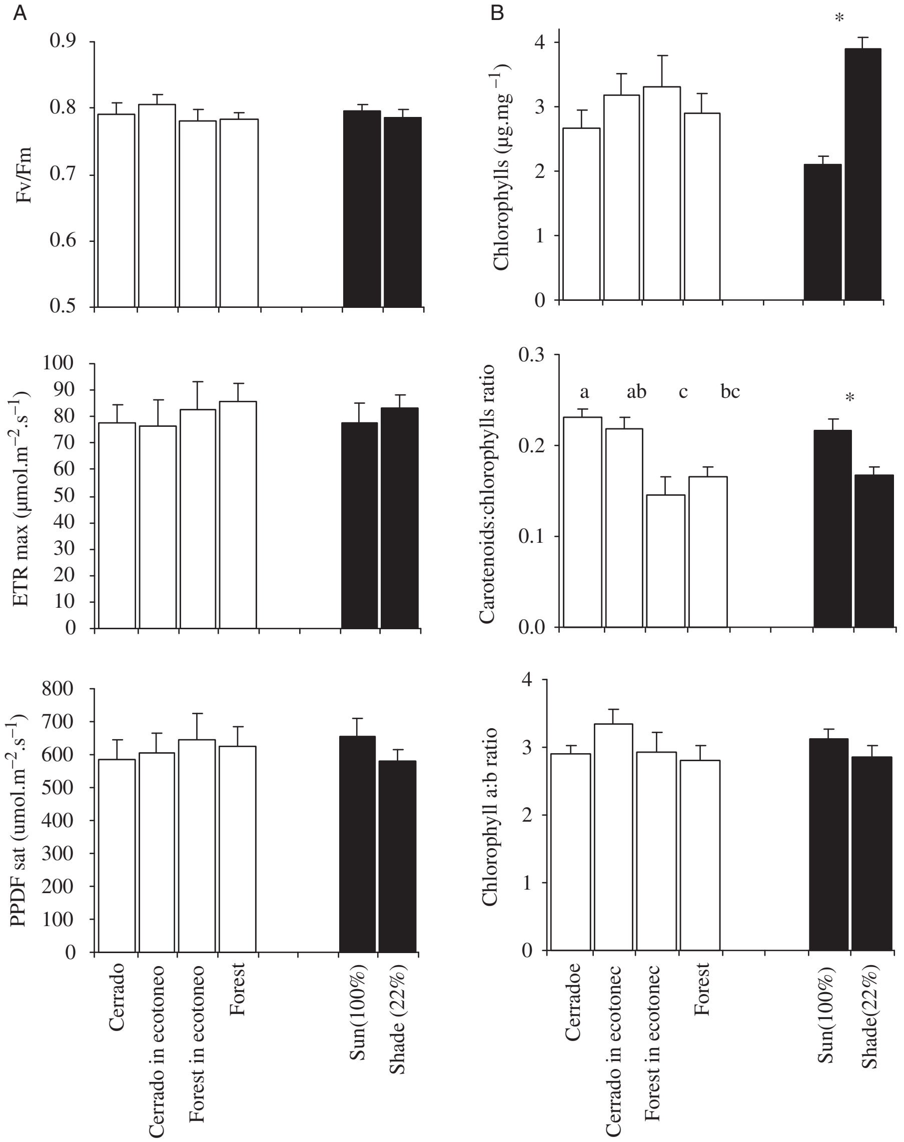

FIGURE 2. Means ± standard error of physiological traits (experiment 1) obtained in 6-mo old individuals of *Plathymenia reticulata* from four populations (cerrado, cerrado in transition, Atlantic Forest in transition and Atlantic Forest) grown under two different light levels (100% and 22% of ambient sunlight). In (A), traits related to photosynthetic performance are present: potential quantum yield of photosystem II (F_v/F_m), photosynthetic electron transport rate (ETR_{max}) and saturating photosynthetically active photon flux density ($PPFD_{sat}$); and in (B), leaf pigments contents are presented: chlorophyll, carotenoids:chlorophyll ratio, chlorophyll *a*:*b* ratio. Letters above white bars indicate comparisons among populations while asterisks above black bars indicate comparisons among light treatments. Alphabetical order of letters corresponds with ranking mean value and absence of letters or asterisk indicates no significant differences considering 95% confidence interval.

root:shoot ratio, ETR_{max} and $PPFD_{sat}$ (final mean RDPI between 0.17 and 0.24). Traits as internode length, RGR, F_v/F_m, carotenoids:chlorophyll ratio and chlorophyll *a*:*b* ratio were comparatively less plastic (final mean RPDI between 0.03 and 0.14). Comparisons of phenotypic plasticity among populations rendered significant differences for seven out of 15 traits. For shoot length, LAR and NAR plasticity were higher in forest core population, while for leaf pigments contents (chlorophyll, carotenoids:chlorophyll ratio, chlorophyll *a*:*b* ratio) and slenderness index, higher plasticity was found either in forest core or ecotonal populations. Internode length, dry mass, shoot:root ratio, SLA, RGR and traits of chlorophyll fluorescence (F_v/F_m, ETR_{max} and $PPFD_{sat}$) did not show differences in plasticity among populations.

DISCUSSION

ECOTYPIC DIFFERENTIATION IN PLASTICITY IN RESPONSE TO LIGHT.— Comparisons of phenotypic plasticity in response to light among populations of *P. reticulata* showed that, when considering traits' indices that significantly differed among population, lower values were recurrently found in cerrado core. This result indicates that, as predicted, comparatively higher plasticity in response to light is

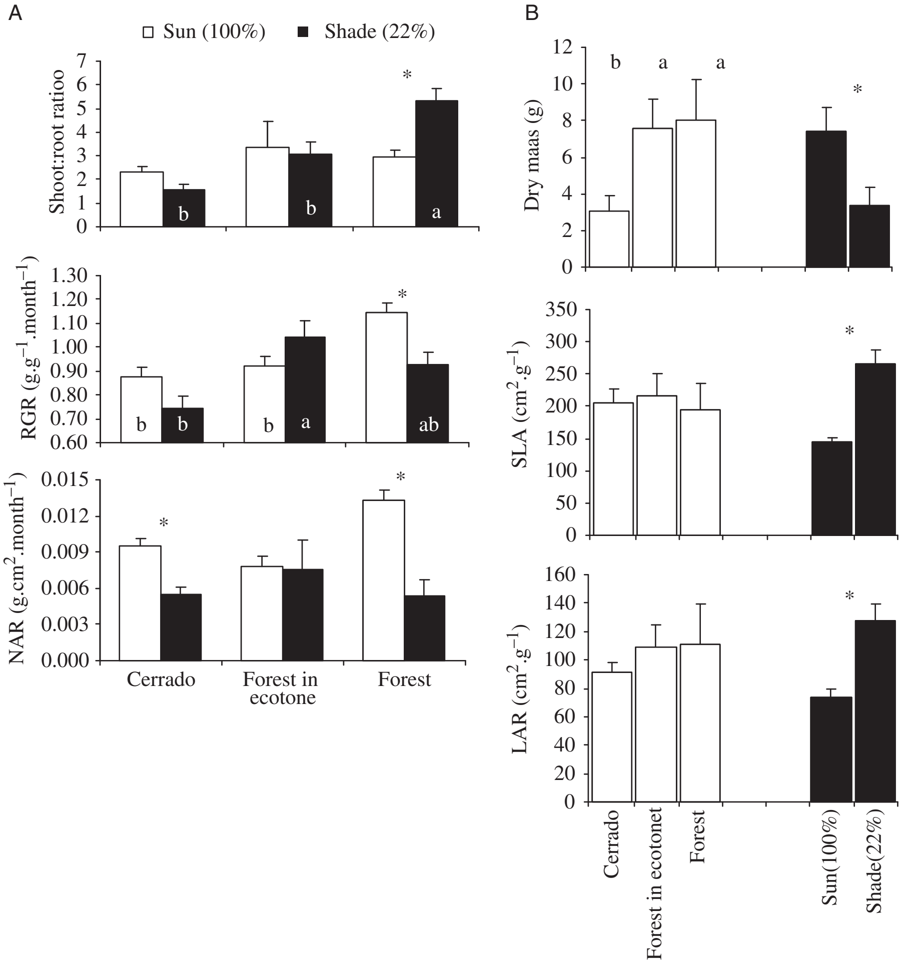

FIGURE 3. Means ± standard error of morphological measurements (experiment 2): shoot:root ratio, relative growth rate, net assimilation rate, dry mass, specific leaf area and leaf area ratio. Data were obtained in 6-mo old individuals of *Plathymenia reticulata* from three populations (cerrado, Atlantic Forest in transition and Atlantic Forest) grown under two different light levels (100% and 22% of ambient sunlight). In (A), there is significant population × light treatment interaction, letters inside bars indicate differences among population within each light treatment while asterisk indicates differences between light treatment within each population. In (B), there is no significant population × light treatment, letters above white bars indicate comparisons among populations while asterisks above black bars indicate comparisons among light treatments. Alphabetical order of letters corresponds with ranking mean value and absence of letters or asterisk indicates no significant differences considering 95% confidence interval.

found in the forest population than in the cerrado population. This pattern can be interpreted as habitat-based selection for plasticity. Higher plasticity for the morphological traits (shoot length and LAR) and NAR in response to light in forest individuals suggests higher efficiency in exploiting this limiting resource than plants from cerrado. Morphological plasticity has been linked to an enhanced capacity to survive and grow in the understory (Valladares *et al.* 2000b) as a particular mechanism to optimize resource acquisition in plants (West-Eberhard 1989). The evolutionary advantage and the benefits of plasticity are better understood than its disadvantages, limits and costs (DeWitt *et al.* 1998), but lower plasticity in cerrado may be a feature related to the stress resistance syndrome (Chapin *et al.* 1993). Cerrado vegetation occurs in nutritionally poor acid soils in a climate characterized by a seasonal drought. As pointed out by Grime and Mackey (2002) in unproductive habitats high plasticity is unlikely to be sustainable as it may promote high growing under temporally favorable circumstances that may not be maintained once conditions deteriorate. Thus, low morphological plasticity to light is likely to be found in cerrado plants, as already demonstrated for evergreen woody plants in both tropical and Mediterranean ecosystems (Valladares *et al.* 2000a, b).

For some traits, such as the pigment content, both populations from the ecotonal site showed higher plasticity than the cerrado core area. The bordering areas between Atlantic Forest and cerrado were spatially dynamic in response to climatic changes during the late Pleistocene and Holocene (Behling 2002, Silva *et al.* 2008), so this high plasticity might be important for the maintenance of *P. reticulata* populations in these areas.

In order to test the hypothesis that phenotypic plasticity is greater in habitats with a more heterogeneous light environment,

TABLE 3. *Plasticity indexes (RDPI) of morphological and physiological traits obtained in* Plathymenia reticulata *from four populations.*

Traits	Cerrado	Cerrado in ecotone	Forest in ecotone	Forest	*F*	χ^2
Shoot length^{np}	0.17^{b}	0.16^{b}	0.15^{b}	0.29^{a}	—	86.69^{***}
Slenderness index^{np}	0.11^{c}	0.20^{b}	0.22^{a}	0.21^{ab}	—	56.62^{***}
Internode length^{np}	0.14	0.14	0.10	0.13	—	ns
Dry mass	0.45		0.32	0.45	ns	—
Shoot:root	0.21		0.21	0.29	ns	—
SLA	0.33		0.21	0.31	ns	—
LAR	0.23^{b}		0.22^{b}	0.42^{a}	9.62^{***}	—
RGR^{np}	0.10		0.06	0.10	—	ns
NAR	0.27^{b}		0.24^{b}	0.44^{a}	6.26^{**}	—
F_v/F_m^{np}	0.03	0.03	0.02	0.02	—	ns
ETR_{max}^{np}	0.13	0.18	0.21	0.14	—	ns
PPFD_{sat}	0.18	0.17	0.20	0.19	ns	—
Chlorophyll	0.26^{b}	0.35^{b}	0.52^{a}	0.30^{b}	10.49^{***}	—
Carotenoids:chlorophyll^{np}	0.10^{b}	0.17^{a}	0.17^{a}	0.11^{b}	—	15.70^{**}
Chlorophyll *a*:*b*	0.08^{b}	0.13^{a}	0.16^{a}	0.12^{a}	—	9.67^{*}

Characters marked with ‘np’ are nonparametric, medians are presented and compared by Kruskal–Wallis (χ^2-value) and *post-hoc* Holm test, for the others, means are presented and compared by ANOVA (*F*-values) and *post-hoc* Tukey test. Levels of significance are $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Alphabetical order of letters corresponds with ranking mean or median value.

LAR, leaf area ratio; NAR, net assimilation rate; ns, not significant; RGR, relative growth rate; SLA, specific leaf area.

we assumed that functional light heterogeneity experienced by *P. reticulata* saplings in the forest was higher than in the cerrado. This assumption differs from that of Hoffmann and Franco (2003) who assumed greater light heterogeneity in cerrado savannas than in forests. Without a specific determination of the functional light heterogeneity experienced by plants in each of these habitats, the hypotheses of both the present work and those by Hoffmann and Franco (2003) can be proposed. However, in support of our assumption, it is unlikely that saplings from cerrado *senso stricto* experience a greater gradient in light resource than those in the forest, because the very low levels of light availability observed in the forests are probably not found in the cerrado, while both ecosystems have zones of similarly high levels of irradiance. The higher plasticity among forest populations, which we assume to have a more heterogeneous light environment, reflects a similar pattern to that reported by Balaguer *et al.* (2001) who investigated Mediterranean oak populations from habitats with different levels of light heterogeneity.

Hoffmann and Franco (2003) explored macroevolutionary processes across cerrado and forests by comparing congeneric pairs of savanna and forest plant species. They concluded that plasticity was higher in cerrado species, although exceptions were reported. Considering that many cerrado species occur in several microhabitats, from grasslands to woodlands, higher plasticity in some cerrado species is likely to be explained at least in part by the fact that these species occur over a wider range of environmental conditions and not because savanna in cerrado is more heterogeneous with regard to light. Thus, the apparent contradictory results found here can be reconciled with those of Hoffmann and Franco (2003) by considering that their results may be interpreted as an evidence that species occurring over a wider range of light environments show higher plasticity in response to light than the ones that occur over a narrower range, has already being reported by Popma *et al.* (1992).

ECOTYPIC DIFFERENTIATION IN FUNCTIONAL TRAITS.—Besides showing differentiation in phenotypic plasticity, the results of this work also provide evidence of ecotypic differentiation in functional traits of *P. reticulata* populations from cerrado and forest. Individuals from all populations invested in growing taller by elongating stems when exposed to shade, a known shade escape response related to the shade avoidance syndrome (Kurepin *et al.* 2006). These shade responses, however, were much more evident in forest than in cerrado populations. In general, forest habitats are more limited by light than by any other resource (Chazdon *et al.* 1996, Pearcy 2007) and our results show that forest populations of *P. reticulata* are more adapted to cope with it than the cerrado populations.

Higher biomass accumulation and higher RGR in individuals from forest core and ecotone could be related to a higher competitive ability in these populations when compared with cerrado. Higher growth rates in individuals from forest may be advantageous by promoting quick occupation of the available space within the crowded vegetation (Poorter & Garnier 2007). On the other hand, in cerrado, lower growth is related to a stress resistant syndrome, typical of plants specialist to low-resource environment (Chapin *et al.* 1993). Besides lower growth rate, under shade conditions, lower shoot:root ratio was another characteristic exhibited by *P. reticulata* from cerrado that is frequently related to stress resistant syndrome (Chapin *et al.* 1993). Differences in shoot:root ratio have been considered the most striking difference between cerrado and forest species (Hoffmann & Franco 2003). Higher investment in aerial growth in forest plants should be related to shade avoidance while greater investment in roots in cerrado plants is interpreted as a strategy to capture nutrient and water (Hoffmann & Franco 2003).

Our results reinforce the notion that light directly affects leaf pigment content (Rosevear *et al.* 2001). Individuals submitted to shade improved light interception by showing higher chlorophyll concentration (Johnson *et al.* 1997) while the ones submitted to sun maximized photoprotection by showing higher carotenoids:chlorophyll ratio (Demmig-Adams & Adams 1992). These data could explain the absence of differences in photoinhibition among individuals submitted to sun and shade treatments. The pigment content results also show a genetic influence on pigment features because the cerrado population showed higher carotenoids:chlorophyll ratio than forest ones at both light treatments. Concerning chlorophyll fluorescence traits, independently of light treatment, we could expect forest individuals to show higher levels of photoinhibition as shade-tolerant plants that show low capacity to dissipate excess light energy (Reich *et al.* 2003, Valladares & Niinemets 2008). In contrast, cerrado individuals should have higher light saturation levels, according to common features of sun species (Valladares & Niinemets 2008). In this work, however, no significant differences between populations in chlorophyll fluorescence traits were observed. This agrees with Nicotra *et al.* (1997) who reported that physiological traits related to photosynthetic performance are under strong stabilizing pressure and may show little or no variation among genotypes.

Previous work compared congeneric pairs of savanna and forest species and showed that despite some significant differences, variation among genera still accounted for a large proportion of the total variance, indicating high levels of phylogenetic inertia in several traits related to grow (Hoffmann & Franco 2003) and to stem and leaf hydraulic characteristics (Hao *et al.* 2008). Indeed, data from *P. reticulata* populations show that many traits are highly conserved within the ecotypes but, on the other hand, significant differences with regard to other important adaptive traits point to the same direction of the differences frequently reported when species from forest and open environments are compared.

In conclusion, data obtained in the present study suggest that the ecotypic differentiation of the populations of *P. reticulata* involves differentiation not only in certain functional traits but also in the plasticity of these traits to light. Populations from cerrado core showed lower plasticity than forest core, suggesting an overall more conservative resource use strategy in more stressful environment of cerrado. Populations from ecotonal site showed also higher plasticity, that can be important for the maintenance of *P. reticulata* in these temporally and spatially heterogeneous areas.

ACKNOWLEDGMENTS

This work was supported by FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais). M. F. Goulart received a fellowship from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). J. P. Lemos-Filho and M. B. Lovato received research fellowships from CNPq. This work was in partial fulfillment of requirements for M. F. Goulart's doctoral thesis in Ecology and Wildlife Conservation and Management, Universidade Federal de Minas Gerais, Brazil.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

FIGURE S1. Brazil and Minas Gerais State main biomes, with the approximate location of the studied populations of *P. reticulata*.

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