 Springer

Leaf traits and water relations of 12 evergreen species in Costa Rican wet and dry forests: patterns of intra-specific variation across forests and seasons

Sybil G. Gotsch · Jennifer S. Powers · Manuel T. Lerdau

Abstract This study examined variation in leaf traits and water relations in 12 evergreen and semideciduous woody species that occur in both seasonal wet and dry forests in Costa Rica and compared intra-specific leaf–trait correlations to those found in inter-specific global studies. The following traits were measured in both forests across seasons for 2 years: leaf nitrogen (N), leaf carbon (C), specific leaf area (SLA), toughness, cuticle thickness, leaf thickness, and leaf lifespan (LLS). Leaf water potential (LWP) and water content (LWC) were measured as indices of plant available water. Canopy openness, soil moisture, and herbivory were also measured to compare environmental variation across sites. Although species contributed the greatest amount to variation in traits, season, forest, and their interaction had a large influence on patterns of intra-specific leaf–trait variation. Leaf traits that contributed most to variation across sites were C, LWP, leaf thickness, and SLA. Traits that contributed most to variation across seasons were leaf toughness, LWP, and LWC. Furthermore, leaf traits were more correlated (i.e., number and strength of correlations) in the dry than in the wet forest. In contrast to results from global literature syntheses, there was no correlation between LLS and N, or LLS and SLA. Both light and water availability vary seasonally and may be causing variation in a number of leaf traits, specifically those that relate to water relations and leaf economics. Strong seasonality may cause leaf–trait relationships at the local scale to differ from those documented in continental and global-scale studies.

S. G. Gotsch (✉)
Department of Ecology and Evolution, State University
of New York at Stony Brook, Stony Brook,
NY 11790, USA
e-mail: sybilgotsch@gmail.com

J. S. Powers
Department of Ecology, Evolution and Behavior,
and Plant Biology, University of Minnesota,
1987 Upper Buford Circle,
St. Paul, MN 55108, USA
e-mail: powers@umn.edu

M. T. Lerdau
Department of Environmental Sciences and Biology,
University of Virginia, Clark Hall, 291 McCormick Rd,
PO Box 400123, Charlottesville, VA 22904-4123, USA
e-mail: mlerdau@virginia.edu

Present Address:
S. G. Gotsch
Department Natural Resource Ecology and Management,
Iowa State University, 339 Science II, Ames,
IA 50011-3221, USA

Introduction

All plants in natural environments, regardless of their life form and habitat, are bound by the same fundamental need to balance growth, defense, and reproduction. However, there are many ways that plants achieve this balance. Many studies have shown that leaf traits such as nitrogen (N) content, lifespan, photosynthetic rate, and specific area (mass per unit area) correlate well across major biomes, functional groups, and species, but much less is known about how leaf traits vary within species and across habitats or seasons (Grime 1977; Coley et al. 1985; Kitajima et al. 1997; Reich et al. 1997, 1999; Wright et al. 2004, 2005a, b).

The discovery of fundamental axes of variation in leaf traits has been useful in modeling efforts because it collapses variation found in nature to simple relationships that reflect underlying biological trade-offs. Indeed, these relationships are often used in models investigating the effects of changes in climate and land use on carbon (C) and N cycling in plant communities (Moorcroft et al. 2001; Bonan et al. 2002; Kaplan et al. 2003). For example, studies across many species and habitats have found that plants generally exhibit a positive relationship between specific leaf area (SLA) and maximum photosynthetic rate (A_{max}) (Wright et al. 2004). Although some major axes of trait variation in plants across the globe have been identified, there is still a great deal of variation in leaf traits that these trade-offs cannot explain (Wright et al. 2005b; Bonser 2006; Westoby and Wright 2006). Additional variation in leaf traits may be due to spatial or temporal variation in environmental conditions at the local scale (Kitajima et al. 1997). Most tropical forests have some degree of seasonality; predictable changes in environmental factors are likely to influence traits that are important in the ability of species to withstand stressful periods. Since there is a cost to the expression of traits, it is likely that plants exhibit optimal expression, (i.e., greater SLA during period of greatest photosynthetic activity or greater toughness when herbivore abundance peaks) especially in markedly seasonal environments. These within-species patterns are potentially very important because many taxa occur across more than one biome, and within-taxon variation may be orthogonal to cross-taxon variation (Clark 2010).

One of the challenges facing efforts to refine and improve models of leaf–trait relationships involves the role of this within-species trait variability. We examined patterns of intra-specific variation in leaf traits and leaf–trait correlations of 10 evergreen and two semi-deciduous species that co-occur in both a seasonal tropical dry forest and a less seasonal wet forest in Northwestern Costa Rica (dry forest and wet forest throughout the text). We tested the hypothesis that there is significant covariation in leaf traits within species, and across forests and seasons, and that these patterns of trait covariation would be congruent across species. Specifically, we predicted that seasonal variation in leaf traits would be significant in both forest types, but that this variation would be greater in the dry forest. Furthermore, we predicted that seasonality would be a greater source of variation in physiological leaf traits, while species would determine a greater amount of the variation in morphological traits. We also hypothesized that this covariation would cause leaf–trait relationships to differ from those documented in global datasets due to differences that seasonal stress may have on important traits such as leaf lifespan (LLS). Finally, we expected the stress of the dry forest to result in more significant trait correlations and stronger correlations when compared to the less seasonal wet forest. It has been suggested that plants undergoing stress may have increased levels of phenotypic integration (Schlichting 1986, 1989a, b), i.e., greater number of trait correlations and strength of those correlations; we expected greater integration in the more stressful site. Our study is the first to focus on leaf–trait variation and correlations across seasons and biomes using the same species. By holding species constant, we can examine the degree to which traits vary from wet forest to dry forest and from the wet season to the dry season at the population level. Moreover, by studying species that occupy distinct environments such as a highly seasonal, dry forest and a less seasonal, wet forest, we can also examine patterns of correlations in traits which may vary as a function of the degree of environmental stress.

We measured a suite of chemical and structural leaf traits and water relations measures: SLA, leaf N, leaf C, LLS, leaf thickness, cuticle thickness, and leaf thickness. We measured water potential and leaf water content (LWC) to compare water availability and water stress of individuals across forests and seasons

(throughout the text we will refer to these water relations measures as "traits" for simplicity). We chose these traits because they reflect aspects of leaves' nutrient economy, adaptations to water stress, defensive strategies, and water status. Last, we also quantified variation in three important environmental variables that differ across these biomes and may affect leaf traits: soil water content, canopy openness, and herbivory.

Materials and methods

Study sites

We studied trees in two forest habitats in the Área Conservación Guanacaste in Northwestern Costa Rica: the dry forest was located in the Santa Rosa sector (10°50′29.28″ N, 8.5°37′10.20″ W, 285 m above sea level) and the wet forest was located in the Maritza sector on the flanks of Volcán Orosi (10°57′25″ N, 8.5°29′42″ W, 500 m above sea level). The two sites are approximately 20 km apart. This protected area contains 120000 ha of dry and wet forest, as well as abandoned pastures and secondary forest in early stages of regeneration. In both the seasonal wet and dry forests there are areas of old growth as well as secondary forest regenerating from previous land use (Powers et al. 2009). Mean annual rainfall is 1528 mm at Santa Rosa, and 3269 mm at Maritza (Area Conservación Guanacaste, http://www. acguanacaste.ac.cr/ and Stroud Water Research Center, http://www.stroudcenter.org/ meteorological records). In both forests the majority of the rain falls in the wet season between May and November. The forest at Maritza is predominantly evergreen, while the forest in Santa Rosa is a mixture of evergreen and deciduous trees. Although precipitation has a seasonal distribution in wetter forest, this site maintains higher surface soil moisture, humidity, and cloud cover throughout the year, while the drier site experiences extreme seasonal changes in these variables (Gotsch 2006).

There are large differences in air temperature from the wet to the dry forest. However, the maximum temperature occurs toward the end of the dry season (April) in both sites. The maximum air temperature in the wet forest is 27°C while in the dry forest temperatures can reach 44°C. In addition to high dry season temperatures, there is also greater seasonal variation in air temperature in the dry forest (Area Conservación Guanacaste, http://www.acguanacaste. ac.cr/ and Stroud Water Research Center, http:// www.stroudcenter.org/ meteorological records). Although strong trade winds, lower relative humidity, and high irradiance accompany the dry season in both sites, the higher soil moisture and cloud cover year-round, as well as the lower temperatures in the wet forests create a buffer to dry season evaporative demand that is not present in the dry forest.

Species

The 12 woody plant species included in this study are common in both wet and dry forests in Northwestern Costa Rica (Table 1, for simplicity we refer to them by genus). Species were generally associated with one habitat type (i.e., early regeneration or old growth forest). However, we located and sampled individuals from most species in different habitats to provide a range of light environments. Two species (*Alibertia* and *Malvaviscus*) are shrubs while the others are trees. Two species are found predominantly in mature forests (*Ardisia* and *Brosimum*), three species are predominantly found in regenerating forests (*Brysonima*, *Guazuma*, and *Roupala*), and seven species are present in many age classes (*Alibertia*, *Casearia*, *Cupania*, *Malvaviscus*, *Miconia*, *Ocotea*, and *Manilkara*). Ten of the species are evergreen, while two species are characterized as semideciduous (*Malvaviscus* and *Guazuma*), i.e., they maintain only a partial canopy in the dry season (Janzen 1983; Gotsch, pers. observ.). These species encompass a large range of phylogenetic diversity at the family level: two of the families are considered basal (Lauraceae and Proteaceae) to the eudicot groups while the other families are more recent in origin (Soltis et al. 2005). A wide variety of herbivores feed on these species, including Lepidopteran, Coleopteran, Homopteran, Orthopteran, and Hemipteran larvae (http://janzen.sas.upenn.edu/caterpillars/database.lasso).

Between 10–15 juvenile and young adult trees of each species were identified and marked in both forests. There was a minimum distance of 30 m between individuals of the same species, although every attempt was made to sample trees that had greater distance between them. Within each forest type, distances of 500 m to a few kilometers often separated individuals of a species. On a few

Table 1 Species information for focal trees in this study

Species	Family	Growth type	Forest type
Alibertia edulis	Rubiaceae	Understory shrub	Mature/late regeneration
Ardisia revoluta	Myrsinaceae	Canopy tree	Mature
Brosimum alecastrum	Moraceae	Canopy tree	Mature
Brysonima crassifolia	Malpighiaceae	Early succession tree	Early regeneration
Casearia sylvestris	Flacourtiaceae	Understory tree	Late regeneration
Cupania guatemalensis	Sapindaceae	Understory tree	Mature/late regeneration
Guazuma ulmifolia	Sterculiaceae	Early succession tree	Early regeneration
Malvaviscus arboreus	Malvaceae	Understory shrub	Early regeneration
Manilkara chicle	Sapotaceae	Canopy tree	Mature/late regeneration
Miconia argentea	Melastomataceae	Understory tree	All types
Ocotea veraguensis	Lauraceae	Understory tree	Mature/late regeneration
Roupala montana	Proteaceae	Early succession tree	Early regeneration

occasions trees died and additional individuals were marked and added to the study.

Biotic and abiotic variables

We measured the seasonal variation in biotic and abiotic variables that may affect leaf characteristics (soil moisture, canopy openness, and herbivory). These measurements, together with leaf water potential (LWP) (described in leaf trait measures section) and rainfall (collected at the biological stations), were used to determine the intensity of seasonal drought and herbivore stress for the 12 species. All measurements were taken during 2004 and 2005.

Soil moisture was measured gravimetrically on soil cores from the top 15 cm of mineral soil (i.e., excluding the litter layer) from 10 locations (two samples per location) in each forest type. Three to four locations were measured in each of the different habitat types (early regeneration, secondary forest, old growth) in the dry and wet forests. Samples were taken once in the middle of the wet and dry seasons for 2 consecutive years. Soil samples were extracted using a 5 cm diameter punch tube soil corer and placed in metal tins in the field to prevent soil water loss. The tins were placed at 50°C and reweighed every day after 3 days until they reached a constant weight.

Canopy openness was quantified in each site across seasons to track seasonal changes in light availability in wet and dry forests. Canopy openness was measured at 120 cm using a 24-mm digital camera in 10 locations representative of the range of habitats in each forest. These measurements were taken once in the wet and dry season for 2 consecutive years. Images were analyzed using ImageJ, and averages were taken over the different habitat types for each forest (the accuracy of this method is described in Engelbrecht and Herz 2001).

Herbivory was measured every month on the same 10–15 individuals of the 12 species in each forest type for 2 years. Assessment of the entire crown was made visually on a four-point scale (0 = 0%, 1 = 0–25%, 2 = 25–50%, 3 = 50–75%, and 4 = 75–100% activity), and values were transformed to the midpoint of the percentage range. Herbivory was estimated as the percentage of damage to the newly expanded leaves.

Leaf traits

To determine the spatial (i.e., across forests) and temporal (across seasons) patterns of intra-specific variation in leaf traits, 10 leaf traits were measured repeatedly in the populations of marked individuals for 2 years. Leaves were measured in the middle of the wet season (August–September) and the middle of the dry season (January–February) in both forests. Leaf trait measures included cuticle thickness, leaf thickness, toughness, SLA, LWC, LLS, predawn/midday LWP, and total leaf C and N. For all leaf trait measures the youngest fully expanded leaves were used. Fresh leaves were collected, bagged, and stored

in a refrigerator until measured (later the same day or the following morning). In an earlier study we determined that trait values of stored leaves did not differ significantly from fresh leaves (Gotsch, unpublished data). Sun leaves were measured for trees that were growing in the sun (most cases) while shade leaves were used for trees growing predominantly in the shade. For all species, both individuals in open and shaded environments were measured so we could determine the effect that light environment as well as forest type and season had on leaf–trait variation.

Leaf cuticle thickness and overall leaf thickness were determined by hand sectioning fresh leaves. One section per leaf (near the middle) was stained with Sudan III solution. Adaxial cuticle thickness and total leaf thickness were measured with an optical micrometer in the middle of the leaf halfway between the edge and the midrib, to avoid biasing measurements toward thicker midribs. These measurements were made at multiple points along the leaf cross-section and reported as averages per leaf.

Leaf toughness was determined using a leaf penetrometer following the specifications in Feeney (1970) and Coley (1983). The leaf penetrometer measures the mass needed to puncture a leaf using a 3 mm diameter rod. The leaf was secured between two pieces of thick Plexiglas, through which a 3 mm diameter hole had been drilled. A lightweight platform was placed on top of the leaf and weight was added in small increments to a beaker on the platform until the platform with the weight pierces the leaf. Leaf toughness measured in this manner shows a strong correlation with susceptibility to herbivory (Coley 1983).

To measure SLA and LWC, 4–8 hole punches of a constant area were taken per leaf. Leaf punches were used after finding no significant difference between the values of these samples and that of the whole leaf (without the petiole). Samples were weighed wet and then dried at 50°C for 3 days and weighed again (dry weight). SLA was calculated as the area of the leaf punches divided by the dry weight. LWC was calculated in the following way: ((fresh weight-dry weight)/dry weight) × 100.

Leaf lifespan was determined by marking four leaves per tree in different ordinal directions (10 trees per species per forest) with colored wire as new leaves expanded. A different colored wire was used to mark leaves each month until 40 leaves per species per forest were marked. Presence/absence data were collected every month, and average LLS per tree was calculated. In general, these species flushed leaves at different times of year in the two forest types (Gotsch, unpublished data); leaves were generally flushed in the middle of the dry season in the wet forest and in the beginning of the wet season in the dry forest. As a result the wet forest leaves are always approximately 2 months older than the dry forest leaves at any given measurement time which may add to variation seen across seasons.

Water potential was measured using a PMS portable pressure chamber (Model 1000). Leaves were collected between 5 and 6:30 AM for predawn water potential and between 11:30 AM and 1:00 PM for midday water potential. Leaves were collected by placing a plastic bag over the leaf (to slow transpiration) and the petiole was cut at the base with a sharp straight edge. Four leaves were collected per tree (sun leaves if possible) and placed in a cooler with ice and then transferred to a refrigerator until measurements were made. An initial study confirmed that the LWP did not differ significantly from fresh leaves using this method (Gotsch, unpublished data). Pre-dawn and midday water potential measurements were attempted on the same day for each tree unless environmental conditions (e.g., midday rain) made this impossible. In the case of midday rain, the corresponding PM measurements were made on the next sunny midday.

Foliar C and N were determined for a subset of all trees/shrubs. Leaves were collected and dried at no greater than 40°C (ambient) in the field and were redried at 60°C prior to analysis in the lab. Leaf samples were ground using a Thomas-Wiley Intermediate mill (Thomas Scientific, Swedesboro, NJ). C and N were analyzed on four individuals per species (five leaves per individual) per forest across four consecutive seasons. Individuals in both open and closed light environments were sampled. Samples were analyzed for total C and N content with a CHN analyzer (CE Elantech, Lakewood, NJ) in the Department of Ecology and Evolution's Functional Ecology Research and Training Laboratory at the State University of New York at Stony Brook.

Data analysis

We conducted a multivariate analysis of variance (MANOVA) to determine if there were differences in soil water content and canopy openness across forests

and seasons. Soil water content and canopy openness were arcsine transformed to achieve normality. The effects tested in this analysis were "forest," "season" and their interaction. Statistical analyses for canopy openness and soil moisture were performed using JMP statistical software (version 7.0 for Windows).

We performed MANOVAs and examined canonical analyses for all leaf traits that were measured seasonally to determine whether leaf traits varied significantly across forests and seasons. An attempt was made to follow the same trees in all seasons, but, as noted in the "Materials and Methods" section, some individuals died and were replaced. As a result of this sampling, leaf trait variables across seasons were analyzed using a MANOVA and Repeated Measures MANOVA. All leaf traits were $\log_{10}$ transformed except for LWC, which was arcsine transformed to obtain a normal distribution. The main effects for all analyses were "forest," "season," "year," "light environment," (full sun, dry season sun, and shade) and "species," as well as the interactions of the main effects. Species was considered a random factor in the analyses. A canonical analysis was performed along with the MANOVA to determine how individual traits influenced the variation measured across the main effects and the interaction terms. Changes in LLS within species in the wet and dry forest were analyzed using ANOVA. Leaf trait MANOVAs and ANOVAs were conducted using SAS version 9.1 (SAS Institute, Cary NC, USA).

Patterns of trait correlations were examined by calculating the pairwise Pearson-Product correlation coefficients. There is not a widely accepted method for analyzing patterns of multiple trait correlations; therefore we examined the patterns of correlations in the two forests using a variety of methods. We examined the average strength of the pairwise correlations (Pearson's product moment coefficients), the variance of the eigenvectors in a principal components analysis, and we also conducted a contingency analysis of the frequency of correlations in different groups of character types. All of these methods yielded identical results (results not shown); therefore, we will only discuss the results for the average strength of the pairwise correlations. In order to examine the relationships among LLS, SLA and leaf N (three of the most commonly reported variables in the leaf economics spectrum), averages for the seasonally measured traits were taken across

seasons and a single averaged value was calculated for SLA and leaf N for each species in the two forests. In addition, we performed an ANCOVA on SLA and leaf N to determine if the relationship between these traits was significantly different across forests. All analyses on trait correlations were performed using JMP statistical software (version 7.0 for Windows).

Results

Environmental variables

Soil moisture (0–15 cm) and canopy openness varied significantly across forests ($P < 0.0001$) and seasons ($P < 0.0001$), although the interaction between these two factors was not significant ($P = 0.3217$, MANOVA in Electronic Supplementary Material, S1). In the dry forest the soil was consistently drier than in the wet forest. In the dry forest soil water varied from 12.5% in the dry season to 37.7% in the wet season, while in the wet forest soil water content varied from 25.5% in the dry season to 52.2% in the wet season (Fig. 1). Between successional habitats within each forest there were no significant differences in soil moisture (results not shown). Canopy openness in the largely deciduous dry forest showed a 30% decrease from the wet season to the dry season (Fig. 1). Such large seasonal variation in canopy openness was restricted to the dry forest. In the largely
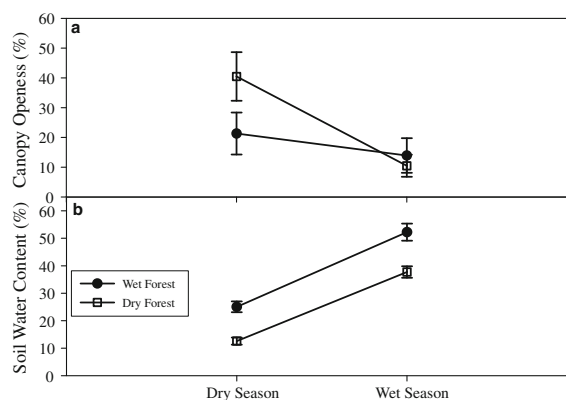

Fig. 1 Canopy openness (**a**) and gravimetric soil water content (**b**) in the wet and dry seasons in a more and less seasonal forest. Each value is an average of two wet and two dry seasons. *Error bars* represent the standard error of the mean

evergreen wet forest, canopy openness was 8% higher in the wet season compared to the dry season, which was not a statistically significant difference. Herbivory peaked in the dry forest in June (early in the wet season). This peak corresponded to an average canopy leaf area loss of 20% (Electronic Supplementary Material, S3). Trees in the wet forest experienced low levels of herbivory throughout the year with the exception of *Roupala montana*, which experienced a large peak in herbivory in the wet season, exclusively from leaf-cutter ants (Gotsch, pers. observ.).

Response measures

When analyzing all leaf traits together via MANOVA (Table 2) and Repeated Measures MANOVA (not shown), the main effects of forest, species, season and year, and their interaction terms were all significant ($P < 0.0001$). Light environment (whether a tree was growing in full sun, dry season sun, or shade) did not have a significant effect on leaf–trait variation (results not shown) and was removed from the analysis. The effects that contributed most to the total variation of the model were species (46.5%), season (26.7%), forest (5.3%), and the interaction between forest and season (6.6%).

Although species explained more of the total variability in traits than the other main effects

(Table 2), seasonal variation, and variation due to forest type also contributed significantly to leaf–trait variation. The most predictable patterns of seasonal variation were for predawn and midday LWP. Water potential decreased in all species in both forests from the wet season to the dry season, and this decrease was much greater in the dry forest (Fig. 2g, h).

Seasonal variation in SLA, LWC, and toughness differed in the two forest types. For most species, SLA increased from the dry season to the wet season in the dry forest. These same species maintained similar SLA across seasons in the wet forest (Fig. 2a). Trees in the dry forest generally experienced an increase in LWC in the wet season in the dry forest while leaves in the wet forest maintained similar LWC across seasons. In contrast, most species in the wet forest exhibited an increase in leaf toughness from the dry season to the wet season while individual species tended to behave differently in the dry forest (Fig. 2b).

In general, leaf N varied modestly, but significantly, in both forests but in different directions (Fig. 2f, inset). In the dry forest, leaf N increased for most species from the dry season to the wet season while species in the wet forest exhibited inconsistent responses (Fig. 2f). The overall pattern in the wet forest is likely driven by strong seasonal variation in

Table 2 MANOVA on log10 transformed data for leaf toughness, cuticle thickness, leaf thickness, leaf water potential (predawn and midday), SLA, LWC, leaf N, and leaf C

Effect	*F*-value	% variation	Num DF	*P*-value
Species	449.94	46.6	11	<0.0001
Forest	50.89	5.3	9	<0.0001
Season	257.85	26.7	9	<0.0001
Year	32.29	3.3	9	<0.0001
Forest × season	63.65	6.6	9	<0.0001
Forest × year	40.36	4.2	9	<0.0001
Forest × species	18.33	1.9	11	<0.0001
Season × species	27.49	2.8	11	<0.0001
Year × species	8.62	0.9	22	<0.0001
Forest × year × species	9.49	1	21	<0.0001
Forest × season × species	7.58	0.8	11	<0.0001

Species was considered a random factor in this analysis. The *F*-value and degrees of freedom were calculated using Roy's greatest root statistic. Although the *F*-values and degrees of freedom varied between the different test statistics, the *P*-values were identical. The percentage of the total variation attributed to each effect was calculated by dividing the *F*-value for each effect but the total *F*-value
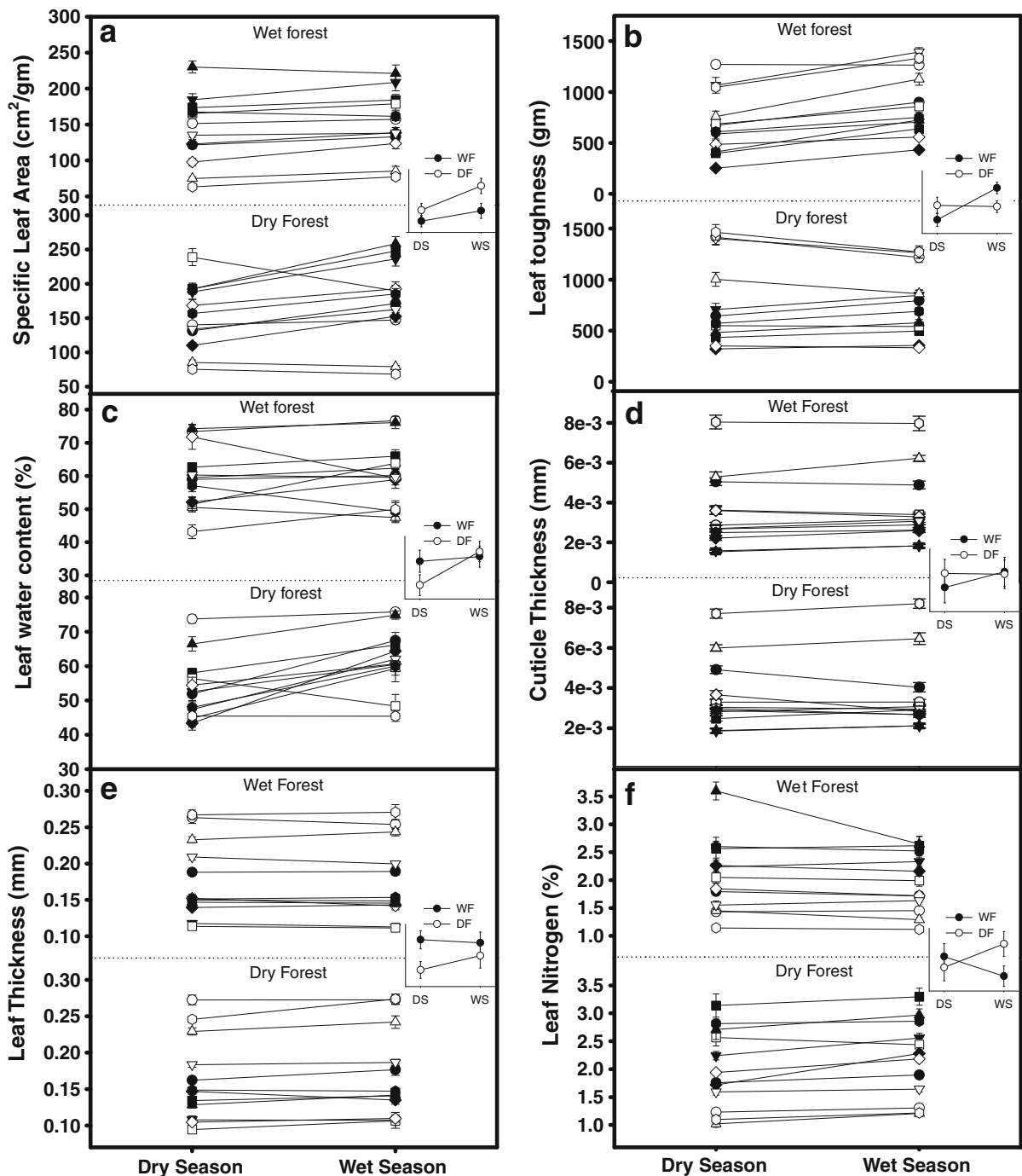
Fig. 2 Leaf traits for 12 species measured in wet and dry forests in the wet and dry seasons in NW Costa Rica. Season in which the measurements were made is noted on the horizontal axis. *Error bars* represent the standard error of the mean. *Insets* represent values for each trait across forests and seasons (species averaged)

one species, *Malvaviscus arboreus*. This species has a short LLS and may be producing leaves with greater photosynthetic capacity in the dry season.

Leaf lifespan also varied significantly across forests ($F = 52.47$, $P < 0.0001$). Trees retained leaves for 8–14 months in the wet forest and
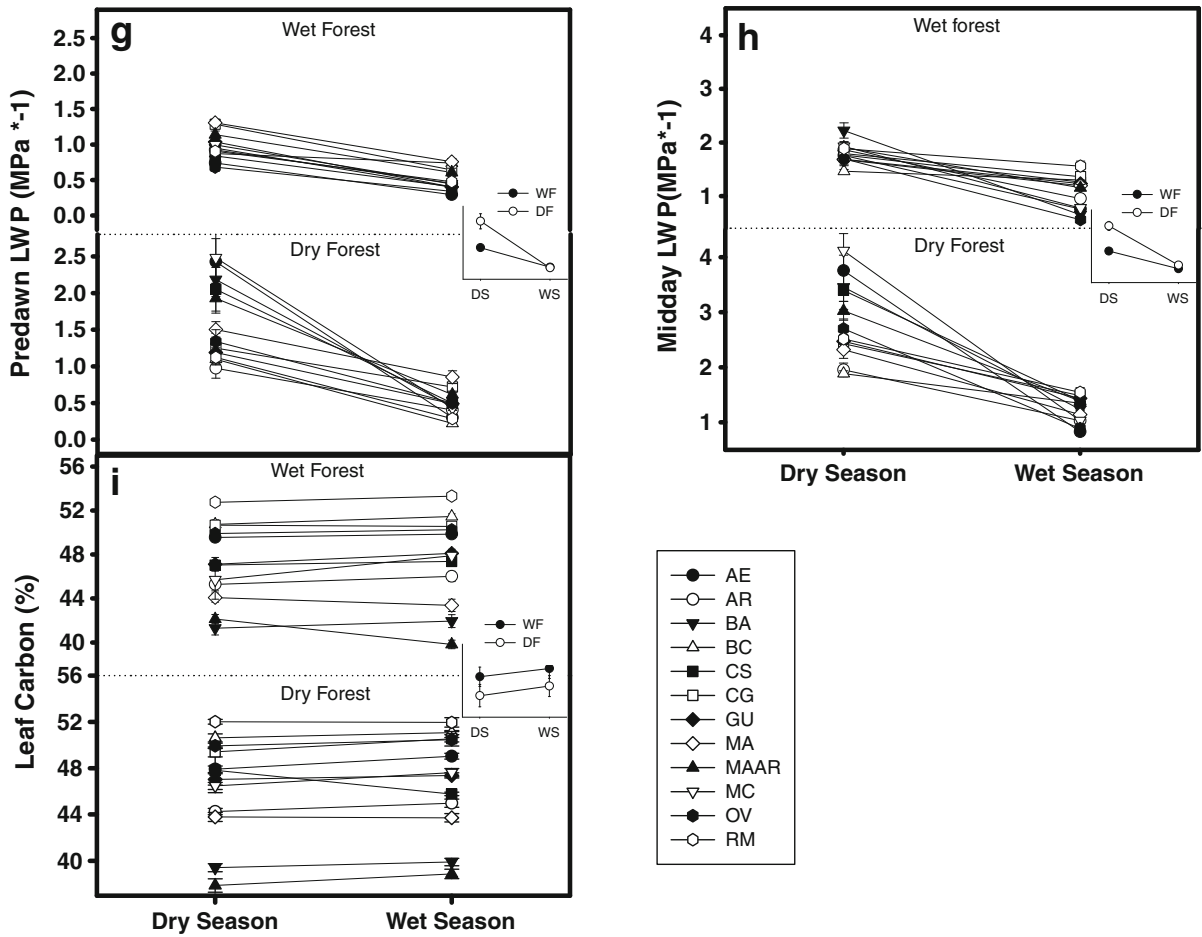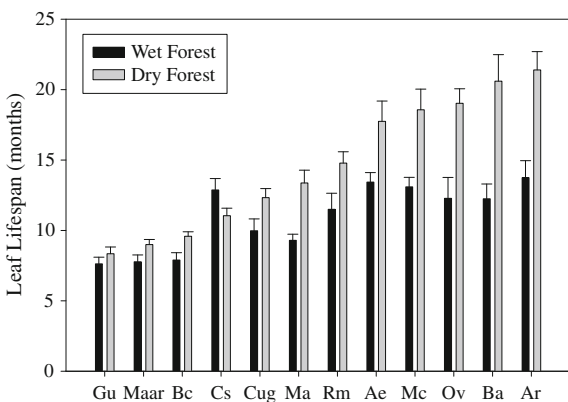
Fig. 2 continued

9–24 months in the dry forest (Fig. 3). This difference was greater in the longest lived leaves and was almost negligible for the shortest lived leaves (Fig. 3). There was little seasonal variation in leaf C within species in either forest type (Fig. 2i); in general leaf C was greater in the wet forest (Fig. 2i, inset).

Most species exhibited little variation in cuticle thickness (Fig. 2d) and leaf thickness (Fig. 2e) either across seasons or forests. Inter-specific variation in these traits may indicate that factors such as phylogeny, successional status or shade tolerance are more important in their expression. Although the majority of trait variation was determined by species, the effects of forest and season also contributed significantly to the variation in many traits indicating the additional importance of spatial and temporal

Fig. 3 LLS for 12 species in seasonal wet and dry forest. On the *x*-axis are the species codes (*first letter* is the first initial of the genus name and the *second letter* is the first initial of the species name) in order of increasing LLS. *Error bars* represent the standard error of the mean. See Table 1 for a list of the species. On the *y*-axis is the LLS in months

environmental heterogeneity on trait variation. (Table 2; Fig. 2a–i).

Correlation of leaf traits

There were many significant pairwise correlations between traits in both forests, although there were more significant correlations in the dry forest (Table 3). The correlations that were significant in the dry forest but not in the wet forest were between LWP and toughness, cuticle thickness, and leaf thickness (Table 3). In both forests (across seasons), there was a positive correlation between SLA and leaf N (Table 3; Fig. 4) as expected. In both forests this correlation was strong (0.88 DF vs. 0.77 WF), and the difference in correlation strength across forests was not statistically significant (ANCOVA,

Table 3 Significant correlation coefficients ($P < 0.05$, calculated in JMP version 5.1 for Windows) among leaf traits using species means in the dry forest and wet forest pooled across seasons

Variable	By variable	Wet forest	Dry forest
TOUGH	LLS	0.69	ns
CT	TOUGH	0.61	ns
LT	TOUGH	0.77	0.77
LT	CT	0.80	0.79
WPAM	TOUGH	ns	−0.62
WPAM	CT	ns	−0.83
WPAM	LT	ns	−0.82
SLA	CT	−0.86	−0.89
SLA	LT	−0.70	−0.81
SLA	WPAM	ns	0.87
N	TOUGH	−0.68	−0.63
N	CT	−0.83	−0.85
N	LT	−0.79	−0.73
N	WPAM	ns	0.78
N	SLA	0.77	0.88
C	CT	0.82	0.66
C	SLA	−0.66	ns
C	LWC	ns	0.66

The arcsine transformation was used for LWC. LLS and WPPM are missing from this table because their trait combinations were not significant in either site

TOUGH $\log_{10}$ transformations were used leaf toughness, *CT* cuticle thickness, *LT* leaf thickness, *WPAM* predawn leaf water potential, *WPPM* midday leaf water potential, *SLA* specific leaf area, *N* leaf nitrogen, *C* leaf carbon, *LLS* leaf lifespan

results not shown). In the dry forest there were also more strong negative correlations than in the wet forest. Although the numbers of trait correlations were greater in the dry forest, these differences were not statistically significant. The average strength of the significant pairwise correlations was similar in both forests ($r^2 = 0.77$ in the dry forest, $r^2 = 0.75$ in the wet forest) indicating that across all traits measured in this study, patterns of correlations were generally the same across the two forest types. Surprisingly, LLS was not correlated with any variable in either forest.

We compared relationships between SLA, leaf N and LLS in this study to correlations reported in global datasets by plotting the bi-variate relationships for both forests together with the axis of variation documented for the "leaf economics spectrum." This comparison was plotted to determine whether the direction of the significant variation detected in the MANOVA was along the axis previously reported. SLA and leaf N (Fig. 4), were positively correlated in both forests. For six of the 12 species, this relationship was in the same direction as the linear relationship documented across species by Wright et al. (2004). On the other hand, neither correlations for LLS and SLA, nor LLS and leaf N were significant (Table 3). In fact, the variation from the wet to dry forest was often in a direction other than that of the documented axis of variation (Figs. 5, 6).

Discussion

In this study we found significant intra-specific trait variation across seasons and forest types. Although most traits varied significantly by species as other studies in this region have found (Powers and Tiffin, in press), season and forest explained a large portion of the variation in many traits, indicating that, in general, species responded to environmental variation in a coherent fashion. As predicted, populations growing in the dry forest showed greater overall seasonal variation in leaf traits than populations in the wet forest (Fig. 2), and different types of traits varied in patterns of temporal (seasonal) and spatial (forest type) variation. Traits reflecting plant water status (water potential and LWC) showed particularly strong seasonal trends that are likely driven by changes in light and water availability. SLA and leaf
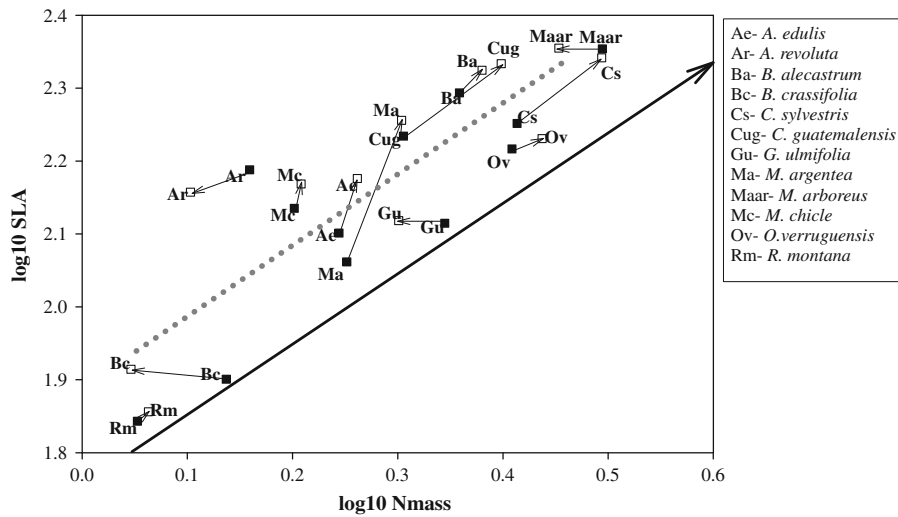
Fig. 4 Leaf SLA and N for 12 species from wet forest (*black*) and dry forest (*open*). The wet and dry season trait values are pooled for each forest. For the 12 species included in this study there was large variation in the species relationships between SLA and leaf N in the two forests. The *bold black line* represents the relationship found in the global datasets for these two variables (Wright et al. 2004) while the *dotted line* represents the relationship found in this study, which was not significantly different across forests
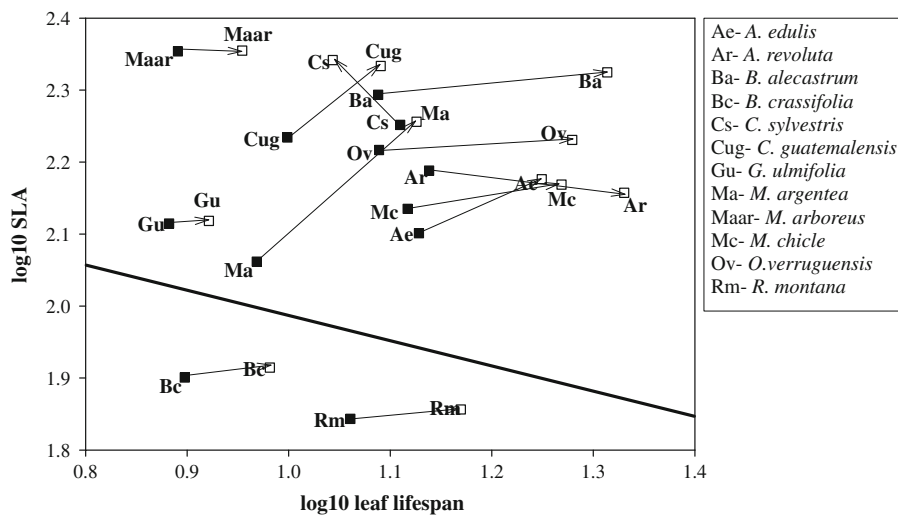

Fig. 5 Leaf SLA (log10) and LLS (log10) for 12 species from wet forest (*black*) and dry forest (*open*). The wet and dry season trait values are pooled for each forest. The *bold black line* is the relationship found in a global dataset for these two variables (Wright et al. 2004)

N, which are well correlated with C gain and maximum assimilation rate, showed both seasonal and spatial differences, which may be due to both water and light availability. Last, traits related to structural defenses (i.e., leaf toughness and cuticle thickness), which we hypothesized might be correlated with herbivory rates, did not vary in concert with leaf damage, which varies seasonally; however, further studies must be conducted to specifically address which other environmental factors are driving the patterns of variation in these traits. In another study on these same species, we found that leaves are primarily produced in the dry season in the wet forest and in the beginning of the wet season in the dry
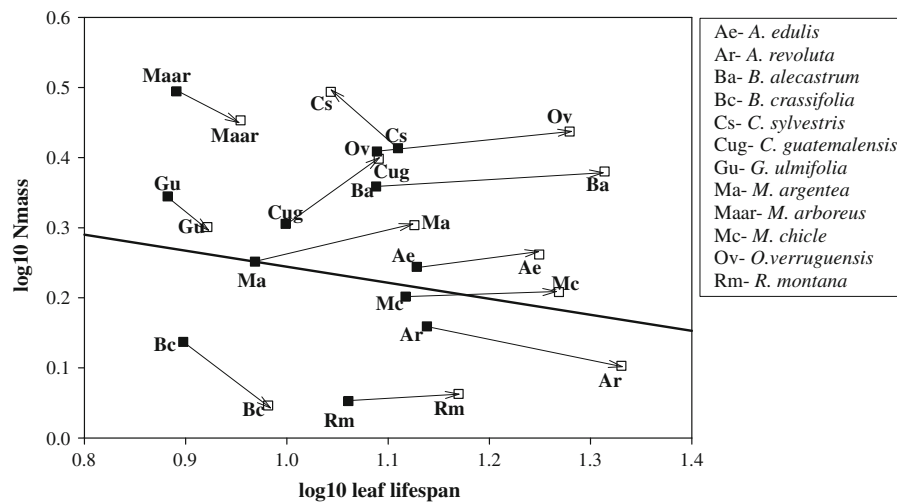
Fig. 6 Leaf Nmass (log10) and LLS (log10) for 12 species from wet forest (*black*) and dry forest (*open*). The wet and dry season trait values are pooled for each forest. The *bold black line* is the relationship found in a global dataset for these two variables (Wright et al. 2004)

forest (Gotsch, unpublished data). Ontogeny may also have an effect on variation in leaf traits over time (Janzen and Waterman 1984), although the patterns of variation seen in this study indicate that season is also important.

Specific leaf area and leaf N are two important traits in the form and functioning of plants. These traits are related to structural and physiological properties of leaves such as maximum assimilation rate, and generally correlate with ecosystem processes such as decomposition and palatability to herbivores (Field and Mooney 1986; Reich et al. 2003; Santiago 2007; Kurokawa and Nakashizuka 2008). The patterns of variation for these two traits in the seasonal wet and dry forests in NW Costa Rica suggest that there are coarse differences in the photosynthetic capacity of trees in more and less seasonal forests. Dry forest trees may generally have leaves with a higher photosynthetic capacity (SLA and N), but restrictions in growth due to water stress (as indicated by LWP) could limit photosynthetic activity to the wet season. Seasonal variation in SLA in dry forest trees may be a response to seasonality in water or light availability. Although across species leaves are often smaller and have lower SLA under water limitation (Givinish 1987; Cunningham et al. 1999), within evergreen species we found that SLA is generally higher in the dry forest, in particular during the wet season. These results underscore the

importance of observing seasonal trends in leaf traits in seasonal forests. For example if we had examined SLA in either the wet season or the dry season across forests our results would have been inconsistent with patterns established in the literature (i.e., low SLA with low water availability). However, when assessed seasonally we can see that in each forest (especially in the dry forest), when water is less available, there is a decrease in SLA.

We also hypothesized that the magnitude of seasonal variation in environmental factors would alter the correlations among traits in different habitats. Although there were more significant correlations in the dry forest, these differences were minor, and analyses of the correlation matrices for the wet and dry forest did not reveal a significant difference. Our results indicate that tree species that traverse ecological gradients may adapt to a new environment by altering their trait means rather than by altering patterns of leaf–trait correlations (i.e., phenotypic integration).

As noted above, one of our goals was to determine whether leaf–trait relationships across biomes and species are the same as leaf–trait relationships across biomes *within* species. In our sample of tropical forest tree species growing in both wet and dry habitats, we found some of the same relationships between leaf traits documented across biomes and functional groups (e.g., broadleaf evergreens and

Author's personal copy

herbaceous plants) as in other studies but also some important differences (Reich et al. 1992; Reich et al. 1999; Wright et al. 2005a). In both of the forests we studied in NW Costa Rica, there was a significant, positive relationship between SLA and leaf N, as seen in the global datasets. Furthermore the direction of the variation from the wet to the dry forest was mostly along the axis documented previously (Fig. 4). However, unlike the global datasets, there was no overall relationship between LLS and SLA, or LLS and N concentration (see Reich et al. 1991; Wright et al. 2005a). In these two cases the variation in traits across populations was often in a direction opposite the axis of inter-specific variation found in global datasets (Figs. 5, 6). In previous studies examining *inter*-specific variation in leaf traits across rainfall gradients in Australia and Panama, species in drier regions tended to have shorter lifespans (Reich et al. 1999; Santiago et al. 2004; Wright et al. 2002, 2005a); we found the opposite pattern in our examination of *intra*-specific variation in leaf traits in wet and dry forests. Our data imply that global patterns in leaf–trait relationships may not appear at local to regional scales because of seasonal variation in leaf traits and because the processes controlling leaf–trait correlations within taxa may not be the same as those controlling them across taxa. Considerations of distinct leaf–trait relationships in more and less seasonal tropical forests should be included in future attempts to define global patterns of leaf function and in the applications of these patterns in large-scale models.

Conclusions

Most comparative studies of leaf traits focus on patterns that emerge from inter-specific comparisons across biomes or strong environmental gradients and do not speak to the significance of intra-specific variation in response to the environment. Recent studies on the regulation of biodiversity have highlighted, however, the potential significance of intra-specific variability (Clark 2010). In this study we found that a significant portion of trait variation (up to 30%) was explained by environmental variation across seasons and differences in forest type. Understanding patterns of variation at this level will add to basic research in evolutionary ecology regarding the

mechanisms behind intra-specific variation such as local adaptation, plasticity, and phenotypic integration and will contribute to our understanding of the controls of biological diversity. Furthermore, understanding patterns of variation within species across seasons and habitats is essential for improving large-scale vegetation models and adding more realistic mechanisms to these models.

References

Bonan GB, Levis S, Kergoat L, Oleson KW (2002) Landscapes as patches of plant functional types: an integrating concept for climate and ecosystem models. Glob Biogeochem Cycles 16(2):1029

Bonser SP (2006) Form defining function: interpreting leaf functional variability in integrated plant phenotypes. Oikos 114(1):187–190

Clark JS (2010) Individuals and the variation needed for high species diversity in forest trees. Science 327(5969):1129–1132

Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol Monogr 53(2):209–233

Coley PD, Bryant JP, Chapin FS (1985) Resource availability and plant antiherbivore defense. Science 230(4728):895–899

Cunningham S, Summerhays B, Westoby M (1999) Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. Ecol Monogr 69:569–588

Engelbrecht BMJ, Herz HM (2001) Evaluation of different methods to estimate understorey light conditions in tropical forests. J Trop Ecol 17:207–224

Feeney P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51(4):565–581

Field C, Mooney HA (1986) The photosynthesis-nitrogen relationship in wild plants. In: Givnish T (ed) On the economy of plant form and function. Cambridge University Press, Cambridge, pp 25–55

Gianoli E (2004) Plasticity of traits and correlations in two populations of *Convolvulus arvensis* (Convolvulaceae)

differing in environmental heterogeneity. Int J Plant Sci 165(5):825–832

Givinish T (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. New Phytol 106:131–160

Gotsch S (2006) Leaf form and function in the seasonal forests of northwestern Costa Rica. Doctoral Dissertation, State University of New York at Stony Brook, Stony Brook

Grime JP (1977) Evidence for existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111(982):1169–1194

Janzen DH (ed) (1983) Costa Rican natural history. University of Chicago Press, Chicago

Janzen DH, Waterman PG (1984) A seasonal census of phenolics, fibre and alkaloids in foliage of forest trees in Costa Rica: some factors influencing their distribution and relation to host selection by Sphingidae and Saturniidae. Biol J Linn Soc 21:439–454

Kaplan JO, Bigelow NH, Prentice IC, Harrison SP, Bartlein PJ, Christensen TR, Cramer W, Matveyeva NV, McGuire AD, Murray DF, Razzhivin VY, Smith B, Walker DA, Anderson PM, Andreev AA, Brubaker LB, Edwards ME, Lozhkin AV (2003) Climate change and arctic ecosystems: 2. Modeling, paleodata-model comparisons, and future projections. J Geophys Res Atmos 108(D19):8171

Kitajima K, Mulkey SS, Wright SJ (1997) Seasonal leaf phenotypes in the canopy of a tropical dry forest: photosynthetic characteristics and associated traits. Oecologia 109(4):490–498

Kurokawa H, Nakashizuka T (2008) Leaf herbivory and decomposability in a Malaysian tropical rain forest. Ecology 89:2645–2656

Moorcroft PR, Hurtt GC, Pacala SW (2001) A method for scaling vegetation dynamics: the ecosystem demography model (ED). Ecol Monogr 71(4):557–585

Nicotra AB, Chazdon RL, Schlichting CD (2007) Patterns of genotypic variation and phenotypic plasticity of light response in two tropical *Piper* (Piperaceae) species. Am J Bot 84(11):1542–1552

Pigliucci M, Kolodynska A (2006) Phenotypic integration and response to stress in *Arabidopsis thaliana*: a path analytical approach. Evol Ecol Res 8:414–433

Powers JS, Tiffin P (in press) Plant functional type classifications in tropical dry forests in Costa Rica: leaf habit versus taxonomic approaches. Funct Ecol

Powers JS, Becknell JM, Irving J, Perez-Aviles D (2009) Diversity and structure of regenerating tropical dry forests in Costa Rica: geographic patterns and environmental drivers. For Ecol Manag 258:959–970

Reich PB, Uhl C, Walters MB, Ellsworth DS (1991) Leaf life-span as a determinant of leaf structure and function among 23 Amazonian tree species. Oecologia 86(1):16–24

Reich PB, Walters MB, Ellsworth DS (1992) Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. Ecol Monogr 62(3):365–392

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. Proc Natl Acad Sci USA 94(25):13730–13734

Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. Ecology 80(6):1955–1969

Reich PB, Buschena C, Tjoelker MG, Wrage K, Knops J, Tilman D, Machado JL (2003) Variation in growth rate and ecophysiology among 34 grassland and savanna species under contrasting n supply: a test of functional group differences. New Phytol 157(3):617–631

Santiago LS (2007) Extending the leaf economics spectrum to decomposition: evidence from a tropical forest. Ecology 88:1126–1131

Santiago LS, Kitajima K, Wright SJ, Mulkey SS (2004) Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. Oecologia 139(4):495–502

Schlichting CD (1986) The evolution of phenotypic plasticity in plants. Annu Rev Ecol Syst 17:667–693

Schlichting CD (1989a) Phenotypic integration and environmental change. Bioscience 39:460–464

Schlichting CD (1989b) Phenotypic plasticity in Phylox II. Plasticity of character correlations. Oecologia 78:496–501

Schlichting CD, Pigliucci M (1998) Phenotypic evolution—a reaction norm perspective. Sinauer Associates, Sunderland, pp 191–226

Soltis P, Soltis D, Edwards C (2005) Angiosperms. Flowering plants, version 03 June 2005. http://tolweb.org/Angiosperms/20646/2005.06.03 in the tree of life web project, http://tolweb.org/

Westoby M, Wright IJ (2006) Land-plant ecology on the basis of functional traits. Trends Ecol Evol 21(5):261–268

Wright IJ, Westoby M, Reich PB (2002) Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf life span. J Ecol 90(3):534–543

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The world-wide leaf economics spectrum. Nature 428(6985):821–827

Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Garnier E, Hikosaka K, Lamont BB, Lee W, Oleksyn J, Osada N, Poorter H, Villar R, Warton DI, Westoby M (2005a) Assessing the generality of global leaf trait relationships. New Phytol 166(2):485–496

Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Groom PK, Hikosaka K, Lee W, Lusk CH, Niinemets U, Oleksyn J, Osada N, Poorter H, Warton DI, Westoby M (2005b) Modulation of leaf economic traits and trait relationships by climate. Glob Ecol Biogeogr 14(5):411–421