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PATTERNS OF GENOTYPIC VARIATION AND PHENOTYPIC PLASTICITY OF LIGHT RESPONSE IN TWO TROPICAL *PIPER* (PIPERACEAE) SPECIES[1]

ADRIENNE B. NICOTRA, ROBIN L. CHAZDON,[2] AND CARL D. SCHLICHTING

Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269

Patterns of phenotypic plasticity and genotypic variation in light response of growth and photosynthesis were examined in two species of rain forest shrub that differ in ecological distribution within the forest. We further examined correlations among photosynthetic and growth traits. We hypothesized that the pioneer species, *Piper sancti-felicis*, would display greater phenotypic plasticity than the shade-tolerant species, *Piper arieianum*. We further proposed that, in both species, genotypic effects would be more apparent in growth-related traits than photosynthetic traits due to more concentrated selection pressure on gas-exchange traits. *P. sancti-felicis* did not demonstrate greater phenotypic plasticity of light response. Although many of the traits measured had significant genotype effects, neither species showed any significant effects of genotype on light response of photosynthesis, suggesting little genetic variation for this trait within populations. A principal components analysis clearly illustrated both species and light effects, with the treatments dividing neatly along the axis of the first principal component and the species separating along the second principal component axis. Results indicated general similarities between the species in their trait correlation structure and level of integration among traits, but characteristic differences were observed in the patterns of change between low and high light. Both species had more correlations than expected within groups of growth-related or photosynthetic traits; strong correlations of traits between these two groups were underrepresented. The similar pattern of genetic variation and phenotypic integration observed in these two congeners may be due more to their close phylogenetic relation than to their ecological distributions.

Key words: genetic variation; light acclimation; phenotypic plasticity; *Piper*; Piperaceae; reaction norm; trait correlation.

Responses of plant populations to environmental change clearly depend upon the interaction between individual phenotypic plasticity and genetic variation. Ultimately, the significance of phenotypic plasticity relative to genetic variation in a given trait may be dictated by the scale of heterogeneity in the habitat, by life-history characteristics of the species, or by the nature of the trait under consideration. Although considerable research has focused on phenotypic plasticity of light response in tropical forest species (see reviews by Chazdon et al., 1996; Strauss-Debenedetti and Bazzaz, 1996), few studies consider patterns of genetic variation in these responses (Hogan et al., 1994; Hogan, 1996). Moreover, interspecific studies of phenotypic plasticity seldom consider the genetic composition of their study sample (Chazdon and Field, 1987; Walters and Field, 1987). Only recently have studies of genetic variation in natural populations considered the plasticity, or reaction norms, of the genotypes in question (e.g., Zangerl and Bazzaz, 1983; Sultan and Bazzaz, 1993a, b; Pigliucci and Schlichting, 1995, 1996).

Studies of plant ecotypic differentiation have widely demonstrated genetic variation in allocational and growth related traits (Galen et al., 1991; McGraw and Antonovics, 1983; Bennington and McGraw, 1995; Sultan 1995). Physiological traits, such as maximum photosynthetic rate or water use efficiency, are generally presumed to be under stronger stabilizing selection pressures than allocational or growth traits. As such, physiological traits—although phenotypically plastic—may show less genetic variation than characters that receive less concentrated selection pressure (e.g., Scheiner, Gurevitch, and Teeri, 1984; Levin, 1988, Sultan and Bazzaz, 1993a).

If plasticity is viewed as a characteristic of a single trait, as opposed to a whole-organism level phenomenon (Sultan, 1987), selection may act directly upon that plasticity (Schmalhausen, 1949; Bradshaw, 1972; Schlichting, 1986; Via et al., 1995). Plasticity thus has a direct genetic component (estimated by the genotype by environment interaction). There is not, however, any consistent correlation between amounts of genetic variation and phenotypic plasticity (Schlichting, 1986; Sultan, 1987; and references therein). In habitats where spatial and temporal variation occur on a fine scale relative to an organism's life span, selection may favor evolution of plastic phenotypes, rather than genetic differentiation (Bradshaw, 1965; Sultan, 1987; Levin, 1988). Thus, pioneer species, occurring in relatively heterogeneous environments, are generally found to be more plastic in light response characters than shade-tolerant species (Bazzaz, 1979; Bazzaz and Carlson, 1982; but see Strauss-Debenedetti and Bazzaz, 1996). Plasticity in a given trait may decrease the likelihood of selection for genetic differentiation (e.g., ecotype formation) or, conversely, if genotypes converge in plastic responses, adaptive plasticity may actually support maintenance of genetic diversity (Sultan, 1987).

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[2] Author for correspondence.

Finally, patterns of plasticity and genetic variation may be influenced by the level of integration between the trait under consideration and other traits (Schlichting, 1986, 1989a). If two traits are correlated, selection favoring one will also affect the other. If positively correlated, the traits will respond in the same direction; if negatively correlated, responses to selection pressures will be opposed, indicating the potential for a constraint on selection response. If the correlation structure of traits is environment dependent, then the outcome of selection will reflect the conditions under which the organism developed (Schlichting, 1989a, b). Levels of integration between traits will most likely depend upon the sort of trait under consideration. Berg (1960) originated the concept of correlation pleiades, in which functionally related traits show the strongest patterns of correlation. Thus, for example, traits related to growth response may be expected to be tightly correlated with one another, but not with traits related to photosynthetic response.

We examined patterns of genotypic variation and phenotypic plasticity among light response traits in two tropical species of the genus *Piper* (Piperaceae). We examined norms of reaction (phenotypic response patterns) for randomly selected genotypes in light environments representative of the species' natural habitats. *Piper arieianum* C. DC and *P. sancti-felicis* Trel. grow in different light environments within the rain forest, and have different plastic responses to light (Chazdon, 1992). Forest understory species, such as *P. arieianum*, are generally less plastic in photosynthetic light response than shade-intolerant species (Bazzaz, 1979; Björkman, 1981; Bazzaz and Carlson, 1982). Such shade-adapted species typically have lower photosynthetic rates, lower dark respiration rates, and lower light compensation points than shade-intolerant species, even when they are grown at high light (Björkman, 1981; Bazzaz and Carlson, 1982; Strauss-Debenedetti and Bazzaz, 1991). In contrast, species that grow in high-light conditions, such as the pioneer shrub *Piper sancti-felicis*, often exhibit a high degree of plasticity in photosynthetic rates (Chazdon et al., 1996; Strauss-Debenedetti and Bazzaz, 1996).

We expected that *P. sancti-felicis*, the pioneer species, would exhibit a greater degree of plasticity in response to light variation than the shade-tolerant species, *P. arieianum*, as has been shown in previous work (Chazdon, 1992; Chazdon and Kaufman, 1993). The specific objectives of this study were: (1) determine whether both species showed more genetic variation in biomass and growth-related characters than in leaf-level photosynthetic traits; (2) examine whether patterns of genetic variation could be related to patterns of phenotypic integration among traits; and (3) determine whether the species differed in levels of genetic variation and phenotypic integration as a function of the environments they inhabit.

MATERIALS AND METHODS

Piper arieianum and *P. sancti-felicis* are common shrubs in Costa Rican lowland rain forest. *Piper arieianum* inhabits the understory of primary and secondary forest and small gaps, whereas *P. sancti-felicis* is most often found in clearings, large gaps, and shrubby secondary growth. The species differ in the plasticity of light response characters. Photosynthesis of *P. sancti-felicis* closely tracks spatial variation in light availability from 1 to 50% of full sun, whereas *P. arieianum* shows a plastic response to light availability only between 1 and 20% full sun (Chazdon, 1992; Chazdon and Kaufman, 1993).

Plant material for cuttings was collected from five genotypes per species at La Selva Biological Station in the Atlantic lowlands of Costa Rica. The genotypes constituted a random sample of individuals and habitats for each species, and were at least 500 m from one another. Each cutting consisted of a single unbranched stem with two nodes. The lower node was leafless, and the upper node had one leaf pruned to ~20 cm^2 of leaf area. Ten cuttings/genotype were established on a mist bench in November 1990. In March 1991 they were potted into 3.8-L plastic pots containing a 1:1 mix of sand and sifted alluvial soil. Pots were fertilized, and placed in one of two shadehouses under neutral-density shadecloth. Within each genotype, cuttings were ranked by initial biomass at time of potting, and were then assigned to shade treatments in alternating order. Shade treatments were chosen to represent understory (2% full sun) and gap (33% full sun) conditions. Preliminary ANOVA analyses demonstrated that there were no species or light treatment differences in initial cutting size.

In October 1991, after 6 mo of growth, sufficient cuttings had survived to yield six individuals per light treatment in each of four genotypes of *P. arieianum* and three genotypes of *P. sancti-felicis*. Maximum photosynthetic rate was measured in the laboratory on a recently expanded leaf of each plant using a LI-COR 6200 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska). Saturating light levels were obtained using a 150-W halogen bulb; light levels were adjusted using screen filters. To minimize variation in CO_2 concentration within and among days, an external air source was created by pumping ambient air into a waterbed mattress early each morning. During measurements, CO_2 concentrations were maintained between 340 and 370 μL/L, relative humidity ranged from 60 to 85%, and leaf temperatures did not exceed 34° C. Specific leaf mass and chlorophyll *a* and *b* concentrations were measured for each leaf following photosynthesis measurements. Chlorophyll was extracted following the method of Moran and Porath (1980). Chlorophyll *a* and *b* concentrations were then determined according to the equations of Inskeep and Bloom (1985). Plants were harvested and separated into leaves, stems, and roots. Leaf area was measured with a LI-COR leaf area meter. Plant parts were dried at 68°C for 72 h, and weighed to the nearest 0.001 g.

Data analyses—Seven biomass- or growth-related dependent variables were considered: leaf mass ratio (LMR, leaf biomass/total biomass), stem mass ratio (SMR, stem biomass/total biomass), root mass ratio (RMR, root biomass/total biomass), total biomass, root to shoot ratio (RSR), specific leaf mass (SLM, grams of leaf per square centimetre of leaf), and leaf area ratio (LAR, leaf area/total biomass). Six photosynthetic leaf-level dependent variables were also considered. These were: maximum photosynthetic capacity on both area and mass bases [P_{max}(area), in μmoles per square metre per second and P_{max}(mass), in μmoles per gram per second], stomatal conductance at maximum photosynthetic capacity (Cond, in μmoles per square metre per second), chlorophyll *a*:*b* ratio, and chlorophyll content on both area and mass bases [Chl(area), in μmoles per square metre, and Chl(mass), in μmoles per gram]. The variables SLM, P_{max}(area), Cond, chlorophyll *a*:*b* ratio, root:shoot ratio, and total biomass were log transformed prior to analysis to meet the assumptions of normality. There were three trait pairs with strong correlations in both species and in the two light treatments, and one trait of each pair was removed prior to analysis to ameliorate collinearity.

Data were analyzed using two different analysis-of-variance models. In each model, log (initial cutting biomass) was used as a covariate whenever the covariate explained a significant amount of variation. When the covariate was not significant, ANOVA was used instead of ANCOVA. The first model included data from both species, and examined the main effects of light, species, and genotype, as well as the light-by-species and light-by-genotype interactions. Using a second model, individual analyses were conducted for each species to investi-

TABLE 1. Results of ''random effects'' ANOVA/ANCOVA for *P. arieianum* and *P. sancti-felicis*. The model incorporates light, species, and genotype effects, where genotype is a random factor nested within species. Species effect was tested over genotype MS; light and light-by-species interactions were tested over light-by-genotype MS.

Variable	Log(initial biomass) (df = 1)		Light (df = 1)		Species (df = 1)		Genotype (df = 6)		Light-by-species (df = 1)		Light-by-genotype (df = 6)		Error	
	MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*	df	MS
Log(total biomass)	0.763	***	0.867	*	0.713	NS	0.315	***	0.205	NS	0.081	NS	67	0.035
Leaf area ratio	10 820	***	274 035	***	1995.9	NS	3063	**	30.25	NS	1496	NS	67	832
Stem mass ratio	0.011	**	0.029	*	0.031	NS	0.016	***	0.003	NS	0.004	NS	67	0.001
Leaf mass ratio	0.072	***	0.757	***	0.003	NS	0.035	***	0.001	NS	0.006	NS	67	0.004
Log(root : shoot)	0.118	**	2.058	**	0.051	NS	0.060	**	0.026	NS	0.043	*	67	0.015
Log(specific leaf mass)	—	—	0.827	***	0.013	NS	0.010	*	0.009	NS	0.018	**	68	0.004
Chlorophyll content by area	—	—	457 491	**	22 758	NS	23 804	**	41 812	NS	15 242	*	68	6002
Log(chlorophyll *a* : *b*)	—	—	0.003	NS	0.016	NS	0.004	***	0.001	NS	0.001	**	68	0.000
Log(photosynthetic rate, by area)	—	—	0.202	*	0.215	*	0.021	NS	0.000	NS	0.023	NS	68	0.023
Log(conductance)	—	—	0.034	NS	0.282	NS	0.092	**	0.009	NS	0.025	NS	68	0.027

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

gate genotypic effects and light-by-genotype interactions (main effects of light and genotype). For all analyses, genotype was considered as a random factor nested within species, because genotypes were selected as random representatives of the population. The inclusion of a random effect in the ANOVA leads to more conservative *F* tests for several main effects and interactions. *F* tests were carried out as follows: species was tested over genotype, and light and light-by-species interaction effects were both tested over the light-by-genotype interaction term.

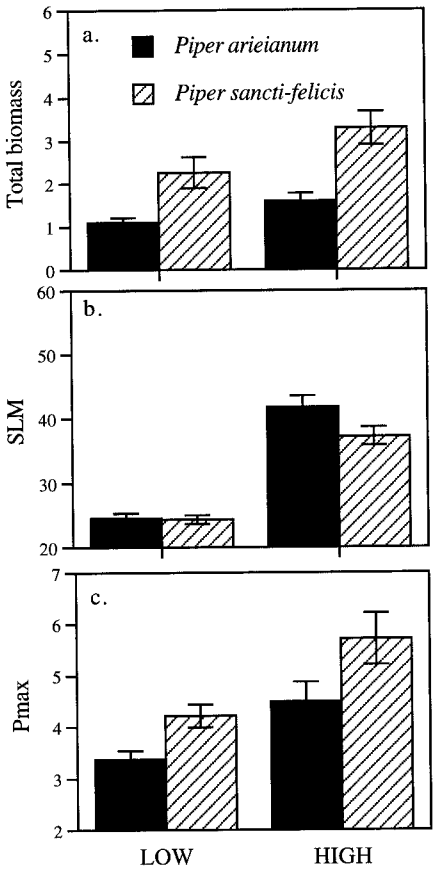

Fig. 1. Effect of light on growth-related and photosynthetic traits in *P. arieianum* (solid bars) and *P. sancti-felicis* (hatched bars). Bars are mean values for all individuals within each species and light treatment, ± 1 standard error: (a) total biomass (g); (b) specific leaf mass (SLM, (g/cm^2); (c) maximum photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$).

To examine species and treatment effects from a multivariate perspective, a principal components analysis (PCA) was conducted on genotype means for each species in each light environment ($n = 14$; 2 light levels × 7 genotypes). The data were not coded by either species or treatment so that the analysis would be blind to these factors.

Pearson correlations between sets of traits for each species within each light treatment were examined to investigate the integration of traits. Single correlations reached significance (uncorrected) for values of the correlation coefficient (r) ranging from ≈ 0.41 to 0.47 for a P level of 0.05 (depending upon sample size). For graphical purposes we used minimum r of 0.50, which is conservative relative to the significance levels for single correlations. For making contrasts between environments, a difference between correlation coefficients of 0.50 was considered significant. Comparisons between observed and expected numbers of significant trait correlations among growth and photosynthetic traits were made using *G* tests. Expected values were calculated by multiplying the proportion of significant correlations ($r \geq 0.50$) for a given species/treatment combination by the total number of pairwise correlations possible within that category (e.g., 21 among growth traits). Because some expected values were <5, we combined the two within-group sets of correlations in contrast to those between groups. The results were nearly identical, so we present the more detailed analysis.

RESULTS

Species, light, and genotype effects—The first ANOVA model revealed a significant effect of light on most of the traits examined (Table 1, Fig. 1). The exceptions to this pattern were chlorophyll *a*:*b* ratio, which showed significance only in the light-by-genotype interaction term, and conductance, which showed no significant variation with light. Plants were larger, had greater root/shoot ratios, and lower LAR when grown under high light (Fig. 1a, RSR and LAR not shown). High-light leaves of both species were thicker (greater specific leaf mass), and had lower chlorophyll content [Fig. 1b, Chl(area) not shown]. Notably, although maximum photosynthetic rate was significantly greater at high light (Fig. 1c), stomatal conductance showed no significant variation with light level (not shown).

In the first ANOVA model, the genotype effect is significant for all the growth-related characters, and for all leaf-level traits except maximum photosynthetic rate. The

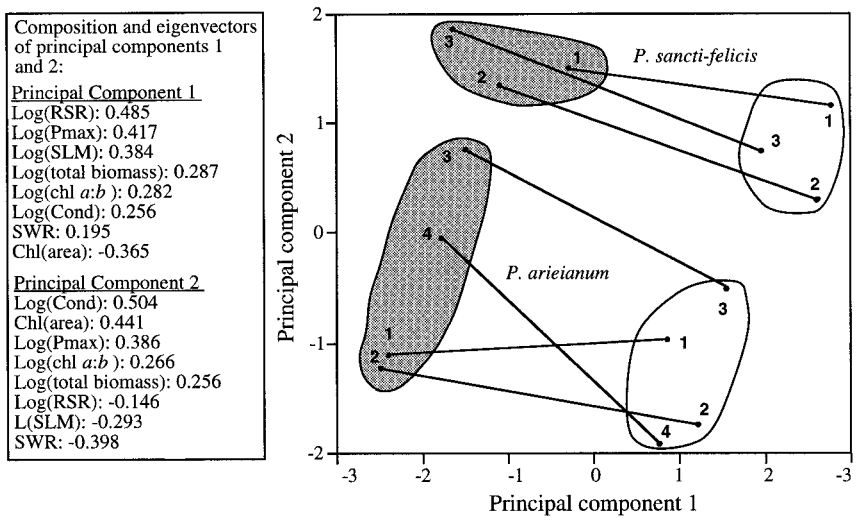

Fig. 2. Principal components diagram. Each point represents a single genotype in a single light environment. Composition of each component is given in legend. To simplify interpretation, clusters of genotypes within species and treatments have been circled. Shaded circles contain points representing genotypes at low light. Variables are: L(SLM)= log(specific leaf mass); L(Cond)= log(conductance); L[P_{max}(area)]=log(maximum photosynthetic rate on an area basis); L(Chl *a*:*b*)=log(chlorophyll *a*:*b* ratio); Chl(area)=chlorophyll/unit area; L(RSR)=log(root:shoot ratio); L[Total(g)]=log(total biomass); and SMR=stem mass ratio.

model yielded a significant effect of species only for maximum photosynthetic rate—the one trait not significantly affected by genotype (Fig. 1c). There were no significant light-by-species interactions (Table 1).

In contrast, the PCA clearly separated both species and treatment in PCA space (Fig. 2). The first two principal components of the PCA explained 63% of the variation in the data. The first component separated the light treatments in PCA space, and was strongly weighted by biomass-related traits and P_{max}. The second component was composed of photosynthetic traits, with biomass traits receiving negative weightings. This component distinguished the species in PCA space.

Genotypic effects within species—As would be expected based upon the previous analysis, light significantly affected all of the biomass-related traits in *P. arieianum* (Table 2A). There were fewer significant effects of light on photosynthetic characters; only chlorophyll content showed a significant main effect of light, while the chlorophyll *a*:*b* ratio showed a significant light-by-genotype interaction.

TABLE 2. Results of ''random effects'' ANOVA/ANCOVA for (A) *Piper arieianum,* and (B) *Piper sancti-felicis.* Model includes light and genotype effects and interactions between these factors. Light effect was tested over light-by-genotype MS.

	Log(initial biomass)		Light		Genotype		Light-by-genotype		Error	
Variable	MS	*P*	MS	*P*	MS	*P*	MS	*P*	df	MS
A) *Piper arieianum*	(df = 1)		(df = 1)		(df = 3)		(df = 3)			
Log(total biomass)	0.250	**	0.151	*	0.226	***	0.015	NS	39	0.025
Log(root : shoot)	0.036	**	0.928	**	0.017	NS	0.023	NS	39	0.014
Log(leaf area)	—	—	1.717	NS	0.345	**	0.317	**	40	0.060
Log(specific leaf mass)	—	—	0.615	*	0.008	NS	0.028	**	40	0.004
Chlorophyll content by area	—	—	136 089	*	34 121	**	11 396	NS	40	6731
Log(chlorophyll *a* : *b*)	—	—	0.004	NS	0.003	***	0.002	**	40	0.000
Log(photosynthetic rate, by mass)	—	—	9314	NS	2195.220	NS	2109.030	NS	40	1743
Log(photosynthetic rate, by area)	—	—	0.113	NS	0.033	NS	0.035	NS	40	0.021
Log(conductance)	—	—	0.005	NS	0.111	*	0.041	NS	40	0.029
B) *Piper sancti-felicis*	(df = 1)		(df = 1)		(df = 2)		(df = 2)			
Log(total biomass)	0.547	**	0.845	NS	0.454	***	0.188	*	27	0.049
Log(root : shoot)	0.554	***	2.205	**	0.071	NS	0.011	NS	27	0.035
Log(leaf area)	—	—	0.130	NS	0.774	***	0.266	*	28	0.061
Log(specific leaf mass)	—	—	0.281	**	0.012	*	0.003	NS	28	0.003
Chlorophyll content by area	—	—	328 267	NS	8328	NS	21 011	*	28	4960
Log(chlorophyll *a* : *b*)	—	—	0.000	NS	0.005	***	0.001	NS	28	0.000
Log(photosynthetic rate, by mass)	—	—	2825	NS	2110	NS	427	NS	28	3299
Log(photosynthetic rate, by area)	—	—	0.093	*	0.004	NS	0.004	NS	28	0.025
Log(conductance)	—	—	0.032	*	0.060	NS	0.001	NS	28	0.024

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

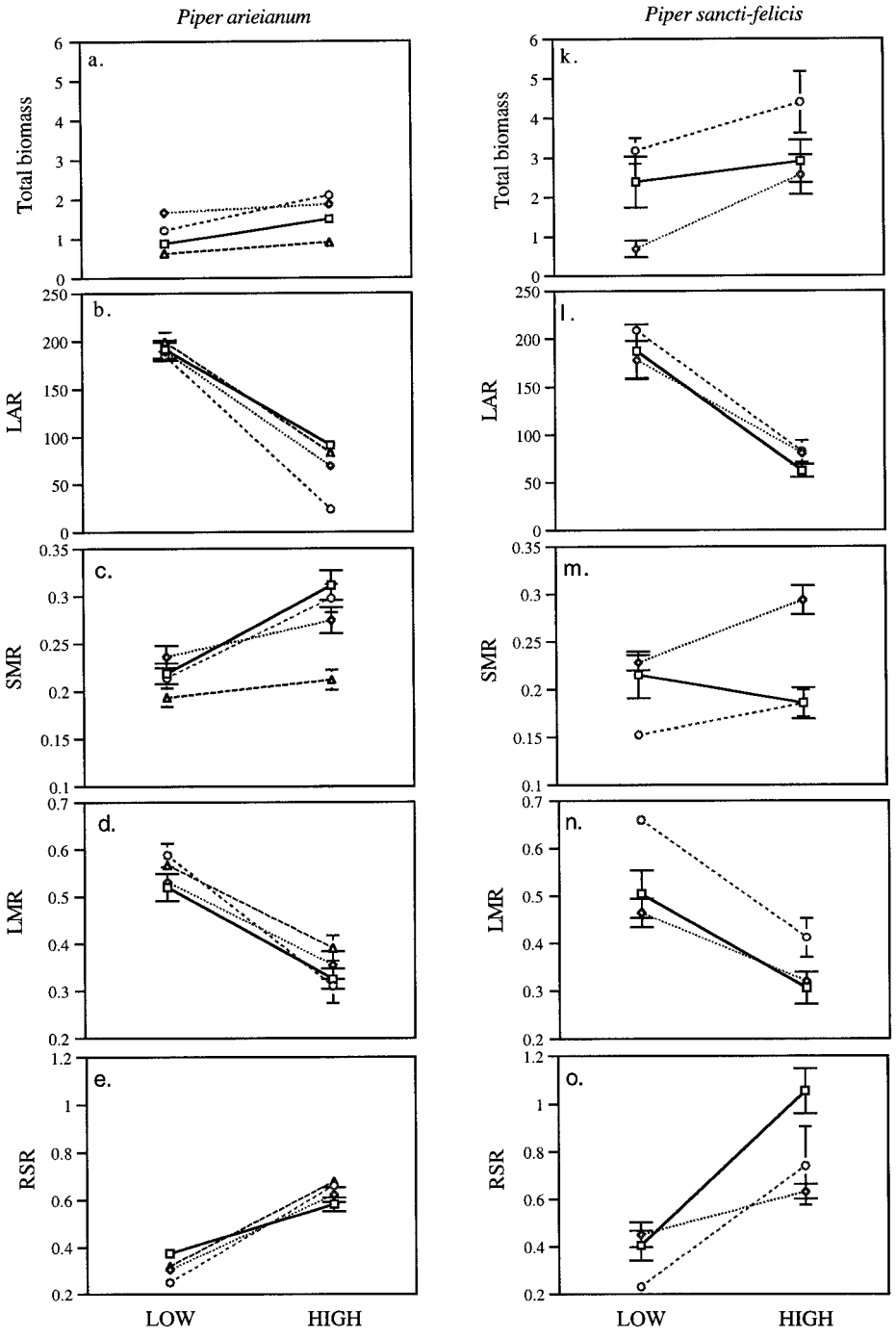

Fig. 3. Genotypic reaction norms of light response for *P. arieianum* and *P. sancti-felicis.* Lines are the mean ± 1 SD for each genotype plotted against light environment. The plots allow the response of each trait to light availability to be examined on an individual genotype basis. Growth-related traits: (a, k) total biomass (g); (b, l) leaf area ratio (LAR); (c, m) stem mass ratio (SMR); (d, n) leaf mass ratio (LMR); (e, o) root/shoot ratio (RSR); (f, p) specific leaf mass (SLM, g/cm^2). Photosynthetic traits: (g, q) chlorophyll content per unit area (μmol/m^2); (h, r) chlorophyll *a*:*b* ratio; (i, s) maximum photosynthetic rate (μmol·m^{-2}·s^{-1}); (j, t) stomatal conductance (mol·m^{-2}·s^{-1}).

There were significant genotype effects for three of the six biomass-related traits in *P. arieianum* (Table 2A). Among the photosynthetic traits, genotype significantly affected chlorophyll traits and stomatal conductance, but not maximum photosynthetic rate (Table 2A).

Reaction norms for these traits illustrate the significant light and genotype effects (Fig. 3). The light responses of the growth traits are particularly clear in LAR, LMR, RSR, and SLM, but less so for total biomass and SMR (Fig. 3a–f). The crossing pattern in the reaction norms for total biomass, LAR, and SLM reflect significant light-by-genotype interactions in these traits (Fig. 3a, b, f; Table 2A). Among the photosynthetic traits, only the effect of light on chlorophyll content by area is clear (Fig. 3g–

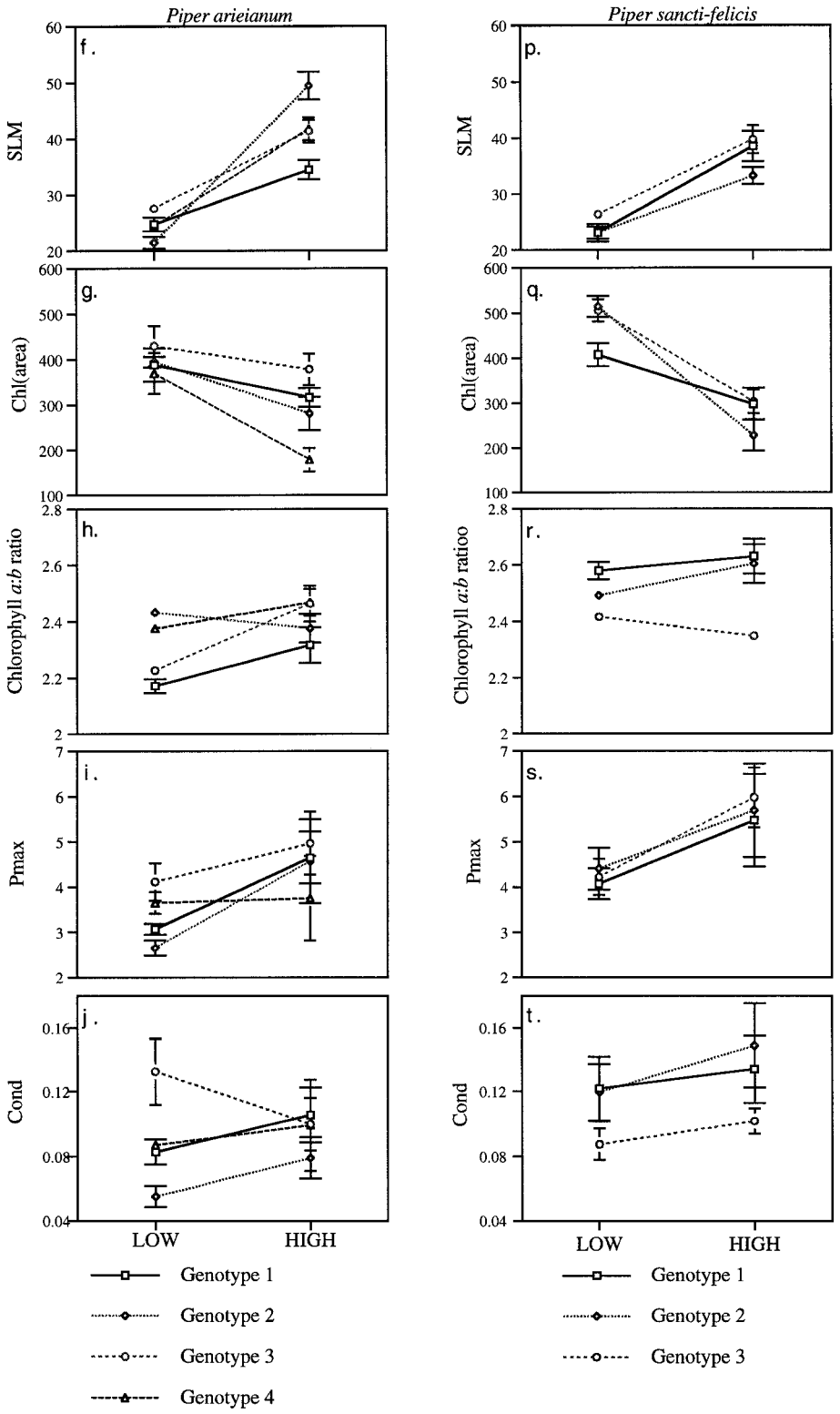

Fig. 3. Continued.

j). For the other photosynthetic traits, weak light response and the consistently overlapped error bars explain the lack of significant genotype and light effects.

In *P. sancti-felicis*, light significantly affected all growth-related characters, although these were significant only in the interaction term for total biomass, SMR, and RSR (Table 2B). The effect of light on chlorophyll content was significant in the light-by-genotype interaction, but there was no light effect on chlorophyll *a*:*b* ratio. Light treatment significantly influenced both max-

TABLE 3. Pearson correlation matrices for *P. arieianum* and *P. sancti-felicis* in each light environment. Correlations significant at $P < 0.05$ are in boldface. Underlined values represent cases where the correlation coefficient differs by >0.50 across treatments, while double underline indicates a shift in sign as well.

A) *P. arieianum*	Chl(area)	Chl(mass)	LChl *a*:*b*	Pmax	LPmax	LCond	RMR	SMR	LMR	LAR	LTOT	LSLM
High light												
Chl(mass)	**0.87**											
LChl *a*:*b*	−0.15	−0.26										
Pmax	**0.65**	**0.67**	−0.08									
LPmax	**0.60**	0.38	0.05	**0.85**								
LCond	0.41	0.36	0.05	**0.86**	**0.86**							
RMR	−0.25	−0.04	−0.08	0.10	−0.03	0.16						
SMR	**0.66**	**0.62**	−0.29	0.34	0.31	0.18	−0.26					
LMR	−0.25	−0.41	0.28	−0.34	−0.20	−0.28	−**0.70**	−**0.50**				
LAR	−0.20	−0.01	−0.15	−0.08	−0.25	−0.15	−0.35	−0.11	0.39			
LTOT	0.20	0.03	0.02	−0.08	0.02	−0.19	0.04	0.38	−0.30	−0.36		
LSLM	−0.26	−**0.67**	0.21	−0.37	0.09	−0.13	0.11	−0.26	0.29	−0.34	0.21	
LRSR	−0.26	−0.05	−0.07	0.10	−0.03	0.16	**0.99**	−0.26	−**0.71**	−0.35	0.03	−0.11
Low light												
Chl(mass)	**0.82**											
LChl *a*:*b*	−0.12	0.07										
Pmax	0.08	0.21	0.02									
LPmax	0.09	−0.09	−0.17	**0.83**								
LCond	0.07	−0.31	−0.37	0.17	**0.56**							
RMR	0.01	0.09	−0.14	−0.41	−0.48	−0.19						
SMR	0.08	0.06	0.09	−0.24	−0.24	−0.04	−0.24					
LMR	−0.07	−0.13	0.07	**0.54**	**0.60**	0.21	−**0.78**	−0.42				
LAR	−0.16	−0.01	0.09	0.38	0.27	−0.09	−**0.55**	−0.31	**0.71**			
LTOT	0.11	0.05	0.27	−0.26	−0.22	−0.13	−0.41	0.45	0.09	−0.05		
LSLM	0.02	−**0.54**	−0.37	−0.26	0.31	**0.65**	−0.18	0.01	0.16	−0.19	0.11	
LRSR	0.00	0.09	−0.08	−0.45	−**0.53**	−0.21	**0.99**	−0.21	−**0.79**	−**0.54**	−0.40	−0.20

Chl(area) = Chlorophyll per unit leaf area; Chl(mass) = Chlorophyll per unit leaf mass; Chl *a*:*b* = Chlorophyll *a*:*b* ratio; P_{max} = photosynthetic capacity per unit leaf mass; LP_{max} = Log(P_{max}); LCond = Log(Stomatal Conductance); RMR = root mass ratio; SMR = shoot mass ratio; LAR = leaf area ratio; LTOT = log(total biomass); LSLM = log(specific leaf mass); LRS = log(root:shoot ratio).

imum photosynthetic rate and stomatal conductance (Table 2B).

The genotype effect on growth-related traits in *P. sancti-felicis* was significant for all traits except LAR (Table 2B). In contrast, for photosynthetic traits, there was a significant effect of genotype only on the chlorophyll *a*:*b* ratio, and a significant light-by-genotype interaction only for chlorophyll content (Table 2B).

There were significant light-by-genotype interactions for total biomass, RSR, and SMR in *P. sancti-felicis* (Fig. 3k, m, o). The significant interaction term for SMR could result from the apparent lack of response in two of the three genotypes (Fig. 3m). The genotypic differences are clear for all the biomass-related traits except LAR (Fig. 3k–p). Reaction norms also reveal the source of the significant effects of light on chlorophyll content, maximum photosynthetic capacity, and conductance (Fig. 3q–t). For chlorophyll content and chlorophyll *a*:*b* ratio, crossing and diverging reaction norms explain the significant genotype effect (Fig. 3q, r). There was a clear increase in maximum photosynthetic rate at high light, and no significant genotype effect in this trait (Fig. 3s). Stomatal conductance shows a similar pattern (Fig. 3t).

Trait correlation structure—Although one trait from each of three trait pairs was excluded from the ANOVAs because of strong correlations, we chose not to exclude any traits from the analysis of phenotypic integration because these traits exhibited either (a) different patterns of correlation with other traits, or (b) different patterns of change across treatments (Table 3). Although the correlation structures of these two species are broadly similar, the fine-scale relationships among traits, and the ways that those relationships respond to environmental change, are distinctive. Examination of the correlation structure for each species indicated an overall level of integration for the traits considered of ~15–20%. Of a possible 78 correlations, 14 reached values of *r* of 0.50 in both low and high light in *P. arieianum* (Fig. 4a, b), and 17 in each treatment in *P. sancti-felicis* (Fig. 4c, d). Examination of the distribution of these correlations reveals that they are both species and environment specific. Only nine ''significant'' correlations are shared between species in the high-light treatment and six in the low light. Only seven correlations are shared between the two light treatments for *P. arieianum*, and only nine for *P. sancti-felicis*.

TABLE 3. Extended.

B) *P. sancti-felicis*	Chl(area)	Chl(mass)	LChl *a*:*b*	Pmax	LPmax	LCond	RMR	SMR	LMR	LAR	LTOT	LSLM
High light												
Chl(mass)	**0.86**											
LChl *a*:*b*	−0.03	0.10										
Pmax	**0.57**	**0.73**	0.35									
LPmax	**0.64**	**0.60**	0.29	**0.90**								
LCond	0.28	0.43	**0.54**	**0.82**	**0.72**							
RMR	0.22	0.02	0.19	−0.04	0.09	0.12						
SMR	−0.49	−0.18	0.43	0.22	0.02	0.33	−0.40					
LMR	0.13	0.11	**−0.51**	−0.12	−0.11	−0.37	**−0.74**	−0.31				
LAR	−0.02	0.19	−0.38	0.00	−0.18	−0.26	**−0.84**	0.03	**0.85**			
LTOT	0.05	−0.20	−0.26	−0.13	0.12	−0.05	0.27	−0.35	−0.03	−0.34		
LSLM	0.04	−0.47	−0.20	−0.44	−0.06	−0.34	0.29	−0.45	0.03	−0.40	0.34	
LRSR	0.18	−0.13	−0.11	−0.11	0.16	0.02	**0.70**	**−0.51**	−0.36	**−0.66**	**0.87**	0.44
Low light												
Chl(mass)	**0.65**											
LChl *a*:*b*	−0.30	−0.04										
Pmax	−0.01	0.32	0.41									
LPmax	0.31	0.23	0.24	**0.85**								
LCond	0.08	0.11	0.47	**0.60**	**0.67**							
RMR	−0.30	−0.02	**0.56**	0.17	−0.01	0.19						
SMR	−0.37	−0.13	0.47	0.35	0.18	0.43	**0.70**					
LMR	0.35	0.07	**−0.57**	−0.26	−0.07	−0.31	**−0.95**	**−0.89**				
LAR	0.05	0.08	−0.43	0.02	0.02	−0.04	**−0.72**	−0.32	**0.61**			
LTOT	−0.07	**−0.68**	−0.20	−0.31	0.00	−0.21	−0.41	−0.24	0.37	0.01		
LSLM	**0.55**	−0.26	−0.35	−0.41	0.10	−0.04	−0.37	−0.34	0.39	−0.02	**0.65**	
LRSR	−0.17	**−0.72**	0.04	−0.28	−0.00	−0.16	0.06	0.05	−0.06	−0.40	**0.88**	**0.56**

The two species exhibit nearly identical patterns of heterogeneity of the distribution of correlation coefficients ≥ 0.50 (Table 4), although only the pattern for *P. sancti-felicis* is significant. There are more significant correlations among photosynthetic traits in the high-light environment than would be expected given overall integration levels (Fig. 4 a, c). Growth traits also tended towards stronger integration than expected (more so in the low-light environment). Correlations between growth and photosynthetic traits were substantially underrepresented, although their frequency increases in the low-light environment.

These correlation data also allow us to address the issue of changes in correlation structure. We identified those pairs of traits for which the correlation coefficients differed by more than 0.50 between the two light environments (Table 3), and those that changed sign from positive to negative across treatments. For *P. arieianum*, 15 correlations had shifts of this magnitude. Eight of those involved a change of sign; of these seven were for correlations between the two character groups. The majority of these shifts (ten for magnitude and six for sign) were for traits correlated with aspects of maximum photosynthetic rate. For *P. sancti-felicis*, ten correlations had shifts of 0.50 or more and four of those changed sign; these shifts were mostly for relationships among growth traits. Correlations with root mass ratio accounted for five of the magnitude shifts and all four of the sign changes; the relationship between root mass ratio and shoot mass ratio changed from high positive under low light (0.70) to quite negative (−0.40) under high light.

DISCUSSION

Species differences and light effects—The PCA clearly illustrated both species and light effects, with the treatments dividing neatly along the axis of the first principal component, and the species separating along the second principal component axis. This level of species differentiation was not equally apparent in the univariate analyses. In fact, the results generated by the first analysis-of-variance model, that which considered light, species, and genotype effects, are quantitatively, but not qualitatively different, from those generated by previous studies on these species (Chazdon, 1992). Whereas the studies agree with regard to light effects, the present study showed very few species effects. Because this was a small-scale study consisting of two species and a total of only seven genotypes, it is impossible to determine whether these conflicting results actually demonstrate a lack of species effect, or whether model 1 simply does not have the power to discern species differences. The use of a nested model here means that the effect of species is tested over the random effect of genotype. The

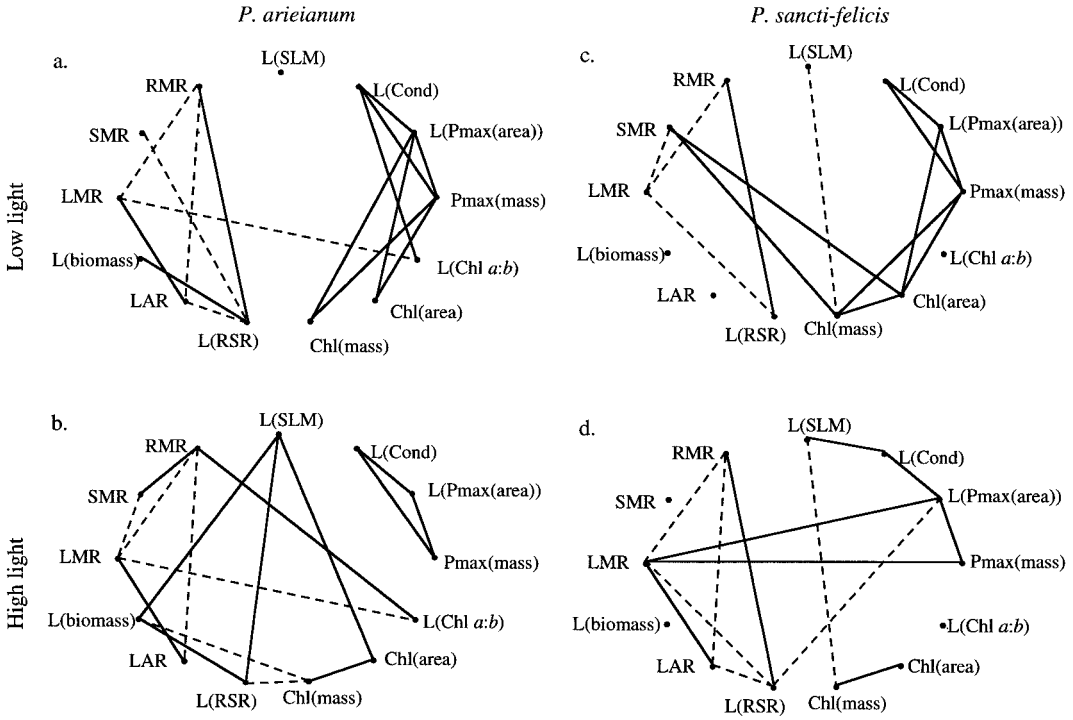

Fig. 4. Phenotypic correlations between traits for *P. arieianum* and *P. sancti-felicis*: (a, b) *P. arieianum* high- and low-light treatments, respectively; (c, d) *P. sancti-felicis* high- and low-light treatments, respectively. All correlations >0.5 are shown (see text for explanation). Solid lines depict positive correlation; dashed lines are negative correlations. Six of the 13 variables were log transformed to meet assumptions of normality. For definition of variables, see Fig. 3.

associated decrease in power may cause the model to yield very few significant species effects or species-by-light interactions. However, the significant effects of genotype demonstrated in models 1 and 2, and depicted in Fig. 2 invalidate an analysis that disregards genotype.

Given that the results qualitatively concur with those of previous studies on these and closely related species (including some of the same genotypes used by Chazdon, 1992), it is likely that the lack of significant species effects is due to the power of the model and the sample size of the study. The patterns of plasticity in total biomass, SLM, and P_{max} depicted in Fig. 1, replicate those of Chazdon (1992) closely.

We predicted that genotypes of *P. sancti-felicis* would show greater plasticity of light response than those of *P. arieianum.* With respect to photosynthetic traits, this was partially confirmed. There was a significant light effect on the chlorophyll *a*:*b* ratio for *P. arieianum,* but not for *P. sancti-felicis.* However, in *P. sancti-felicis* light treatment significantly affected both photosynthetic rate and conductance, but not in *P. arieianum.* Overall, the results of the single species analysis demonstrate that *P. sancti-felicis* had significant light or light-by-genotype effects for nine out of ten traits, compared to eight out of ten traits in *P. arieianum.*

By comparing the species' *F* ratios for each trait, one can compare the magnitude of the light responses in the species. *F* ratios were greater for *P. sancti-felicis* in only five of the ten traits. Likewise, the coefficients of variation (*cv*) for each trait can serve as an index of plasticity. *P. arieianum* had a greater *cv* for six of the ten traits. Thus, under these experimental regimes, the plasticity of light response was similar in the two species.

TABLE 4. Analysis of heterogeneity of correlations among and between growth and photosynthetic traits in the two light treatments. (A) *P. arieianum,* (B) *Piper sancti-felicis.* Tabled values are: observed/expected; df = 5.

Species/treatment	Among growth traits	Among photosynthetic traits	Between groups
A) *Piper arieianum*			
High light	4/3.77	7/2.69	3/7.54
Low light	6/3.77	3/2.69	5/7.54
$G = 10.48$, $P < 0.10$			
B) *Piper sancti-felicis*			
High light	7/4.58	9/3.27	1/9.16
Low light	8/4.58	4/3.27	5/9.16
$G = 24.22$, $P < 0.001$			

Genotypic effects within species—The two *Piper* species were remarkably similar with regard to patterns of genetic variation. Each species possessed significant genetic variation for six or seven of the ten traits measured. We also predicted that gas-exchange traits would show less genetic variation than growth-related traits, due to the effects of strong stabilizing selection. In accordance with this prediction, we found that neither species had a significant genotypic effect for maximum photosynthetic rate. *P. sancti-felicis* lacked a significant genotype effect for maximum stomatal conductance as well.

Few studies have examined genetic variation in physiological traits. Among those that have, some have found

little or no genetic variation in leaf-level maximum photosynthetic rates, even when there is genetic variation in whole-plant growth rates (Scheiner, Gurevitch, and Teeri, 1984; Garbutt, 1986; Sultan and Bazzaz, 1993a; Hogan et al., 1994). Likewise, Antlfinger (1981) found that the genetic component of variation was greater in morphological characters than physiological characters, although photosynthetic rate was not directly measured. Among inbred lines of the annual herb *Polygonum arenastrum*, however, genotypes differed significantly in maximum rates of photosynthesis, maximum stomatal conductance, and daily water-use efficiency (Geber and Dawson, 1990).

Our study was limited to only two species and seven genotypes, yet the resulting patterns suggest several areas for further inquiry. In a habitat that is heterogeneous on a temporal scale shorter than an organism's life span, genetic variation will be less favored than phenotypic plasticity, or acclimation potential (Bradshaw, 1965). If a genotype is superior at all levels of heterogeneity, it should be selectively favored. Alternatively, if reaction norms of the genotypes in a population cross, and the habitat is heterogeneous on a fine temporal scale, then different genotypes will be favored under different conditions, and genetic variation will be selectively maintained (Sultan, 1987; Sultan and Bazzaz, 1993b). The lack of a significant light-by-genotype interaction for those reaction norms that do not cross (e.g., Figs. 3i, l) may be due to lack of power in the ANOVA design. Although the genotype-by-light interaction was significant for fewer than half of the traits considered in this study, in general, the reaction norms of each species tended to cross and overlap (e.g., Fig. 3f, g), rather than be consistently parallel (e.g., Fig. 3r). This pattern of genotype response suggests the hypothesis that genetic variation, along with phenotypic plasticity, is being selectively maintained in these populations.

Trait correlation structure—Patterns of trait correlations can be examined to reveal overall levels of integration among traits (e.g., Schlichting, 1986, 1989a, b; Waitt and Levin, 1993; Cheplick, 1995). It has been suggested that an intermediate level of integration may be most beneficial to a species, as it would allow flexible, but still coordinated, responses to environmental change (Schlichting, 1989a). Some studies of closely related species have found large differences in integration, potentially related to habitat heterogeneity (Schlichting, 1989b). The ideal level of integration may also depend upon the environments in which a species occurs. For the two closely related species of *Piper* considered in this study, overall integration levels were similar, around 20%, despite their preferences for different habitats. The overall patterns of trait correlation differ though, as do the specific responses to high vs. low light. A substantial fraction of the correlations shifted in magnitude, and some in sign as well. These patterns of changing correlations have been observed in other studies as well (e.g., Andersson and Shaw, 1994; Bennington and McGraw, 1995; Pigliucci, Whitton, and Schlichting, 1995).

Also revealed by analysis of the trait correlation structure was a pattern of greater phenotypic integration among related traits (growth and photosynthesis) than between traits in these groups. This phenomenon was originally postulated by Berg (1960) who suggested that, in general, traits that are functionally related should have higher intercorrelations. Studies on a variety of organisms have detected similar patterns related both to functional and developmental relationships (e.g., Cheverud, 1982; Pigliucci et al., 1991; Waitt and Levin, 1993; Cane, 1993; Conner and Sterling, 1995). The PCA also generally supported this pattern. PC2 (which separated the species) was primarily based upon physiological traits, with biomass-related traits as a group receiving negative weightings. PC1 was based largely upon biomass-related traits, but P_{max} was also a large part of this component.

The results of the analysis-of-variance models demonstrate that if a single model is to incorporate genetic and species-level factors, with genotype as a random factor, then either many species relative to the number of genotypes within species, or many genotypes of few species, will be needed to obtain sufficient analytical power. If a species-level analysis is conducted in the absence of genotype data, then the role of genetic differences in the responses of the species is ignored. In a study with few genotypes, a single aberrant genotypic response may overpower an otherwise consistent treatment effect. For example, the divergent behavior of *P. arieianum* genotype 3 with regard to light response of stomatal conductance (Fig. 3j) may have led to the lack of light response in both the single-species model (Table 2A) and the joint model (Table 1).

Conclusions—Despite the small number of genotypes considered, the results of this study illustrate some clear patterns with regard to phenotypic plasticity and genetic variation. Although *P. arieianum* and *P. sancti-felicis* are found in different ecological positions and have been shown to differ in plasticity of light response (Chazdon, 1992), they have similar patterns of genetic variation and phenotypic integration. Correlations among traits (Fig. 4) are similar for the species, as are the patterns of genotypic effects demonstrated by reaction norms and ANOVA. This suggests that the pattern of genetic variation and phenotypic integration in these two congeners may be due more to their close phylogenetic relation than to their ecological distributions. In addition, the results demonstrate that the relationship between genetic variation and phenotypic plasticity is likely to be influenced as much by the type of trait under consideration (e.g., whole plant vs. leaf level), as by the ecological position of the species under consideration.

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