THE INFLUENCE OF CANOPY POSITION, POLLINATOR SYNDROME, AND REGION ON EVOLUTIONARY TRANSITIONS IN POLLINATOR GUILD SIZE[1]

R. D. Sargent[2] and J. C. Vamosi[3]

Department of Integrative Biology, University of California, Berkeley, California 94720-3140, U.S.A.; and
Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada

Little is known about how ecological context influences the probability of transitions in the extent of pollinator specialization. One unexplored hypothesis suggests that transitions to environments with different light conditions should be accompanied by transitions in pollinator guild because of the combined effects of exposure to a new pollinator community and the different relative costs associated with pollinator attraction in the understory and the canopy. Using data from literature surveys, we compiled a data set of habitat light availability (e.g., canopy vs. understory), pollinator identity, and guild size for 481 angiosperm species representing four broad regions (India, Neotropics, paleotropics, and Canada). Phylogenetic independent contrasts were used to assess the degree to which transitions in canopy position are associated with transitions in the usage of particular pollinators and pollinator guild size. We further examined the degree to which each of these traits tends to be evolutionarily labile versus evolutionarily conserved. Our analysis demonstrates that species that tend to occupy the same position in the canopy are more closely related than expected by chance, as are species that follow traditional pollinator syndromes (e.g., bee or bird), but species that have exceptionally wide pollinator guilds (e.g., are visited by bees, flies, and moths) are widely scattered across the angiosperm phylogeny. Transitions to generalist pollination appeared to be strongly associated with beetle and fly pollination and with position in the canopy above the forest floor.

Keywords: pollination, phylogenetic niche conservatism, phylogenetic independent contrasts, light conditions.

Introduction

Plant evolutionary biologists have a particular interest in the frequency (Wilson et al. 2006), directionality (Armbruster and Baldwin 1998; Castellanos et al. 2004; Wilson et al. 2006), and ecological context (Sargent and Otto 2006; Vamosi et al. 2006; van der Niet et al. 2006) of evolutionary transitions in plant-pollinator interactions. Such interactions are fundamental to understanding how selection operates on floral traits, mating systems, and inflorescence architecture. Furthermore, plant-pollinator interactions are hypothesized to play an integral role in angiosperm speciation (Grant 1994; Dodd et al. 1999; Fulton and Hodges 1999; Bradshaw and Schemske 2003; Johnson 2006). One unknown and virtually unexplored area is the degree to which the size of a plant's pollinator community (or guild) is determined by heritable traits that can evolve versus a highly labile state that depends mainly on the environment in which a plant lives (e.g., Waser et al. 1996; Fenster and Dudash 2001).

The argument that pollination is labile with respect to environmental influences suggests that the environment itself is labile with respect to phylogeny (i.e., sister species or populations are likely to experience differing environments and thus different pollinator communities). Whether this is true has not been adequately explored.

Certainly, habitat specialization is widely held to have influenced the diversification of angiosperm species and traits (e.g., Fine et al. 2004; Rajakaruna 2004). Shifts in habitat specialization and pollinator guild size may be evolutionarily correlated because of selection pressure imposed by a new pollinator community upon transition to a novel habitat (Grant and Grant 1965; Johnson 1996; von Hagen and Kadereit 2003; van der Niet et al. 2006).

As part of their evolutionary history, or indeed, even within a few generations, plant populations are likely to face transitions to conditions with differences in light availability. Because of its effect on the rate of photosynthesis, light availability affects the amount of resources available to the plant, and, of particular importance to pollination biologists, can affect the amount of resources available for floral rewards. Not surprisingly, plants growing in low-light conditions exhibit a reduction in floral nectar production (Boose 1997), fruit set (Niesenbaum 1993), and pollination (Bertin and Sholes 1993; Larson and Barrett 2000). However, it remains unclear whether transitions to low-light environments should be accompanied by transitions to plant specialization or generalization. One hypothesis suggests that plants that are adapted to understory habitats will experience strong selection to restrict their costly floral rewards to only a small number of faithful (i.e., specialized) pollinators (Feinsinger 1983). However, whether such faithful pollinators exist in low-light environments remains questionable. Low light availability affects the pollinator community and visitation rates (Herrera 1995) and is associated with high pollen limitation (Larson and Barrett 2000). Furthermore, forest understory

habitats may contain disproportionately more generalist (indiscriminate) than specialist pollinators (Bawa 1990). Thus, a second hypothesis purports that low-light environments may constrain the pollination of species to be rather generalized. Interestingly, both hypotheses suggest that transitions to a habitat with a different light availability will be accompanied by shifts in a plant's pollinator guild. Accordingly, community surveys of the pollination ecology of understory plants provide support for a correlation between habitat and plant-pollinator interactions (e.g., Schemske 1981; Sakai et al. 1999; Kay and Schemske 2003; Mayfield et al. 2006). However, no study has tested whether there is a correlation between transitions in habitat light availability and transitions in pollinator guild size.

The evolutionary history of a trait has important implications for the assessment of across-species ecological patterns. When traits are phylogenetically clustered or conserved, traditional statistical tests for associations between traits among taxa will fail the assumption of independence. Thus, when conducting tests of ecological patterns, it is vital to take phylogenetic relatedness into account (Felsenstein 1985). However, the importance of correcting for phylogeny depends on how likely it is that the traits influencing pollinator composition are species or clade specific. For example, if pollinator composition changes with light environment and light environment is so labile as to change from population to population or from sister species to sister species, then correcting for phylogeny loses importance. Finally, difficulties arise in assigning causal relationships to global patterns. For instance, the composition of pollinators within the guild may be correlated with guild size (e.g., a species that includes birds as pollinators may have smaller guild size than one that includes flies because of the nectar quantities required to attract each), yet ultimately, guild size and composition may depend on regional affinities of the pollinators themselves (e.g., bird pollination may be more common in Neotropical regions than in paleotropical regions; Momose et al. 1998). Identifying and dealing with such confounding influences remains a central goal of comparative biology.

In addition to the statistical considerations, information about phylogenetic distribution can give us clues about the evolutionary constraints inherent to a particular trait or set of traits. The tendency for closely related species to have similar sets of ecological characteristics is known as phylogenetic niche conservatism (Wiens and Graham 2005). When ecological traits such as pollinator guild size and habitat associations are phylogenetically conserved, species may be more limited in their ability to adapt to novel habitats or, in the case of pollination mode, novel biotic interactions. Reproductive traits such as sexual system and pollination syndrome have previously been demonstrated to exhibit some phylogenetic niche conservatism (Fox 1985; Chazdon et al. 2003; Wilson et al. 2006). Conversely, the extent to which pollinator-mediated speciation is responsible for the diversification of angiosperms relies on plant-pollinator interactions being relatively labile with respect to phylogeny (Goldblatt and Manning 2006; Thomson and Wilson 2008), yet we are unaware of any studies that have set out to specifically examine the degree of phylogenetic conservatism in the size of a species' pollination guild.

Using literature surveys, we assembled information about primary habitat and pollination guild size for 481 angiosperm species. Most available studies of pollinator guild size were on tropical plant species. We used phylogenetic independent contrasts to address (1) the degree to which plant habit, pollinator syndrome, and plant-pollinator interactions tend to be phylogenetically labile versus conserved and (2) the evidence for evolutionary associations among pollinator specialization, canopy position, pollinator syndrome, and region.

Methods

Data Collection

We used the Web of Science (http://apps.newisiknowledge .com)to search for articles containing the search terms "pollinat* and understory" or "pollinat* and canopy or gap." We found a total of 39 articles containing information pertinent to our study. Most of these studies were performed in the tropics and can be pooled into four main regions: paleotropical ($N = 285$ plant species), Neotropical ($N = 73$), tropical India ($N = 105$), and temperate (largely Canadian; $N = 15$). We were interested in species that clearly inhabited a particular light environment (e.g., understory, forest floor, gap, canopy, etc.). We accomplished this by assigning the following light regime values to the habitats listed in published studies. Forest floor (very low light) = 1; understory (low light) = 2; midrange canopy, such as species that are epiphytes, emergents in the subcanopy (moderate light) = 3; and canopy/gap (high light) = 4.

We then determined the extent of pollination specialization by surveying studies that monitored the identity of animal species that visited a particular plant species. Regrettably, much variation exists in the literature in the precision of pollinator identification. Some studies identified pollinators to the species level, whereas many others listed pollinators as, e.g., "Coleoptera" or, more vaguely, "diverse insects." In an attempt to merge this variation into a coherent meta-analysis, we considered each insect order as a separate group of pollinators. Our designation of pollinator guild size was designed to adhere as closely as possible, given the constraints of the data, to the "functional group" definition of pollinator specialization outlined by Fenster et al. (2004). For example, if a species is pollinated by only four species of flies, it was categorized as having a pollinator guild size of 1, whereas a species pollinated by bees and flies was categorized as having a guild size of 2. Plants that had been recorded as visited by diverse insects were given a guild size of 3 because that tended to be the highest number of insect orders pollinating a single plant species. In cases where pollinators were identified only as "insect species," we assumed that each species was a different order. Wind-pollinated plant species were excluded. Because of the difficulties associated with determining the degree to which an animal visiting a flower is actually involved in pollination (i.e., transfer of self or outcross pollen to the stigma of a conspecific flower), we considered a species to be a pollinator if it was reported to visit the flowers of a particular plant species. With this data set we performed ANOVA tests to determine (1) whether a cross-species (i.e., with no phylogenetic correction) relationship existed between light index and guild size and (2) whether particular pollinator types were associated with species that had many or few other pollinating types visit. Finally, we also performed Pearson contingency tests to determine (3) whether particular

orders of pollinators (which may have a higher likelihood of specializing) predominated in certain habitats and regions.

Phylogenetic Independent Contrasts

A backbone phylogeny of the species in the database was created using the program Phylomatic (Webb and Donoghue 2005). Species were placed on the backbone using the angiosperm maximally resolved seed plant tree (Stevens 2001). Generic relationships within families were further resolved where possible (a list of phylogeny sources is available from the authors). We used the function BLADJ, implemented in the program PHYLOCOM (Webb et al. 2006), to estimate branch lengths from fossil-calibrated node ages on the tree (Wikstrom et al. 2001). Data and phylogeny are available from the authors upon request.

To determine whether there is phylogenetic conservatism in our two traits, we examined whether related species were more similar in terms of light regime and pollinator breadth than expected by random chance. We used a commonly employed method for detecting phylogenetic signal that quantifies the similarity of close relatives but does not make any statement or assumption about the processes that lead to phylogenetic signal (Blomberg and Garland 2002). We used standardized phylogenetic independent contrasts (PICs), implemented using the Analysis of Traits (AOT) tool in the program PHYLOCOM (Webb et al. 2006), to test the hypothesis that transitions in the size of a plant species' pollination guild vary predictably with transitions in light regime. Although maximum likelihood and Bayesian methods are available to address correlated evolution and may be more powerful for small data sets, recent studies have found these methods to be very sensitive to taxon sampling (Nosil and Mooers 2005) and differential diversification (i.e., if the traits in question influence speciation or extinction; Vamosi et al. 2003; Maddison 2006). PICs, on the other hand, have been found to be relatively robust and conservative (Oakley and Cunningham 2000; Martins et al. 2002). Our estimate of phylogenetic signal calculates the average magnitude of contrasts across the tree and compares it to a null expectation generated using 10,000 randomizations of trait values across the tips of the tree. Tests were conducted coding the data as continuous variables (see light and guild index in "Data Collection"). Because the cross-species comparison revealed a trend for forest floor species to have specialized pollinators (only one visiting order), we include the results of an a posteriori Wilcoxon signed-rank test that examines whether transitions to the forest floor habitat (i.e., with binary coding of the habitat as forest floor or not) results in consistent decreases in the pollination guild index.

Results

Our literature survey included a total of 481 species in 275 genera and 91 families. To give an overview of the families and trait distribution in our study, we illustrate average trait values by family in figure 1. Ten families account for 238 (49.2%) of species in the data set: Zingiberaceae (40 species), Rubiaceae (37), Euphorbiaceae (33), Dipterocarpaceae (28), Annonaceae (24), Fabaceae (17), Lauraceae (17), Malvaceae

(17), Costaceae (13), and Verbenaceae (12). We obtained primary habitat and pollinating species (based on visitation) data for all species in the data set. The majority of the species are tropical in distribution.

Analyses of Uncorrected Data

Most species (81.2%) were pollinated by only one functional group, confirming the results of previous studies demonstrating that tropical plant species tend to exhibit a high degree of specialization on a particular species or related group of pollinators (reviewed in Ollerton et al. 2006). It is important to note that finding a high proportion of specialists reflects the method of categorizing specialization by functional group (here, approximated as taxonomic order) rather than by individual pollinating species (Fenster et al. 2004). Nearly half the species in the data set were recorded as being found in forest floor or understory habitats. The remaining species were found in some sort of open habitat (table 1). The number of pollinating orders tended to increase with the amount of light in the environment, with forest floor species displaying the highest incidence of specialization for pollination ($F_{3,477} = 3.53$, $P = 0.015$). A Tukey test identified the key significant difference to be between canopy/gap species and forest floor species. The probability of encountering certain pollinators in the different light environments could have profound effects on our perception of pollinator specialization. Table 2 displays the major pollinating taxa represented in our data set. Hymenoptera, Lepidoptera, Coleoptera, and Diptera represented 87% of the 613 recorded visitors to the 481 species. Note that more than one species within a given order may have visited but that this is recorded as only one visiting order in our data set. We also display the expected and observed frequencies of these pollinators in each light environment. Lepidoptera, for example, appear as more common visitors to the forest floor species (light environment 1 in table 2), while birds were observed more than expected in the understory (light environment 2). Canopy position preferences of the pollinators themselves may create bias for where pollinator specialization occurs (i.e., no pollination by Thysanoptera, Hemiptera, birds, or mammals occurred in forest floor species; forest floor species can have a maximum guild index of 4, while species in other habitats can have a guild index of up to 8). Finally, plant species that were observed to employ certain pollinators were also seen to have varying degrees of specialization. Plant species employing birds had fewer other pollinators ($F_{1,479} = 6.35$, $P = 0.01$), while the opposite was true for plant species visited by Hymenoptera ($F_{1,479} = 22.15$, $P < 0.001$), Lepidoptera ($F_{1,479} = 18.91$, $P < 0.001$), Diptera ($F_{1,479} = 296.16$, $P < 0.001$), Coleoptera ($F_{1,479} = 36.19$, $P < 0.001$), and Hemiptera ($F_{1,479} = 17.19$, $P < 0.001$). Plant species that included mammals and thrips in their pollinator guild were not significantly different in their specialization from those that did not. Contingency tests of all pollinator types revealed highly significant differences in frequency between the regions ($P < 0.001$ for all; fig. 2). Pollinator breadth was significantly higher (i.e., more generalized) in India than in Neotropical and paleotropical regions ($F_{3,470} = 7.75$, $P < 0.001$) and was surprisingly higher than that of temperate regions, although not significantly so.
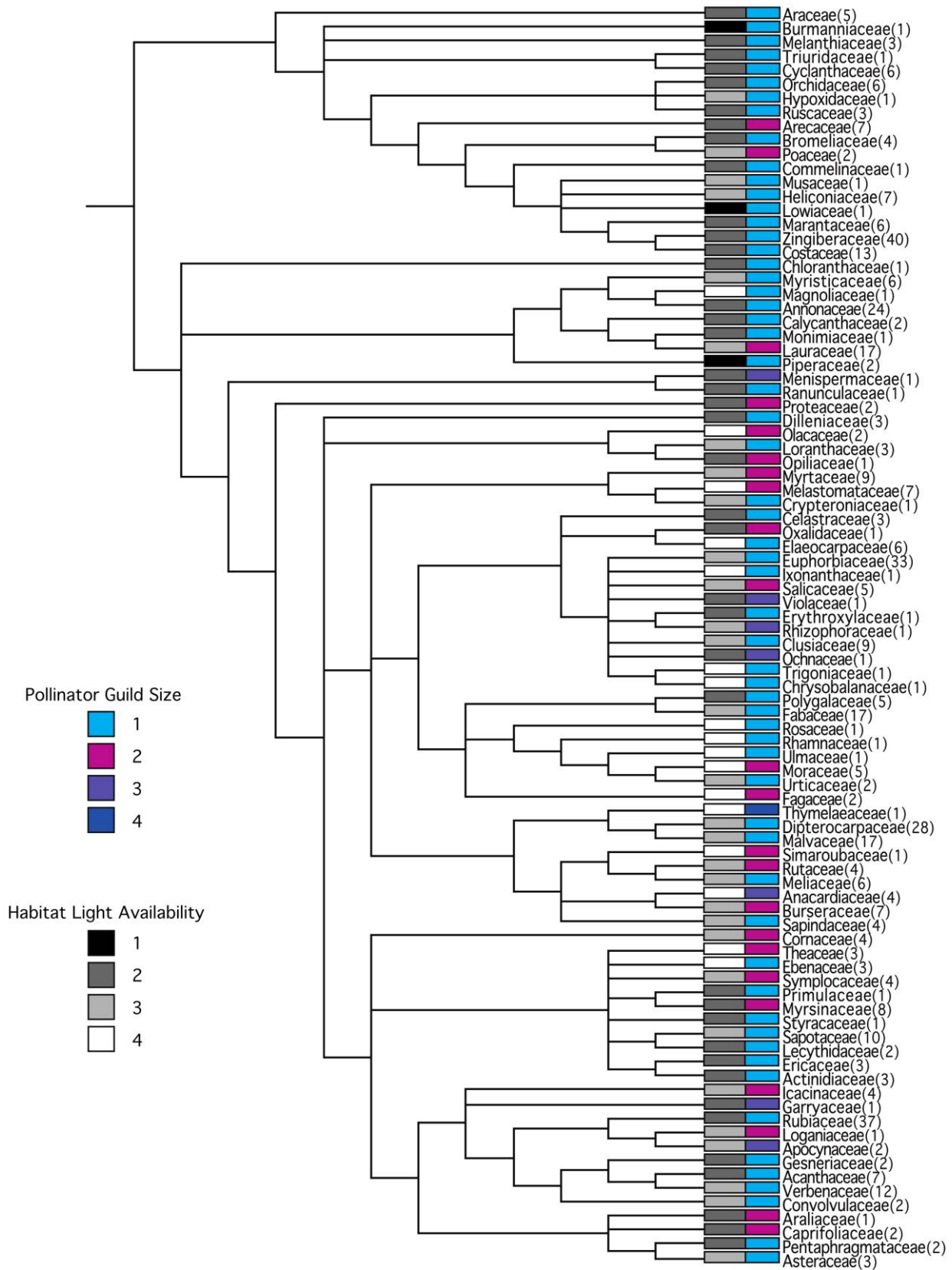
Fig. 1 Phylogenetic relatedness, average pollinator guild size, and habitat light availability for angiosperm families used in the analyses. Numbers in parentheses indicate the number of species included in the analysis.

Table 1

Summary of the 481 Species Used in Phylogenetic Analysis

Canopy layer	Light index	N	No. pollinator orders (±SE)
Forest floor	1	41	1.02 ± .11
Understory	2	208	1.32 ± .05
Liana	3	21	1.23 ± .16
Epiphyte	3	5	1.00 ± .33
Subcanopy	3	47	1.38 ± .11
Emergent	3	33	1.14 ± .16
Gap	4	52	1.33 ± .10
Canopy	4	74	1.53 ± .09

Note. Canopy layers were divided into four ordinal light environments (light index). Because the majority of information regarding pollinator identification "on the wing" is in the form of, for example, "stingless bees/beetles," we necessarily had to reduce our specialization index to the ordinal level (and in the cases of birds and mammals, to the class level). Cross-species analysis reveals significant differences in the pollinator breadth of the different light levels ($F_{3,477} = 3.53$, $P = 0.015$), with a Tukey test identifying the key significant difference to be between canopy/gap species and forest floor species. The mean pollination guild index for all species in the analysis is 1.32 ± 0.74.

Phylogenetic Comparative Tests

The significant result of the cross-species analysis above may be to some degree caused by the nonindependence of related species. We tested the association between habitat and size of pollinator guild using phylogenetic independent contrasts and found no correlation between these two traits (fig. 2; $F_{1,311} = 0.589$, $P = 0.443$). The results of phylogenetic tests for correlations between guild size and pollinator types were similar to those from the nonphylogenetic analysis (table 3). Inclusion of Hymenoptera, Lepidoptera, and Diptera in a plant's pollinator pool was associated with increases in pollinator guild size, while the inclusion of birds displayed a nonsignificant trend toward decreases in pollinator guild size. By contrast, none of the pollinator types showed a phylogenetic association with canopy position. The trend for increased pollinator breadth in India was marginally significant in a phylogenetic analysis ($P = 0.05$), possibly because of a dearth of bird pollination ($P =$

0.01). Canopy position exhibited strong phylogenetic conservatism ($P = 0.001$), while size of pollination guild did not ($P = 0.112$). However, all pollination syndromes tested (table 3), with the exception of Diptera, displayed strong phylogenetic signal ($P < 0.001$). Habitation of the forest floor also appeared to be phylogenetically conserved ($P = 0.020$), and the forest floor habitat was marginally associated with fewer pollinators (one-tailed Wilcoxon-signed rank test, $P = 0.059$, df = 31).

Discussion

Examining the geographical and ecological context under which transitions to pollinator specialization are favored can provide insight into the selective forces contributing to pollinator specialization. We found little support for the hypothesis that a plant's primary light environment is correlated with the size of a plant's pollination guild, especially when phylogeny is accounted for. Instead, we found strong patterns of association between generalization, region, and pollinator identity. The finding that the regional pollinator pool is an important determinant of pollinator guild size has consequences for plant evolution. For instance, the selection pressures imposed by generalized pollination syndromes are considered to be too diffuse to precipitate speciation (Waser et al. 1996), and therefore we might predict that clades with traits (e.g., in terms of pollinator identity or region) associated with generalist pollination will have fewer species per clade.

Our results suggest that forest floor species tend to have a smaller number of pollinators than canopy or gap species, providing some support for the idea that extremely shaded conditions select for specialization. Plant species in shaded environments have been hypothesized be more specialized due to selective pressures to reduce wastage of floral resources on inefficient pollinators (Feinsinger 1983). Conversely, plants in shady habitats may tend to be more generalized because such habitats tend to be dominated by generalist insect pollinators (Bawa 1990). Thus, we provide some evidence consistent with Feinsinger's (1983) hypothesis. However, because we did not observe strong correlations between canopy position and pollination guild size, a variety of light environments appear to provide the necessary conditions for transitions toward greater or smaller pollinator breadth.

Table 2

Presence of the Major Pollinating Taxa in Canopy Layers 1–4 and the Mean Guild Index in Plant Species That Employ Each Taxon

Pollinator	N	Layer 1 (8.5%)	Layer 2 (43.2%)	Layer 3 (22.0%)	Layer 4 (26.2%)	Guild index
Hymenoptera	257	22 (8.6%)	114 (44.4%)	46 (17.9%)	75 (29.2%)	1.46 ± .05[**]
Lepidoptera[*]	84	9 (10.7%)	26 (30.9%)	27 (33.3%)	21 (25.0%)	1.63 ± .08[**]
Coleoptera[*]	127	9 (7.1%)	45 (36.0%)	41 (32.3%)	32 (25.2%)	1.65 ± .06[**]
Diptera	73	2 (2.7%)	40 (54.8%)	13 (17.8%)	18 (24.7%)	2.39 ± .07[**]
Thysanoptera	12	0	6 (50.0%)	1 (8.3%)	5 (41.7%)	1.42 ± .21
Hemiptera	8	0	3 (37.5%)	4 (50.0%)	1 (12.5%)	2.38 ± .26[**]
Birds[*]	57	0	30 (52.6%)	16 (28.1%)	11 (19.3%)	1.09 ± .10[*]
Mammals	10	0	4 (40.0%)	4 (40.0%)	2 (20.0%)	1.00 ± .23

Note. Mean guild index refers to the number of pollinating orders utilized. Guild indexes differing significantly when the pollinator was present versus absent are marked with asterisks. See table 1 for explanation of canopy layers.

* $P < 0.05$ (Pearson contingency test).
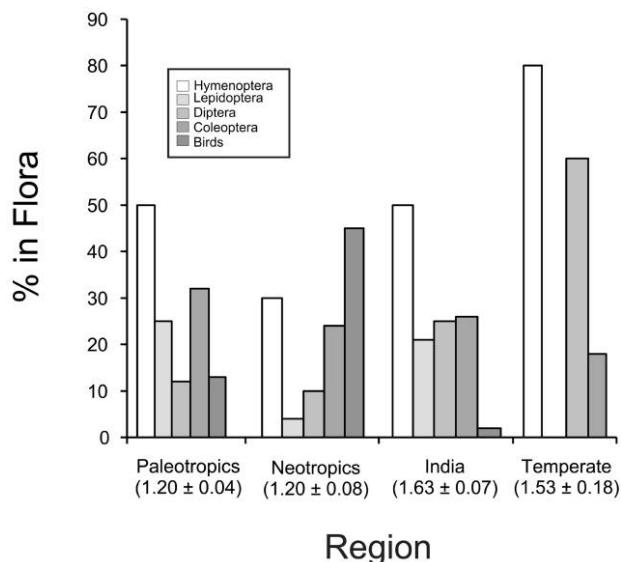
** $P < 0.001$.

Fig. 2 Distribution of pollinator visitation in geographical regions (percentage of species visited by particular pollinating orders). Contingency tests of all pollinator types revealed highly significant differences in frequency between the regions ($P < 0.001$). Numbers below the X-axis indicate the mean pollinator guild size ($\pm$SE) of species for the region.

Pollinator Guild Size, Identity, and Region

It is important to note that we do find that certain light environments are depauperate in pollinator diversity, possibly because of pollinator preferences for certain light intensities (Bawa 1990; Bertin and Sholes 1993; Bishop and Armbruster 1999). Thus, forest floor species could appear to attract only a small number of pollinators, not because of floral adaptations but because a smaller suite of pollinators is available at this low light level. This finding is supported by the observation that shaded habitats have lower pollinator fauna species richness than unshaded habitats in Alaska (Bishop and Armbruster 1999) but is in contrast to the findings of other studies (e.g., Bawa et al. 1985) that have found a greater diversity of pollinators at low levels of the canopy strata.

Interestingly, our results suggest that pollinator identity strongly influenced pollinator guild size, with bee, moth, beetle, and fly pollination being associated with increased pollinator guild size and bird pollination with decreased pollinator guild size. Region also exerted a strong influence, with the tropical species of India displaying significantly higher guild size than Neotropical and other paleotropical regions, perhaps because of a reduction in the amount of pollination by birds. Incidentally, our phylogenetic global analysis finds little evidence to substantiate the claims of Devy and Davidar (2003) that Neotropical countries such as Venezuela and Jamaica (two countries that figure heavily in our data set) show a paucity in the number of pollinator guilds and a high proportion of species skewed toward generalization.

Somewhat paradoxically, the pollinators of forest floor species often included Lepidoptera—a group that was associated with relatively generalist species in our study—but not birds, which are associated with specialization. This finding is consistent with the hypothesis that forest floor plants are less likely to be bird pollinated because high amounts of light are needed to produce the copious amounts of nectar required to attract birds (Stiles 1978). However, our phylogenetic analysis revealed reduced significance of the association between Lepidoptera pollination and low canopy position, indicating that the pattern can most likely be attributed to disproportionate representation in the data set of certain families with Lepidoptera-pollinated forest floor species. Nevertheless, further studies that combine the knowledge that certain pollinators (1) may prefer particular light intensities and (2) may be more likely to influence plant traits in such a way as to exclude other pollinators (Kato 1996, 2005) are warranted. In particular, the similarity of the association of beetles and flies with generalization is intriguing because there are few common foraging characteristics between these pollinators other than their propensity to visit flowers with an open floral form (Devy and Davidar 2003). However, having an open floral form may reduce the potential for adaptive trade-offs in floral morphology that have been shown to contribute to the evolution of specialization (Muchhala 2007), which might help to explain why the possession of radially symmetrical flowers is associated with reduced species richness (Sargent 2004).

Phylogenetic Conservatism

Plant canopy position exhibited significant phylogenetic conservatism. Thus, closely related species in our sample were more

Table 3

Phylogenetic Signal and Correlations with Habitat and Guild Size for Pollinator Types with $N > 20$

Pollinator	Signal (P)	Correlation with canopy position			Correlation with guild size		
		C	df	P	C	df	P
Hymenoptera	.001	.08	90	.623	.22	90	.025
Lepidoptera	.001	−.23	48	.186	.52	48	.001
Coleoptera	.001	.10	64	.327	.78	64	<.0001
Diptera	.158	−.10	60	.438	1.16	60	<.0001
Bird	.001	.29	25	.109	−.13	25	.117

Note.   Positive correlations indicate that the inclusion of a particular pollinator is associated with higher levels in the canopy or increases in guild size (e.g., plant species that are visited by bees are more likely to be visited by other pollinators as well compared to species that are not visited by bees). C = mean contrast value (e.g., the mean difference in trait X [e.g., canopy position] between sister groups that differ in whether bees act as pollinators). In two-tailed Wilcoxon signed rank tests, C was significantly different from zero (Vamosi and Vamosi 2005).

likely to have similarities in their light environment than distantly related species. This result is consistent with previous research demonstrating that a plant species' light environment and shade tolerance tend to be evolutionarily conserved rather than labile (Prinzing et al. 2001; Niinemets and Valladares 2006). The degree to which plant ecological traits tend to be conservative in their evolution is an important and somewhat controversial topic in plant ecology (reviewed in Webb et al. 2002; see also Silvertown et al. 2006) and may have implications for the mode and pace of plant speciation (reviewed in Wiens and Graham 2005). It is somewhat surprising that light environment would tend to be phylogenetically conserved because the amount of light that a plant receives tends to vary at a much smaller spatial scale than other environmental gradients (Prinzing et al. 2001). Our result may be a reflection of the fact that a species' position in the canopy most likely reflects ecological gradients beyond simple differences in the amount of light received. For example, particularly in tropical species, an epiphytic habit is correlated with occurrence in the canopy layer, and if epiphyte habitat itself is phylogenetically conserved, it would tend to inflate the degree to which light environment exhibits phylogenetic signal.

In contrast to canopy position, we found no evidence that the size of a plant's pollinator guild is phylogenetically conserved; closely related species were as likely to have similar pollinator guild sizes as distantly related species. To our knowledge, this is the first statistical test of the phylogenetic conservatism of pollinator guild size. We did, however, find that the inclusion of a particular pollinator order was strongly conserved, which is consistent with the idea that pollination systems exhibit niche conservatism (Wilson et al. 2006; Thomson and Wilson 2008). Our results suggest that lineages with specialized pollination systems may experience switches in the identity of their main pollinators rarely when compared to the frequency with which plant species increase or decrease their pollinator breadth.

This finding is significant because theories of pollinator-driven speciation rest on the assumption that pollinator syndromes are evolutionarily labile and are prone to change relatively rapidly upon exposure to different pollinator environments (reviewed in Johnson 2006). Paradoxically, our results, in conjunction with results reported by Wilson et al. (2006), suggest that, at least for the species represented in our study, pollinator syndromes may be relatively conserved. Because the evolutionary lability of pollinator syndromes has implications for our understanding of the mechanisms of plant speciation, we suggest that further investigations of the degree to which pollinator syndromes tend to exhibit phylogenetic conservatism are warranted.

Finally, it is important to note that the method that we used to define the size of a plant species' pollinator guild is only one of several possible methods (reviewed in Fenster et al. 2004). Our pollinator guild data do not distinguish between pollination and visitation, which may have different implications for natural selection and speciation (Fenster et al. 2004; Maad and Nilsson 2004). Other workers in this field (e.g., Waser et al.

1996) favor an approach that would have many of our "specialized" species categorized as generalists, which would have influenced the phylogenetic pattern of specialization. Most comparative studies of pollinator specialization to date have been forced to interpret visitation data as pollination data because of the scarcity of data sets that have recorded pollination (e.g., Ollerton et al. 2006; Wilson et al. 2006). Our approach represents a middle ground between methods advocated by Waser et al. (1996) and Fenster et al. (2004). Unfortunately, a methodology for classifying a plant's pollinator guild that avoids the pitfalls of lumping together some pollinators that may in fact influence floral evolution differently (sensu Fenster et al. 2004) while separating others that may not (sensu Waser et al. 1996) has yet to be described. We feel that our methodology reduces the subjectivity associated with classifying pollinators into functional groups while maintaining the philosophy behind it.

Controversy surrounds the role of pollinator interactions in the diversification of the angiosperms. Our results suggest that evolutionary transitions between specialized and generalized interactions are not uncommon, are not subject to phylogenetic constraint, and are (largely) not limited to certain light environments. However, certain pollinators were associated with more specialized pollination than others, and whether a particular pollinator type was included within a plant's suite of visitors did have a strong phylogenetic signal. These findings may have implications for plant speciation. Undoubtedly, speciation accompanied by floral shifts for specialized pollinator types (e.g., bird vs. bee) has occurred (reviewed in Thomson and Wilson 2008). Our data suggests that (1) the majority of plant speciation has occurred without shifts in pollinator types (as evidenced by the strong phylogenetic signal for the majority of pollinators) and (2) further instances where pollinator-mediated speciation has occurred will most likely be found within clades that include bird pollination and do not include pollination by beetles and flies. To our knowledge, although isolated cases exist to substantiate the claim that transitions to certain pollinators or positions in the canopy may subsequently influence the diversification rates within lineages or regions (e.g., Kay et al. [2005] found high rates of speciation in hummingbird-pollinated *Costus* lineages), large-scale phylogenetic examinations of speciation rates among clades with contrasting pollinators and/or locales have yet to be conducted. We assert that formal phylogenetic tests of the association between specialized pollination and angiosperm speciation are an important goal of this field.

Acknowledgments

Literature Cited

Armbruster WS, BG Baldwin 1998 Switch from specialized to generalized pollination. Nature 394:632.

Bawa KS 1990 Plant-pollinator interactions in tropical rain forests. Annu Rev Ecol Syst 21:399–422.

Bawa KS, SH Bullock, DR Perry 1985 Reproductive biology of tropical lowland rain forest trees. 2. Pollination systems. Am J Bot 72: 346–356.

Bertin RI, ODV Sholes 1993 Weather, pollination and the phenology of *Geranium maculatum*. Am Midl Nat 129:52–66.

Bishop JA, WS Armbruster 1999 Thermoregulatory abilities of Alaskan bees: effects of size, phylogeny and ecology. Funct Ecol 13:711–724.

Blomberg SP, T Garland 2002 Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. J Evol Biol 15:899–910.

Boose DL 1997 Sources of variation in floral nectar production rate in *Epilobium canum* (Onagraceae): implications for natural selection. Oecologia 110:493–500.

Bradshaw HD, DW Schemske 2003 Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. Nature 426:176–178.

Castellanos MC, P Wilson, JD Thomson 2004 "Anti-bee" and "pro-bird" changes during the evolution of hummingbird pollination in *Penstemon* flowers. J Evol Biol 17:876–886.

Chazdon RL, S Careaga, C Webb, O Vargas 2003 Community and phylogenetic structure of reproductive traits of woody species in wet tropical forests. Ecology 73:331–348.

Devy MS, P Davidar 2003 Pollination systems of trees in Kakachi, a mid-elevation wet evergreen forest in Western Ghats, India. Am J Bot 90:650–657.

Dodd ME, J Silvertown, MW Chase 1999 Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. Evolution 53:732–744.

Feinsinger P 1983 Coevolution and pollination. Pages 283–310 *in* DJ Futuyma, M Slatkin, eds. Coevolution. Sinauer, Sunderland, MA.

Felsenstein J 1985 Phylogenies and the comparative method. Am Nat 125:1–15.

Fenster CB, WS Armbruster, P Wilson, MR Dudash, JD Thomson 2004 Pollination syndromes and floral specialization. Annu Rev Ecol Evol Syst 35:375–403.

Fenster CB, MR Dudash 2001 Spatiotemporal variation in the role of hummingbirds as pollinators of *Silene virginica*. Ecology 82: 844–851.

Fine PVA, I Mesones, PD Coley 2004 Herbivores promote habitat specialization by trees in Amazonian forests. Science 305:663–665.

Fox JF 1985 Incidence of dioecy in relation to growth form, pollination and dispersal. Oecologia 67:244–249.

Fulton M, SA Hodges 1999 Floral isolation between *Aquilegia formosa* and *Aquilegia pubescens*. Proc R Soc B 266:2247–2252.

Goldblatt P, JC Manning 2006 Radiation of pollination systems in the Iridaceae of sub-Saharan Africa. Ann Bot 97:317–344.

Grant V 1994 Modes and origins of mechanical and ethological isolation in angiosperms. Proc Natl Acad Sci USA 91:3–10.

Grant V, KA Grant 1965 Flower pollination in the phlox family. Columbia University Press, New York.

Herrera CM 1995 Microclimate and individual variation in pollinators: flower plants are more than their flowers. Ecology 76:1518–1524.

Johnson SD 1996 Pollination, adaptation and speciation models in the Cape flora of South Africa. Taxon 45:59–66.

——— 2006 Pollinator-driven speciation in plants. Pages 295–309 *in* LD Harder, SCH Barrett, eds. Ecology and evolution of flowers. Oxford University Press, New York.

Kato M 1996 Plant-pollinator interactions in the understory of a lowland mixed dipterocarp forest in Sarawak. Am J Bot 83:732–743.

——— 2005 Ecology of traplining bees and understory pollinators in ecological studies. Pages 128–133 *in* DW Roubik, S Sakai, AAH Hamid Karim, eds. Pollination ecology and the rain forest. Vol 174. Springer, New York.

Kay KM, PA Reeves, RG Olmstead, DW Schemske 2005 Rapid speciation and the evolution of hummingbird pollination in Neotropical *Costus* subgenus *Costus* (Costaceae): evidence from nrDNA ITS and ETS sequences. Am J Bot 92:1899–1910.

Kay KM, DW Schemske 2003 Pollinator assemblages and visitation rates for 11 species of Neotropical *Costus* (Costaceae). Biotropica 35:198–207.

Larson BMH, SCH Barrett 2000 A comparative analysis of pollen limitation in flowering plants. Biol J Linn Soc 69:503–520.

Maad J, LA Nilsson 2004 On the mechanism of floral shifts in speciation: gained pollination efficiency from tongue- to eye-attachment of pollinia in *Platanthera* (Orchidaceae). Biol J Linn Soc 83:481–495.

Maddison WP 2006 Confounding asymmetries in evolutionary diversification and character change. Evolution 60:1743–1746.

Martins EP, JAF Diniz, EA Houseworth 2002 Adaptive constraints and the phylogenetic comparative method: a computer simulation test. Evolution 56:1–13.

Mayfield MM, DD Ackerly, GC Daily 2006 The diversity and conservation of plant reproductive and dispersal functional traits in human-dominated tropical landscapes. J Ecol 94:522–536.

Momose K, T Yumoto, T Nagamitsu, M Kato, H Nagamasu, S Sakai, RD Harrison, T Itioka, AA Hamid, T Inoue 1998 Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. Characteristics of the plant-pollinator community in a lowland dipterocarp forest. Am J Bot 85:1477–1501.

Muchhala N 2007 Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. Am Nat 169:494–504.

Niesenbaum RA 1993 Light or pollen: seasonal limitations on female reproductive success in the understory shrub *Lindera benzoin*. J Ecol 81:315–323.

Niinemets U, F Valladares 2006 Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. Ecol Monogr 76:521–547.

Nosil P, AO Mooers 2005 Testing hypotheses about ecological specialization using phylogenetic trees. Evolution 59:2256–2263.

Oakley TH, CW Cunningham 2000 Independent contrasts succeed where ancestor reconstruction fails in a known bacteriophage phylogeny. Evolution 54:397–405.

Ollerton J, SD Johnson, AB Hingston 2006 Geographical variation in diversity and specificity of pollination systems. Pages 283–308 *in* NM Waser, J Ollerton, eds. Plant-pollinator interactions: from specialization to generalization. University of Chicago Press, Chicago.

Prinzing A, W Durka, S Klotz, R Brandl 2001 The niche of higher plants: evidence for phylogenetic conservatism. Proc R Soc B 268: 2383–2389.

Rajakaruna N 2004 The edaphic factor in the origin of species. Int Geol Rev 46:471–478.

Sakai S, M Kato, T Inoue 1999 Three pollination guilds and variation in floral characteristics of Bornean gingers (Zingiberaceae and Costaceae). Am J Bot 86:646–658.

Sargent RD 2004 Floral symmetry affects speciation rates in angiosperms. Proc R Soc B 271:603–608.

Sargent RD, SP Otto 2006 The role of local species abundance in the evolution of pollinator attraction in flowering plants. Am Nat 167: 67–80.

Schemske DW 1981 Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. Ecology 62:946–954.

Silvertown J, K McConway, D Gowing, M Dodd, MF Fay, JA Joseph, K Dolphin 2006 Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc R Soc B 273:39–44.

Stevens PF 2001 Angiosperm phylogeny website, version 7. http://www.mobot.org/MOBOT/research/APweb/.

Stiles FG 1978 Ecological and evolutionary implications of bird pollination. Am Zool 18:715–727.

Thomson JD, P Wilson 2008 Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. Int J Plant Sci 169:23–38.

Vamosi JC, TM Knight, JA Steets, SJ Mazer, M Burd, T-L Ashman 2006 Pollination decays in biodiversity hotspots. Proc Natl Acad Sci USA 103:956–961.

Vamosi JC, SP Otto, SCH Barrett 2003 Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. J Evol Biol 16: 1006–1018.

Vamosi SM, JC Vamosi 2005 Endless tests: guidelines to analyzing non-nested sister-group comparisons. Evol Ecol Res 7:567–579.

van der Niet T, SD Johnson, HP Linder 2006 Macroevolutionary data suggest a role for reinforcement in pollination system shifts. Evolution 60:1596–1601.

von Hagen KB, JW Kadereit 2003 The diversification of *Halenia* (Gentianaceae): ecological opportunity versus key innovation. Evolution 57:2507–2518.

Waser NM, L Chittka, MV Price, NM Williams, J Ollerton 1996 Generalization in pollination systems, and why it matters. Ecology 77:1043–1060.

Webb CO, DD Ackerly, SW Kembel 2006 Phylocom: software for the analysis of community phylogenetic structure and trait evolution, version 3.40. http://www.phylodiversity.net/phylocom.

Webb CO, DD Ackerly, MA McPeek, MJ Donoghue 2002 Phylogenies and community ecology. Annu Rev Ecol Syst 33:475–505.

Webb CO, MJ Donoghue 2005 Phylomatic. http://www.phylodiversity .net/phylomatic.

Wiens JJ, CH Graham 2005 Niche conservatism: integrating evolution, ecology and conservation biology. Annu Rev Ecol Evol Syst 36:519–539.

Wikstrom N, V Savolainen, MW Chase 2001 Evolution of the angiosperms: calibrating the family tree. Proc R Soc B 268:2211–2220.

Wilson P, MC Castellanos, AD Wolfe, JD Thomson 2006 Shifts between bee and bird pollination among penstemons. Pages 47–68 *in* NM Waser, J Ollerton, eds. Plant-pollinator interactions: from specialization to generalization. University of Chicago Press, Chicago.