CHAPTER 17

Floral characters and species diversification

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Outline

The burgeoning of phylogenetic information during the past 15 years has focused much interest on whether specific features of clades enhance or hinder the evolution of species diversity. In the angiosperms many of the traits thought to affect clade diversity are floral in nature, because of their association with reproduction and thus species isolation. Therefore, we briefly review mechanisms by which floral traits can affect diversification. We then consider the possible influences of four specific traits by comparing the species diversity of a clade possessing a trait with that of its sister clade that lacks the trait. Clearly, this approach requires correct identification of sister groups, so that changes in phylogenetic reconstruction can have profound effects on these analyses. Here we use a recent supertree analysis of the angiosperms, which includes nearly all described families, along with other phylogenies to reexamine a number of floral traits thought to affect diversification rates. In addition, because many of the previous analyses employed a statistical test that has since been shown to be misleading, we use a suite of signed-rank tests to assess associations with diversification. We find statistical support for the positive effect of animal pollination and floral nectar spurs and a negative effect of dioecious sexual system on diversification, as proposed previously. However, our results for the effect of bilaterally symmetric flowers on species diversity are equivocal. We discuss several factors that will aid in future analyses and the need for both more detailed phylogenetic analyses and more studies on floral biology.

17.1 Introduction

The angiosperms are the most abundant and diverse group of plants on Earth today. Since their first appearance in the fossil record during the early Cretaceous (ca. 130 Ma, Crane *et al*. 2004), they have colonized almost every habitat on the planet, and now number approximately 260,000 extant species (Soltis and Soltis 2004). These myriad species vary impressively in morphology, life history, chemistry, and reproductive biology. Especially striking is the floral diversification, which fossils show began among early angiosperms (Friis *et al*. 2000), and therefore must have occurred concurrently with their radiation and rise to ecological dominance. Flowers exhibit an amazing variety of sizes, shapes, colours, arrangements, scents, rewards, and sexual systems, from the tiny self-fertilizing flower of *Arabidopsis thaliana* to the intricate flowers of *Ophrys* orchids, which mimic a female mate for an unsuspecting male wasp, to the enormous putrid inflorescence of the corpse flower, *Amorphophallus titanium*.

Which factors promote angiosperm diversification, especially the role of floral traits and sexual systems, is an enduring question and its resolution

is a central goal of plant evolutionary biology. Darwin puzzled over the apparent sudden appearance of the angiosperms in the fossil record, clearly finding it a challenge to his view of "extremely gradual evolution" and forever labelled the phenomenon with his famous quotation as "an abominable mystery" (Darwin 1903). Knowledge of the timing and pace of angiosperm diversification has progressed considerably with both the discovery of fossil flowers (e.g., Dilcher and Crane 1984; Crane *et al*. 1995; Gandolfo *et al*. 1998) and molecular-based phylogenies (e.g., Qiu *et al*. 2000; Zanis *et al*. 2002). These findings have revealed an increasingly detailed view of patterns of floral diversification.

During the past 10 years or so, phylogenies have been used extensively to assess whether species diversity occurs non-randomly among clades (Sims and McConway 2003) and whether particular traits may be responsible for these patterns. Although studies have reported that changes in angiosperm diversity correlate with several traits, including the rates of molecular evolution (Barraclough *et al*. 1996; Barraclough and Savolainen 2001), latex and resin canals (Farrell *et al*. 1991), herbaceous growth habit (Dodd *et al*. 1999), and climbing habit (Gianoli 2004), floral traits have been implicated most commonly. This apparent evolutionary importance of floral traits is perhaps not surprising, because a correlation between the rise and diversification of angiosperms and the diversification of pollinating insects has long been recognized (Crepet 2000). As much of this volume attests, aspects of both sexual system and floral morphology can affect how a plant reproduces and with which other plants it mates. Thus, these traits are natural subjects for investigating their effects on species diversity.

Comparative studies have identified several floral characters that affect rates of angiosperm diversification, including animal pollination (Dodd *et al*. 1999), floral nectar spurs (Hodges and Arnold 1995; Hodges 1997a, b), bilateral symmetry (Sargent 2004), and a dioecious sexual system (Heilbuth 2000) (Plate 7). However, most previous analyses of the effects of floral traits on species diversity either used now-discredited statistical methods (Dodd *et al*. 1999; Hodges and Arnold 1995; Hodges 1997a, b) or relied largely on angiosperm phylogenies that lacked representatives of many families and were constructed mostly with plastid-gene sequences (Dodd *et al*. 1999; Heilbuth 2000; Sargent 2004). Clearly, incorrect statistical techniques can cause misinterpretations about diversification hypotheses. The limited taxon sampling in phylogenies can both lead to errors in inferring sister-group relationships and the timing of the origin of a trait of interest and reduce the sample of replicate origins of a key trait. Finally, because plastids do not undergo recombination, plastid genes are inherited essentially as a single locus, so that sequences of different genes provide limited independent phylogenetic information. Consequently, phylogenetic information from plastid genes should be combined with data from other loci for a robust phylogeny based on multiple independent lines of evidence. These problems all call for a reanalysis of the role of floral traits in angiosperm diversification.

Here we reanalyse four purported floral correlates of angiosperm diversity—animal pollination, floral nectar spurs, bilateral symmetry, and the dioecious sexual system—using both more complete phylogenetic analyses and appropriate statistical tests. We use a recently constructed and nearly comprehensive supertree of angiosperm families derived from 46 source trees (Davies *et al*. 2004), along with other phylogenies at lower taxonomic levels, and family circumscriptions consistent with APGII (2003). For each character we identify phylogenetically independent contrasts and compare the species richness of the sister clades composing each contrast. We discuss our findings in light of hypotheses for how these traits affect diversification, and suggest avenues for future work to clarify the mechanisms responsible for any correlations.

17.2 How might floral traits affect diversification?

For a trait to affect diversification rates, it must influence the probability of speciation, extinction, or both. Speciation involves the evolution of reproductive isolation and is generally initiated by geographical isolation (Coyne and Orr 2004).

Therefore, a trait that promotes the colonization of new habitats, limits dispersal between populations, or increases the propensity or ability to mate with phenotypically similar individuals is a good candidate for a trait that might affect diversification by increasing speciation. Certain traits may affect speciation, but in almost all cases these effects are thought to be coincidental to adaptation to local conditions or genetic divergence in isolation. Thus, although natural selection may cause evolution in these traits, they are not selected for reproductive isolation or higher diversification *per se* (Chapter 16). Conversely, some traits may be selected in certain environments and during short periods, but predispose their possessors to a higher chance of extinction. A trait that leads a population to experience greater demographic stochasticity or lower adaptive genetic diversity (see Chapter 2) would be a good candidate for a trait affecting diversification through extinction. Unfortunately, without a detailed phylogeny and fossil record, speciation and extinction are exceedingly difficult to tease apart. Thus analysis of diversification requires a hypothesis for how a trait affects either speciation, extinction, or both, which can be tested more directly than by simply assessing its association with the overall diversification rate.

Animal pollination, bilateral floral symmetry, and nectar spurs have all been suggested to enhance speciation rates for seemingly similar reasons, namely, their likely effects on the specificity of mating among plants. Animal pollination is one of the most striking features of angiosperms, with plants using an incredible array of insects, birds, and mammals to disperse pollen. Because successful pollen transfer is so important to fitness, pollinators exert selection on floral traits (reviewed in Fenster *et al*. 2004 and most chapters in this volume). Spatial and temporal variation in pollinator assemblages can promote evolutionary divergence in floral traits among populations (Chapters 8, 15, and 16), and plant populations adapted to different suites of pollinators may be less likely to mate with each other (Thompson 1994). This avenue for reproductive isolation is not available to abiotically pollinated lineages, which depend on wind or water to transfer pollen. Dodd *et al*. (1999) compared the diversity of sister clades using the methods of Slowinski and Guyer (1993) and found a strong overall pattern that animal pollination was associated with more rapid diversification than abiotic pollination. This finding is bolstered by an extensive body of empirical research on the role of plant–pollinator interactions in speciation (reviewed in Stebbins 1974; Grant 1981; Coyne and Orr 2004; Chapter 16).

Similarly, floral nectar spurs may further promote specialization on different pollinators; affecting reproductive isolation, and thus diversification (Hodges and Arnold 1995; Hodges 1997a, b). The presentation of nectar at the base of a relatively long, thin tube requires a match between the pollinator and the floral morphology, limiting the number of pollinating species that can manipulate the flower successfully. Hodges and Arnold (1995) and Hodges (1997a, b) found an association between the evolution of floral nectar spurs and higher diversification in both a comparative study among angiosperms and a detailed study of columbines (*Aquilegia*).

In contrast, bilateral floral symmetry, or zygomorphy, may affect diversification somewhat differently. Compared with radially symmetric, or actinomorphic, flowers, zygomorphy constrains the orientation of pollinators while they visit flowers, thereby enhancing the precision of pollen exchange between pollinators' bodies and the sexual organs of flowers (Neal *et al*. 1998; Sargent 2004). This increased precision could affect reproductive isolation if it promotes specialization by different pollinators on different types of zygomorphic flowers or if flowers diverge in the location of pollen placement on a pollinator (Chapter 16). Zygomorphy may also limit the number or type of pollinating species that manipulate a species' flowers effectively, which may increase the variance in pollinator assemblages and hence selection on floral traits across the landscape. Although examples of pollen placement affecting reproductive isolation between species visited by the same pollinator are known (Brantjes 1982; Grant 1994; Kay 2006; Chapter 16), the importance of such shifts in speciation remains to be clarified. Nevertheless, in a sister-group study among angiosperms, Sargent (2004) found accelerated

diversification in lineages with bilaterally symmetric flowers.

Conversely, a trait may increase the chances of extinction, even if it is favoured by selection in the short term (Chapter 2). The dioecious sexual system, with separate male and female individuals, may be an example of this. Dioecy has evolved multiple times and occurs in approximately 6% of angiosperm species (Renner and Ricklefs 1995). In a comparative study across angiosperms, Heilbuth (2000) found a striking association between dioecy and lower species richness of sister clades. Dioecy may retard diversification for several reasons. Because they lack the reproductive assurance of being able to self-pollinate, dioecious plants may have a higher risk of dying without reproducing and may have a lower colonization ability (Baker 1954; Bawa 1980; Chapter 12). However, self-incompatible species should be subject to the same constraint and have lower species diversity than their self-compatible sister taxa, but no such association has been found (Heilbuth 2000). Vamosi and Otto (2002) proposed that differential selection on male and female flowers can lead one sex to become more showy than the other (typically male), resulting in poor pollination during years of low pollinator abundance. Dioecious plants can also suffer increased variance in both pollination and seed dispersal, because, unlike hermaphrodites, not every individual is a potential mate and only females disperse seeds (Heilbuth *et al*. 2001; Wilson and Harder 2003). Because reproductive success varies nonlinearly with pollination and seed dispersal, this increased variance can reduce the average reproductive performance of dioecious species relative to that of otherwise similar hermaphroditic species (Wilson and Harder 2003).

17.3 Common tests for key innovations

Discovery of the mechanisms by which particular traits influence speciation and extinction is fundamental, but phylogenetically based comparative studies are necessary to identify the importance of a trait to angiosperm diversification in general. Many traits can influence diversification in some circumstances, but certain traits have been suggested to act as key innovations, allowing their possessors to diversify rapidly and create new niches. Such effects should be relatively consistent across lineages in which they evolve. Ideally, identification of key innovations requires knowledge of the evolutionary relationships among taxa and the timing of all critical events, such as speciation, extinction, and the origins of the trait of interest. Unfortunately, barring an exceptionally detailed fossil record, these factors are usually incompletely known, dictating the use of less powerful statistical tests for an association between a trait and diversification rate.

The simplest technique for testing whether a trait alters diversification compares the numbers of species in two sister taxa differing in the trait of interest. By definition, sister groups are the same age, so any difference in species numbers must be a result of differences in rates of speciation and/or extinction. These differences are compared to a null model of equal diversification to determine whether they are sufficiently large to indicate a change in the diversification rate with the origin of the putative key innovation or whether they occurred stochastically during speciation and extinction (Sanderson and Donoghue 1994, 1996). Such an analysis can be implemented with only a rudimentary phylogeny showing sister-group relationships, and may therefore be feasible in many diverse and poorly characterized lineages; however, it has low statistical power and can detect only extremely large differences in diversification rates (Sanderson and Donoghue 1996).

More powerful inferences can be drawn for traits that evolve repeatedly, which can provide replicated evidence for changes in diversification. Most simply, numbers of species between pairs of sister clades can be compared with a sign test. However, the sign test ignores the magnitude of differences in species numbers, and thus provides limited statistical power. Consequently, the sign test should be used only when the relative sizes and not the species numbers of sister groups are known. Perhaps the most commonly used method has been that of Slowinski and Guyer (1993), which compares the difference in species richness between individual sister groups with a null model based on random speciation and extinction, and then combines probabilities from multiple

comparisons. However, several researchers have identified severe shortcomings with this method (e.g., de Queiroz 1998; Goudet 1999; McConway and Sims 2004). Vamosi and Vamosi (2005) recently reviewed and compared statistical tests for sister-group comparisons and showed clearly that the Slowinski–Guyer method is prone to type I errors, because a few large differences in species numbers can result in a significant test statistic, regardless of the direction or magnitude of the remaining contrasts. This problem is especially severe for datasets in which some sister-group comparisons have large differences in species counts that favour the hypothesis, but other sister group comparisons have large differences in the opposite direction. Such a dataset results in a U-shaped frequency distribution of the proportion of species in each sister-group pair possessing the trait of interest. In these cases, the Slowinski–Guyer method can give the nonsensical result that the trait both promotes and retards diversification significantly. For these reasons, Vamosi and Vamosi (2005) recommended against the use of the Slowinski–Guyer method, and suggested more suitable, less biased techniques. They also recommended that plots of the data accompany any statistical tests, making it possible to check visually for data with a U-shaped frequency distribution.

Instead of the Slowinski–Guyer test, contrasts between sister clades can be analysed using a Wilcoxon signed-rank test. Vamosi and Vamosi (2005) reviewed various methods for calculating the contrasts to be tested (also see Isaac *et al*. 2003). "Simple" contrasts based on the absolute difference in species numbers between sister clades may seem straightforward, but do not account for the overall species richness of the pair and can be misleading. For example, Vamosi and Vamosi showed that simple contrasts of 1020 versus 1010 species and 20 versus 10 species result in the same test statistic, but represent two intuitively contrasting cases. Two alternative methods of calculating the contrasts avoid this problem. "Proportional" contrasts are calculated as the proportion of all species in the sister group represented by the clade possessing the trait of interest minus 0.5, so that it ranges from -0.5 to $+0.5$. For example, the proportional contrast between clade A with 10 species and clade B with 5 species shown in the hypothetical phylogeny of Fig. 17.1 equals 0.167. This test is prone to errors if the proportion of species with the trait of interest for each contrast has a U-shaped distribution. In this case, tests for effects of either character state can result in significant test statistics. In contrast, this approach applied to a data set with an L-shaped frequency distribution, in which most of the large contrasts in species counts fall in the same direction, will yield a significant result for only one of the character states. Finally, "log" contrasts compare sister-group diversity based on the ratio of the log number of species in the larger group to that for the smaller group. In this case, the contrast between A and B in Fig. 17.1 equals 1.43. Log contrasts may favour small or young sister groups, and therefore should be used cautiously if replicate sister groups differ systematically in phylogenetic age according to the direction of their contrast. Tests based on log contrasts yield the same result if a specific character state promotes or retards diversification; however, the direction of the effect can be identified from a plot of the contrast distribution (Vamosi and Vamosi 2005). Isaac *et al*. (2003)

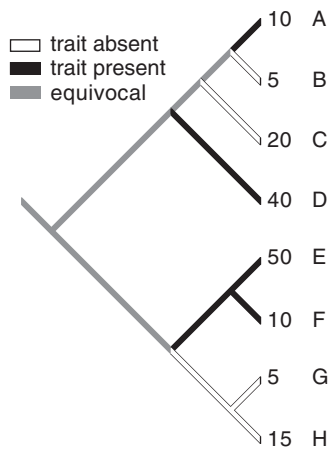

Figure 17.1 Hypothetical phylogenetic tree illustrating the mapping of both the character state for a trait (present, absent or equivocal) and the species numbers for each clade (A–H) at the tips of each branch. The construction of specific contrasts is explained in the text.

used a simulation study to examine the performance of these three contrast methods and recommended using either log contrasts, when sister groups are of similar age, or proportional contrasts.

Note that all techniques based on the Wilcoxon signed-rank test necessarily rely on phylogenies, which in themselves constitute hypotheses. Thus, a sister-group analysis should ideally incorporate the confidence in each phylogenetic hypothesis. Of particular concern are large-scale phylogenies with limited sampling and ability for analysis of statistical support. Incomplete sampling can impact the nature of the comparisons profoundly. For instance, in Fig. 17.1, A and B are sister clades and A, possessing the trait of interest, is also more species rich. However if B was not sampled for the phylogenetic analysis, one would erroneously conclude that A and C are sister clades and that the clade lacking the trait is more diverse. Studies based on comparisons of multiple sister groups implicitly assume that such errors are not biased in one direction or another.

17.4 Methods

Our general approach in reanalysing the effects of the four key traits on diversification was to review the plant groups identified in previous studies, identify new groups that could be added, update the taxonomic circumscriptions to reflect APGII (2003), and update the phylogenetic information according to Davies *et al*. (2004) and other available phylogenies for lower taxonomic ranks. Independent contrasts between groups possessing and lacking the trait of interest were identified starting at the tips of the phylogeny and proceeding towards the root. Nested contrasts were removed from higher-level contrasts, so that no group was used in more than one contrast. For example, in Fig. 17.1 we would calculate a contrast between A and B, and then remove that contrast from the tree and calculate another contrast between C and D. We used the consensus tree presented by Davies *et al*. (2004) for family-level and higher contrasts. This tree includes several unresolved nodes, which causes uncertainty about the appropriate sister group. In such cases, we used the species counts from the clades that would be most conservative with regard to the hypothesis of contrasting diversification rates (i.e., the results are biased against finding an effect). Because families are arbitrary constructs and do not represent a well-defined evolutionary unit, we used total species numbers in our higher-level contrasts, instead of averaging across the species richness of the constituent families, as is typically done in nested contrasts of continuous variables. For example, in the contrast of E and F versus G and H in Fig. 17.1, we would contrast species counts of 60 and 20, rather than the average counts of 30 and 10.

17.4.1 Trait datasets

We first compared species richness between sister clades with biotic and abiotic pollination at the family level and higher. We used the data of Dodd *et al*. (1999), with species counts taken from Davies *et al*. (2004). Additional pollination information was obtained from Watson and Dallwitz (2005), or from literature searches on the ISI Web of Science using the word ''pollination'' and the family name in the topic field. Pollination mode for each family was coded as either primarily biotic, primarily abiotic, both modes present, or unknown, and we excluded families in the latter two categories from analyses.

To assess the role of zygomorphy in diversification, we expanded the dataset constructed by Sargent (2004). Character state determinations were taken from Sargent (2004), Watson and Dallwitz (2005), Takhtajan (1997), and Mabberley (1997). Families were considered zygomorphic if they were described as primarily having zygomorphic, bilaterally symmetrical, irregular or bilabiate corollas, whereas actinomorphic families were described as having radially symmetrical, polysymmetric, or regular corollas. Only animal-pollinated families are considered in this analysis, because the hypothesis for how floral symmetry affects diversification depends on plant–pollinator interactions. To be conservative in finding an effect, Sargent (2004) subtracted actinomorphic genera from zygomorphic families, but did not subtract zygomorphic genera from actinomorphic families. As

this method could bias the results, we did our analysis both with and without these subtractions.

For nectar spurs, many contrasts between spurred and non-spurred groups occur within families. Therefore we searched the literature to find as many spurred taxa as possible, regardless of rank, and to determine their putative sister clade. Spurred lineages surveyed previously by Hodges (1997a, b) and Hodges and Arnold (1995) were reviewed for more recent evidence regarding sister-group relationships. In addition, we searched the literature for phylogenetic data identifying sister groups for additional spurred lineages that were unavailable in previous analyses. We excluded groups possessing nectarless spurs, which may not function in the hypothesized manner, and groups with flowers described as only saccate. We included the Marcgraviaceae, which does not have spurs within flowers, but rather highly modified floral bracts that form elaborate extrafloral nectaries (Ward and Price 2002), which pollinators probe to access to nectar in a similar manner to probing nectar spurs. We also considered whether each group is zygomorphic or actinomorphic (e.g., Plate 7d versus 7e) to test whether spurs correlate with diversity for the subset of instances in which nectar spurs evolved independently of floral symmetry.

For our analysis of dioecy, we reviewed the dioecious taxa identified by Heilbuth (2000). Lineages were considered dioecious if most or all of the species exhibit separate sexes on different individual plants, whereas lineages were considered non-dioecious if most or all of the species exhibit both sexes on the same individual plants. To avoid inflating the number of species in the non-dioecious sister group, we either subtracted any dioecious genera from the non-dioecious families, or subtracted the estimated number of dioecious species, if this information was available. Information on dioecious genera was taken from Mabberley (1997), Takhtajan (1997), and the database of Renner and Ricklefs (1995).

17.4.2 Analyses

For each trait of interest, we first constructed a frequency distribution of the proportion of species from each sister group possessing the trait of interest to examine qualitatively whether the data exhibited a U-shaped distribution. We then performed one-tailed Wilcoxon signed-rank tests on simple, proportional and log contrasts (see Section 17.3). For datasets including monotypic groups, we added one to all species numbers before log-transformation. For each contrast, we assigned a positive to contrasts matching our hypothesis and a negative to those opposing it. We excluded cases for which the focal and sister clades have equal species numbers, because they are uninformative.

17.5 Results

17.5.1 Pollination mode

Of 379 families included in this analysis, we identified 39 with abiotic pollination, 202 with animal pollination, 17 with both modes present, and 121 for which there is insufficient information. For these data we found 16 independent contrasts between pollination modes. Because the animal-pollinated clade contained more species than the abiotically pollinated clade for 11 of the 16 contrasts (Electronic Appendix 17.1, http://www.eeb.utoronto.ca/EEF/), the frequency distribution of the proportion of biotically pollinated species in sister groups was strongly L-shaped (Fig. 17.2a). Indeed, regardless of the contrast measure used, animal pollination seems to promote significantly higher diversification (Table 17.1). A notable exception to this pattern is the contrast between the animal-pollinated Bromeliaceae and an abiotically pollinated clade including the Poaceae, Juncaceae, and Typhaceae.

17.5.2 Floral symmetry

We found 22 independent contrasts in floral symmetry among animal-pollinated angiosperms (Electronic Appendix 17.2). Only 16 of these contrasts involve the predicted higher species richness in clades with asymmetric flowers and the frequency distribution of the proportion of species in sister groups with asymmetric flowers is distinctly U-shaped, with most contrasts being either strongly positive or strongly negative (Fig. 17.2b).

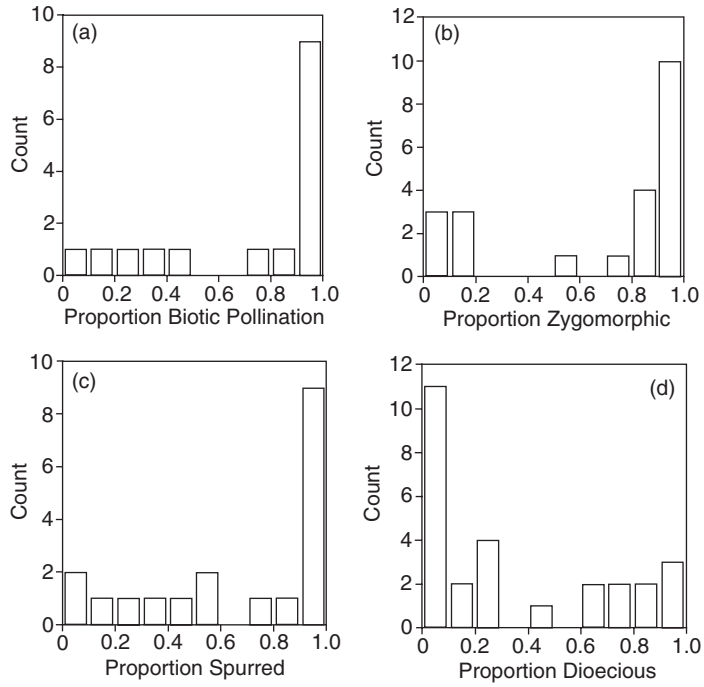

Figure 17.2 Frequency distributions of the proportion of species in a sister group represented by the clade exhibiting (a) biotic pollination, (b) zygomorphic floral symmetry, (c) presence of floral nectar spurs, and (d) dioecious sexual system.

Table 17.1 Results of Wilcoxon signed-ranks tests for the effects of four reproductive characters on diversification.

Character	Method of Ranking		
	Difference in species numbers	Proportion	Log (high)/ log (low)
Biotic pollination	$P = 0.033$	$P = 0.005$	$P = 0.005$
Zygomorphy	$P = 0.035$	$P = 0.009$	$P = 0.010$
Spurs	$P = 0.137$, ns ($P = 0.010$)	$P = 0.019$ ($P = 0.007$)	$P = 0.007$ ($P = 0.007$)
Dioecy	$P = 0.019$	$P = 0.025$	$P = 0.020$

For each character, we considered three methods for calculating the difference in diversity between clades, as described in the text. For floral nectar spurs, the results in parentheses represent tests that considered only comparisons for which both sister groups have the same floral symmetry. Probabilities represent the results of one-tailed tests for each comparison, because test comparisons addressed a specific directional hypothesis.

The statistical tests for proportional and log contrasts are both significant (Table 17.1); however, the U-shaped distribution makes the statistical results suspect. Furthermore, the sister group for one contrast, with 10 zygomorphic families including Marantaceae (total of 1549 spp., Electronic Appendix 17.2), was unresolved and could involve either the relatively species-rich Arecaceae (2500 spp.), or the relatively species-poor Rapataceae (80 spp.), or both combined. In our analysis (Electronic Appendix 17.2) we used the Rapataceae as the sister group, as this relationship supported the hypothesis of greater diversity in the zygomorphic clade; however, use of either other possible sister group reduces statistical support for zygomorphic flowers promoting diversification and amplifies the U-shaped distribution.

17.5.3 Floral nectar spurs

We found 16 independent origins of floral nectar spurs for which the sister group can be identified (Electronic Appendix 17.3). For 12 cases, the spurred group includes more species than its sister clade. A one-tailed Wilcoxon signed-rank test of simple contrasts rejected an association higher

diversification with nectar spurs, whereas tests of proportional and log contrasts are highly significant (Table 17.1). Furthermore, the proportion of species with nectar spurs in sister groups has a distinctly L-shaped frequency distribution (Fig. 17.2c) that is consistent with spurs promoting diversification. Restriction of the dataset to consider only comparisons for which the sister groups have the same floral symmetry resulted in 10 independent comparisons, of which 9 have more species in the spurred group compared with its sister clade. Each of the signed-rank tests for this restricted analysis detected significantly greater diversity in spurred clades (Table 17.1).

17.5.4 Dioecy

We identified 29 independent contrasts in sexual system among angiosperms (Electronic Appendix 17.4). For 18 contrasts, the dioecious clade has lower species richness, whereas the opposite is true for 9 contrasts. Two comparisons, Barbeyaceae versus Dirachmaceae, and Myricaceae versus Juglandaceae, involve equivalent species numbers between sexual systems, and we excluded them from the analysis. The frequency distribution for the proportion of species in sister groups with a dioecious sexual system is L-shaped (Fig. 17.2d), with dioecious clades being less diverse than hermaphroditic clades. Regardless of the contrast measure used, dioecy is associated with significantly lower diversification rates (Table 17.1).

17.6 Discussion

With one exception, our reanalysis supports previous findings that the evolution of floral traits can alter subsequent species richness within clades. Like previous analyses, we found that the evolution of animal pollination (Dodd *et al*. 1999) and floral nectar spurs (Hodges and Arnold 1995; Hodges 1997a, b) enhanced species diversification, whereas the evolution of dioecy retarded diversification (Heilbuth 2000). In contrast, our results cast some doubt on Sargent's (2004) conclusion that the evolution of bilaterally symmetric flowers affects diversification. The overall similarity of our results to previous analyses occurred despite our use of a different phylogenetic tree, which represents angiosperm families much more completely, and new statistical methods. The general robustness of these results to new analyses provides strong support to the conclusion that a variety of floral traits thought to affect the likelihood of speciation or extinction contribute to species diversification. However, note that every trait that we considered had contrasts that span the full range of outcomes (Fig. 17.2). Thus, the effect of any of these traits on diversification is likely to be context dependent, with other factors influencing specific cases. Also, the general pattern found for any trait need not explain the true causal factor for diversity in any specific contrast, even those strongly supporting the general trend. As we emphasize below, even when a multiple sister-group analysis supports a hypothesis for diversification, these correlations should represent starting points of more thorough phylogenetic and population analyses.

In contrast to the expectation that changes in phylogenetic reconstruction should not favour one hypothesis or another, the difference between our results and those of Sargent (2004) suggest that even a strong association between the evolution of trait and subsequent diversity should be treated cautiously. Such caution is especially necessary in the absence of additional data supporting the functional hypothesis (see below). In this specific case, we found that although most contrasts support the hypothesis that the evolution of zygomorphic flowers enhances diversification, a substantial number of the contrasts support the exact opposite conclusion.

The results for flower symmetry should be treated prudently, as zygomorphy may influence diversification in some instances, but not others. As noted in Section 17.2, zygomorphy may influence reproductive isolation by constraining the orientation of pollinators during flower visitation, encouraging precise placement of pollen on their bodies. However, some species with actinomorphic flowers may have other traits that restrict the position of pollinators while they visit flowers, such as inflorescence architecture and flower orientation. For example, bees visiting flowers

arranged in a raceme inflorescence (e.g., *Chamerion angustifolium*) do so primarily from the bottom towards the top and so approach each flower from a similar angle (Routley and Husband 2003), especially if the flower face is roughly vertical. Similarly, hummingbirds visiting *Aquilegia formosa* do so by probing the nectar spurs in a precise manner causing their chin to brush against the anthers and stigmas. Thus, not all zygomorphic taxa may have more precise pollen placement or pollinator specificity than their actinomorphic sister groups. We encourage studies concerning whether the evolution of zygomorphy enhances diversity in specific cases and especially studies of how zygomorphy may enhance pollinator and pollen placement specificity and reproductive isolation, which remain uncertain, as it has never been tested explicitly.

Speculation about how a trait affects rates of species diversification points to a general problem with tests such as those performed here. Associations of diversification with specific traits are, of course, just associations. Any additional factor that co-varies with the trait of interest could be the true causal factor for increased diversification, even if it is not recognized. Thus, although identification of characters that associate significantly with diversification is an important first step, additional analyses of how a particular trait affects speciation or extinction are essential for testing whether a true causal relationship exists.

One such analysis requires more detailed phylogenetic sampling for specific examples of the origin of a trait. For instance, von Hagen and Kadereit (2003) examined a detailed phylogeny for *Halenia*, which possesses floral nectar spurs. Simple sister-group analysis shows that *Halenia* has many more species than its non-spurred sister group; however, von Hagen and Kadereit (2003) showed that diversification did not follow the evolution of nectar spurs immediately. Rather, diversification seems to have increased after the invasion of South America by a subclade of the genus. Although contradicting an immediate diversification effect of nectar spurs, this pattern is consistent with a general hypothesis of a key innovation (Simpson 1953), which considers two factors, the origin of the trait and the ecological context in which it evolves. If the evolution of nectar spurs promotes diversity by facilitating pollinator transitions, this role can be played only in the presence of a diverse pollinator fauna. Perhaps *Halenia* encountered a sufficient pollinator diversity for nectar spurs to affect diversification only after invading South America (von Hagen and Kadereit 2003). Unfortunately, little is known about the pollination biology of this group, so that this latter hypothesis remains untested.

A second type of phylogenetic analysis explores how a trait may affect diversification. For instance, the hypothesized effects of both animal pollination and floral nectar spurs on diversification involve an increased likelihood of transitions to novel pollinators, thereby promoting reproductive isolation and thus speciation (Hodges and Arnold 1995; Dodd *et al*. 1999). Therefore, a species-level phylogeny should reveal frequent transitions to novel pollinators, especially for recent radiations for which extinction is less likely to influence clade diversity. Unfortunately, few species-level phylogenies are currently available for entire groups, particularly for those in which a trait correlated with species diversification has evolved recently (although see Beardsley *et al*. 2003; Kay 2005; Whittall 2005). In addition, although transitions between major pollinator types (e.g., bee and hummingbird) are most convincing, transitions between different species within a major pollinator type may also provide reproductive isolation. Thus, detailed knowledge of pollination in multiple species will be needed for a full analysis.

Lack of phylogenetic information also restricted our analyses because the sister-group relationships could not be determined for many groups. This difficulty is especially problematic for our review of floral nectar spurs, because spurs are commonly generic, rather than family, traits and genus or species-level phylogenetic information is comparatively rare. Consequently, we had to exclude many groups from our analyses, reducing the power of our tests. In addition to reducing the number of comparisons available for simple tests, such as those described here, this lack of phylogenetic information precludes more detailed analyses needed to tease apart the effects of multiple characters.

Many characters probably affect the rate of either speciation or extinction. For instance, Vamosi and Vamosi (2004) performed nested analyses of the effect of dioecy, while also controlling for woody versus herbaceous growth habit, tropical versus temperate distribution, and fleshy versus dry fruits. Based on the detailed analysis of dioecious genera and their non-dioecious sister lineages, Vamosi and Vamosi found significantly slower diversification in dioecious groups, which is ameliorated somewhat for clades with a tropical distribution and/or fleshy fruits. They attributed these results to opposing effects on extinction, with dioecy increasing the risk of extinction, whereas fleshy fruits, a tropical distribution, and possibly woody growth reduce the risk of extinction. This untangling of multiple factors was possible only with a large number of sister-group comparisons derived from a fairly comprehensive character database and phylogenetic information below the family level.

We attempted a similar analysis for floral nectar spurs, because 6 of 16 sister-group comparisons coincide with a change from actinomorphic to zygomorphic flowers. Comparisons within symmetry classes found even stronger support for the association of spurs with higher diversification rates (Table 17.1). Although these specific tests involve fewer comparisons, they are more robust, because a potentially confounding factor has been excluded.

More detailed phylogenetic information would enhance the study of diversification in several other ways. With more complete taxon sampling and information on the timing of lineage divergence, more powerful methods can be used to detect changes in diversification rates. Sanderson and Donoghue (1994) developed a maximum-likelihood approach that employs the diversities of three branches of a clade and determines which models of changes in diversification best fit these diversities. Wollenberg *et al*. (1996) proposed a method for comparing branching patterns in empirical trees to those generated by a stochastic model of speciation and extinction. Ree (2005) also proposed using stochastic models of speciation, but allowed for uncertainty in the tree topology and for multiple gains and losses of the putative key innovation. As yet, such analyses tend to be performed on specific groups for which the necessary phylogenetic information is available (e.g., spur evolution in *Halenia* described above). More detailed tests, such as these, performed on multiple groups that have evolved the same trait would be especially useful for exposing how particular traits affect the timing and tempo of diversification.

Last, tests of a key-innovation hypothesis can focus on whether a particular trait actually affects speciation and/or extinction. For example, Fulton and Hodges (1999) showed that aspects of nectar spurs in *Aquilegia* (spur length and orientation) affect pollinator visitation and pollen removal (and therefore, presumably, pollen dispersal) and Hodges *et al*. (2004) showed that nectar spur colour affects pollinator visitation. Such studies link variation in nectar spurs directly to pollinator behaviour and reproductive isolation. Several other studies suggest that nectar spurs affect pollinator visitation or pollen dispersal, including studies of orchids (Nilsson 1988; Johnson and Steiner 1997) and *Epimedium* (Suzuki 1984). More such studies are needed, particularly between sister species, to test fully how proposed key innovations affect speciation or extinction. Studies such as these are particularly amenable in hybrid zones (Chapter 18) between species that differ in the trait of interest.

Our review also revealed the remarkable lack of knowledge about the floral biology of most plant species and, therefore, the need for more studies of floral biology. This lack of information hindered our tests of hypothesized associations of floral traits with diversification rates. For example, we found no information on the dominant mode of pollination for over 30% of plant families (121 out of 379). This paucity of information probably resulted in fewer independent contrasts in our data set, inaccurate estimates of species numbers in some sister clades, and misidentification of some sister-group relationships. Although these problems need not have biased our tests in favour of increased diversification, we note again how new information can alter the interpretation of associations with diversity, as we found for zygomorphy. The Eriocaulaceae illustrate this point. Although this family is listed as having either

biotic or abiotic pollination (Watson and Dallwitz 2005), the pollination system of the family was first reported only recently, for two species of *Syngonanthus* (Ramos *et al*. 2005). This study found clear evidence of animal pollination in these species, despite contrary predictions of other authors, and suggests that other species that have been considered abiotically pollinated in the family may actually be animal pollinated as well. Future studies targeting families with poorly known pollination systems and sexual systems will be especially fruitful avenues for research.

We conclude that there is strong evidence that a number of floral characters have affected the species diversity of many angiosperm lineages. However, determining how these characters may have stimulated these changes remains elusive, and these characters can explain only some of the numerous shifts in diversification rates that have been detected during angiosperm evolution (Davies *et al*. 2004). Thus, other floral characters must also be considered with comparative, detailed phylogenetic, and population studies. For example, exploration of the effects of other sexual systems on diversification will probably be a fruitful avenue of research. Many authors have suggested that the evolution of self-pollination is an evolutionary dead-end leading to extinction (Barrett *et al*. 1996; Schoen *et al*. 1997) and self-incompatibility would be expected to have similar effects to dioecy, though no effect has been detected in sister-group analysis (Heilbuth 2000). Other floral structures and features that may enhance specific pollinator visitation and therefore specialization include the evolution of tubular flowers, and specific floral attractants and rewards, such as fragrances and oils. Thus, future comparative research aimed at understanding the evolutionary dominance and diversity of the angiosperms will provide many fruitful avenues for investigating the ecology and evolution of flowers.

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