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A phylogenetic analysis of pollination mode and the evolution of dichogamy in angiosperms

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ABSTRACT

Dichogamy, the temporal separation of male and female function, is widespread among angiosperms, yet its causes and consequences are not well understood. Two forms of dichogamy exist: protandry, in which pollen dispersal precedes stigma receptivity, and protogyny, in which the reverse occurs. Species-level comparative studies show that protandrous species tend to be pollinated by bees or flies, whereas protogynous species tend to be wind- or beetle-pollinated. This suggests a functional role for pollinators in the evolution of dichogamy. We mapped dichogamy and pollination characters onto a phylogeny of angiosperm species. Using the program Discrete, two models of evolutionary change (one allowing only for independent evolution and the other allowing correlated evolution of the two traits) were fit to the phylogeny to test for correlations. Log-likelihood ratio tests and Monte Carlo simulations support a correlated model for the evolution of the type of dichogamy and the form of pollination, demonstrating that the previously reported correlations are robust to phylogenetic correction. However, pollination mode was not found to affect transitions between protandry and protogyny. Rather, an examination of transition rates revealed that the rate of transitions between biotic and abiotic pollination depends on whether a species is protandrous or protogynous. Additionally, we found more support for a role of pollination in the evolution of protogyny from non-protogyny than in the evolution of protandry from non-protandry. This study calls into question some previous findings regarding the role of pollinators in the evolution of dichogamy.

Keywords: correlated evolution, dichogamy, Discrete, pollinator, protandry, protogyny.

INTRODUCTION

Dichogamy has long been considered a mechanism that prevents inbreeding in hermaphrodite plants (Darwin, 1877; Faegri and van der Pijl, 1978). Recent studies suggest that the function of dichogamy may entail more than inbreeding avoidance (Lloyd and Webb, 1986; Griffin *et al.*, 2000; Harder *et al.*, 2000; Fetscher, 2001; Mallick, 2001; Routley and Husband, 2003). Here we examine the evidence for correlated evolution between the form of dichogamy and pollination mode in angiosperms.

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Previous studies have suggested a role for pollinator-mediated selection in the evolution of dichogamy (Wyatt, 1983; Barrett, 2003). Indeed, three comparative studies have noted a correlation across species in the mode of pollination and the form of dichogamy. A general survey of angiosperms (Bertin and Newman, 1993) and a survey of British flora (Lloyd and Webb, 1986) found protandry to be more prevalent than protogyny and observed that protandry was more common in biotically pollinated systems and protogyny more common in wind-pollinated systems. The prevalence of protandry is consistent with the hypothesis that the evolution of protogyny is subject to a developmental constraint, as it requires a reversal in the usual order of the development of floral organs (Waller, 1988). However, aspects of reproductive function may also affect the incidence of alternate forms; Wyatt (1983) found protogyny to be twice as common as protandry among species pollinated by wind, beetles and wasps as opposed to bees and flies.

Protogyny is hypothesized to be an ancestral trait in angiosperms (Endress, 1997), as it tends to be clustered in basal angiosperm families (Henslow, 1888; Bawa and Beach, 1981; Thien *et al.*, 2000). Protogyny is widely thought to have evolved as a means of preventing self-pollination (Lloyd and Webb, 1986; Bertin, 1993). In a protogynous flower, ovules may be fertilized by pollen from other sources before the flower presents its own pollen (Bawa and Beach, 1981; Bertin, 1993; Griffin *et al.*, 2000). With protogyny, ovules that remain unfertilized by external sources can be fertilized by self-pollen, assuring some fertilization for self-compatible species (Herlihy and Eckert, 2002). Because pollen can remain viable for a period of time in many species, protandry would appear to be less effective as an adaptation to avoid self-pollination within flowers (Bertin, 1993; Barrett, 2003). Rather, protandry, when combined with particular inflorescence architectures and stereotypical pollinator behaviour, may be an adaptation that reduces self-pollination among flowers (geitonogamy) and the associated reduction in pollen available for export (Jordan, 2000; Harder *et al.*, 2001; Routley and Husband, 2003; for an exception, see McKone *et al.*, 1995).

The occurrence of both forms of dichogamy correlates with a variety of ecological conditions (such as latitude and altitude), mating system, pollination mode and flower size (for a review, see Bertin and Newman, 1993). Ecological correlates of protandry and protogyny are difficult to interpret, because both traits also have strong associations with certain plant families, so that many of the patterns described above appear to be at least partly explained by phylogenetic relatedness. In several cases, one form of dichogamy appears to be characteristic of an entire family. For example, protogyny is more common than protandry in monocots (Lloyd and Webb, 1986).

Previous studies of the correlates of dichogamy have used species as the unit of comparison, without formal consideration of the evolutionary relationships among these species. Comparative analyses examining correlated characters across species can pose problems because statistical tests rely on the assumption that observations (data points) are independent. However, data obtained from related species are not independent because the species may have inherited the trait from a common ancestor, rather than having evolved the trait independently, as comparative tests assume (Felsenstein, 1985).

Here we test whether transitions between forms of dichogamy evolve in association with specific modes of pollination after accounting for phylogenetic relatedness. We use Pagel's (1994) maximum likelihood program (Discrete) to test the direction of transitions between protandry, protogyny and adichogamy and between biotic and abiotic pollination. In addition, we introduce a new application of Discrete that allowed us to test the hypothesis that pollination mode drives the evolution of dichogamy rather than dichogamy driving the evolution of pollination mode.

MATERIALS AND METHODS

To test for across-species associations of traits requires a phylogeny, preferably one that is well resolved (Pagel, 1994). We used Soltis and colleagues' (2000) phylogeny (their figures 1–12) of relationships among angiosperm taxa. For each hypothesis tested, we pruned the tree according to the species for which we had the necessary data. We then mapped character data for both traits (i.e. pollination mode and dichogamy type) onto the tree. Although this study involves an incomplete sampling of angiosperm taxa, Discrete does not require a complete phylogeny, because it infers the most likely transition rates along those branches that are included in the phylogeny of sampled taxa. As long as the taxa are sampled randomly with respect to the traits of interest (as we expect to be true in this study), incompleteness of a phylogeny does not provide evidence for a correlation if, in fact, the traits have evolved independently. If, on the other hand, the traits truly evolved in a correlated manner, the power to detect this correlation is reduced by using a less complete phylogeny.

Data collection

We collected dichogamy and pollinator data for as many species from the angiosperm phylogeny of Soltis *et al.* (2000) as we could find. Much of the data came from a database kindly provided by R. Bertin. The remaining data were found through literature searches. When data for these species were unavailable, we substituted data from closely related species whose phylogenetic position is expected to be similar and for which data were available ($n = 8$). The substituted species were identified using genus- and family-level phylogenies (see the Appendix). If no family phylogeny or dichogamy/pollination data existed for a species within the same family, the species was omitted from the analysis. Depending on the set of characters being tested, different versions of the 'pruned' tree were used to test hypotheses. Of the 560 angiosperm species used to build Soltis and colleagues' (2000) tree, we obtained sufficient data for 170 species (Appendix).

Testing for correlated evolution

We used Discrete (Pagel, 1994) to test for correlated evolution between type of dichogamy and pollination mode. Discrete uses maximum likelihood to estimate instantaneous rates of evolution between combinations of states (Fig. 1). The method estimates transition rates between traits with two discrete states using a continuous-time Markov model. Because the model calculates transition probabilities across all possible character states at each node, hypothesis testing does not require the assignment of ancestral states, which are often difficult to infer (Schluter *et al.*, 1997).

Discrete calculates the likelihood of two models of evolutionary change for the traits. The four-parameter independent transition model assumes that the two traits (type of dichogamy and pollination mode) evolve independently. We used a modified independent model that did not allow simultaneous changes in both traits but assumed that the

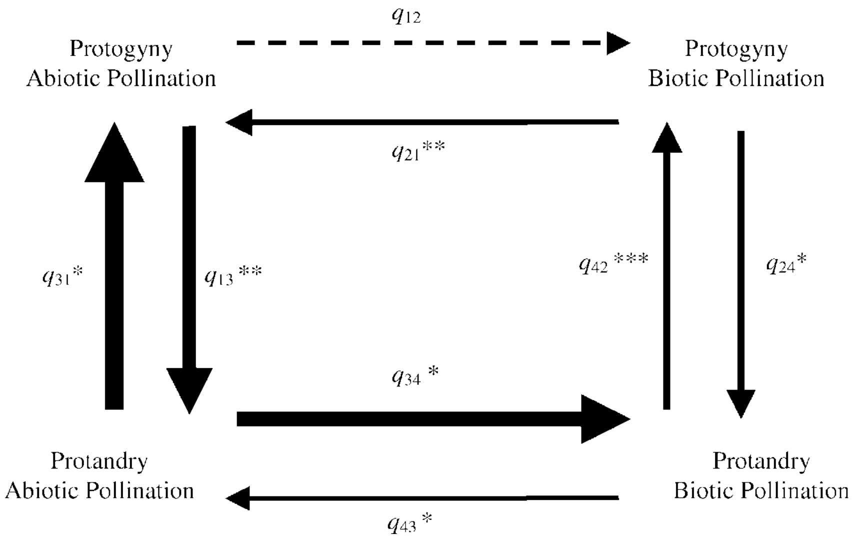

Fig. 1. Transition rates between two forms of dichogamy and two types of pollinators. The q_{ij}'s indicate transition rate parameters; in this example, 1 = protogyny, abiotic pollination; 2 = protogyny, biotic pollination; 3 = protandry, abiotic pollination; 4 = protandry, biotic pollination. Dashed lines indicate transition rates that are not significantly different from zero ($P > 0.1$). Line thickness indicates the $\log_e$ of the relative size of the transition rates in the full (LD_8) dependent model with the highest likelihood ratio. Asterisks indicate that the transition rate differs from zero: * marginal significance ($P < 0.1$); ** significance at $P < 0.01$; *** significance at $P < 0.001$.

transition rates between states of trait X are independent of the state of trait Y and vice versa (Fig. 1: $q_{12} = q_{34}$; $q_{21} = q_{43}$; $q_{42} = q_{31}$; $q_{13} = q_{24}$), leaving four parameters. The subscripts for the transition rates indicate the beginning and end states for the X (dichogamy type) and Y (pollination mode) traits, where 1 = 0,0; 2 = 0,1; 3 = 1,0; 4 = 1,1. Discrete identifies the most likely parameter combination consistent with the observed traits of the species, yielding a log-likelihood estimate, $L(I_4)$, for the independent model. The eight-parameter dependent transition model involved no restriction on transition rates, yielding a log-likelihood estimate, $L(D_8)$. The likelihood ratio, ($LR = 2(L(D_8) - L(I_4))$), measures the extent to which the dependent model fits the data better than the independent model. The significance of the likelihood ratio can be tested either by comparing it to the χ^2 distribution (d.f. = 4) or by Monte Carlo simulation. The search for a maximum likelihood parameter combination was repeated a minimum of 20 times to decrease the chance of accepting a local rather than a global maximum likelihood.

Testing for directionality of the correlation

Here we introduce a new method that uses the transition rates estimated by Discrete to detect directionality in the correlated evolution of two traits. In its original version (Pagel, 1994), Discrete tested for directionality by fixing two transition rates equal to each other (e.g. $q_{12} = q_{34}$) and determining whether this restriction significantly reduced the log-likelihood estimate $L(D_7)$ compared with that of the unrestricted model $L(D_8)$. If the likelihood ratio test finds that the restricted model differs significantly from the full model, the alternative hypothesis is accepted (i.e. $q_{12} \neq q_{34}$), which is interpreted to mean that the state of trait X influences the direction of evolution of trait Y (Pagel, 1994). However, it is possible that the rate (the frequency of transitions between the two states) rather than the direction of transitions is influenced by the other trait. For example, imagine that transitions from wind pollination to biotic pollination along protandrous branches occur at a much higher rate than along protogynous branches (e.g. $q_{34} = 10$ and $q_{12} = 1$). One could interpret this to mean that selection for biotic pollination is higher with protandry than with protogyny. However, if the reverse transition rate, biotic pollination to wind pollination, is also higher with protandry than with protogyny (e.g. $q_{43} = 10$ and $q_{21} = 1$), then the above interpretation would be incorrect. Instead, one should conclude that form of dichogamy does not influence the direction of evolution of pollination mode, but that protandry causes transitions in both directions to occur at a higher rate than protogyny.

To account for the possibility that transition rates are affected by the state of the other trait, Pagel modified Discrete for us to test for directionality in evolution using a rate contingency test. Specifically, under the null hypothesis that trait X (e.g. form of dichogamy) does not influence the direction of evolution of trait Y (e.g. pollination mode), we expect $q_{12}q_{43} = q_{21}q_{34}$ even if trait X influences the rate of transitions among states of Y (Fig. 1). Similarly, one can test whether trait Y influences the direction of evolution of trait X by restricting $q_{13}q_{42} = q_{31}q_{24}$. Note that this reduces the four directionality tests described by Pagel (1994) to two rate contingency tests.

We also tested the importance of individual transition rate values (q_{ij}) to the overall likelihood of the dependent model by setting each parameter to zero, rerunning the model, and determining whether this restriction significantly reduced the log-likelihood estimate $L(D_7)$ when compared with that of the unrestricted dependent model $L(D_8)$.

Hypothesis testing

The motivation for this study was to test for a correlation between dichogamy and pollination mode while controlling for phylogenetic relatedness among species. The Discrete program tests for correlated evolution between traits with two discrete categories only. Unfortunately, the traits of interest, mode of pollination and dichogamy, do not easily fall into dichotomous categories. For example, species may be protandrous, protogynous, heterodichogamous (populations consisting of both protandrous and protogynous individuals) or adichogamous (flowers lacking appreciable temporal separation of anther and stigma presentation). Because of this constraint, we used Discrete to test a series of hypotheses that considered different groupings of species. For each hypothesis, different subsets of the data shown in the Appendix were used. Where correlated evolution was detected between two traits, we tested the importance of individual transition rates as described above and in Table 4.

1. Protandry versus protogyny and biotic versus abiotic pollination

The species for which dichogamy and pollination data were available (Appendix) were identified as protandrous or protogynous species. The mode of pollination for these species was determined to be primarily biotic (bee, beetle, bird, fly, mammal, moth or wasp) or abiotic (wind or, in one case, water). Because some of the species were adichogamous, heterodichogamous or obligate selfers, the sample size for this test was reduced to 126.

2. Protandry versus non-protandry and biotic versus abiotic pollination

Species were divided into those that displayed protandry and those that were adichogamous, protogynous or heterodichogamous. Species were placed into the pollinator categories biotic or abiotic (described previously). The sample size for this test was 167 (excluding autogamous species).

3. Protogyny versus non-protogyny and biotic versus abiotic pollination

Species were divided into those that displayed protogyny and those that were adichogamous, protandrous or heterodichogamous. Species were placed into the pollinator categories biotic or abiotic (described previously). The sample size for this test was also 167 (excluding autogamous species).

4. Dichogamy versus adichogamy and bird/autogamous versus other pollination

Species were divided into those that displayed dichogamy (including heterodichogamy) and those that were adichogamous. Pollination categories were grouped into two categories: species that are bird-pollinated or obligately autogamous and other species (insect-, mammal- or wind-pollinated). Obligate autogamy has been hypothesized to be associated with adichogamy (Runions and Geber, 2000). Similarly, bird pollination is not as effective at reducing geitonogamy in dichogamous species (Harder *et al.*, 2001), and thus selection for dichogamy could be reduced if a species experiences a change from insect to bird pollination (see Discussion). The sample size for this test was 170.

RESULTS

Because all previously reported correlations are based on samples of angiosperm taxa that differ from our own, we first examined whether dichogamy was associated with pollination mode within our data set regardless of phylogenetic considerations. As in previous studies, protogyny was more common among species exhibiting abiotic (primarily wind) pollination and protandry was more common among biotically pollinated species (Table 1, $\chi^2 = 15.1$, d.f. = 1, $P < 0.001$).

1. Protandry versus protogyny and biotic versus abiotic pollination

A likelihood ratio test found that dichogamy type (protandry or protogyny) and pollination mode (biotic or abiotic) did not evolve independently (Table 2, $P < 0.001$). Monte Carlo simulations (100 replicates) confirmed that a model of correlated evolution fits the data better ($P = 0.01$). The transition rates predict that protogyny and abiotic pollination should become positively associated over time (as they are in the raw data; Table 1), as should protandry and biotic pollination. We found no effect of pollination mode (biotic or abiotic) on the direction of evolution of the type of dichogamy (protandry or protogyny; Table 3, $P = 0.15$). In contrast, dichogamy type (protandry or protogyny) affected the direction of evolution of pollination mode ($P = 0.018$). Specifically, the transition rates from abiotic to biotic pollination along protandrous branches (q_{34}) and from biotic to abiotic pollination along protogynous branches (q_{21}) were significantly higher than expected from the opposite transition rates (Table 3, Fig. 1). Table 4 shows the likelihood of the eight possible alternative models when compared with the dependent model. The phylogeny examined

Table 1. Type of pollination and direction of dichogamy for species used in the current study and by Bertin and Newman (1993)

	Protandry[a]	Protogyny[a]	Adichogamy[a]	Heterodichogamy[a]	Protandry[b]	Protogyny[b]
Abiotic	2	12[c]	1	3	17	133
Insect	62	43	27	3	865	437
Bird	3	1	5	1		
Autogamy	0	0	3	0		
Mammal	1	2	1	0		
Total	68	58	37	7	882	570

[a] Species from the current study. [b] Species taken from Bertin and Newman 1993. [c] All of these species are wind-pollinated except *Zostera marina*, which is water-pollinated.

Table 2. Results from tests of independence of two characters using Discrete

Test	LI_4	LD_8	LR (P)	P
Protogyny/protandry *vs* abiotic/biotic	−110.8	−101.4	18.8 (<0.001)	0.01
Protandry/other *vs* abiotic/biotic	−151.3	−149.0	4.5 (0.34)	0.56
Protogyny/other *vs* abiotic/biotic	−134.5	−128.9	11.2 (0.024)	0.02
Dichogamy/adichogamy *vs* bird/other	−134.5	−129.1	10.7 (0.03)	N/A

Note: P-values for likelihood ratio tests (LR) are based on a χ^2 distribution with d.f. = 4. P-values for Monte Carlo simulation are based on 100 replicates.

provides the least evidence for transitions from abiotic to biotic pollination among protogynous species (Fig. 1, Table 4).

2. Protandry versus non-protandry and biotic versus abiotic pollination

We were unable to reject the hypothesis that protandry versus non-protandry evolved independently of pollination mode (biotic or abiotic; Table 2, $P = 0.34$). Monte Carlo simulations (100 replicates) confirmed that a correlated model of evolution fails to fit the data better ($P = 0.56$).

3. Protogyny versus non-protogyny and biotic versus abiotic pollination

Protogyny versus non-protogyny did not evolve independently from pollination mode (biotic or abiotic; Table 2, $P = 0.025$). Monte Carlo simulations (100 replicates) confirmed that a model of correlated evolution fits the data better ($P = 0.02$). The transition rates predict that protogyny and abiotic pollination should become positively associated over time (as they are in the raw data; Table 1), as should non-protogyny and biotic pollination. A rate contingency test (Table 3) found no evidence that protogyny versus non-protogyny affects the direction of the evolution of pollination mode (biotic or abiotic) or that pollination mode (biotic or abiotic) affects the direction of evolution of dichogamy.

4. Dichogamy versus adichogamy and bird/autogamous versus other pollination

Adichogamy versus dichogamy did not evolve independently of pollination mode (bird/autogamy versus other pollination modes; Table 2, $P = 0.03$). The rate contingency tests

Table 3. Comparison of transition rates between type of pollination and form of dichogamy

Test	Hypothesis	*LR*	*P*	Description
Protogyny/protandry *vs* abiotic/biotic	$\frac{q_{12}}{q_{21}} \neq \frac{q_{34}}{q_{43}}$	5.56	0.018 $\frac{q_{12}}{q_{21}} < \frac{q_{34}}{q_{43}}$	Dichogamy type (protandry (1) or protogyny (0)) affects the direction of evolution of pollination mode (biotic (1) or abiotic (0))
	$\frac{q_{13}}{q_{31}} \neq \frac{q_{24}}{q_{42}}$	2.06	0.15	Pollination mode (biotic or abiotic) has no effect on the direction of evolution of dichogamy type (protandry or protogyny).
Protogyny/ adichogamy *vs* abiotic/biotic	$\frac{q_{12}}{q_{21}} \neq \frac{q_{34}}{q_{43}}$	0.38	0.54	Dichogamy type (non-protogyny (0) or protogyny (1)) has no effect on the direction of evolution pollination mode (biotic (0) or abiotic (1))
	$\frac{q_{13}}{q_{31}} \neq \frac{q_{24}}{q_{42}}$	1.18	0.28	Pollination mode (biotic or abiotic) has no effect on the direction of evolution of dichogamy type (non-protogyny or protogyny)
Dichogamy/ adichogamy *vs* bird/other	$\frac{q_{12}}{q_{21}} \neq \frac{q_{34}}{q_{43}}$	2.1	0.15	Presence of dichogamy (adichogamy (1) or dichogamy (0)) has no effect on the direction of evolution of the pollination system (bird/autogamy (0) or other (1))
	$\frac{q_{13}}{q_{31}} \neq \frac{q_{24}}{q_{42}}$	2.1	0.15	Pollination mode (bird/autogamy or other) has no effect on the direction of evolution of dichogamy (adichogamy or dichogamy).

Note: The null hypothesis is that the specified transition rates are equal. The test statistic (e.g. $-2(L(D_8) - L(q_{ab}/q_{ba} = q_{cd}/q_{dc}))$ has an approximate chi-square distribution with one degree of freedom. *LR* = likelihood ratio.

suggested that the type of pollination affected the direction of evolution of dichogamy more than the converse, but neither test was significant (Table 3). The transition rates predict that adichogamy and bird pollination should become positively associated over time (as they are in the raw data; Table 1), as should dichogamy and other pollination modes. Note that when the correlations are tested separately (i.e. bird versus other pollination modes or autogamy versus other pollination modes), neither correlation is significant, reflecting a loss of power.

DISCUSSION

Associations between dichogamy and pollination appear to be the product of convergent evolution rather than artefacts of phylogenetic relatedness. Tests revealed that the pollination mode (abiotic or biotic) in angiosperms was evolutionarily correlated with the type of dichogamy (protandry or protogyny). Specifically, species that are abiotically pollinated

Table 4. Likelihood values for models in which one transition rate is excluded, compared with the likelihood of the full eight-parameter model of dependent evolution between dichogamy (protandry or protogyny) and pollinator type (biotic or abiotic)

Model description	$L(D_7)$	Likelihood ratio	P
$q_{12} = 0$	101.7	0.440	0.5
$q_{13} = 0$	105.0	7.12	0.008**
$q_{21} = 0$	104.8	6.72	0.01**
$q_{24} = 0$	103.1	3.26	0.07*
$q_{31} = 0$	103.3	3.62	0.06*
$q_{34} = 0$	104.0	5.24	0.02*
$q_{42} = 0$	107.2	11.5	< 0.001***
$q_{43} = 0$	103.1	3.32	0.07*

Note: Asterisks indicate level of significance (see Fig. 1).

(primarily via wind) are more likely to be protogynous, whereas biotically pollinated species are more likely to be protandrous, confirming the results of previous studies (Wyatt, 1983; Lloyd and Webb, 1986; Bertin and Newman, 1993). However, only the evolution of protogyny versus non-protogyny correlates with pollination mode, with protogyny being strongly correlated with abiotic pollination (Table 1, Fig. 2). We found that the proportions of biotically and abiotically pollinated species are similar among protandrous and adichogamous species (Table 1, Fig. 2). It is therefore not surprising that the association between abiotic pollination and protogyny is strong when contrasted against adichogamous and protandrous species, whereas the association between biotic pollination and protandry disappears when contrasted against adichogamous and protogynous species. Several previous studies compared only protandrous and protogynous species, and did not compare either to adichogamous species (e.g. Wyatt, 1983; Lloyd and Webb, 1986). Bertin and

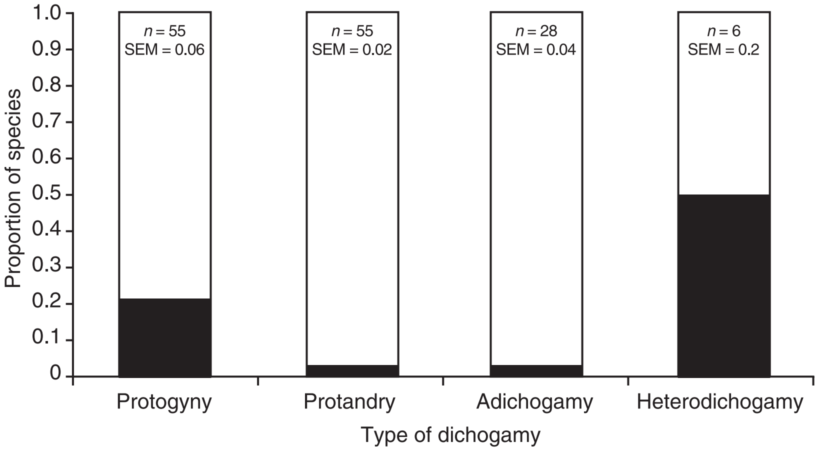

Fig. 2. Proportion of species from the Appendix with abiotic (solid portion) and biotic (open portion) pollination as a function of the form of four types of dichogamy. SEM = standard error of the mean.

Newman (1993) calculated the mean degree of dichogamy (using a continuous scale ranging from protandry through adichogamy to protogyny) for species with different types of pollinators and determined that, on average, insect-pollinated species fell between protandry and adichogamy, because insect-pollinated species frequently fall into both categories. This is consistent with our results that transitions from non-protandry to protandry do not depend on pollination mode, in contrast to transitions from non-protogyny to protogyny.

The tests of the causes of the correlations reveal new information about the evolutionary forces acting on dichogamy. We found strong support for correlated evolution between protogyny and abiotic pollination. This correlation is most often explained by strong selection for protogyny in wind-pollinated taxa to prevent self-fertilization (Lloyd and Webb, 1986; Barrett, 2003). However, we found no evidence that either biotic or abiotic pollination drives increased transitions from protogyny to non-protogyny or the reverse. This could be due to reduced power to detect the cause of correlated evolution because of our use of an incomplete phylogeny. The only significant contingency test found that transitions from biotic to abiotic pollination were more likely among protogynous species than among protandrous species. One explanation for this result is that protogynous species are perhaps less likely than protandrous species to suffer from increased self-fertilization upon a switch to wind pollination.

The results of the rate contingency test are not, however, robust to the inclusion/exclusion of different sub-samples of species. In particular, we tested whether the removal of *Zostera*, the only water-pollinated species in our analysis, altered the result that transitions from biotic to abiotic pollination were more likely among protogynous species than among protandrous species. We found that the result was sensitive to the inclusion of *Zostera* and was no longer significant when *Zostera* was excluded. Rather, in the absence of *Zostera*, there was significant evidence for the hypothesis that the state of the pollination system affects which type of dichogamy evolves. The reason that the results of the rate contingency test are particularly sensitive to the presence of *Zostera* might be because *Zostera* is an abiotically pollinated, protogynous species that falls in a large clade of biotically pollinated, protogynous species. Its phylogenetic position thus shifts the evidence in favour of the hypothesis that the form of dichogamy drives the pollination system, rather than the reverse. In contrast to the results of the rate contingency test, there was significant evidence for correlated evolution regardless of whether or not *Zostera* was included in the analysis.

The association between bird pollination or autogamy and adichogamy provides evidence that the evolutionary interaction between dichogamy and pollination mode is specific to particular types of pollinators and/or floral forms. The movement patterns of bird pollinators on inflorescences differ from those of insects. Insects visiting vertical inflorescences tend to consistently start at either the bottom (in the case of many bees, wasps and moths) or top of the inflorescence (flies) and move upwards or downwards, respectively (Harder *et al.*, 2001). In contrast, hummingbirds have been shown to move upwards or downwards on inflorescences with approximately equal frequency (Healy and Hurly, 2001). Because the movement of bird pollinators among early developing and late developing flowers is less stereotypical, selection on dichogamy to reduce self-pollination among flowers on a plant (geitonogamy) is less effective. This reasoning explains, perhaps, why fewer bird-pollinated species are dichogamous. It is also predicted that obligately autogamous plants should reduce the temporal separation in anther and stigma development to increase the probability of self-fertilization (Runions and Geber, 2000). In our study, all three of the obligately autogamous plants were adichogamous. These results should be treated with caution, however, because there were not many bird-pollinated or obligately autogamous plant species within our study, and their association with adichogamy is not significant when investigated separately.

We were unable to detect any effect of pollination mode (abiotic or biotic) on the transition from non-protandry to protandry (or the reverse), suggesting that this transition may not be as dependent on pollination by animals as has been previously hypothesized (Wyatt, 1983; Lloyd and Webb, 1986; Bertin and Newman, 1993). Indeed, in this and previous studies the association between protandry and biotic pollination is weaker than the association between protogyny and abiotic pollination (Table 1, Fig. 2). A possible explanation for a lack of correlation between protandry and biotic pollination is that forcing the data into the false dichotomy of biotic pollination (which included many types of insects as well as mammals and birds) versus abiotic pollination may have obscured the true patterns. For example, selection by beetle pollinators may be as different from that of bee pollinators as they both are from abiotic pollination. Unfortunately, there is no program currently available to detect correlated evolution between characters with more than two states.

One limitation of our methodology is that the tests performed assume that the traits do not affect speciation or extinction rates. However, wind pollination is associated with lower species diversity than biotic pollination (Eriksson and Bremer, 1992; Dodd *et al.*, 1999); if this pattern were due to higher extinction of wind-pollinated and non-protogynous species, extinctions rather than transition rate differences could account for the correlations we found. A further caveat of all correlative studies is that the traits in question may in fact be correlated with other traits that are actually responsible for the observed patterns. Other traits that may be correlated with dichogamy include latitude (e.g. protogynous species tend to be found in alpine zones), breeding system (e.g. protogyny is more common among self-compatible species), floral traits (e.g. short-lived flowers are more likely to be protandrous) and many others (Bertin and Newman, 1993). Clearly, we have omitted several potential correlates by focusing only on pollination system. However, of the correlates listed in a thorough review by Bertin and Newman (1993), one-third are traits related to the pollination system. Indeed, the type of pollinator is widely invoked as part of the functional explanation for the existence and form of dichogamy (Wyatt, 1983; Lloyd and Webb, 1986). Future studies should expand the focus to other candidate traits. A final caveat is that the type of dichogamy has not always held up to experimental validation. Griffin *et al.* (2000) discovered that *Aquilegia canadensis*, a species considered protogynous in the literature, was functionally adichogamous. If researchers are more likely to label wind-pollinated species as protogynous, such a bias could contribute to the correlations observed in this study.

In conclusion, this study has used phylogenetic evidence to confirm the existence of an association between dichogamy and pollination type. Across the phylogeny of angiosperms, we find little support for the hypothesis that the type of pollinator drives selection for either protandry or protogyny. Rather, our results suggest that the form of dichogamy may influence the pollination mode that evolves. We also present results that call into question the validity of previous reports of a correlation between biotic pollination and protandry. Protandry does not appear to be significantly more correlated with biotic pollination systems than all other breeding systems considered together. Instead, the dichogamy type with an unusual pollination mode is protogyny (and perhaps heterodichogamy), which differs from all other forms in being commonly (but not predominantly) found in wind-pollinated systems.

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APPENDIX

Species and traits used for analyses. Numbers in parentheses indicate the number of species in the genus that were found to have the dichogamy and pollinator combination listed out of total number of species in the genus (from Mabberley, 1997). It is possible that more species in the genus fit the pattern; the dichogamy data (in particular) are difficult to find and thus the values should be interpreted as the minimum for the genus. † Indicates species that were substituted for species on Soltis and colleagues' (2000) tree; original species is indicated in parentheses.

Family	Species	Type of dichogamy	Primary pollinator
Acanthaceae	*Justicia squarrosa*	Protandry	Bee
	Thunbergia grandiflora	Adichogamy	Bee
Agavaceae	*Agave* sp.	Protandry	Insect
Aizoaceae	*Tetragonia expansa* † (*Delosperma echinatum*)	Protogyny	Insect
Alliaceae	*Allium fistulosum*	Protandry	Bee
Amaranthaceae	*Amaranthus retroflexus* † (*Celosia argentea*)	Protogyny	Wind
Annonaceae	*Annona* sp. (11/137)	Protogyny	Beetle
	Asimina triloba	Protogyny	Fly
Apiaceae	*Apium graveolens*	Protandry	Fly
Apocynaceae	*Vinca major* † (*Apocynum androsaemifolium*)	Adichogamy	Fly
	Nerium oleander	Adichogamy	Insect

APPENDIX—*continued*

Family	Species	Type of dichogamy	Primary pollinator
Araceae	*Spathiphyllum friedrichsthalli*	Protogyny	Bee
Araliaceae	*Hedera helix*	Protandry	Insect
	Panax quinquefolius	Protandry	Bee/fly
	Iriartea sp. (2/2)	Protandry	Bee
Arecaceae	*Sabal* sp. (3/16) † (*Phoenix canariensis*)	Protogyny	Insect
Aristolochiaceae	*Asarum canadense*	Protogyny	Fly
	Aristolochia sp.	Protogyny	Fly
Balsaminaceae	*Impatiens capensis*	Protandry	Bee
Begoniaceae	*Begonia* sp. (3/900)	Protandry	Insect
Betulaceae	*Alnus* sp. (2/25)	Protogyny	Wind
Bignoniaceae	*Campsis radicans*	Protandry	Bird
	Catalpa speciosa	Adichogamy	Insect
Blandfordiaceae	*Blandfordia grandiflora*	Protandry	Bee
Boraginaceae	*Hydrophyllum appendiculatum*	Protandry	Bee
	Borago officinalis	Protandry	Bee
Brassicaceae	*Brassica* sp. (5/35)	Protogyny	Bee/fly
Bromeliaceae	*Aechmea lasserii*	Adichogamy	Bird
	Tillandsia ixioides † (*Glomeropitcairnia penduliflora*)	Protandry	Bird
	Pitcairnia altensteinii † (*Puya raimondii*)	Adichogamy	Insect
Buxaceae	*Buxus sempervirens*	Protogyny	Bee/fly
Cabombaceae	*Brasenia schreberi*	Protogyny	Wind
	Cabomba caroliniana	Protogyny	Insect
Calycanthaceae	*Calycanthus floridus*	Protogyny	Beetle
	Chimonanthus fragrans † (*Idiospermum australiense*)	Protogyny	Beetle
Campanulaceae	*Campanula trachelium*	Protandry	Insect
	Lobelia angulata	Protandry	Bee
Caprifoliaceae	*Sambucus nigra*	Adichogamy	Insect
	Symphoricarpos alba	Adichogamy	Insect
	Viburnum opulus	Adichogamy	Insect
Caryophyllaceae	*Stellaria media*	Protandry	Fly
Celastraceae	*Euonymus europaeus*	Protandry	Fly
Chloranthaceae	*Chloranthus spicatus*	Protogyny	Thrips
	Sarcandra sp. (2/2)	Protogyny	Thrips
Chrysobalanaceae	*Maranthes polyandra* † (*Licania tomentosa*)	Protandry	Insect
Cistaceae	*Helianthemum* sp. (4/110)	Protogyny	Bee/fly
Clusiaceae	*Hypericum perforatum*	Protandry	Insect
Colchicaceae	*Colchicum autumnale*	Protogyny	Bee/fly
Combretaceae	*Quisqualis indica*	Adichogamy	Moth/bee
Commelinaceae	*Tradescantia virginiana*	Protandry	Bee
Convallariaceae	*Convallaria majalis*	Protandry	Bee

APPENDIX—*continued*

Family	Species	Type of dichogamy	Primary pollinator
Convolvulaceae	*Ipomoea purpurea*	Protogyny	Bee
	Convolvulus arvensis	Heterodichogamy	Insect
Coriariaceae	*Coriaria myrtifolia*	Protogyny	Wind
Costaceae	*Costus spiralis*	Adichogamy	Bird
Cucurbitaceae	*Cucumis sativus*	Protandry	Insect
	Cucurbita pepo	Protandry	Insect
Cyclanthaceae	*Cyclanthus bipartus*	Protogyny	Beetle
	Sphaeradenia hamata	Protogyny	Beetle
Cyperaceae	*Cyperus* sp. (2/300)	Protogyny	Wind
Datiscaceae	*Datisca glomerata*	Protogyny	Wind
Degeneriaceae	*Degeneria vitiensis*	Protogyny	Beetle
Dipsacaceae	*Dipsacus silvestris*	Protandry	Bee/fly
Dipsacaceae	*Scabiosa* sp.	Protandry	Insect
Droseraceae	*Drosera* sp. (2/110)	Adichogamy	Bee/fly
Elaeagnaceae	*Elaeagnus angustifolia*	Protandry	Insect
Elaeocarpaceae	*Elaeocarpus hookerianus*	Protandry	Bee
Ericaceae	*Arbutus unedo*	Protogyny	Insect
Euphorbiaceae	*Euphorbia* sp. (9/2000)	Protandry	Bee/fly
Fabaceae	*Bauhinia ungulata*	Protogyny	Bat
Fagaceae	*Quercus* sp. (5/400)	Protogyny	Wind
Frankeniaceae	*Frankenia* sp. (2/81)	Protandry	Insect
Geraniaceae	*Geranium sanguineum*	Protandry	Bee/fly
Geraniaceae	*Pelargonium* sp. (14/280)	Protandry	Fly
Graminae	*Zea mays*	Heterodichogamy	Wind
Gunneraceae	*Gunnera* sp. (2/40)	Protogyny	Wind
Haloragidaceae	*Myriophyllum exalbescens*	Protogyny	Wind
Heliconiaceae	*Heliconia* sp. (10/~150)	Adichogamy	Bird
Hydrangeaceae	*Philadelphus coronarius*	Protogyny	Insect
Illiciaceae	*Illicium parviflorum*	Protogyny	Fly
Iridaceae	*Gladiolus* sp. (2/195)	Protandry	Bee/fly
Juncaceae	*Juncus effuses*	Protogyny	Wind
Juglandaceae	*Carya* sp. (3/14)	Heterodichogamy	Wind
	Juglans sp. (3/21)	Heterodichogamy	Wind
Lamiaceae	*Clerodendrum thomsoniae*	Protandry	Bee/fly
	Lamium amplexicaule	Adichogamy	Autogamy
Lauraceae	*Cinnamomum camphora*	Protogyny	Insect
Lentibulariaceae	*Pinguicula vulgaris*	Adichogamy	Autogamy
Liliaceae	*Clintonia borealis*	Protogyny	Bee
	Scilla sp. (2/40)	Adichogamy	Bee
Limnanthaceae	*Floerkea proserpinicoides*	Adichogamy	Autogamy
	Limnanthes douglassii	Protandry	Bee
Linaceae	*Linum* sp. (3/36)	Protandry	Bee/fly
Loasaceae	*Caiphora laterita*	Protandry	Bee
Lowiaceae	*Orchidantha inouei*	Adichogamy	Beetle
Lythraceae	*Lythrum salicaria*	Adichogamy	Bee

APPENDIX—*continued*

Family	Species	Type of dichogamy	Primary pollinator
Magnoliaceae	*Magnolia tripetala*	Protogyny	Beetle
Malvaceae	*Theobroma cacao*	Adichogamy	Fly
	Tilia americana	Protandry	Fly
Marantaceae	*Calathea timothei*	Adichogamy	Bird
Meliaceae	*Swietenia mahagoni*	Protandry	Insect
Moraceae	*Ficus* sp. (20/750)	Protogyny	Insect
	Morus alba	Adichogamy	Wind
Musaceae	*Musa acuminata*	Protogyny	Mammal
Myrsinaceae	*Ardisia escallenoides*	Protogyny	Bee
Nelumbonaceae	*Nelumbo lutea*	Protogyny	Insect
Nyctaginaceae	*Mirabilis jalapa*	Protogyny	Insect
Nymphaceae	*Nuphar variegata*	Protogyny	Insect
	Nymphaea odorata	Protogyny	Insect
Oleaceae	*Jasminum* sp. (2/200)	Protandry	Insect
Onagraceae	*Clarkia xantiana*	Protandry	Bee
	Chamerion angustifolium	Protandry	Bee
Orchidaceae	*Cypripedium calceolus*	Adichogamy	Bee
Oxalidaceae	*Oxalis stricta*	Adichogamy	Bee
Paeoniaceae	*Paeonia californica*	Protogyny	Insect
Papaveraceae	*Dicentra spectabilis*	Adichogamy	Bee
Parnassiaceae	*Parnassia palustris*	Protandry	Fly
Passifloraceae	*Passiflora quadrangalis*	Protandry	Bee
Pedaliaceae	*Proboscidea louisianica*	Protandry	Bee
	Sesamum indicum	Adichogamy	Insect
Piperaceae	*Piper nigrum*	Protogyny	Wind
Plantaginaceae	*Plantago lanceolata*	Protogyny	Wind
Plumbaginaceae	*Limonium* sp.	Protandry	Bee
Poaceae	*Oryza sativa*	Protandry	Wind
Polemoniaceae	*Cobaea scandens*	Protandry	Bat
	Gilia sp. (8/25)	Protandry	Bee/fly
	Phlox sp. (5/67)	Protandry	Moth
Portulacaceae	*Portulaca* sp. (2/40)	Adichogamy	Insect
Primulaceae	*Anagallis tenella*	Adichogamy	Insect
	Androsace sp. (4/100)	Adichogamy	Insect
Ranunculaceae	*Coptis trifoliate*	Protandry	Insect
	Ranunculus sardous	Protogyny	Bee/fly
Resedaceae	*Reseda* sp. (2/60)	Protandry	Bee
Rhamnaceae	*Ceanothus thrysfloris*	Protandry	Insect
	Rhamnus frangula	Protandry	Insect
	Trevoa quinquenenia	Protandry	Bee
Rhizophoraceae	*Brugiera exaristata*	Protandry	Bird
Rosaceae	*Prunus persica*	Adichogamy	Insect
Rubiaceae	*Cephalanthus occidentalis*	Protandry	Insect
Rutaceae	*Cneorum pulverulentum*	Adichogamy	Bee/fly
	Citrus paradisi	Protandry	Insect

APPENDIX—*continued*

Family	Species	Type of dichogamy	Primary pollinator
Sapindaceae	*Acer saccharum*	Heterodichogamy	Insect
	Aesculus pavia	Protogyny	Insect
Sapotaceae	*Manilkara bahamaens*	Protogyny	Bird
Sarraceniaceae	*Sarracenia flava*	Adichogamy	Bee
Scrophulariaceae	*Digitalis* sp. (5/19)	Protandry	Bee
	Pedicularis lanceolata	Protandry	Bee
	Scrophularia sp. (25/200)	Protogyny	Wasp
	Verbascum thapsus	Protogyny	Bee
	Veronica beccabunga	Protogyny	Fly
Solanaceae	*Nicotiana tabacum*	Protogyny	Bee
	Solanum sp. (11/1700)	Protandry	Insect
Sparganiaceae	*Sparganium* sp. (2/14)	Protandry	Wind
Sterculiaceae	*Sterculia chica*	Adichogamy	Fly
Staphyleaceae	*Staphylea trifoliate*	Protogyny	Bee/fly
Strelitziaceae	*Phenakospermum henakospermum*	Adichogamy	Bat
	Ravenala madagascariensis	Heterodichogamy	Bird
Stylidiaceae	*Stylidium graminifolium*	Protandry	Bee
Tecophilaeceae	*Cyanella alba*	Protandry	Insect
Thymelaecaeae	*Thymelaea hirsuta*	Protandry	Insect
Tofieldiaceae	*Tofieldia calyculata*	Protogyny	Insect
Trilliaceae	*Trillium* sp. (2/42)	Protandry	Bee
Tropaeolaceae	*Tropaeolum* sp. (2/87)	Protandry	Bee
Urticaceae	*Pilea pumila*	Protogyny	Insect
Valerianaceae	*Valeriana* sp. (2/200)	Protandry	Fly
Velloziaceae	*Barbacenia flava*	Adichogamy	Bird
Verbenaceae	*Phyla incisa*	Adichogamy	Insect
Vitaceae	*Vitis vinifera*	Adichogamy	Insect
Winteraceae	*Drimys brassiliensis*	Protogyny	Insect
Zingiberaceae	*Zingiber*	Heterodichogamy	Insect
Zosteraceae	*Zostera marina*	Protogyny	Water