

# USING PHYLOGENETIC APPROACHES FOR THE ANALYSIS OF PLANT BREEDING SYSTEM EVOLUTION

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■ **Abstract** Until recently, studies of plant reproductive systems have been at the population level, using microevolutionary approaches. The development of cladistic approaches, combined with the emergence of molecular systematics, has resulted in an explosion of phylogenetic studies and an increase in interdisciplinary approaches combining ecological and systematic methodology. These new approaches offer the possibility of testing explicit hypotheses about the number of evolutionary transitions in reproductive characters and the evolutionary relationship of these characters to changes in the environment. Character mapping may be especially useful for detecting convergent evolution. In a number of cases, character mapping has provided new insights into the evolution of plant breeding systems and pollination biology, especially in suggesting the number of times evolutionary transitions have taken place, indicating where there have been reversals and suggesting when preadaptation has been important. The insights provided by character mapping are determined by a number of factors, including the degree of confidence in phylogenies underlying these studies and the identification of appropriate outgroups. Assumptions about character coding, character ordering, inclusion vs. exclusion of characters that are mapped on trees in the data matrix, and weighting of characters will have profound effects on interpretation of character evolution. Highly labile characters that evolve frequently and have the potential to undergo reversals may make it difficult to detect the pattern of character evolution. Characters that are very strongly correlated with each other or with ecological shifts may make prediction of cause and effect using phylogenetic approaches difficult because changes in characters and ecological shifts will occur, apparently simultaneously, on the same branches. Results from microevolutionary studies have been used in several cases to weight transitions, suggesting that results of phylogenetic studies may not provide fully independent assessments of character evolution. While not a simple cure to understanding problems that have been studied only in the realm of microevolutionary studies, phylogenetic approaches offer clear potential for providing new insights for evolutionary studies.

## INTRODUCTION

It is only recently that phylogenetic approaches have been used to address questions about the evolution of plant reproductive systems and plant-pollinator interactions. Although many population-level studies have been comparative in nature, fewer attempts have been made to incorporate detailed historical analysis of characters important to population biologists. Conversely, systematic treatments and monographs have rarely treated information on plant reproductive systems in a manner permitting explicit historical analysis.

The development of cladistic approaches that define monophyletic groups on the basis of shared, derived character states, combined with the emergence of molecular systematics, has resulted in an explosion of phylogenetic studies and an increased awareness of the need for interdisciplinary approaches combining ecological and systematic methodology (6, 7, 9, 19, 39, 52, 66). These phylogenetic approaches offer the hope of understanding the historical context for the evolution of character evolution, including plant reproductive systems. For example, it may be possible to determine the number of times a character state evolves and whether the character state evolves in response to ecological shifts hypothesized as causative in character evolution. The potential for understanding both the number of times a character state has evolved and the timing of the evolution of that character state relative to environmental shifts is a powerful complement to population-level studies, which provide detailed information but are necessarily limited to one or a few populations, often of a single species. The purpose of this review is to determine how character mapping using phylogenetic trees has been used for the analysis of plant breeding system evolution and plant-pollinator interactions. The assumptions and difficulties associated with the use of character mapping are discussed, using examples from plant reproductive systems, including pollination mechanisms (Table 1). A second goal is to determine how these macroevolutionary approaches have extended our understanding of plant reproductive biology beyond the insights derived from studies of microevolutionary processes. The potential for using phylogenetic studies to guide population level studies is also examined. Several examples of insights from these approaches are addressed in detail, and factors contributing to successful use of character mapping are summarized.

## THE NECESSITY FOR HISTORICAL APPROACHES

Felsenstein (26) recognized that analyses correlating traits of organisms with each other or with ecological features suffered from a potential lack of independence. A single evolutionary transition in the ancestor of several species would produce a pattern of similarity among the descendent species that would confound attempts to understand the evolutionary significance of relationships. For example, if a shift to a novel environment occurred in a lineage at the same time a new

**TABLE 1** Case studies where phylogenetic approaches have been used to reconstruct the evolutionary histories of diverse reproductive systems and plant-pollinator interactions. Case studies are organized by the type of reproductive system or plant-pollinator interaction and are listed alphabetically within categories by family

<b>Genus or lineage</b>	<b>Type of data (morphological, molecular, or combination of both)</b>	<b>Were characters that were mapped included or excluded from the data matrix?</b>	<b>Conclusions based on character mapping</b>	<b>Reference</b>
<i>Androdioecy</i> <i>Datisca</i> (Datisaceae)	Molecular data	Characters not included	Conclusions about the evolution of androdioecy (occurrence of staminate and hermaphroditic individuals in populations) varied greatly depending on molecular data set used to reconstruction phylogeny.	56, 70
Dioecy: <i>Schiedea</i> and <i>Alsindendron</i> (Caryophyllaceae)	Morphological and molecular data	Both	Dimorphism (gynodioecy, subdioecy and dioecy) hypothesized to evolve 1–6 times depending on character coding and whether reproductive system was ordered. Inclusion of reproductive system in data matrix had little effect on results, although inclusion of characters related to dioecy resulted in many more equally parsimonious trees.	60, 67, 75, 81
<i>Lepechinia</i> (Lamiaceae)	Morphological	Both	When breeding system character was included in the data matrix, dioecy hypothesized to evolve on two occasions. When the character was excluded, dioecy hypothesized to evolve on four occasions.	37, 38

(continued)

TABLE 1 (continued)

Genus or lineage	Type of data (morphological, molecular, or combination of both)	Were characters that were mapped included or excluded from the data matrix?	Conclusions based on character mapping	Reference
<i>Freycinetia</i> , <i>Pandanus</i> , <i>Saranga</i> (Pandanaaceae)	Morphological	Included	Dioecy and vertebrate pollination ancestral, broader geographic range found in species with incomplete or wind pollination and facultative apomixis.	15
<i>Siparuna</i> (Siparunaceae)	Molecular	Characters not included	Dioecy has evolved from monoecy.	57
<i>Heterostyly and multi-allelic self-incompatibility</i> Lythraceae	Morphological	Characters included	Five transitions to tristily hypothesized.	34
Pontederiaceae	Morphological and molecular	Characters excluded in morphological analysis	Morphological analysis produced many equally parsimonious trees, preferred tree did not split homostylous and heterostylous species of <i>Eichhornia</i> . In molecular analysis, with equal weighting of characters and assuming characters were unordered, four gains of tristily were possible. With weighting favoring gains of tristily, a single gain of tristily occurred.	24, 45
Multi-allelic self-incompatibility in angiosperms	Morphological	Characters excluded	Using different phylogenetic hypotheses and different systems of coding self-incompatibility produced little evidence for basal self-incompatibility in angiosperms. Self-incompatibility is very unlikely to be homologous in divergent angiosperm lineages.	76

<i>Evolution of self-fertilization vs. outcrossing</i>			
<i>Amsinckia</i> , (Boraginaceae)	Molecular	Characters not included	62
		When the outgroup was assumed to be distylous, and the breeding systems was treated as unordered, four separate transitions to homostyly and selfing were hypothesized. Weighting loss of distyly as more likely produced a similar result. If the outgroup was assumed to be homostylous, and characters were unweighted and unordered, homostyly was hypothesized as basal.	
<i>Schiedea</i> and <i>Alsindendron</i> (Caryophyllaceae)	Morphological and molecular	Both	60, 67, 75, 81
		Two separate transitions in lineage to facultative or obligate self-fertilization. Very wet habitats appear to favor autogamy, perhaps because enclosure of anthers within calyx protects pollen from rainfall and facilitates selfing.	
<i>Scutellaria angustifolia</i> complex (Lamiaceae)	Morphological and molecular (allozyme data)	Not included	55
<i>Cuphea</i> (Lythraceae)	Morphological	Not included	35
		<i>Cuphea</i> section <i>Brachyandra</i> , comprised of mostly self-fertilizing species, is highly polyphyletic, with species distributed to four other sections of <i>Cuphea</i> .	
<i>Linanthus</i> section <i>Leptostiphon</i> (Polemoniaceae)	Molecular	Not included	33, 33a
		Self-incompatibility basal in <i>Linanthus</i> , 3–4 transitions to self-compatibility.	

(continued)

TABLE 1 (continued)

Genus or lineage	Type of data (morphological, molecular, or combination of both)	Were characters that were mapped included or excluded from the data matrix?	Conclusions based on character mapping	Reference
<p><i>Pollination biology: Vertebrate pollination</i>  <i>Aphelandra</i>  <i>pulcherrima</i> complex            (Acanthaceae)</p>	Morphological	Information not given	<p>Pollination by short-billed trochiline hummingbirds derived in complex; pollination by traplining hermit hummingbirds represents the ancestral character state. Shift to use of short-billed hummingbirds as pollinators associated with shift to dry habitats. Morphology of corollas provides further evidence that short corollas have been independently derived.</p>	53
<p><i>Adansonia</i>            (Bombacaceae)</p>	Molecular	Characters excluded	<p>Basal pollination system could not be determined because of diversity of pollinators in closely related genera. Assuming that hawkmoth pollination is basal, mammalian pollination has arisen on two occasions in lineage.</p>	8
<p><i>Erythrina</i>            (Fabaceae)</p>	Morphological and molecular	Characters included	<p>Passerine pollination is ancestral, four transitions to hummingbird pollination hypothesized. Morphological information suggests that floral features associated with hummingbird pollination in these four lineages are not homologous.</p>	10

<i>Parkia</i> (Fabaceae)	Morphological	Characters included	Entomophily basal in genus, with a single shift to bat pollination hypothesized.	49
<i>Insect pollination</i> <i>Abrotanella</i> (Asteraceae)	Morphological	Characters included	White-flowered and purple-flowered species confined to a single clade within genus; purple-flowered species found in sub-Antarctic Islands, a pattern repeated for other primarily New Zealand genera. White coloration is a possible response to fly-dominated pollinator fauna; purple pigment may protect against effects of sunlight.	69
<i>Dalechampia</i> (Euphorbiaceae)	Morphological	Characters not included	Pollination systems very labile, with many independent gains of similar pollination systems. In three lineages where fragrances are collected by euglossine bees, different morphological structures secrete fragrances.	2, 3
<i>Lapeirousia</i> (Iridaceae)	Morphological	Information not given	Mapping of pollination systems indicated a wide range of pollinators with two types of long-tongued fly pollination evolving convergently on several occasions.	32

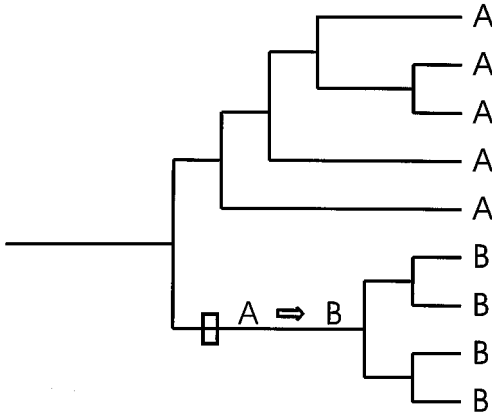
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TABLE 1 (continued)

Genus or lineage	Type of data (morphological, molecular, or combination of both)	Were characters that were mapped included or excluded from the data matrix?	Conclusions based on character mapping	Reference
Subtribes Cyrtopodiinae and Catasetinae (Orchidaceae)	Molecular	Characters not included	Two shifts to pollination by euglossine bees hypothesized; a single shift at the base of the tree is equally parsimonious, but improbable in view of the Old and New World distribution of the Cyrtopodiinae and the New World distribution of the euglossine bees.	14
<i>Disa</i> (Orchidaceae)	Morphological	Characters included	Nineteen pollination syndromes found among 27 species included in study. Many pollination syndromes have evolved on numerous occasions, and autogamy has evolved from outcrossing on three occasions.	42
<i>Bird and insect pollination</i> Nectar spur diversification in <i>Aquilegia</i> (Ranunculaceae)	Molecular	Not included	Evolution of nectar spurs a key innovation promoting species diversification.	40, 41

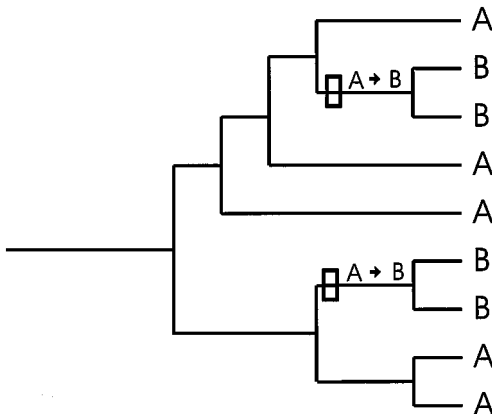


<p><i>Hydrophyty and wind pollination</i></p> <p>Cymodoceaceae</p>	Morphological	Characters included	<p>All taxa in Cymodoceaceae dioecious, leading to hypothesis that the ancestor was also dioecious. Hydrophyty assumed to have evolved at the base of the lineage containing the Cymodoceaceae and two related families (Zosteraceae and Posidoniaceae).</p>	16
Marine angiosperms	Molecular	Characters excluded	<p>Hydrophyty has evolved on several occasions; conclusion of Cox and Humphries (16) resulted from exclusion of many nonmarine sister taxa. Unisexuality is basal in some marine angiosperm lineages, and has not necessarily evolved after hydrophyty.</p>	46
<p><i>Schiedea</i> and <i>Alsinidendron</i> (Caryophyllaceae)</p>	Morphological and molecular	Both	<p>Wind pollination strictly associated with evolution of dimorphism (gynodioecy, subdioecy, and dioecy), and has probably evolved each time there has been a shift to dimorphism (1–6 occasions).</p>	78



**Figure 1** Hypothesized single ecological shift (*open rectangle*) in one branch of lineage, followed by shift from character state A to B. All four derivative species would show the same correlation of habitat and trait, suggesting, in the absence of phylogenetic information, a strong causal basis.

morphological trait appeared, perhaps incidentally, subsequent diversification of the lineage would give the impression of a strong correlation between the novel environment and the trait (Figure 1). Despite the strong correlation, there might not be a causal relationship. In contrast, if species in a lineage colonized the novel environment multiple times and acquired the morphological trait on each occasion, the case for a causal relationship would be much stronger (Figure 2). Subsampling, or



**Figure 2** Ecological shifts (*open rectangles*) occur independently in different portions of the phylogeny, followed in each case by a transition from character state A to B. Evidence that the ecological shift has caused the transition is stronger than for the phylogeny shown in Figure 1.

sampling at different taxonomic levels, would not resolve the problem (26). Only when phylogenetic approaches are used, where the relationship of changes in the trait with the relative timing of shifts in ecology can be specified, can causality be understood. This approach asks whether traits evolve with greater frequency in portions of trees where other traits have changed or where shifts in ecology have been detected (19, 50). The process involves first the construction of a phylogenetic tree using parsimony or maximum likelihood methods (72). The phylogenetic tree is viewed as a hypothesis of evolutionary relationships. Morphological or molecular characters, or a combination of the two types of data, are used to construct the phylogeny. Using the principle of parsimony, traits are mapped (or optimized) on the tree in a manner that minimizes the degree of convergent evolution. Parsimony is the most significant assumption underlying this approach. As emphasized by Felsenstein and others (17a, 26), evolution may occur nonparsimoniously, and transitions in character states could be radically underestimated because of undetected convergent evolution. The possibility that character state changes may be underestimated must be recognized in all studies. Use of maximum likelihood for the reconstruction of character states (17a, 61a) circumvents some of the problems of parsimony. Maximum likelihood methods take into account differences in evolutionary rates and in the lengths of internode branches, and may favor nonparsimonious reconstructions of character state evolution (17a). To date, these approaches have been used only rarely in studies of the evolution of reproductive systems (e.g. 33a, 61).

## CONSTRUCTING PHYLOGENIES

### Morphological and Molecular Data Sets

Construction of the phylogeny is the critical and obvious first step of the process of character mapping using phylogenetic approaches. Morphological data may be an important source of information (22) and often may lead to robust phylogenetic hypotheses. While molecular data sets are sometimes viewed as superior because they are less likely to show convergence, independent gains of morphological traits are often phylogenetically informative (22). This will be true so long as they are recognized as independent gains (31). The introduction of molecular approaches to phylogenetic reconstruction has led to a very rapid increase in the number of phylogenetic analyses, providing the hope that character mapping can answer large-scale questions about trait evolution and cause-effect relationships.

Molecular data are sometimes viewed as the best or only appropriate data for phylogenetic analysis of character evolution because of the difficulties of detecting convergent evolution (homoplasy) using morphological data (31, 65). Concerted convergence has been defined as environmentally induced convergence so strong that entire syndromes of morphological traits converge, with little hope for detecting convergence using morphological characters (31). Although molecular data are often thought to show little homoplasy, this is not always the case. Homoplasy

may occur in older lineages where many molecular changes have accumulated, so that unrelated taxa may occur in close proximity on phylogenetic trees constructed using molecular data (22).

Combined data sets consisting of morphological and molecular data are often employed, particularly when the individual data sets give congruent results. When similar results are obtained from independent data sets, the level of confidence in the phylogenetic hypothesis increases, and often resolution of the branching patterns in the phylogenetic tree is enhanced (22). Adequate phylogenetic resolution using only molecular data may be a problem, particularly for rapidly evolving or young clades where few molecular differences have accumulated. For these groups, morphological data may provide the only phylogenetically informative characters. In contrast, basal portions of clades are often well defined using both morphological and molecular characters because of the time available for divergence for both types of characters.

Future studies will undoubtedly rely heavily on molecular approaches, especially as new regions of the genome become available for molecular analysis. It seems likely, however, that there will be renewed emphasis on morphological characters and phylogenies constructed using combinations of molecular and morphological characters.

## Identification of Appropriate Outgroups

Correct identification of the outgroup, the taxon most closely related to the group under study, is critical for identification of ancestral character states within lineages and is therefore of fundamental importance to character mapping (21, 51). When outgroups cannot be identified with certainty, the outgroup substitution approach may be a satisfactory alternative (21). Using this approach, the ancestral state of the character is determined using a variety of potential outgroups, and the effects of varying the outgroup on character mapping are analyzed. The ability to precisely identify the outgroup provides a powerful means for understanding character evolution. For example, in the Hawaiian silversword alliance (*Argyroxiphium*, *Dubautia*, *Wilkesia*; Asteraceae), molecular data and artificial hybridization have shown with a high degree of probability that the ancestor of the Hawaiian silverswords was closely related to self-incompatible, perennial species of *Madia* and *Raillardia* in California (5). The current widespread self-incompatibility in the silverswords (11) is therefore likely to be the result of colonization by a self-incompatible ancestor of this lineage. Unfortunately, precise outgroup identification is often problematic. Even in remote archipelagoes like the Hawaiian Islands, where the rarity of colonization events means that most lineages are very likely to be monophyletic, it may be difficult to determine the correct outgroup because lineages in Hawaii have often diverged dramatically from continental relatives (e.g. 81).

Several studies illustrate approaches used to address difficulties in outgroup identification. Analysis of the evolution of distylous reproductive systems in *Amsinckia* using phylogenetic approaches is complicated by the difficulty in

identifying the appropriate outgroups (62). To circumvent this problem, different assumptions about the breeding system of the outgroup were used. When the outgroup was assumed to be distylous and the breeding system character was treated as unordered, distyly was hypothesized to be basal in the lineage, with four separate transitions to homostyly and selfing (43, 62). When the outgroup was considered homostylous and loss of distyly was weighted as more likely than a gain (cf. 45), a single transition to distyly at the base of the lineage was most parsimonious. Homostyly was the basal condition in *Amsinckia* only when the outgroup was treated as homostylous and the breeding system character was unweighted and unordered (62).

In a molecular phylogenetic study of *Linanthus* section *Leptosiphon* (Polemoniaceae; 33a), outgroup analysis could not be used to resolve the question of whether self-incompatibility is the ancestral breeding system state. Self-incompatibility is widely distributed in the Polemoniaceae and sporophytically controlled in *Linanthus parviflorus* (33). Because of uncertainty over relationships among sections of *Linanthus*, and between *Linanthus* and other genera of Polemoniaceae, a more distant outgroup (*Navarretia*) was chosen to root the tree. Because this outgroup was not appropriate for determining the basal breeding system condition (33a), no assumptions were made about the basal breeding system condition based on outgroup analysis. When losses and gains of self-incompatibility were weighted equally, or when loss of self-incompatibility was considered more likely than a gain, self-incompatibility proved to be the ancestral character state in *Linanthus*, and selfing evolved on three to four occasions (33a). *Linanthus bicolor*, a widely distributed selfing species, was shown to be polyphyletic in origin. Alternate trees required fewer transitions to self-compatibility but broke apart a clade with 100% bootstrap support and had significantly lower likelihood values using the maximum likelihood approach for constructing phylogenies. When a loss of self-incompatibility was considered slightly less likely than a gain (weighting ratio of 1.2:1), there were five independent origins of self-incompatibility.

The basal condition for pollination systems could not be estimated in baobabs (*Adansonia*, Bombacaceae) based on outgroup comparison because of the wide diversity of pollination systems in closely related genera and the lack of phylogenetic information for these genera (8). If the basal pollination condition in *Adansonia* is assumed to be hawkmoth pollination, then mammalian pollination has arisen on two occasions in the lineage. Morphological features associated with mammalian pollination are diverse, as are the mammals that pollinate these species, and it seems likely that these differences will provide further evidence for independent transitions to mammalian pollination in *Adansonia*.

Difficulties in outgroup identification may eventually be resolved for many lineages as increased information about generic and family level relationships becomes available. Molecular variation is likely to be especially suited to this task, particularly using those sequences whose variation is too limited to provide species-level resolution in many cases, but which may be sufficient for higher level resolution.

## Character Inclusion or Exclusion

Whether characters that will be mapped on trees should be included in the morphological data matrices used to produce trees has been debated extensively. A commonly held view is that inclusion of these characters is circular because taxa possessing a character state would group together and cases of multiple evolution of the character state (homoplasy) would be missed (2,9,65). In contrast, others (18,22,71) argue that traits should be included if they are phylogenetically informative. Even with substantial homoplasy, these characters may be useful in phylogenetic reconstruction, assuming that there are enough unrelated characters that homoplasy can be detected. Luckow & Bruneau (48) concluded that traits under study should always be included in analyses, and they argued that exclusion of characters may lead to weaker phylogenetic hypotheses. They also distinguished between characters coded on a functional basis that may be misleading versus characters that follow the rules of homology.

This distinction is exemplified in Bruneau's (10) investigation of passerine and hummingbird pollination in *Erythrina*. Similar phylogenies were obtained from morphological and molecular approaches. Passerine pollination is the ancestral condition in *Erythrina*, with four hypothesized transitions to hummingbird pollination. Transitions to hummingbird pollination involve shifts from horizontal to upright inflorescences, secund flowers to flowers radially arranged along the inflorescence axis, and standard petals that are open and expose the reproductive structures to standard petals that are folded to form a pseudotube.

Despite similarities among the hummingbird-pollinated species of *Erythrina*, each transition involved differences in calyx and pollen morphology indicating that transitions to hummingbird pollination were not strictly homologous. Deletion of characters associated with the pollination systems of *Erythrina* would have resulted in substantial loss of phylogenetically useful information. Luckow & Bruneau (48) argued that deletion of characters in phylogenetic analysis would always be arbitrary because it would be necessary to know which characters were associated with the adaptation in question at the outset of the analysis. The characters that one might choose to exclude would vary depending on the ecological hypothesis under investigation.

The argument of whether it is better to include or exclude particular characters in morphological data sets is unlikely to have a single answer. Armbruster (3) suggested that characters with a low consistency index (those that evolve or are lost on more than a single occasion in a tree) should be used preferentially in phylogenetic analysis because they would be less likely to interfere with phylogenetic reconstruction. A similar approach would be to determine whether characters should be excluded on a case-by-case basis. If multiple independent origins for a character state are hypothesized, inclusion of the character in the data matrix would be appropriate because inclusion would favor a single origin of the character state, and the bias is in the appropriate direction for a hypothesis of multiple transitions (18). Conversely, including a character would be inappropriate when a single origin is considered likely because this would favor the hypothesized result (18).

In the orchid genus *Disa*, multiple shifts were inferred to occur in several pollination syndromes; apparently enough characters unrelated to these floral syndromes were used in the phylogenetic analysis to prevent all species possessing similar pollination biology from grouping together (42). Johnson et al (42), like de Queiroz (18), viewed the use of a potentially functionally related character as a conservative approach reducing the likelihood of detecting multiple transitions, rather than overestimating them. Regardless of whether characters show single or multiple origins, the actual number of transitions is likely to be underestimated (48). Luckow & Bruneau (48) and de Queiroz (18) have argued that discarding characters with high consistency indices will result in less accurate phylogenies.

The effects of character exclusion will undoubtedly vary depending on the features of lineages under investigation. If the characters in question constitute a small fraction of the total number of characters in the matrix, then character exclusion may have little effect on phylogenetic reconstruction, assuming that the remaining characters contain phylogenetically useful information. Armbruster (3) was able to obtain a highly resolved tree, despite elimination of all floral traits from his analysis. If characters that are phylogenetically informative are few in number, then inclusion of the character will exert undue influence on the tree, and cases of convergence might not be detected. One solution is the inclusion of additional, presumably unrelated characters (10,42,48), assuming that they are available (10).

The minor role of floral characters in establishing phylogenetic relationships in *Schiedea* (Caryophyllaceae) may explain why deletion of the breeding system trait had no effect on estimates of the number of transitions to dimorphism (81). In *Schiedea*, flowers are small and have relatively few characters of phylogenetic significance. In contrast, vegetative characters, which are quite variable, delineate four major clades in the genus. Addition or subtraction of the breeding system character (presence of hermaphroditism, gynodioecy, subdioecy, or dioecy) had relatively little effect on phylogenetic reconstruction (81), presumably because of the overwhelming effect of the vegetative characters. Addition of characters thought to be associated with breeding system, such as the degree of condensation of the inflorescence (a trait associated with wind pollination and dimorphism) resulted in increasing numbers of equally parsimonious, less-resolved trees. The significant impact of adding traits associated with dimorphism indicates that one or several of these traits had a more significant effect on phylogenetic reconstruction than the breeding system character alone (81). Including all characters thought to be related to the evolution of dimorphism, especially inflorescence structure, resulted in changes in tree topology that implied basal positions for hermaphroditic species within clades. This occurred because outgroups were coded as having diffuse inflorescences, in contrast to the highly condensed inflorescences of several dimorphic *Schiedea* species.

One limitation in *Schiedea* for assessing potential convergence in the evolution of dimorphism is the small number of characters directly associated with the shift to dimorphism. Although the evolution of dimorphism might have occurred on several occasions in *Schiedea*, there are few morphological features that might be

used to detect whether changes have occurred independently (are not truly homologous), because evolution of dioecy involves loss or reduction of floral parts rather than modification of parts. In contrast, the complex shifts that occur in *Erythrina* species adapted to hummingbird pollination have taken several routes, permitting detection of different shifts to the derived pollination syndrome (10). In this study of *Erythrina*, the question of failure to detect convergence is somewhat moot with respect to the pollination features since detailed morphological studies demonstrated that with each origin of hummingbird pollination different patterns of floral modification could be detected. Bruneau's study (10) approaches the case where knowing the "true homology" of the character means that each character state appears at a single point on the tree (48). Cases where an in-depth analysis produces this type of result may be rare, but the analysis of *Erythrina* indicates how morphological analyses provide insights into shifts in a complex trait.

Phylogenetic analysis was used to determine whether bat pollination in *Parkia* (Fabaceae) has evolved on separate occasions in the New and Old World (49). This question was motivated by the diverse inflorescence architecture of species from the two geographic areas, and the absence of a close relationship between the New and Old World bat species that pollinate *Parkia*. Another question of interest was whether insect pollination in this genus is the basal condition or secondarily derived from bat pollination. All characters were used in the phylogenetic analysis; exclusion of evidence was projected to weaken the phylogenetic hypothesis, and there was no a priori basis for judging which characters should be excluded because of their potential association with bat pollination (49). In this study, inclusion of characters associated with pollination biology biases results in the opposite direction from the prediction of more than one transition to bat pollination.

In contrast to the expectation that bat pollination might have evolved on several occasions in *Parkia*, phylogenetic analysis indicated that a single transition to bat pollination has occurred, presumably in the New World. Only one character, a change in anther attachment, was perfectly correlated with the shift to bat pollination, although the functional significance of this character was unclear. Few characters in *Parkia* provide obvious clues for understanding why bat pollination evolved because many of the features associated with bat pollination are found in outgroup species. Entomophily is basal in the genus, and a species pollinated by nocturnal bees may represent a preadaptation for bat pollination (49).

With only a single shift to bat pollination hypothesized in *Parkia*, understanding general ecological conditions and morphological features favoring the evolution of this type of pollination will depend on additional phylogenetic studies of other lineages where bat pollination has evolved, as well as microevolutionary studies focused on the functional significance of characters potentially associated with bat pollination.

Despite the different conclusions about the number of transitions to derived pollination states in *Erythrina* and *Parkia*, the studies are similar in that a large number of characters are associated with the floral syndromes under consideration.



In *Erythrina*, these characters indicated slightly different syndromes of features associated with transitions to hummingbird pollination (a lack of homology in the feature), while in *Parkia*, despite the diversity of bat-pollinated flowers, a single origin of the shift in pollination biology was likely. The rich diversity of characters makes these conclusions robust, and the issue of character inclusions vs. exclusion is less relevant than in those studies where reproductive systems or pollination biology have few characters for analysis.

Hart's (37,38) study of the evolution of dioecy in *Lepechinia* (Lamiaceae) was the first use of phylogenetic methods for mapping a reproductive system trait. He found that dioecy has evolved from gynodioecy on two occasions, if the reproductive system was included as a character. Deletion of the breeding system character (dioecy vs. gynodioecy) in *Lepechinia* resulted in an increase in the number of transitions to dioecy from two to four. This result demonstrates the substantial impact of excluding the reproductive character in *Lepechinia*. Although Hart determined the effect of deleting the single breeding system character from his matrix, he did not remove characters such as flower size that are likely to be related to breeding system.

In a phylogenetic analysis of the Pontederiaceae, Eckenwalder & Barrett (24) excluded characters known to be part of the tristylous breeding system from their data matrix. Many equally parsimonious trees resulted, and the authors chose as their preferred tree one that did not split homostylous and heterostylous species of *Eichhornia* because of "the clear relationships among these species." Based on microevolutionary studies, the authors could pair homostylous and heterostylous species of *Eichhornia*, suggesting that homostyly has been derived on several occasions from tristyly. These relationships could not be detected using phylogenetic analysis of morphological characters. The selfing syndrome may have provided enough characters to unite homostylous species in a single clade. This tendency was probably heightened by deliberate exclusion of traits associated with tristyly, as traits associated with tristyly appeared useful for recognizing species pairs (24). Some features of the tristylous reproductive system appear to provide useful data for assessing phylogenetic relationships; the difficulty is in distinguishing these characters from those that result in grouping of species with the same breeding system that may not be closely related.

In summary, approaches to inclusion versus exclusion of characters have been extremely diverse, and there is probably less agreement on the best approach to this problem than any other aspect of character mapping. When inclusion of characters in data matrices appears to result in misleading conclusions about phylogenetic relationships (cf. 71), investigators are likely to delete these characters from matrices, despite the difficulty in determining a priori which characters are problematic. The complexity of the traits that are mapped on trees and the total number of characters used to construct trees will undoubtedly influence approaches taken by investigators. When deletion of a single or small number of characters from a tree has a pronounced effect on the topology of the tree and conclusions about the evolution of a characters, it seems clear that the phylogenetic hypothesis is poorly

supported, and character mapping is unlikely to yield significant insights about character evolution.

## Character Coding, Ordering, and Weighting

How characters are coded and whether characters are ordered may have profound effects on interpretation of character evolution. Morphological characters that are coded as a number of different states will more accurately reflect the diversity within the group in question but usually result in more transitions to derived character states. When characters with multiple states are mapped on branches of a tree, derived character states occur nearer to the terminal branches of the tree. In contrast, when the character state is binary, derived character states may occur at deeper levels of the tree, ultimately resulting in fewer transitions to the derived character state and more reversals near the branch tips. The effects of differences in character coding can be seen in *Schiedea*, where coding the breeding system as four states (hermaphroditic, gynodioecious, subdioecious, and dioecious) rather than two states (hermaphroditic, dimorphic) resulted in a greater number of hypothesized transitions from hermaphroditism to dimorphism (81).

Ordering of character states and binary character coding have similar effects on the estimation of numbers of transitions to derived character states. In *Schiedea*, ordering of the character states implies that dioecy evolves from hermaphroditism through intermediate gynodioecious and subdioecious character states. Given that the character states are defined in large part by the frequency of females in populations, ordering of the character states seems very reasonable. Such an approach may be unrealistic, however, if changes in breeding systems occur rapidly relative to branching events in trees (81). In *Schiedea*, ordering of characters resulted in fewer transitions to gynodioecy and more reversals to hermaphroditism.

Character weighting, where transitions between character states are more likely in one direction than the other, will have major effects on interpretation of character evolution. Using a molecular approach, Kohn et al (45) reconstructed the phylogeny of the Pontederiaceae. As predicted from earlier studies (24), tristylous species of *Eichhornia* were most closely related to monomorphic species. When monomorphic species were forced to form a single clade, trees were 23–26 steps longer. Two optimization schemes were used to map tristily and related characters, including self-incompatibility. In the first scheme, all character states were unordered and weighted equally. In the second approach, loss of tristily (or enantiostily, a second breeding system found within the Pontederiaceae) was favored by a two-fold margin over a gain of either breeding system or an interconversion between them. Not surprisingly, conclusions about breeding system evolution varied substantially, depending on the weighting scheme. With equal weighting and the characters unordered, up to four gains of tristily were possible, and self-incompatibility evolved twice in the family. With unequal weighting, a single gain in tristily occurs, and one to two gains of self-incompatibility are possible. The conclusion from the molecular study that tristily has originated a single time is

based in large part on the assumption that the weighting scheme is appropriate. If a heavy bias in favor of a particular outcome is introduced at the outset of the study, one might argue that there is little point in the phylogenetic analysis, at least for the purpose of character mapping. Kohn et al (45) argue, however, that character weighting is perhaps more reasonable than leaving the characters unweighted, in view of the complexity of tristylly and the low probability that this breeding system evolves very frequently. Using the weighting scheme that favors the loss over the gain of tristylly, self-incompatibility appears to evolve after the evolution of tristylly (45). The evolution of self-incompatibility after tristylly is obtained when losses and gains are equally weighted or when a loss of self-incompatibility is weighted more heavily than a gain. Using different rootings of the tree and different coding schemes for self-incompatibility could change this interpretation (45).

Using morphological phylogenetic data, Graham et al (34) concluded that tristylly in the Lythraceae has evolved on at least five occasions. In this analysis, heterostyly was included in the data matrix. Because of the difficulty in finding a sufficient number of characters, the phylogeny based on these characters was very weakly supported. A weighting scheme that favored loss of heterostyly over gains would presumably have resulted in substantial modifications of the phylogeny, especially in view of the limited number of characters used for the analysis, and fewer transitions to heterostyly. Self-compatibility in the Lythraceae is characteristic of all monomorphic genera, and families related to the Lythraceae are also self-compatible, suggesting that acquisition of tristylly in the Lythraceae has been accompanied by the evolution of self-incompatibility (76). If heterostyly has evolved on separate occasions, then self-incompatibility may also have evolved independently.

These examples illustrate the complexity of issues related to character delimitation and assumptions about these characters. Although the use of phylogenetic trees for the analysis of character evolution may appear to be an objective means of obtaining additional insights into evolutionary processes, it seems clear that in many cases, prior views of character evolution may strongly influence results.

## Limitations of Phylogenetic Hypotheses

The use of phylogenetic approaches for understanding the evolution of plant reproductive biology is clearly limited by the phylogenies underlying the studies. Difficulties in the use of phylogenies have diverse causes (19). Phylogenies may be poorly supported or have topologies that do not reflect the evolutionary history of the lineage. Even when phylogenies are well supported, attempts to map characters may lead to ambiguity in interpretation because of the topology of the tree.

There are many causes for weakly supported phylogenetic hypotheses. Recent evolution of a lineage and reduced phylogenetically informative variation lead to poorly resolved phylogenies. Lineages within the Hawaiian Islands illustrate difficulties resulting from reduced variation. Area cladograms (phylogenies mapped on the geographic distribution of species within a monophyletic lineage) demonstrate

for many lineages that basal portions of radiations occur on the older high islands. The older portions of these lineages are more likely to be resolved than those occurring on the younger islands because of the accumulation of derived character states (74). Alternatively, lineages that colonized the younger islands initially (e.g. *Geranium* [Geraniaceae] and *Tetramolopium* [Asteraceae]) are likely to have less morphological diversity than lineages that have migrated from older to younger islands. Even in cases where there is substantial morphological diversity, molecular differentiation may be limited, as in the case of *Schiedea* (67).

Lineages in the Hawaiian Islands are particularly suitable for illustrating the difficulties resulting from extinction of lineages. Species found on the older islands may well represent the remnants of lineages that were once extensive but that have largely disappeared due to the erosion and subsidence of islands (75). As species become extinct, clades will become compressed, and species that would not have been viewed as closely related and may be very different in morphology will eventually appear as sister taxa. Outgroup analysis could be misleading in these circumstances and could predict unlikely combinations of character states. For example, *Schiedea membranacea*, a species occurring in the basal lineage of *Schiedea* and *Alsinidendron*, is placed in a clade of two *Schiedea* species (*S. membranacea* and *S. verticillata*) sister to highly selfing *Alsinidendron* (*Schiedea* is paraphyletic). On that basis, the breeding system of *S. membranacea* was predicted to be highly selfing (17), a prediction not borne out by studies of outcrossing rates and allozyme variation (17, 79). *Schiedea membranacea* and *S. verticillata* may represent the sole surviving species in a much larger clade that is now largely extinct. Additional information on the breeding system of *S. verticillata* would be useful. If this species is outcrossing, then the prediction that *S. membranacea* would be a selfer is clearly not warranted. *Schiedea verticillata* occurs on Nihoa, a small, largely eroded island occurring 200 km northwest of Kauai. Geological evidence indicates that Nihoa was once as large as Kauai; at one point there may have been many other species related to *S. membranacea* and *S. verticillata* on older Hawaiian Islands before erosion and subsidence, and most of these now extinct taxa may have been outcrossers.

Although the effect of extinction on the process of character mapping is particularly easy to conceptualize for the Hawaiian Islands, where the islands are progressively older to the northwest and extinction rates higher, the same arguments are likely to apply to many clades occurring in continental areas. If basal portions of these clades have suffered from disproportionate extinction, character mapping might result in misleading predictions for expected character states. The occurrence of long branches due to reduced branching could have a similar effect on the reliability of character state assessment, assuming that the probability of change in character states is constant over time and over the phylogeny. Maximum likelihood approaches to character-state reconstruction would minimize some of these problems.

Portions of trees where species have evolved recently may present equally difficult problems for character mapping if characters states are labile (29). For these

characters, there will be considerable ambiguity in predicting points at which character states undergo modification. When predicted character states are equivocal, delayed transitions (DELTRAN) and accelerated transitions (ACCTRAN) mapping procedures will give very different results. With accelerated transitions, equivocal character state changes will occur at deeper branching points of the phylogeny. Delayed transitions of equivocal character states occur closer to the tips of the tree. When character mapping produces equivocal results, character state changes can only be bracketed on the tree, rather than pinpointed with accuracy.

Characters that are likely to be labile are those that may present the greatest difficulties for character mapping (26,29), although these characters might be of considerable interest. Modeling studies indicate, however, that unless clades are very small and transition probabilities are very high, ancestral character states should be determined with high reliability (64). Paradoxically, in portions of clades where species have evolved recently and extinction is unlikely to interfere with interpretation of character evolution, difficulties in resolving phylogenies and the lability of characters of interest may limit the ability to infer ancestral character states. One approach to these difficulties is the use of alternate phylogenetic hypotheses that include trees with different topologies and alternate rootings to determine the range of possible character reconstructions (21).

## INFERRING CAUSE AND EFFECT USING PHYLOGENETIC APPROACHES

One of the potential strengths of phylogenetic approaches is the ability to infer causality by mapping the sequence of events on phylogenetic trees. Donoghue (19), using Maddison's (50) method of asking whether gains or losses of traits are concentrated on portions of trees, investigated the relationship of the evolution of fleshy seeds and dioecy in gymnosperms. An earlier contingency analysis of gymnosperms by Givnish (30) indicated a strong relationship between fleshy seeds and dioecy. This relationship was used to suggest that accelerating fitness gains resulting from increasingly large allocation to female function have selected for separate sexes in gymnosperms. As discussed by Donoghue (19), Givnish's use of contingency analysis implies that each case of fleshy seeds and dioecy represents an independent evolutionary event, an assumption unlikely to be true. When Maddison's phylogenetic approach was used, inconclusive results were obtained because of the very strong correlation between the two traits. The tight correlation of dioecy and fleshy fruits suggests the possibility of a functional relationship, but the order of evolutionary events, critical to testing Givnish's hypothesis, could not be tested with this data set. Maddison (50) applied the same test to the larger data set from Donoghue (19) and obtained ambiguous results, depending on the interpretation of coincidental gains of traits. When all shifts to dioecy occurring on branches with animal dispersal were assumed to take place after the evolution of animal dispersal, the probability of the association occurring by chance was very low ( $P = 0.018$ ).

The distribution of dimorphism in *Schiedea* demonstrates a similar problem. In one of the two clades in the lineage where dimorphism evolves, all character reconstructions indicate unambiguously that the transition to dimorphism occurred at the base of the clade. The transition from mesic to dry habitats, a switch hypothesized as a causative factor in the evolution of dimorphism, occurs on the same branch as the evolution of dimorphism, making it impossible to determine which change occurred first. In a second clade with dimorphism, the uncertainty over the number of transitions to dimorphism makes inferences about causality with respect to habitat difficult. One unambiguous transition to dry habitats occurs in a third clade containing only hermaphroditic species, indicating that habitat shifts do not always lead to the evolution of dimorphism in *Schiedea* (81). Armbruster (2) and Frumhoff & Reeve (29) argued that phylogenetic analyses are likely to help determine the order of historical events only if the selective associations between characters are relatively weak. Paradoxically, the method works least well when relationships are strong and likely to be of the greatest interest to evolutionary biologists.

Using a consensus tree based on morphological characters of the Pandanaceae, Cox (15) concluded that dioecy and vertebrate pollination are ancestral character states and that the distribution of the three genera in the family has been influenced strongly by subsequent modifications of both the breeding system and pollination biology. Vertebrate-pollinated *Freycinetia* is widely distributed in the Pacific region, and its distribution is attributed to increased colonization potential as a result of incomplete dioecy, a derived character state. *Saranga* has retained complete separation of sexes but evolved insect pollination, whereas *Pandanus*, with the widest geographic distribution, is dioecious, wind pollinated, and facultatively apomictic. Interpreting ancestral character states in the Pandanaceae is complicated by the lack of a readily defined outgroup (15). The hypothesis that dioecy evolved as an adaptation protecting against destructive vertebrate pollinators may be more testable once the sister group of the Pandanaceae has been determined.

The significance of presumed key innovations, behavioral or morphological traits that are thought to result in new adaptive radiations can be tested using phylogenetic approaches (e.g. 61). Using a molecular phylogeny for *Aquilegia* and related genera, Hodges & Arnold (41) demonstrated that species diversity is very likely to have increased dramatically after the evolution of nectar spurs. The increased diversity is presumed to result from greater reproductive isolation associated with the presence of nectar spurs. No other derived character states appeared to be associated with diversification. The presumed role of nectar spurs and diversification is bolstered by the occurrence of similar higher diversity in the majority of lineages where nectar spurs have appeared (41).

The rapidity of the radiation in *Aquilegia* results in some difficulties in interpretation: The position of one spurless species of *Aquilegia* is unresolved within the genus (40). Presumably, if greater phylogenetic resolution were obtained, this species would be placed in a basal position in the genus (41), although a secondary reversion to spurless flowers is also possible (40). Because of the limited molecular

diversification in *Aquilegia* (41) and the limited number of morphological traits useful for phylogenetic analysis, it may be difficult to attain any further resolution of the phylogeny.

## DETECTING HOMOLOGY IN REPRODUCTIVE SYSTEMS

The difficulty in detecting convergent evolution (homoplasy) is likely to vary dramatically depending on the breeding system or pollination syndrome question. As complexity in reproductive systems or pollination biology increases, ability to detect homology is likely to increase (cf. 23). Bruneau (10) was able to differentiate different combinations of morphological traits associated with transitions to hummingbird pollination in *Erythrina*, providing a strong rationale in her study for using characters associated with pollination biology in the phylogenetic analysis. Armbruster (3) was able to identify different syndromes of floral characters in *Dalechampia* associated with a shift to pollination by fragrance-collecting, male euglossine bees. Transitions in these cases are not homologous because they have occurred by different routes.

In other lineages, it may not be possible to determine whether there have been independent transitions (except through phylogenetic approaches) because the changes involved are too few in number (i.e., the characters are simple; cf. 23). Independent transitions to dioecy from hermaphroditism or from outcrossing to selfing may be difficult to distinguish because the changes result from loss of relatively small numbers of parts. Renner & Ricklefs (58) for example, have suggested that dioecy is more common among basal angiosperm groups because the greater morphological complexity in more derived lineages lowers the likelihood of modifications leading to unisexual flowers. In other cases, apparent homology may result from lack of knowledge about characters. There are likely to be cases, however, where parallel shifts in reproductive biology cannot be distinguished solely on the basis of morphology because so few characters are involved. For example, the differences obtained from morphological vs. molecular phylogenetic analyses in Pontederiaceae indicate that in the morphological analyses, monomorphic, selfing species were more likely to group together because of reduction in floral parts. In the molecular analysis these monomorphic, selfing species grouped with different tristylous species (24, 45).

For some categories of breeding systems, the assumption of homology in early studies may not have been warranted. For example, the evolution of self-incompatibility has been suggested as causative in the diversification of the angiosperms (82), an argument that assumes homology for self-incompatibility. Using Whitehouse's assumption of homology, self-incompatibility was mapped on basal lineages of angiosperms (76). If self-incompatibility spurred the diversification of the angiosperms, it should be basal in the angiosperms. Phylogenetic analysis was complicated by uncertainty over relationships of basal lineages of flowering plants and lack of information about the distribution of self-incompatibility in some

of these lineages. To accommodate this uncertainty, analyses were run using trees based on different data sets and different assumptions about the distribution of self-incompatibility. Regardless of which trees were used for analysis, and which assumptions were used about the presence of self-incompatibility, there was little evidence for self-incompatibility occupying a basal position in the angiosperms. Self-incompatibility will undoubtedly be discovered in additional groups of basal angiosperms (e.g. 63), but it is unlikely that the conclusion that self-compatibility is basal in the angiosperms will change.

Recent molecular studies of self-incompatibility have now revealed that there are diverse mechanisms underlying this phenomenon (76). It may well be the case that many instances of self-incompatibility represent independent gains of nonhomologous traits. If so, there would be no reason to suggest that a single gain of self-incompatibility among early angiosperms resulted in their diversification.

In general, increased knowledge of the genetic systems and molecular biology underlying breeding systems could provide information useful for understanding evolutionary processes. Dioecy, although relatively simple at the morphological level, may show greater complexity in terms of underlying genetic systems. Nuclear-cytoplasmic systems (27, 68) are well known to govern the dynamics of male sterility in some species, while in other species (1, 25, 36, 76) only nuclear genes are implicated in the expression of male sterility. In the Caryophyllaceae, both nuclear control in *Schiedea* and nuclear-cytoplasmic control in *Silene* are known, indicating the independent evolution of dimorphism in this family. Although morphological information has been used to identify different male-sterility mutations (e.g. 73), in many cases genetic information may be useful for distinguishing potential homology from cases of independent evolution (44). Even more detailed molecular information could be used to provide evidence for the degree of homology in breeding systems. In *Schiedea*, hybridizations among different dimorphic species produce the same segregation patterns of male sterile individuals observed following intraspecific hybridization, indicating that the nuclear male sterility alleles in dimorphic species are allelic (77). Evidence from phylogenetic analysis, however, indicates the likelihood that there have been at least two separate transitions to dimorphism (81). Molecular approaches might be useful for determining whether genes controlling male sterility are identical in different clades within *Schiedea*. Similarly, Hodges (40) suggests that QTL mapping of nectar spurs in *Aquilegia* may be a promising method for determining the degree of homology for this trait in *Aquilegia*.

## INSIGHTS FROM PHYLOGENETIC STUDIES

In this section several examples from Table 1 are used to address the question of what has been learned about the evolution of breeding systems and pollination biology using phylogenetic approaches. In particular, how have phylogenetic approaches advanced our understanding of reproductive and pollination biology in ways that have complemented results of micro-evolutionary studies? These



studies include evolution of diverse reproductive systems (androdioecy, dioecy, heterostyly, multi-allelic self-incompatibility, and self-fertilization) and aspects of pollination biology (vertebrate, insect, and abiotic pollination). Phylogenetic analysis may also be used to guide microevolutionary studies with quantitative genetic and physiological components.

## Origin of Androdioecy in Datisceae

The evolution of androdioecy in *Datisca* (Datisceae) has been investigated intensively by Rieseberg and co-workers (28, 47, 56, 70). These studies demonstrate the crucial role of phylogenetic analysis in determining the evolutionary relationship of androdioecy to other reproductive systems occurring in related taxa. Androdioecy (the presence of male and hermaphroditic individuals in populations) is a potential intermediate step in the evolution of dioecy (12). The stringent conditions for maintenance of males with hermaphrodites in populations is a plausible explanation for the rarity of androdioecy and suggests that this breeding system is unlikely to serve commonly as an intermediate step in the evolution of dioecy (13).

Datisceae consist of three genera, *Datisca*, *Octomeles*, and *Tetrameles*. *Datisca cannabina*, *Octomeles*, and *Tetrameles* are dioecious, while the fourth species in the family, *Datisca glomerata*, is androdioecious. Datisceae were assumed to be monophyletic, and chloroplast restriction site analysis (56) was used to infer that androdioecy was derived from dioecy. More recent analyses using *rbcL* and 18S ribosomal sequences were used to investigate monophyly in Datisceae (70). Based on a consensus of the *rbcL* and 18S data, Datisceae are paraphyletic, with Begoniaceae nested within Datisceae and sister to *Datisca*. The positions of *Octomeles* and *Tetrameles* are unresolved. Reconstruction of breeding systems using the *rbcL* tree indicates that either monoecy or dioecy may be ancestral to androdioecy in *Datisca glomerata*, while the 18S tree indicates that monoecy is basal. Using the consensus tree of the *rbcL* and 18S data, dioecy appears to be ancestral. Because the basal breeding systems in taxa used to polarize breeding systems in *Datisca* have not been studied in detail, these results could change with further study.

Phylogenetic investigations of Datisceae show clearly that androdioecy is not an intermediate step in the evolution of dioecy. In contrast, this breeding system is more likely to be derived from dioecy. Phylogenetic studies therefore lead to the question of conditions that would favor a transition from dioecy to androdioecy, rather than the reverse.

## Evolution of Dioecy in *Schiedea* and *Alsinidendron*

Morphological and molecular phylogenetic approaches were used to address the question of the number of transitions from hermaphroditism to dimorphism in the endemic Hawaiian lineage containing *Schiedea* and *Alsinidendron* (Caryophyllaceae). This example shows the difficulties in assessing phylogenetic relationships for recently evolved groups or portions of groups (60, 67, 75, 81). Dimorphism was

hypothesized to have evolved in two of the four major clades in the lineage. The estimated number of transitions varied widely because of the topology of the clade of *Schiedea* containing *S. globosa* and its relatives (81), and the very weak support for the clade. Accelerated transitions predicted a single transition to dimorphism followed by reversals to hermaphroditism in several species. Use of delayed transitions resulted in multiple shifts to dimorphism. Although the *S. globosa* clade is very poorly supported using both morphological and molecular data, even a strongly supported clade with the same topological relationships would present the same ambiguities in character mapping, because of the distribution of hermaphroditic species throughout the clade.

In the clade containing *S. adamantis* and related species, the occurrence of a reversal to hermaphroditism from a gynodioecious ancestor is strongly supported for *S. lydgatei* (54, 81). *Schiedea lydgatei* is the sole hermaphroditic species in the *S. adamantis* clade and is nested within the clade, which ensures that hermaphroditism will be viewed as a reversal. In contrast to the *S. globosa* clade, morphological and molecular data support the *S. adamantis* clade. Unless there have been multiple, nonparsimonious transitions to dimorphism in the *S. adamantis* clade, there seems little doubt that hermaphroditism in *S. lydgatei* represents a reversal. While there is no reason that reversals to hermaphroditism could not occur, especially from a gynodioecious ancestor, this reversal would not necessarily have been predicted from broad comparative data that generally suggest the derivation of dioecy from hermaphroditism.

Phylogenetic information in *Schiedea* can be used to guide population-level studies in the choice of study species as well as hypotheses. *Schiedea salicaria* and *S. adamantis* are two gynodioecious species occurring within the *Schiedea adamantis* clade. Allocation patterns in hermaphrodites vary in the direction predicted by the frequency of females in populations: in *S. salicaria* (12% females; 80), hermaphrodites and females have equivalent female function (AK Sakai, SG Weller, unpublished), while in *S. adamantis* (39% females; 59), females have substantially greater female function than hermaphrodites (59). Differences in these very closely related species have been used to suggest that the appearance of females in populations of *Schiedea* is largely a function of high inbreeding depression and high selfing rates, rather than initial shifts in patterns of resource allocation (59). Future approaches include using phylogenetic information to guide the choice of species for a quantitative genetic analysis of resource allocation patterns in *Schiedea* to determine the genetic potential for shifts in allocation patterns to male and female function (AK Sakai, SG Weller, and DR Campbell, unpublished).

## Pollination Systems in *Aphelandra*

The evolution of plant-pollinator interactions has been investigated by McDade (53) for the *Aphelandra pulcherrima* complex (Acanthaceae). Species in this complex have red, tubular flowers and are pollinated by either short-billed trochiline hummingbirds or long-billed hermit hummingbirds. Comparative studies in the past have suggested that species pollinated by long-billed hermits, which have

long, curved corollas and produce abundant nectar, are evolutionarily specialized compared to species pollinated by trochiline hummingbirds, which have short, straighter corollas and produce far less nectar. Contrary to expectations, phylogenetic analysis of the *A. pulcherrima* complex indicated that pollination by hermits is ancestral, and species with short-tubed corollas have evolved on two occasions. The use of a phylogenetic approach permitted the explicit framing of questions about the evolution of floral structure and function, and its relationship to pollination in this complex. As McDade (53) emphasized, there is no reason to expect that different patterns of trochiline and hermit pollination would not emerge in other hummingbird-pollinated lineages.

### Evolution of Pollination Biology in *Dalechampia*

In *Dalechampia* (Euphorbiaceae), Armbruster (3) mapped pollination characteristics on a phylogenetic tree produced using morphological data. Pollination systems in *Dalechampia* proved to be evolutionarily labile. The most common pollination syndrome, pollination by resin-collecting bees, evolved on one occasion, although several different resin-collecting bee species visit different species of *Dalechampia*. In contrast, pollination by fragrance-collecting male euglossine bees is less common but has evolved three to four times. In three of the lineages in which there has been a transition to fragrance collecting by euglossine bees, different morphological structures secrete rewards (2,3), adding to the evidence that these transitions represent independent evolutionary events. Pollen collection has evolved in one to two species. *Dalechampia* is a large genus consisting of approximately 120 species, of which 40 were studied in detail (3). Presumably, conclusions about the number of transitions to different pollination systems might change considerably if additional species were added to the analysis.

Resins may first have originated as a defense system for staminate flowers and evolved later to become a reward for pollinators (4). If this evolutionary scenario is correct, resin production was a preadaptation that allowed the eventual use of resins as a reward for pollinators. A second case of preadaptation may have occurred in another species, where a defense system appears to have resulted from modification of morphological structures associated with attraction of pollinators (4). Phylogenetic analysis of *Dalechampia* has provided a powerful tool for the investigation of both transitions in pollination systems and the functions that may have predated current use of structures associated with pollination and defense.

## PREDICTING WHEN PHYLOGENETIC APPROACHES WILL BE USEFUL FOR ANALYSIS OF CHARACTER EVOLUTION

Can available studies (Table 1) be used to predict when phylogenetic approaches are likely to be most useful for analyses of character evolution? Clearly, phylogenetic approaches may be most useful in lineages with well-supported phylogenies

including a large proportion of extant species. Character mapping in lineages in which there has been considerable extinction (or where there are regions of the phylogeny with less branching) may be difficult if extinct sister taxa had different character states than did extant species. Younger lineages may have fewer problems associated with extinction but often have less phylogenetic resolution, making the process of character mapping more difficult.

The analyses cited in this review indicate that breeding systems or pollination syndromes that are not highly labile are most successfully analyzed using phylogenetic approaches. When traits are extremely labile, considerable uncertainty may exist for character states at ancestral nodes, and single transitions followed by reversals are likely to have the same likelihood as multiple transitions to the derived character state (81). Large, well-sampled lineages lend themselves to analysis of character evolution, particularly when traits under study are relatively conservative, because multiple transitions are likely to be correctly interpreted (e.g. 10).

When the outgroup is clearly identified in a study, characters within lineages can be polarized correctly, and the evolutionary relationships of different characters can be assessed correctly. In many other cases, inability to specify a single outgroup means that multiple outgroup comparison is essential. Diversity among potential outgroups for the traits under study will further complicate analysis for the ingroup, unless phylogenies for the outgroups are well defined.

Using phylogenies to determine the order of evolutionary events (and thus provide inferences of causality) is likely to work best when cause and effect are not very strongly related. As the correlation between a modification of the environment, for example, and the evolution of a trait increases, traits will co-occur on branches and the order of their acquisition will be impossible to determine. Inference of a relationship will remain strong, but it will not be possible to determine causality using only phylogenetic approaches. In these cases, the stronger inference for causality may come from micro-evolutionary studies.

The evolution of complex character traits is more likely to be interpreted correctly using phylogenetic analysis, particularly when homoplasy can be reinterpreted as cases of independent gains of superficially similar character states. Complex traits can be weighted so that they are more likely to break down than to evolve independently (cf. 34, vs. 45), although these approaches are then not strictly independent of the microevolutionary studies on which the weighting schemes depend. When traits under study are relative simple (e.g. dioecy), multiple gains are less likely to be distinguishable because there are fewer characters associated with the breeding system. Increasingly detailed knowledge of traits, particularly their molecular basis, will undoubtedly increase the probability of correct assessment of homology. When lack of homology is conspicuous, independent gains of pollination syndromes or breeding systems can be identified without using phylogenetic approaches. It seems more likely, however, that phylogenetic approaches will continue to have a critical role in identifying cases where true homology is more restricted than cursory examination might suggest. While not a simple cure to understanding problems that in the past have been studied only in the realm

of microevolutionary studies, phylogenetic approaches offer clear potential for providing new insights.

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