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The comparative biology of pollination and mating in flowering plants

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SUMMARY

The diversification of many flowering plant families has been attributed to adaptive radiation of pollination and mating systems accompanying changes in ecology and life history. Reproductive traits in seed plants therefore provide a potentially rich source of diversity for comparative and phylogenetic studies. Here we address three topics in reproductive biology: floral allocation strategies, mating systems and life history, and the origin of complex reproductive syndromes using various comparative methods. Results from these studies generally complement and inform those obtained from previous microevolutionary work at the population level. Historical inferences concerning reproductive character evolution in some taxa can be hampered by topological uncertainties in tree reconstruction and a lack of resolution in molecular phylogenies. Future insights into the ecology and evolution of plant reproductive adaptations using comparative approaches will require well resolved phylogenies, particularly at the species level.

1. INTRODUCTION

Among life history traits, reproductive characters that determine mating patterns are perhaps the most influential in governing macroevolution. This fundamental role arises because the mating system (who mates with whom and how often) governs the character of genetic transmission between generations and hence the behaviour of all genes in populations. Important evolutionary processes responsible for the diversification of plant lineages, including reproductive isolation and speciation modes, are closely linked to changes in mating patterns through their effects on the genetic structure and evolutionary dynamics of populations (Stebbins 1974). Indeed the diversity of many angiosperm families, such as the Orchidaceae (Dressler 1981) and Polemoniaceae (Grant & Grant 1965), has been directly attributed to the evolutionary flexibility of their reproductive systems.

Evolutionary responses to ecological conditions are evident in all aspects of angiosperm reproduction, from resource investment in reproductive versus vegetative function, through the structure and arrangement of flowers and their role in pollination, to mating patterns within populations. Hence examples of adaptation can be found in the relatively large reproductive effort of annuals compared with their perennial relatives (Primack 1979), the convergence in flower structure and colour of unrelated species with similar pollinators (Fægri & van der Pijl 1979) and the higher incidence of selfing among colonizing species than among related taxa occupying more stable habitats (Lloyd 1980). Because resource allocation, pollination and mating determine reproductive success in an integrated way, functional correlations between these components of reproduction should be commonplace. These functional linkages require that evolution involves coordinated changes to all aspects of reproduction in concert with life history evolution. Unfortunately these linkages have often been overlooked because of the fragmentation of reproductive biology into subdisciplines specializing on different phases of reproduction.

The preceding perspective implies that between-species comparisons of resource allocation, pollination and mating should provide a rich source of insights on the ecology and evolution of plant reproduction. Although comparative analysis has a venerable tradition among reproductive botanists (e.g. pollination and dispersal syndromes), it was used relatively little during the 1970s and 1980s due to the dominance of species-level microevolutionary approaches that accompanied the growth of population biology. However, recognition of the utility of historical reconstruction and comparative biology for testing ecological and evolutionary hypotheses has recently awakened an interest in phylogenetics among evolutionary ecologists interested in plant reproduction and, as a result, studies increasingly consider a broader range of taxa with diverse life histories.

Reproduction is amenable to evolutionary analysis using both comparative and experimental approaches

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for various reasons. Floral traits are often well documented because of their taxonomic importance, hence surveys of floras and monographs often provide valuable data. Plants display considerable inter- and intraspecific variation in floral traits that influence mating patterns, indicating the evolutionary lability of most reproductive characters. This variation increases the chances of detecting patterns in comparative data as well as providing opportunities for genetic and microevolutionary studies. Theoretical models of the evolution of reproductive systems often make specific predictions of both expected character associations and the likely order of establishment of traits in a phylogeny (e.g. the evolution of dioecy; Givnish 1980, but see Donoghue 1989). In some cases (e.g. the evolution of heterostyly; Charlesworth & Charlesworth 1979; Lloyd & Webb, 1992) models differ concerning proposed evolutionary sequences. Phylogenetic reconstruction facilitates resolution of such conflicts.

In this paper we illustrate how comparative approaches can aid studies of plant reproductive adaptations. We begin by clarifying our perspective on what constitutes an adaptation to avoid the confusion caused by recent disagreements about this concept between workers in phylogenetics and comparative biology, on one hand, and those concerned with mechanisms of selection in contemporary populations, on the other. We then investigate three current issues in plant reproductive biology using comparative approaches that differ in the availability of phylogenetic information. For each case we review the theoretical foundation upon which the problem rests and then ask whether comparative approaches provide insights not obtainable by microevolutionary enquiry. The first problem concerns the allocation of sexual resources as it pertains to the ratio of pollen and ovules produced by flowers. The last two issues relate to mating patterns, beginning with whether particular aspects of life history are associated with selfing and outcrossing, and then proceeding to a specific application of phylogenetic reconstruction to analysing the evolutionary assembly of a complex reproductive adaptation governing the mating system. Rather than present detailed results and analyses for these problems, we emphasize unanswered questions that would benefit from research conducted from a historical perspective and identify difficulties inherent in implementing and interpreting comparative analyses.

2. ADAPTATION, ECOLOGY, EVOLUTION AND COMPARATIVE ANALYSES

Comparative analyses are commonly used to identify and interpret adaptations. The perceived success of this exercise often depends on one's understanding of what adaptations are and how they arise and are maintained. Although we do not intend to join the debate about the 'true' meaning of adaptation (reviewed by Reeve & Sherman 1993), our discussion of reproductive adaptations in plants is less susceptible to misinterpretation if we clarify our use of this concept.

Most generally, an adaptation refers to a trait (or complex of traits) that confers functional advantage over alternate traits in a given environment. Association of phenotype with function recognizes that adaptations have purposes which contribute to an organism's fitness. Consequently, covariation between two characters among taxa informs us about adaptation only to the extent that we understand their functional linkage, rather than, for example, their developmental association. By including the environmental context in our definition we further identify that specific traits do not usually have intrinsic ecological value. For plant reproduction this is evident from the association of intraspecific geographical variation in flower design and mating system with variation in pollinator diversity and abundance (e.g. Grant & Grant 1965).

As a result of their functional role, adaptations are maintained through natural selection, thereby achieving evolutionary relevance. Typically, adaptations originate through natural selection, although this need not be universal. For example, if evolutionary divergence involves Wright's (1977, chapter 13) shifting balance, genetic drift could theoretically produce novel traits which subsequently gain status as adaptations if they are more beneficial than alternative traits. Because the demographic conditions prevalent during the origins of traits are unknown and unknowable, assertion that any particular adaptation arose solely through natural selection, let alone that selection favoured a particular function, cannot be justified.

In the context of comparative biology, adaptations can be recognized in several guises, which are characterized by the extent of phenotypic and functional similarity between taxa (table 1). Adaptation is readily recognized when unrelated species perform similar functions, regardless of whether similar or different traits are involved (interpretations 3 and 7). Similarly, trait divergence in related species is typically accepted as evidence of adaptation (interpretations 5 and 6), especially when it is associated with functional differences. On the other hand, comparisons of traits that perform different functions in unrelated species provide little insight into adaptation (interpretations 4 and 8) in isolation from directed studies of the fitness consequences of phenotype-function associations within each species.

Unlike the preceding cases, trait similarity in related species provokes considerable debate about the interpretation and nature of evidence for adaptation (see Reeve & Sherman 1993). We recognize function as an integral component of an adaptation, which is not secondary to phenotype, so that our definition of adaptation differs from that of Gould & Vrba (1982) by encompassing exaptation (interpretation 2). More fundamental dispute arises in the context of related species that use similar traits to perform similar functions (interpretation 1). Functional and phenotypic similarity between closely related species arises because common ancestory bequeaths related species with similar phenotypes and ecological niches. Resemblance of related species could persist if characters are resistant to selection, even though the species occupy different environments ('phylogenetic inertia': uniovulate florets in Asteraceae may provide one

Table 1. *Evolutionary interpretations of traits based on comparisons of species*

	related species		unrelated species	
	similar functions	different functions	similar functions	different functions
similar traits	1*a* parallel evolution 1*b* phylogenetic niche conservatism 1*c* phylogenetic 'inertia' – 'constraint'	2 exaptation for the species with the novel function	3 phenotypic convergence	4 trait similarity uninformative
different traits	5*a* alternative phenotypic solutions 5*b* trait *differences* non-functional	6 adaptive trait divergence	7 functional convergence	8 interpretation uncertain, possibly adaptation

example). Probably more commonly, niche similarity leads to similar selection, so that related species continue to resemble each other because either environmental stasis results in equivalent stabilizing selection so that mean phenotypes remain unchanged, or similar environmental changes promote parallel evolution. To the extent that selection maintains the competitive advantage of the fit between phenotype and function, similarity of related species is consistent with our conception of adaptation.

The perceived importance of resemblance of related species depends on the perspective of the investigator. Ecologists are particularly interested in the association between form and function, whether it arises from directional or stabilizing selection, so both interpretations 1*a* and 1*b* (but not 1*c*) merit attention. In contrast, for some evolutionary biologists, similarity of related species due to analogous stabilizing selection is less interesting because it lacks the essence of evolution, namely change (e.g. Brooks & McLennan 1991; Harvey & Pagel 1991). Consequently definitions of adaptation proposed by several evolutionary biologists (reviewed by Reeve & Sherman 1993) explicitly exclude interpretations 1*a* and 1*b* (in addition to interpretation 1*c*) from consideration. Furthermore, this rejection of phenotypic similarity among related species as an interesting phenomenon, even if that similarity is maintained by stabilizing selection or parallel evolution, is a fundamental feature of recently developed techniques for analysing comparative data (reviewed by Harvey & Pagel 1991). It is not surprising, therefore, that disagreements arise between proponents of these perspectives concerning the interpretation of comparative data (e.g. Harvey *et al.* 1995; Westoby *et al.* 1995), given their somewhat different conceptions of adaptation.

Regardless of one's perspective on adaptation, analysis of comparative data can present a technical problem. Statistical tests (parametric or non-parametric) that compare a test statistic to a prescribed probability distribution (e.g. F or χ^2) rely on the assumption of independent observations (i.e. $P[X = x_i \cap X = x_j] = P[X = x_i]P[X = x_j]$, where $P[X = x_i]$ and $P[X = x_j]$ are the probabilities that the values of some trait (X) for observations i and j are x_i and x_j, respectively, and $P[X = x_i \cap X = x_j]$ is the joint probability of observations i and j having these trait values). Depending on the question of interest, related species may not be independent observations from the statistical population of species phenotypes due to resemblance inherited from their common ancestor (Felsenstein 1985), leading to rejection of the statistical null hypothesis more often than expected (i.e. too many Type I errors). Evaluation of the independence assumption is often not straightforward in comparative analyses. In particular, phylogenetic relatedness of phenotypically similar taxa is not sufficient information to judge independence: the question under consideration provides the context for this assessment. As an analogy, consider whether identical twins who were separated at birth constitute independent observations. Clearly they do not if the trait of interest is eye colour, whereas they likely are independent subjects with respect to the current values of their bank accounts, even if those values are very similar. Similarly, the inclusion of related species in comparative analyses may or may not cause independence problems, depending on the topic and the relevant traits (see also Westoby *et al.* 1995). Unfortunately, lack of independence cannot be assessed by comparing results from statistical analyses that incorporate phylogeny (reviewed by Harvey & Pagel 1991) with those that do not, because current phylogenetically sensitive techniques define independence based on the phylogeny of taxa, rather than on the histories of the traits of interest. As the twin analogy indicated, trait history is the relevant feature in assessing independence of observations.

3. FLORAL ALLOCATION STRATEGIES

Plant reproduction involves hierarchical allocation of resources among inflorescences, between flowers and within flowers. For species with hermaphrodite flowers, allocation within flowers includes relative investment in pollen and ovules. To the extent that intrafloral allocation to gametes is limited and independent of allocation among flowers and inflorescences, investment in pollen should vary inversely with investment in ovules and seeds. Charnov (1982) proposed that the optimal resolution of this trade-off occurred when investment in pollen and ovules produced equivalent marginal fitness returns through both sex roles. The specific optimum for a given species depends on the relation between allocation in a particular gamete type

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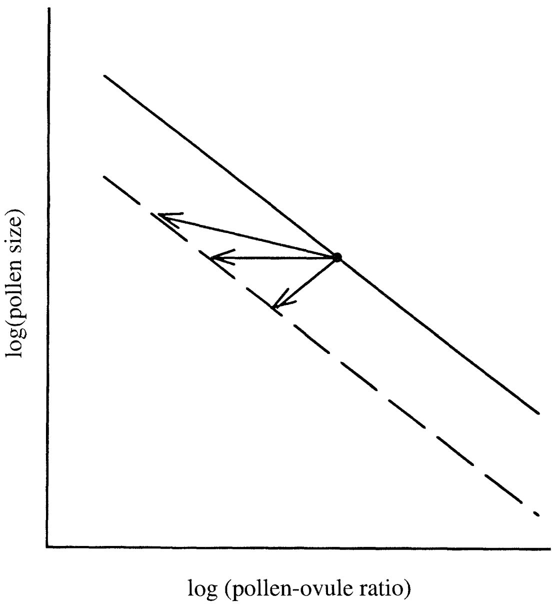

Figure 1. Expected relations between log(pollen size) and log(pollen-ovule ratio) for selfing and outcrossing species with equivalent seed size. The arrows indicate changes in pollen size that could accompany evolution of autogamy from an outcrossing ancestor.

and the realized genetic contributions (sex-specific gain curves). The shapes of these gain curves depend on many aspects of reproduction, including resource availability, pollinator attraction, pattern of pollen export and import, post-pollination processes, inbreeding depression and seed dispersal. As a consequence, species with different reproductive ecologies should produce pollen and ovules in correspondingly different ratios.

Unlike many aspects of plant reproduction, pollen-ovule ratios have been the subject of several comparative analyses. Based on an extensive survey, Cruden (1977) demonstrated that outcrossing species had higher pollen-ovule ratios than predominant selfers. He explained this pattern in terms of pollination efficiency, arguing that pollination is easily realized for selfers and so requires production of few pollen grains per ovule, whereas outcrossing involves the uncertainties that accompany reliance on pollen vectors and necessitates greater pollen production. This perspective was criticized (Charnov 1982) as equating a plant's reproductive success with its seed production (i.e. female function). Although justified, this criticism diverted attention from the importance of pollination and post-pollination processes in determining the shapes of male and female gain curves. Consequently, we believe many ecological and evolutionary influences on sex allocation await intraspecific and comparative analysis.

Investment in pollen involves both the number and size of grains. When the proportion of resources allocated to male function (r) is fixed, the number of pollen grains produced (P) must vary inversely with the resources invested per grain (S), so that $P \propto r/S$. Incorporating this trade-off with a similar relation for ovule and seed production, Charnov (1982) proposed that, for species with equivalent seed size, pollen size should vary with pollen-ovule ratio (P/O) according to

$$\log(S) = \log\left(\frac{r}{1-r}\right) - \log(P/O).$$

Because outcrossers should invest relatively more in male function than selfers (Charnov 1982), $r/(1-r)$ will be greater for outcrossers than for selfers, resulting in an elevated trade-off relation between pollen size and pollen-ovule ratio for outcrossers (Figure 1). Given these relations, how should pollen size differ between selfers and outcrossers? As figure 1 illustrates, both positive and negative differences are possible, so that the answer cannot be found simply by recognizing the size-number relation between pollen size and number and the greater investment in male function by outcrossers. We must also consider functional relations between pollen size and mating system.

At least two features of reproduction suggest that outcrossers might produce larger pollen than selfers. First, large pollen is often associated with long styles (e.g. Williams & Rouse 1990; Kirk 1992), presumably because more resources are required for successful pollen-tube growth and fertilization. Autogamous species typically have small flowers with shorter styles, and so should produce smaller pollen. Second, pollen of outcrossers can be subject to inter-male competition and female mate choice during pollen-tube germination and growth, so that large pollen may convey a competitive advantage, whereas the pollen of selfers need not be as competitive, so selfers should produce smaller, more economical, pollen. Hence details of reproductive biology lead to expectations for associations between pollen-ovule ratio, pollen size and mating system that are not apparent from sex-allocation theory, as currently developed.

We examined these expectations for selected selfing and outcrossing species in the Polemoniaceae. This family of approximately 320 species exhibits considerable variation in life history, growth form, pollination and mating (Grant 1959; Grant & Grant 1965: see also §4). Opportunities for comparative analyses within this family improved recently with the publication of several phylogenetic studies based on DNA sequences of 20–59 species (reviewed by Johnson *et al.* 1996). Based on these phylogenetic trees we selected six pairs of closely related congeners in which one member was autogamous and the other outcrossing (according to Grant & Grant 1965: *Collomia linearis–C. grandiflora*, *C. tinctoria–C. rawsoniana*, *Eriastrum wilcoxii–E. densifolium*, *Gilia leptomeria–G. rigidula*, *Langloisia setosissima–L. punctata*, *Navarretia divaricata–N. intertexta*). Comparison of these pairs indicates that outcrossers have higher pollen-ovule ratios than selfers (mean ± SE difference in $P/O = 204 \pm 59.60$, paired t-test, $t_5 = 3.42$, $P < 0.025$; data from Plitmann & Levin 1990), and outcrossers also produced larger pollen than selfers (mean ± SE difference in log[pollen volume, μm^3] $= 0.63 \pm 0.20$, $t_5 = 3.21$, $P < 0.025$; data from Stuchlik 1967). Interestingly, this pattern also occurs intraspecifically between selfing cleistogamous flowers and outcrossing chasmogamous flower of *Collomia grandiflora* (Lord & Eckard 1984). Despite the small sample, this result implicates pollen size, as well as pollen-ovule ratio and secondary reproductive characters (e.g. corolla size), in mating-system evolution. This conclusion exposes the need for increased attention to interactions between sex allocation, pollination and post-pollination processes, in general, and the functional significance of pollen size, in particular.

4. MATING SYSTEMS AND LIFE HISTORY

Mating systems of seed plants range from obligate outcrossing, through simultaneous outcrossing and selfing (mixed mating), to predominant selfing (autogamy). This diversity is distributed non-randomly with respect to taxonomy and life history (Barrett & Eckert 1990), implying that full understanding of mating-system evolution requires consideration of both phylogeny and ecology. Surprisingly few comparative data have been collected on the ecological and life history correlates of outcrossing and selfing, despite considerable natural history information from groups with known mating systems (reviewed in Lloyd 1980). Moreover, few studies have attempted to reconstruct the phylogeny of outcrossing and selfing using formal cladistic techniques (although see Kohn *et al.* 1996; Schoen and co-workers unpublished).

Theoretical models concerning the relative frequencies of self- and cross-fertilization (reviewed in Uyenoyama *et al.* 1993) have primarily focused on genetic issues, including the transmission characteristics of genes modifying the reproductive system and the relative fitness of selfed and outcrossed individuals. Few models have explicitly considered joint effects of life history and mating system, even though natural history observations suggest associations between the woody growth form and outcrossing, and the annual habit and selfing (reviewed in Lloyd 1980). To assess whether interspecific variation in mating patterns correlates with life history, Barrett & Eckert (1990) surveyed quantitative estimates of selfing rate obtained using genetic markers and classified 129 species according to growth form (annual, herbaceous perennial or woody perennial). They found relatively more outcrossing among woody perennials than among annual and perennial herbs, but noted that the sampled species might not be statistically independent because, for example, the woody perennials included many related species (especially pines and eucalypts).

The recently published *rbc*L phylogeny of seed plants (Chase *et al.* 1993) provides an opportunity to reassess this association. We first surveyed recent literature to update Barrett & Eckert's (1990) dataset, resulting in a sample of 217 species from 43 families. Because the Chase phylogeny primarily depicts relationships between families and is based on different species from those sampled for selfing rates, we sought higher resolution phylogenies for five families (Asteraceae, Fabaceae, Onagraceae, Pinaceae, Poaceae) represented by several species in the selfing-rate data. Based on the inferred patterns of relatedness we constructed phylogenetically independent contrasts (Purvis & Rambaut 1995) between taxa with contrasting growth forms. These contrasts measure the difference in selfing rate between growth forms, which were then tested for consistent deviation from equality with signed-rank tests.

The results from this analysis (figure 2) differed somewhat from those reported by Barrett & Eckert

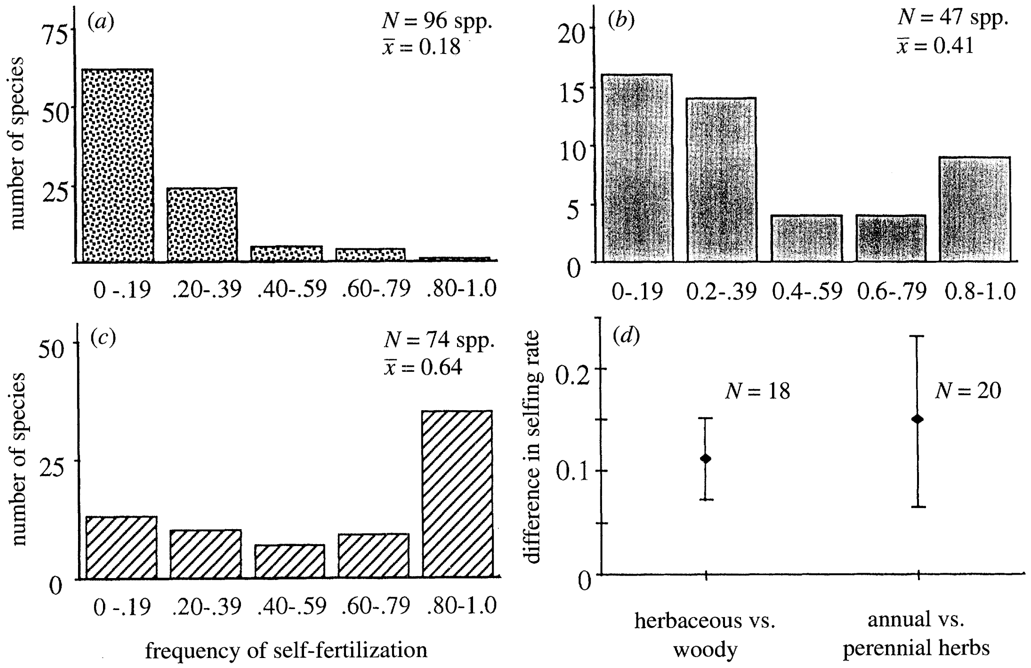

Figure 2. Relations between growth form and selfing rate for 217 species of seed plants. (*a*) Distribution of selfing rate for woody perennials; (*b*) distribution of selfing rate for herbaceous perennials; (*c*) distribution of selfing rate for annual species; (*d*) illustrates the mean (±95% confidence intervals) difference in selfing rate between herbaceous (annual and perennial) versus woody species and annual versus perennial herbs following phylogenetically independent contrasts (N = number of contrasts). See text for further details.

(1990). As with the earlier analysis, we found that woody perennials generally self less than herbs ($P < 0.0005$, $N = 18$). However, our results also reveal that annual herbs tend to self more than perennial herbs ($P < 0.0005$, $N = 20$), whereas Barrett & Eckert found no significant difference between these growth habits. One interpretation of the lack of accord in results for annual and perennial herbs between the two studies is that the earlier analysis suffered from inclusion of non-independent observations. Although this may be true, a second, perhaps more important, interpretation is that the analysis based on contrasts is more powerful because the matching of related species with alternate growth habits provides some control over diverse unmeasured ecological variables that also affect selfing rate. Unfortunately, the relative contribution of phylogeny and ecology as the prime influence on a species' selfing rate cannot be distinguished statistically with opportunistic surveys. A more convincing, though experimentally demanding, assessment requires two explicit contrasts: unrelated annual versus perennial herbs in the same environment to control for aspects of ecology other than those related to life cycle, and related annuals versus perennials from different environments to control for phylogeny.

Growth form incorporates several life-history features that affect the advantages and disadvantages of different mating systems, including longevity, size and breeding frequency. These aspects of life history have important genetic and reproductive consequences. Long-lived species tend to have higher genetic loads resulting in strong inbreeding depression. This may constrain the evolution of selfing and explain why few woody plants are reported with high selfing rates (figure 2*a*). In addition, because long-lived plants are often large and therefore susceptible to geitonogamous selfing, the resulting inbreeding depression may favour evolution of anti-selfing mechanisms (e.g. dioecy, self-incompatibility), which are common among woody plants and more prevalent among perennial than annual herbs. In the face of uncertain conditions during flowering, an annual life cycle is risky unless reproductive success is assured. Selfing provides such assurance, so the high incidence of predominant selfing among our sample of annuals (figure 2*c*) is not surprising. These conditions suggest that life history evolution prompted by altered ecological circumstances likely bears important consequences for mating system evolution, and that the frequency of selfing is particularly responsive to changed conditions.

The evolutionary vagility of mating systems is evident in families with a significant number of herbaceous species, particularly those with a mixture of perennials and annuals (Stebbins 1974). For example, Grant & Grant (1965) noted that in the Polemoniaceae all autogamous species but one were annuals, although not all annuals were selfers, and they recognized that autogamy evolved repeatedly from outcrossing in annual species. With the recent publication of molecular phylogenies (see §3) the responsiveness of mating systems in the phlox family can be established more precisely by reconstructing the likely historical sequence and frequency of these changes. Based on *mat*K sequences for 77 taxa in this family plus two outgroups (L. A. Johnson, unpublished data) we identified 80 equally parsimonious phylogenetic trees with PAUP 3.1.1. (Swofford 1991; program options as in Johnson *et al.* 1996). Although the large number of trees suggests a poorly resolved phylogeny, the trees agreed in the overall historical pattern and disagreed primarily in the resolution of polytomies within four different clades (*Allophyllum*, *Navarretia* and the related *Gilia* sections *Gilia* and *Arachnion*). Onto these trees we mapped life cycle (annual or perennial), whether a taxon reproduced autogamously or not, and the incidence of self-incompatibility (data from Grant & Grant 1965; Plitmann 1994 and assorted floras) with MacClade 3.0 (Maddison & Maddison 1992). Only eight of the 80 trees depicted the combined evolution of *mat*K and mating system with equivalent parsimony: we selected one of these trees (figure 3) to study mating system evolution.

Based on the *mat*K phylogeny, the Polemoniaceae comprises a basal clade of tropical trees and shrubs, the monotypic, shrubby genus *Acanthogilia*, and a large, primarily herbaceous, temperate clade (figure 3; see also Johnson *et al.* 1996). Within the temperature clade the annual life cycle evolved at least seven times and is a basal character for a large clade that includes *Allophyllum*, *Collomia*, *Navarretia* and *Gilia* sections *Gilia* and *Arachnion*. Reversion from an annual to perennial habit apparently occurred at least three times. Autogamy is restricted to the temperate clade and evolved independently from outbreeding at least 14 times, including nine within the *Allophyllum*–*Gilia* (*Arachnion*) clade, whereas there are no unequivocal instances of the opposite transition. Interestingly, self-incompatibility occurs in seven seemingly isolated terminal taxa raising the possibility of multiple origins within the family (see Grant & Grant 1965, page 160).

The Polemoniaceae clearly exhibits evolutionary plasticity of reproductive mode and life history, implying that phylogenetic constraints do not limit opportunities when ecological conditions demand shifts in pollination and mating systems. Self-incompatibility in this family occurs in annual and perennial species with very different pollinators (including specialized flies, bumble bees, hummingbirds, hawkmoths, bats). Hence, there seem to be few limits to the development of self-incompatibility when plants consistently receive sufficient pollen that they can afford to reject self-pollen which would expose their offspring to inbreeding depression. Similarly, recurrent evolution of an annual life cycle, primarily among desert species, suggests considerable vagility of life cycle, at least among herbaceous species. The great diversity of the *Allophyllum*–*Gilia* (*Arachnion*) clade (figure 3) also implies that annual life cycles persist in that lineage because such a life cycle represents a shared, ecologically appropriate solution, rather than a constrained life history. Indeed, the occasional reversion to a perennial life cycle within annual lineages illustrates that life cycle options remain open when they become more suitable for current ecological conditions. This is not to say that the course of evolution is unbounded. For example, the repeated origin of autogamy in lineages of

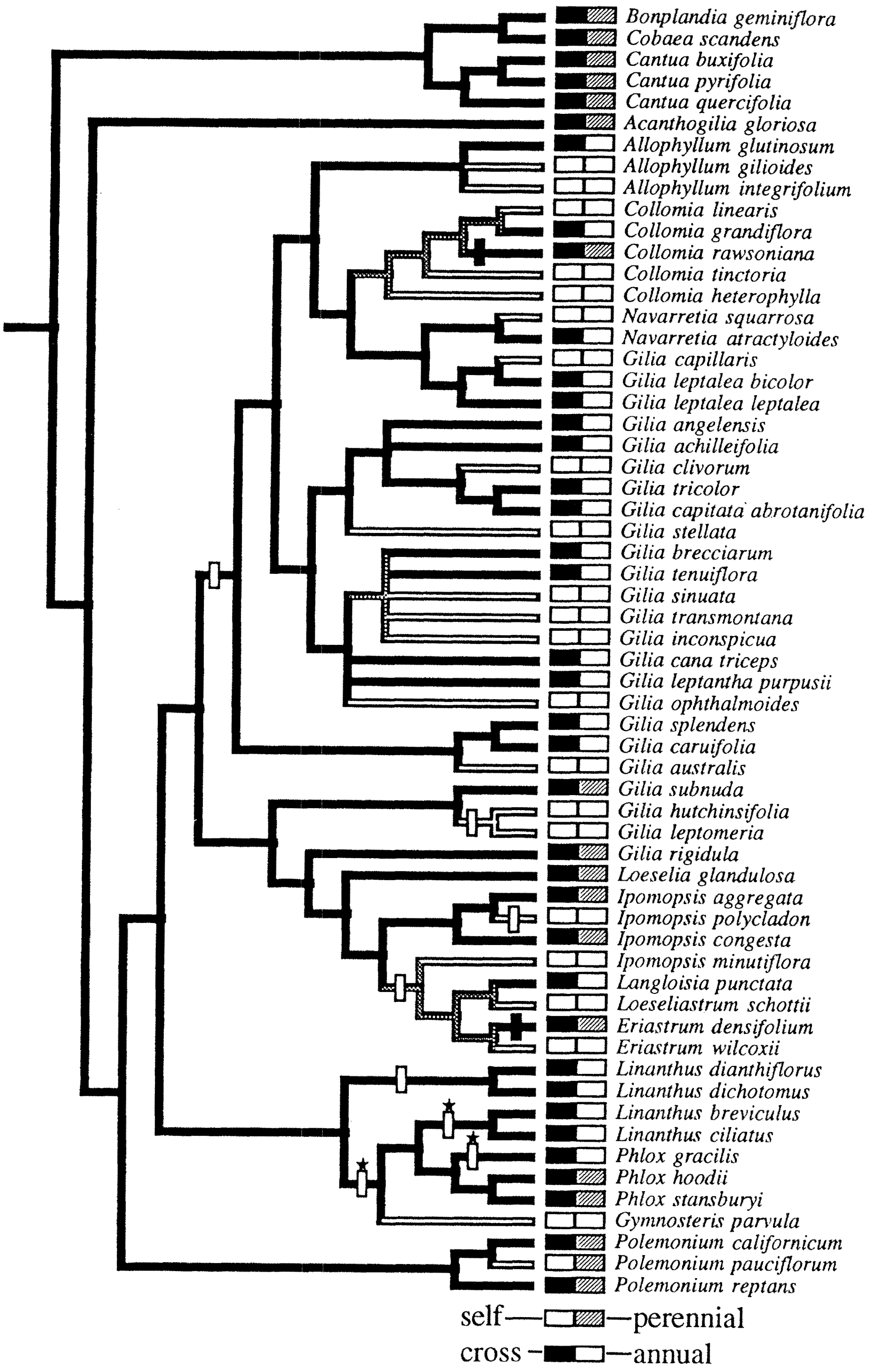

Figure 3. Historical hypothesis of the evolution of annual life cycle and autogamy in Polemoniaceae. The phylogenetic tree is extracted from one of the eight most parsimonious trees based on *mat*K sequence analysis of 77 ingroup and two outgroup taxa (*Sarracenia purpurea*, Sarraceniaceae, *Fouquieria splendens* Fouquieriaceae: sequences provided by L. A. Johnson; consistency index = 0.664, retention index = 0.853), but only ingroup taxa for which the mating system is known are included here. The histories of mating-system and life-cycle transitions were reconstructed independently on the complete tree according to an equally weighted, unordered optimization scheme. Branch shading depicts the inferred sequence of changes in mating system (black branches denote at least partially outbreeding; open branches denote autogamy; hatched branches denote equivocal), whereas bars across branches indicate inferred transitions in life cycle (open bars denote perennial to annual; closed bars denote annual to perennial; stars adjacent to open bars indicate uncertain timing of a transition from perennial to annual.

annuals suggests that the reproductive assurance provided by autogamy is a significant evolutionary influence once species adopt a high risk annual habit (Lloyd 1980). However, the generally terminal occurrence of autogamy in the phylogeny (figure 3) and the absence of reversions to outbreeding imply that reliance on predominant selfing may be an expedient solution with a limited evolutionary future. In this group, at least, the evolution of selfing may be viewed as an 'evolutionary dead end' (Stebbins 1957).

5. THE EVOLUTION OF COMPLEX REPRODUCTIVE ADAPTATIONS

Reproductive adaptations often comprise suites of correlated characters or syndromes that originate independently in different lineages through convergent selection. Comparative methods help determine the correlated structure of syndrome traits, independent of phylogeny; however, inference of the direction and temporal sequence of syndrome assembly requires explicit, optimized mapping of the characters of interest onto cladograms (Maddison & Maddison 1992). Our final example illustrates how this approach helps distinguish between selection models that predict different temporal sequences for the association of traits in a well known reproductive syndrome, heterostyly. A heterostylous population includes two (distyly) or three (tristyly) morphs that differ reciprocally in the relative positions of their male and female reproductive organs (reciprocal herkogamy) and various correlated ancillary polymorphisms of pollen and stigmas (reviewed in Barrett 1992). The floral morphs are usually self- and intramorph incompatible with compatible matings resulting from intermorph pollinations between anthers and stigmas of equivalent height. This particular syndrome exemplifies how the evolution of pollination and mating systems is often functionally linked in complex reproductive adaptations.

Models of the evolution of heterostyly conflict as to whether heteromorphic incompatibility originates before (Charlesworth 1979; Charlesworth & Charlesworth 1979) or after reciprocal herkogamy (Lloyd & Webb 1992) and hence in their respective emphasis on anti-selfing versus improved cross-pollination as selective forces responsible for the evolutionary assembly of the polymorphism. Most heterostylous taxa possess incompatibility, so that self-compatibility has traditionally been viewed as a derived trait in heterostylous groups. However, the number of reported self-compatible species has increased in recent years (Barrett & Cruzan 1994), enabling inference of the sequence of evolutionary events within families containing heterostylous species that differ in the presence of incompatibility, such as Pontederiaceae.

Graham & Barrett (1995) and Kohn *et al.* (1996) reconstructed the phylogenetic history of Pontederiaceae from approximately two-thirds of the species in the family using partial sequences from the chloroplast genes *rbc*L and *ndh*F and chloroplast DNA restriction-site variation. Reproductive traits were mapped onto trees using optimization schemes that differed in the relative weights assigned to shifts in character states. Based on plausible weighting schemes (see below), self-incompatibility appears to have originated after the origin of floral trimorphism. This sequence supports Lloyd & Webb's proposal that reciprocal herkogamy evolves initially to promote more proficient cross-pollination among individuals by more precisely matching pollen dispatch and receipt points on a pollinator's body. Incompatibility develops subsequently, either as a passive consequence of co-adaptation of each class of pollen to the style morph to which it is most proficiently transferred, or as an actively selected anti-selfing device. Several experimental results also support this evolutionary sequence (Barrett & Glover 1985; Kohn & Barrett 1992; Cruzan & Barrett 1996).

Although this concordance between phylogenetic and functional evidence appears satisfying, several issues concerning the historical reconstructions warrant consideration. Topological uncertainties in tree reconstruction can alter historical inferences concerning character evolution. For example, in the Pontederiaceae study root position is uncertain and some near-optimal root positions alter the inferred polarity of floral characters. A second issue concerns the choice of character optimization scheme. The conclusion that heteromorphic incompatibility arose after floral trimorphism depends on an unequally, rather than an equally, weighted optimization scheme. Kohn *et al.* (1996) defend their choice of unequal weighting ($\geqslant 2:1$) based primarily on the extreme rarity of trimorphic incompatibility in the angiosperms and its developmental and genetic complexity, which make its origin improbable relative to its evolutionary dissolution (see Barrett 1993). In contrast, with equal weighting Kohn *et al.* (1996) found the highly unlikely pattern that tristyly evolved up to four times in Pontederiaceae. Virtually all phylogenetic studies on the evolution of plant reproductive traits have treated character shifts as equally likely, presumably because this procedure is simpler and avoids subjectivity involved in character weighting. However, with complex characters (e.g. floral syndromes such as tristyly) this may not be wise. When biological evidence indicates an unequal probability of possible transitions between reproductive traits, workers should at least explore how the character optimization employed influences historical reconstructions.

6. CONCLUDING REMARKS

Comparative biology and phylogenetic reconstruction provide means for identifying and testing functional hypotheses; however, their reliance on phylogenetic trees currently limits their applicability to many issues in reproductive biology. Phylogenetic trees based on morphological and molecular characters both present problems for analysis of reproductive characters. Phylogenetic trees derived from morphology often rely heavily on floral traits, so that circularity can be a serious problem in studying the evolution of reproductive characters (Eckenwalder & Barrett 1986; Wyatt 1988). In contrast, phylogenies based on molecular characters often lack resolution at the species level. Hodges & Arnold (1994) reported a striking example of this problem in their study of adaptive radiation of pollination syndromes in *Aquilegia* (Ranunculaceae). Despite remarkable diversification of floral traits associated with the pollination systems of closely related species, little molecular divergence among the 27 species was evident in sequence variation from both nuclear (ITS) and chloroplast (*atb*B-*rbc*L spacer) regions. A major priority for biologists interested in phylogenetic reconstruction will be the search for genes that allow finer resolution at the species level. Until such genes are found, the availability of well resolved phylogenies will hamper comparative analysis of reproductive characters in some groups.

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