



A comparative analysis of pollen limitation in flowering plants

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We conducted a comparative analysis to determine life history and ecological correlates of pollen limitation among 224 species of animal-pollinated flowering plants. To test predictions concerning the occurrence of pollen limitation, species were classified categorically for six life-history traits (self-compatible vs. self-incompatible, autogamous vs. non-autogamous, specialized vs. unspecialized floral morphology, nectariferous vs. nectarless, monocarpic vs. polycarpic, herbaceous vs. woody) and two ecological conditions (presence in open vs. forested habitats and temperate vs. tropical biomes). Pollen limitation of species in contrasting categories was compared using non-parametric tests (TIPs analysis) and phylogenetically-independent contrasts (PICs). The results of TIPs and PICs analyses were generally congruent, although fewer significant contrasts were evident with PICs, probably because of low statistical power. Overall the results suggest that the influence of phylogenetic history on the intensity of pollen limitation was not particularly strong. Nonetheless, significant variation in the degree of pollen limitation was demonstrated among seven angiosperm families suggesting some phylogenetic component to the phenomenon. With both TIPs and PICs, pollen limitation was less intense in self-compatible and autogamous species. TIPs analysis demonstrated that herbaceous, nectariferous, and temperate species were less likely to be pollen-limited, but using PICs this could only be corroborated for those that were self-incompatible. None of the traits were singularly unambiguous predictors of pollen limitation, possibly reflecting the stochastic nature of pollinator service.

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ADDITIONAL KEY WORDS:—animal pollination – insufficient pollination – life-history traits – phylogenetically-independent contrasts – self-compatibility – autogamy.

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INTRODUCTION

Angiosperms commonly mature fewer fruits and seeds than the flowers and ovules they produce. Two proximate ecological mechanisms account for this pattern: insufficient pollen delivery to stigmas and limited resources for maturation of fruits and seeds (Bierzychudek, 1981; Haig & Westoby, 1988; Campbell & Halama, 1993; Burd, 1994; Vaughton & Ramsey, 1995). Pollen limitation is demonstrated empirically when supplemental pollination of flowers increases their female fertility compared to open-pollinated controls. A survey of 258 species of angiosperms indicated that 62% were pollen limited at some times or in some locations (Burd, 1994). However, the specific ecological mechanisms responsible for insufficient pollen delivery have rarely been investigated, and we have limited ability to predict the fertility of individual plants or species in different environments (Johnston, 1991; Johnson & Bond, 1997; Larson & Barrett, 1999a). One approach to predicting the intensity of pollen limitation among species is to use the techniques of comparative biology (Harvey & Pagel, 1991; Silvertown *et al.*, 1997). These methods can provide evidence that particular life-history traits and ecological conditions are associated with pollen limitation.

There have been two previous comparative surveys of the factors associated with variation in female fertility among angiosperm species (Sutherland, 1986; Burd, 1994). In common with much ecological research these investigations did not consider the phylogenetic relationships among the taxa considered. The availability of a family-level phylogeny for the angiosperms (Chase *et al.*, 1993) allows the use of phylogenetically independent contrasts (hereafter PICs), to accommodate the potential problems posed by the historical non-independence of species (Felsenstein, 1985; Harvey & Pagel, 1991; Purvis & Rambaut, 1995). This method uses a phylogenetic tree to limit statistical comparisons to sister taxa and assumes that trait differences between taxa (PICs) have evolved since their common origin, and hence are independent of one another.

However, there is currently debate about whether tests for correlated trait evolution should consider species independently (TIPs) or whether PICs should always be used in comparative studies (Harvey *et al.*, 1995; Westoby *et al.*, 1995; Mazer, 1998). Although TIPs analyses may inflate statistical degrees of freedom and increase the likelihood of Type I error, PICs assume a particular evolutionary history and imply that phylogeny has a greater influence on trait expression than selection in current environments (Westoby *et al.*, 1995, 1996; Barrett *et al.*, 1996; Mazer, 1998). However, recent meta-analyses of evolution in vertebrates have found a high concordance between the results of TIPs and PICs (Ricklefs & Starck, 1996; Price, 1997),

suggesting that the effect of common ancestry is not generally large enough to invalidate conclusions based on TIPs, particularly when data are available for a large number of species distributed among many separate evolutionary lineages. Nonetheless, these reviews advocate use of both types of analysis because differences between them may provide insights into the tempo of evolutionary change in the traits under consideration.

In this study we use both TIPs and PICs to test several hypotheses concerning the association between pollen limitation and various life-history and ecological traits in 224 species from 64 families of flowering plants. A variety of intrinsic and extrinsic factors likely contribute towards pollen limitation. Pollen limitation could be an adaptive consequence of the possession of particular reproductive traits that optimize fitness in a particular environment. Alternatively, insufficient pollen delivery to stigmas and reduced fertility could arise because of the stochastic nature of the pollination process, especially under ecological conditions that limit pollinator activity (Burd, 1995). While in reality both factors are likely to play a role in determining levels of pollen limitation (Haig & Westoby, 1988; Burd, 1994), comparisons between the results of PICs and TIPs may aid in distinguishing between their relative importance. Here we considered four general hypotheses concerning conditions that are likely to be associated with variation in the intensity of pollen limitation among flowering plants.

Hypothesis 1: Species with the capacity to self-fertilize exhibit less pollen limitation than species that cannot self-fertilize. The first prediction is that self-compatible species exhibit less pollen limitation than those that are self-incompatible because an additional source of pollen, self-pollen, can potentially contribute to their fertility. Burd's (1994) analysis supported this prediction. Self-compatibility provides reproductive assurance only if plants can self-pollinate autonomously giving rise to autogamous self-fertilization (Lloyd, 1992; Lloyd & Schoen, 1992). Autogamy has often been proposed as adaptive where pollinator visitation is unreliable (Hagerup, 1951; Baker, 1955; Motten, 1986; Bond 1994). Therefore, this hypothesis predicts that autogamous species exhibit less pollen limitation than self-compatible species that are not capable of autonomous self-pollination.

Hypothesis 2: Species with short life spans exhibit less pollen limitation than longer-lived species. Pollen limitation may greatly reduce the lifetime fitness of short-lived species, so selection should be stronger on traits minimizing pollen limitation. In contrast, fitness is accrued over multiple years in longer-lived plants so reduced fertility in one year may not lower fitness to the same extent as in short-lived species (Calvo & Horvitz, 1990; Primack & Hall, 1990; Zhang & Wang, 1994). Two predictions arise from these considerations: (i) monocarpic species exhibit less pollen limitation than polycarpic species, and (ii) herbaceous species exhibit less pollen limitation than woody species. The latter prediction is less certain since it assumes that herbs have shorter lifespans than woody species, which may not be the case for herbs with extensive clonal propagation.

Hypothesis 3: Species with flowers visited frequently and by a diversity of pollinators exhibit less pollen limitation than those visited infrequently. Two predictions arising from this hypothesis are that (i) species with 'unspecialized' floral morphology exhibit less pollen limitation than those with 'specialized' flowers, and (ii) nectariferous species are less pollen limited than those with nectarless flowers. Classification of species with respect to

the first prediction is less straightforward than for the second, because of contrasting views of generalization vs. specialization in pollination systems (see for example Waser *et al.*, 1996), and the assumption that these conditions are associated with differences in visitation rate. Nevertheless, for our analysis we attempted a classification based on floral morphology and the accessibility of floral rewards (see Methods). McCall and Primack (1992) reported higher insect visitation rates to open vs. tubular flowers in deciduous woodland and alpine tundra in North America, and in the fynbos of South Africa, providing support for the importance of floral morphology in influencing visitation rates. With respect to the second prediction, higher visitation rates were reported for nectariferous vs. nectarless spring-blooming herbs in North Carolina (Motten, 1986), and higher reproductive success has been documented for nectariferous vs. nectarless orchids in the Cape region of South Africa (Johnson & Bond, 1997) and for orchids in general based on a survey of fruit set in 117 species (Neiland & Wilcock, 1998).

Hypothesis 4. Species that occur under contrasting ecological conditions exhibit different levels of pollen limitation. Despite the large numbers of studies of pollen limitation, there have been remarkably few attempts to demonstrate that contrasting ecological conditions are associated with different degrees of pollen limitation between species or populations within species (see Burd, 1994). With this deficiency in mind, we explored two contrasting environmental circumstances (open vs. closed habitats and tropical vs. temperate biomes) that could potentially affect pollinator visitation rates and hence pollen limitation. We made two predictions for these contrasts. First, we predicted that species occurring in open habitats would be less pollen-limited than those of forested habitats. Insect thermoregulatory capacity is higher in open than shaded habitats so visitation rates to flowers may be greater in the former (Heinrich, 1979). Second, we predicted that species occurring in temperate biomes exhibit less pollen limitation than those in tropical biomes. Plants that occur in tropical regions often occur at lower densities than those in temperate regions (Baker, 1959; Fedorov, 1966). Plants at low densities are usually visited less frequently than those at higher densities (Antonovics & Levin, 1980). At the outset we considered both predictions to be relatively weak in direction, since the communities represented in both contrasts are diverse and vary greatly in biotic and abiotic conditions. The proposed associations with visitation rates and density are therefore unlikely to hold for all situations.

METHODS

Pollen limitation index

Using experimental data from the literature, we calculated a pollen limitation index: $L = 1 - (P_o/P_s)$, where P_o is the percent fruit set of open-pollinated controls and P_s is the percent fruit set by plants that received supplemental cross pollen. $L = 0$ indicates no pollen limitation in the population under study. The index was given a lower bound of zero because negative indices (greater fertility from natural than supplemental pollination) likely result from experimental or Type I statistical error (Young & Young, 1992; Burd, 1994) and are not informative in the current context. Percent fruit set was used as the measure of fertility because pollen limitation has been reported most often in terms of fruit set. The next largest data set, seeds per

fruit, was too small for meaningful comparative tests. Nevertheless, pollen limitation based on percent fruit set and seeds per fruit for 83 spp. in the data set correlate significantly ($r=0.37$, $P<0.001$).

Most data used to calculate pollen limitation were obtained from Burd's (1994) survey, and involved comparisons of fruit set for 207 spp. in 70 families. We supplemented this information with published and unpublished data available since the publication of his review for an additional 34 spp. including four new families (Appendix). For the entire data set, pollen limitation was measured during more than one year or location for 31 and 29 spp., respectively. For these species, we pooled data for different years or sites to give a single measure of pollen limitation.

Trait categorization

The self-compatibility of each species was classified categorically according to information in the original papers. In some cases the authors simply stated whether or not a species was self-compatible, and this categorization was used for the analyses. Otherwise, we classified a species as self-incompatible if fruit set after self-pollination was less than 1/20 of that following cross-pollination (following Burd, 1994). To validate this cut-off value, we also performed a separate analysis in which self-incompatible species were defined as those that set less than 1/2 as many fruit after self-pollination as after cross-pollination.

We coded each species for the other traits under investigation based on information in the original papers or Mabberley (1987). It was not possible to code the potential for autogamy, the presence or absence of nectar, and habitat type for every species. Autogamous species were defined as those with greater than 20% fruit set in bagged relative to outcrossed treatments. *Agave*, *Argyroxiphium* and palms are not truly woody, so they were excluded from the herbaceous-woody comparison. Temperate species were broadly defined to include those in the Mediterranean, the Cape Province of South Africa, New South Wales in Australia, and a few montane and arctic species. Floral specialization was classified according to whether floral morphology restricts pollinator access to floral rewards (nectar). 'Specialized' flowers were defined as those with narrow, tubular flowers or complex morphologies (e.g. Orchidaceae) that can only be accessed by a subset of the pollinator fauna. 'Unspecialized' flowers were those with broad open tubes, bowl-shaped flowers or small clusters of flowers (e.g. Asteraceae) that could potentially be visited by a wide range of pollinator groups. The pollen limitation index and trait codings for each species in the analysis are available from the authors upon request.

Several of the traits that we examined clearly covary with one another. For example, self-compatibility is a requirement for autogamy, but not all self-compatible species are autogamous. Hence, in the comparison of self-compatibility vs. self-incompatibility, autogamous species were removed to isolate the effect of self-compatibility on pollen limitation. Similarly, woody species were removed from the contrast between monocarpic and polycarpic species because only polycarpic species are woody and hence the comparison should involve only herbs. Finally, autogamous species were removed from the comparison between herbaceous and woody species because as yet no woody species are known that are predominantly autogamous (Barrett *et al.*, 1996; Morgan & Schoen, 1997).

Comparative analysis using TIPs

A comparative analysis was first conducted in which each species was treated as an independent observation. The pollen limitation indices for each trait were plotted as histograms and analysed for uniformity using chi-square tests. We tested the predictions outlined in the introduction with one-tailed non-parametric Wilcoxon two-sample tests (Sokal & Rohlf, 1995). As discussed above, our expectations for tests of herbaceous vs. woody species, species with 'specialized' vs. 'unspecialized' floral morphology, tropical vs. temperate species and species occurring in forested vs. open habitat were less certain. In these cases, asymmetrical critical regions and directed tests were used (Rice & Gaines, 1994). Directed tests provide much of the increased power of a one-tailed test, but allow detection of significant results in the 'unexpected' direction. All statistical analyses were conducted using JMP (Version 3.0.2, SAS Institute, 1994).

Since data on pollen limitation were available for multiple species ($N > 6$) in seven of the angiosperm families surveyed (Amaryllidaceae, Asteraceae, Ericaceae, Fabaceae, Liliaceae, Orchidaceae, Scrophulariaceae), we also used one-way ANOVA to investigate the extent to which familial membership influenced mean pollen limitation.

Phylogenetic placement of taxa

To compute PICs, the phylogenetic relationships among species in the data set were determined (Purvis & Rambaut, 1995). Taxa were coded phylogenetically by first positioning families according to the Chase *et al.* (1993) *rbcL* phylogeny of seed plants, as used in several recent comparative analyses (Barrett *et al.*, 1996; Rees, 1996; Silvertown & Dodd, 1996). Ten families in the Burd data set (Cactaceae, Cistaceae, Cochlospermaceae, Guttiferae (Clusiaceae), Hippocastanaceae, Hippocrateaceae, Loasaceae, Salicaceae, Staphyleaceae, and Thymelaeaceae) were not located on the tree, so they were excluded, leaving 224 species. Species placed in the Vivianaceae, Lobeliaceae, and Amaryllidaceae (*Alstroemeria*) by Burd (1994) were instead placed in the Geraniaceae, Campanulaceae, and Alstroemeriaceae, respectively, to correspond with nomenclature used for the Chase *et al.* phylogeny (Cronquist, 1981). Some families were polyphyletic, but genera from the data set were consistently placed in one clade either because they were present on the Chase *et al.* (1993) tree or were closely allied to a genus that was (Mabberley, 1987; J. E. Eckenwalder, pers. comm.). This was only problematic in Liliaceae, so for this family the tree was modified by grafting the Chase *et al.* (1995) *rbcL* phylogeny of Liliaceae onto the original tree to facilitate placement of families in this group. In particular, genera of Liliaceae *s.l.* were split into Alliaceae, Convallariaceae, Liliaceae, Melanthiaceae, Trilliaceae and two clades of the polyphyletic Uvulariaceae.

Given the familial tree, relationships between species were coded to minimize the number of polytomies. PICs can be calculated despite polytomies, but the power of tests is reduced (Purvis & Rambaut, 1995). Resolution of the phylogeny was facilitated by recently published phylogenies for the Amaryllidaceae (Meerow, 1995), Asteraceae (Bremer, 1994), Fabaceae (Doyle, 1995), and Orchidaceae (Dressler, 1993). Phylogenies were unavailable for most families, but placement of taxa according to traditional tribes (from Dahlgren *et al.*, 1985; Mabberley, 1987) and genera provided

additional structure. Most families were represented by few taxa, so the final tree had no polytomies containing more than four genera.

Comparative analysis using PICs

A comparative analysis was conducted using PICs based on the inferred tree. PICs were computed using the computer program CAIC (Purvis & Rambaut, 1995). Branch lengths were set equal, which assumes a punctuational mode of evolution that generally performs better in simulations than other models (Purvis & Rambaut, 1995). Branch lengths reported in the Chase *et al.* (1993) phylogeny were not used because *rbcL* evolution occurs at different rates among angiosperm lineages (Clegg, 1993). Because the pollen limitation index was bounded at zero and one, analyses with continuous independent variables did not meet assumptions of the independent contrasts method regardless of transformations (Garland *et al.*, 1992; Díaz-Uriarte & Garland, 1996). Analyses with categorical predictor variables ('Brunch' option) did meet these assumptions and were used instead.

With categorical variables, the independent contrasts method compares the pollen limitation indices of pairs of taxa that differ in the predictor variable and are phylogenetically independent of other pairs (see Purvis & Rambaut, 1995 for details). Consistently higher indices in taxa with one character state indicate that evolution of this character is associated with increased pollen limitation. PICs were normally distributed (Wilk-Shapiro $P > 0.05$) so we used a single-sample *t*-test to assess whether a contrast deviated significantly from zero (Purvis & Rambaut, 1995). Such a deviation indicates that changes from one character state to another are accompanied by a consistent shift in pollen limitation.

RESULTS

Comparative analysis using TIPs

The mean pollen limitation of angiosperm species in the analysis was 0.40 (SE = 0.022). The TIPs analysis supported seven of the eight predictions outlined in the introduction. Species that are self-compatible, autogamous, monocarpic, herbaceous, nectariferous or that occur in open habitats or temperate regions exhibited significantly less pollen limitation than species with contrasting character states (Table 1; Fig. 1). Species with 'specialized' flowers did not experience different levels of pollen limitation than those with 'unspecialized' flowers ($P > 0.30$). Pollen limitation varied extensively in all traits considered (Fig. 1), indicating that single traits explain only a small proportion of the variation in pollen limitation.

There were significant differences among the seven families in mean pollen limitation ($F_{6,98} = 11.66$, $P < 0.0001$; Fig. 2), with values ranging from 0.11 to 0.68. Pollen limitation in Fabaceae and Orchidaceae was significantly higher and at least double the magnitude of that found in Asteraceae, Ericaceae, Liliaceae and Scrophulariaceae (Tukey-Kramer HSD, $P < 0.05$).

TABLE 1. Mean pollen limitation (\pm SE) and sample sizes for traits considered in the TIPs analysis. Pollen limitation is expected to be lower in the trait listed first for each hypothesis. Distributions of the index for each class of species are given in Fig. 1

Hypothesis	Trait	Mean pollen limitation	Standard error	Sample size
1a.	Self-compatible	0.31	0.03	102
	Self-incompatible	0.59	0.04	66
1b.	Autogamous	0.16	0.04	35
	Non-autogamous	0.38	0.03	97
2a.	Monocarpic	0.16	0.05	14
	Polycarpic	0.42	0.02	124
2b.	Herbaceous	0.32	0.03	102
	Woody	0.52	0.04	82
3a.	Unspecialized flowers	0.38	0.03	130
	Specialized flowers	0.42	0.03	94
3b.	Nectariferous	0.35	0.03	91
	Nectarless	0.47	0.05	42
4a.	Temperate	0.30	0.02	139
	Tropical	0.56	0.04	85
4b.	Open habitats	0.33	0.03	82
	Forested habitats	0.42	0.03	108

Comparative analysis using PICs

PICs confirmed two of the seven patterns found in the comparative analysis based on TIPs. There was less pollen limitation in self-compatible and herbaceous species (Table 2). Pollen limitation was marginally less frequent in autogamous than non-autogamous species ($N=26$, $P<0.057$). A less stringent definition of self-incompatibility (self-compatibility index less than 0.50) confirmed the finding that self-compatible species were less likely to be pollen-limited (37 contrasts, \bar{X} (SE)=0.086 (0.026), $t=3.26$, $P<0.001$). None of the other comparisons were significant, but in all cases the trends were in the predicted direction (Table 2). In general, the results using PICs paralleled those using TIPs and the differences observed probably reflect loss of statistical power.

PICs were also conducted using subsets of the data to provide better control over confounding variables. For example, among the species surveyed there was an association between self-compatibility and both herbaceousness and occurrence in temperate habitats (for herbs, $X^2=21.61$, $P<0.0001$; for temperate species, $X^2=18.45$, $P<0.0001$; based on G -tests of independence). PICs were thus conducted for these classes using only self-incompatible species. Although PICs between self-compatible herbs and woody species were nonsignificant (20 contrasts, $t=0.891$), they were significant for self-incompatible species (10 contrasts, \bar{X} (SE)=0.064 (0.027), $t=2.35$, $P_{\text{dir}}<0.03$). This result corroborates the lowered pollen limitation of herbs relative to woody species *de facto*, and indicates that the result is not just a reflection of their tendency to be self-compatible. Similarly, self-incompatible temperate species were less pollen-limited than tropical species (12 contrasts, \bar{X} (SE)=0.090 (0.016), $t=5.61$, $P_{\text{dir}}<0.0001$) (Fig. 3). Although there was no direct association between self-compatibility and the presence of nectar (for species data, $X^2=0.004$), self-incompatible nectariferous species were also less likely to be pollen-limited than nectarless species (8 contrasts, \bar{X} (SE)=0.040 (0.022), $t=1.80$, $P_{1\text{-tailed}}<0.06$).

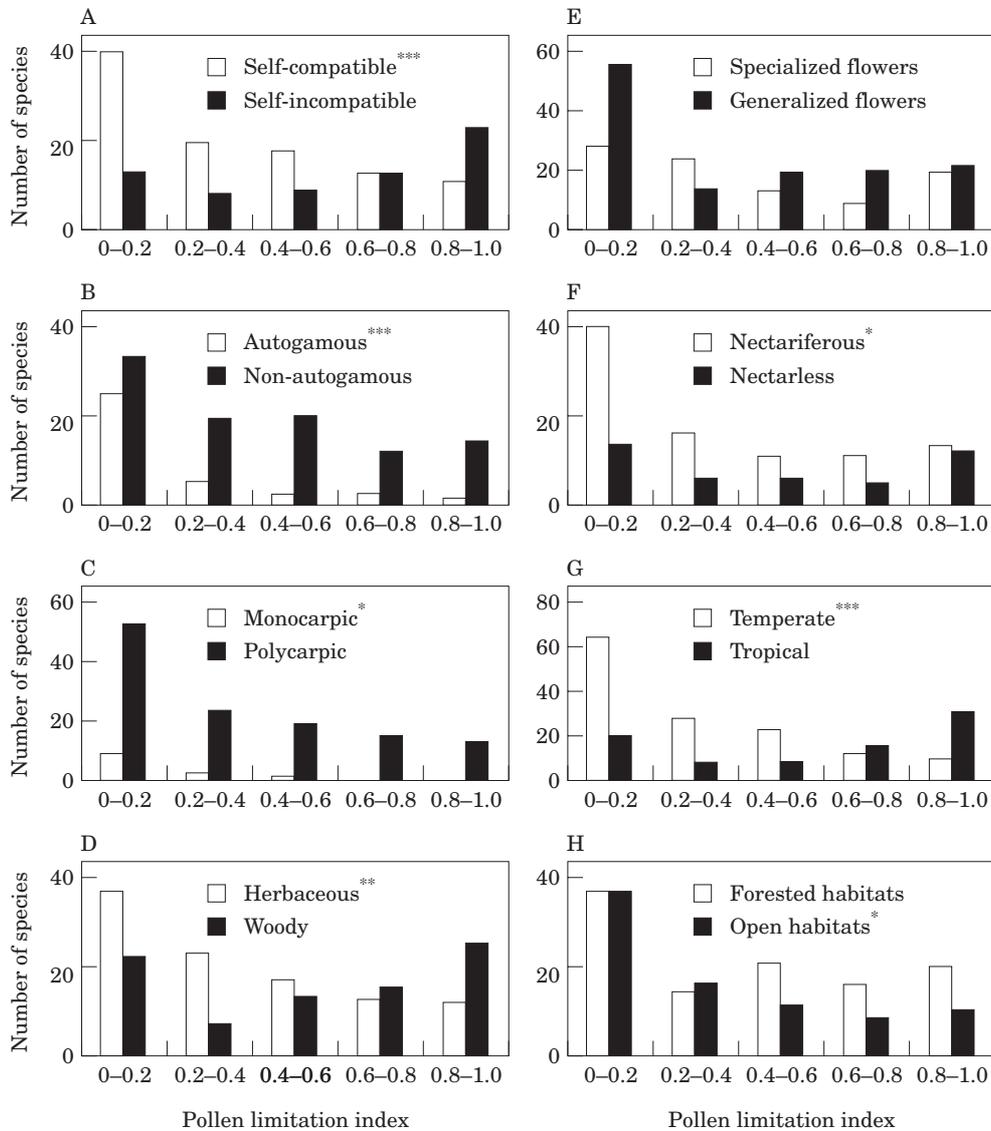


Figure 1. Frequency distributions of pollen limitation indices for species (TIPs) with contrasting life-history traits and that occur in different ecological conditions. (A) self-compatible vs. self-incompatible; (B) autogamous vs. non-autogamous; (C) monocarpic vs. polycarpic; (D) herbaceous vs. woody; (E) specialized flowers vs. generalized flowers; (F) nectariferous vs. nectarless flowers; (G) temperate vs. tropical; and (H) forested vs. open habitats. Sample sizes are given in Table 1. Each of the distributions, except for nectarless species, is non-uniform ($P < 0.05$) based on X^2 tests with four degrees of freedom. Pollen limitation is less severe (lower indices) in the classes indicated by asterisks (***) $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$) based on one-tailed and directed Wilcoxon two-sample tests.

DISCUSSION

Our comparative analysis using phylogenetically-independent contrasts (PICs) revealed several general influences on variation in pollen limitation in angiosperms.

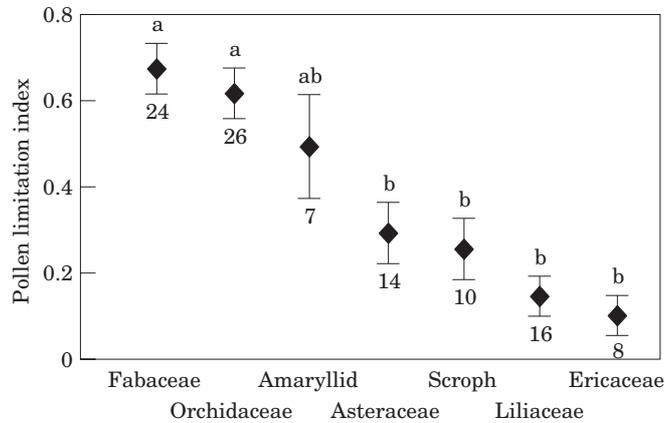


Figure 2. Mean pollen limitation (\pm SE) in seven angiosperm families. Sample sizes are given below the bars, and statistically homogeneous groupings based on a Tukey-Kramer HSD are indicated by the same letter (ab) above the bars.

TABLE 2. Results of tests using PICs of eight predictions concerning traits associated with pollen limitation. The number and mean value (\pm SE) of contrasts are presented, as well as t -statistics and P -values for tests of the predictions. Positive contrasts support the stated predictions. See text and Fig. 3 for tests of hypotheses 2b, 3b and 4a for self-incompatible species only

Hypothesis	Prediction for intensity of pollen limitation	Number of contrasts	Value of contrast (mean \pm SE)	t -statistic	P -value	Hypothesis confirmed?
1a.	Self-incompatible>self-compatible	38	0.057 \pm 0.024	2.390	0.011	Yes
1b.	Non-autogamous>autogamous	26	0.038 \pm 0.023	1.637	0.057	Marginal
2a.	Polycarpic>monocarpic	9	0.031 \pm 0.043	0.725	0.245	No
2b.	Woody>herbaceous	21	0.054 \pm 0.028	1.933	$P_{dir} = 0.042$	Yes
3a.	Specialized>unspecialized flowers	23	0.010 \pm 0.023	0.443	$P_{dir} = 0.331$	No
3b.	Nectarless>nectariferous	24	0.004 \pm 0.026	0.171	0.433	No
4a.	Tropical>temperate	28	0.047 \pm 0.030	1.576	$P_{dir} = 0.081$	No
4b.	Forested>open habitats	33	0.043 \pm 0.027	1.603	$P_{dir} = 0.074$	No

Self-compatibility and autogamy were associated with reduced pollen limitation, presumably because the capacity for self-fertilization decreases reliance on cross-pollination by pollinators. Pollen limitation did not differ between herbaceous vs. woody, nectariferous vs. nectarless and temperate vs. tropical species, except when self-compatible species were excluded from the analysis. Several additional factors influencing pollen limitation were found when species were treated independently (TIPs). Below, we discuss these results, compare PICs and TIPs analyses, and suggest other traits not considered in our analyses that may also affect pollen limitation.

Self-compatibility and reduced pollen limitation

The hypothesis that pollen limitation is reduced in species capable of self-fertilization was strongly supported by both TIPs and PICs. Self-fertilization in

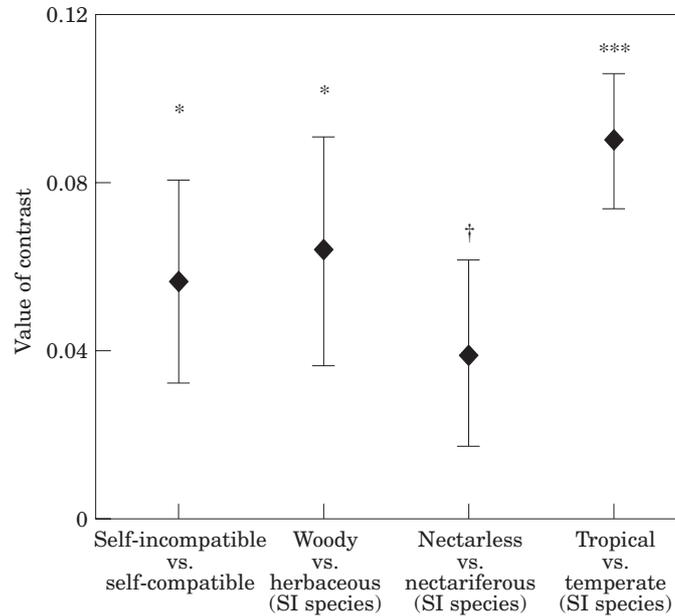


Figure 3. Mean difference (\pm SE) in pollen limitation of species with different attributes following PICs. Positive contrasts indicate that the class listed first for each comparison has more intense pollen limitation (greater pollen limitation index) than the other class. The latter three comparisons are for self-incompatible (SI) species only. See Table 3 and text for number of contrasts for each comparison. *** $P < 0.0001$, * $P < 0.05$, † $P < 0.06$, based on t -tests for a difference from zero.

plants can be mediated by pollinators or it can occur autogamously (Lloyd & Schoen, 1992). The reduced pollen limitation in self-compatible species supports the view that self-fertilization can increase plant fertility by minimizing dependence on pollinators for pollen transfer between plants. Autogamous species depend less on pollen vectors, and are therefore less likely to be pollen-limited than non-autogamous self-compatible species. This conforms with the well-appreciated benefit of autogamy as a means of reproductive assurance when pollination is uncertain (Baker, 1955; Lloyd, 1980).

Self-compatibility also appears to buffer pollen limitation independently of other life-history traits that are possessed by a plant. For example, among self-compatible species there were no differences using PICs for contrasts involving herbaceous vs. woody, nectariferous vs. nectarless, or temperate vs. tropical species. However, these comparisons were significant for self-incompatible species, indicating that these traits are more likely to be associated with pollen limitation in self-incompatible than self-compatible species.

TIPs, but not PICS, demonstrated less pollen limitation in monocarpic than polycarpic species. Most monocarpic species in the analysis are annuals (68.8%, $N = 16$), so this result supports the hypothesis that pollen limitation is less likely in species with short life spans. This result did not arise because of an association between monocarpy and self-compatibility ($X^2 = 0.95$, $P > 0.30$), suggesting that monocarpic species have low pollen limitation for reasons other than self-compatibility alone. The failure to detect a relationship using PICs was most likely associated with the small number of contrasts ($N = 9$) available for this analysis.

Herbaceous species demonstrated less pollen limitation than woody species using TIPs, and using PICs, for those that were self-incompatible. The shorter life span of herbs is only one explanation for this result. As stated earlier, herbaceous species in the survey tend to be self-compatible, but this confounding influence was removed by excluding herbs with this condition from the analysis. Reduced pollen limitation in herbs may alternatively reflect differences in display size relative to woody species. The larger display sizes of many woody species may increase overall visitation rates compared to herbs, but the visitation rate per flower may actually be lower in woody species compared with herbs resulting in insufficient pollen deposition to initiate fruit set. Lower per flower visitation rates in plants with large displays have often been reported, but unfortunately few studies have involved woody species (reviewed in Harder & Barrett, 1996).

Other factors influencing pollen limitation

Neither TIPs nor PICs revealed a difference between pollen limitation in species with 'specialized' versus 'unspecialized' floral morphologies. This result may reflect a variety of problems associated with this particular analysis. First, pollinator specialization may not necessarily be reflected by obvious differences in floral morphology (Herrera, 1988; McCall & Primack, 1992). Hence, our attempt to classify species based on the relationship between morphology and reward accessibility may have little functional significance for pollen limitation. Second, our comparison was based on the assumption of differences in visitation rates between these two floral classes, but this assumption may be unreasonable. Third, visitation rates may sometimes be unrelated to pollen limitation. For example, in some specialized pollination systems sufficient pollen may be deposited on stigmas during a single visit to ensure full seed set. Unfortunately, knowledge of the pollination systems of most species in the survey was too rudimentary to directly classify them with respect to pollinator type(s), which may have been more informative for predicting pollen limitation. Habitat-specific or sister-species comparisons between plants that rely on a small suite of pollinators versus those pollinated by generalists might provide a more powerful test of our hypothesis.

Analyses using TIPs and PICs with self-incompatible species demonstrated that species that were nectariferous exhibit less pollen limitation than those without nectar. This corroborates the common assertion that rewards have a measurable influence on pollinator attraction (Simpson & Neff, 1983). However, in a separate analysis of orchid species in our survey, we found no difference between levels of pollen limitation in nectariferous vs. nectarless species ($df = 24$, $t = 0.53$, $P > 0.60$), but see Johnson and Bond (1997) and Neiland and Wilcock (1998). Comparisons of pollen limitation in nectariferous and co-occurring 'deceitful' sister species that share similar pollinators could provide a more direct test of the importance of rewards for pollinator visitation. Alternatively, experimental manipulation of rewards by the removal or addition of nectar might be a more useful approach for determining the extent to which rewards influence the likelihood of pollen limitation.

Pollen limitation was marginally lower in species of open habitats compared to those of forested habitats, based on TIPs and PICs. The absence of a strong difference between these classes may reflect the artificial dichotomy between open and forested habitats, because many habitats in which the species in our survey

occurred were likely intermediate between these extremes. In addition, some insect foraging may be relatively unaffected by the microclimatic differences between these habitats. This issue could be examined by comparing insect foraging behavior and pollen limitation in populations of a species occurring in habitats that are explicitly contrasted.

TIPs analysis and PICs using self-incompatible species demonstrated that temperate species exhibit less pollen limitation than tropical species. The ecological mechanisms responsible for this difference are unknown. While it is possible that low density conditions and greater dependence on specialized pollinators in some tropical habitats (e.g. tropical forests, Bawa, 1990) may contribute to this result, other factors must also play a role. To determine the mechanistic basis for the observed result, pollen limitation in tropical-temperate sister taxa could be compared in relation to their densities and pollinator foraging rates.

TIPs versus PICs

A comparison of TIPs and PICs can provide insight into the evolutionary lability of life-history traits (Price, 1997). In general, our TIPs and PICs analyses led to broadly similar conclusions, implying that TIPs analyses were not strongly influenced by common ancestry. Rather, ecological conditions in contemporary environments appear to affect the degree of pollen limitation within lineages more strongly than phylogeny. It is also possible that because the species in our survey were distributed widely among 64 angiosperm families, the influence of non-independence was not especially marked in our data set. This is not to say that phylogeny has no effect on the intensity of pollen limitation, since our analysis of seven angiosperm families provided clear evidence that some differed in their overall level of pollen limitation (Fig. 2). For example, in orchids the intensity of pollen limitation was five times greater than in ericads. This result is not unexpected given the specialized floral morphology of orchids and the extremely low insect visitation rates that are characteristic of many members of the family, especially those with nectarless flowers (Neiland & Wilcock, 1998). In contrast, many members of the Ericaceae have relatively unspecialized flowers and are visited by a wide range of insects, especially bees (Reader, 1975).

Our results indicate that the ability to predict pollen limitation using the traits considered was not greatly strengthened by the use of PICs. First, the mean value of significant PICs ranged from 0.04 to 0.09. Given that the average magnitude of pollen limitation for the traits considered was 0.40, this difference is relatively small. Second, the variances of the contrasts were relatively large, with two-tailed confidence limits typically spanning zero. Lastly, the percentage of PICs that were in the wrong direction ranged from 29% in the herbaceous vs. woody comparison to 44% in the comparison of monocarpic vs. polycarpic species.

Limitations of the analysis

A major limitation of most comparative analyses to date is that interactions between traits cannot be fully investigated. We examined interactions in a preliminary logistic regression analysis of the TIPs data. Eight main effects and their alternative

states were considered: pollination treatment, self-compatibility, herbaceousness, monocarpy, specialization of floral morphology, presence of nectar, biome and habitat. The interactions between the latter seven traits and pollination treatment were assessed to determine whether they influenced the intensity of pollen limitation, when other main effects were controlled. The results were largely inconclusive, because of a general absence of significant interaction effects. Rees (1996) used multiple regression to consider interactions between multiple continuous variables in PICs analyses, but acceptable techniques for considering interactions between categorical traits are still under investigation (see Martins & Hansen, 1997).

Interpretation of our results depends in part on how well our measure of pollen limitation characterizes pollen limitation in the species studied. There are two instances in which the index may not accurately depict pollen limitation. First, some of the studies in the survey did not conduct pollen supplementations at the whole-plant level, so the indices for these species may be confounded with resource limitation (Zimmerman & Pyke, 1988). This may be relatively insignificant, however, because all flowers on plants were supplemented with pollen in 66% of the species for which this could be evaluated ($N=108$, excluding woody species). Second, the index concerns fruit set, which in some cases may not be the most appropriate measure to assess pollen limitation. Some authors consider seed set per fruit to be a more adequate descriptor of pollen limitation in certain circumstances (Snow, 1986; Jennersten & Nilsson, 1993). However, for species in which both fruit and seed measures of pollen limitation have been measured, Burd (1994) has shown that overall fertility is more often determined by maturation of whole fruits than with the number of seeds within fruits.

Another deficiency of our pollen limitation index is that it gives no indication of the stochasticity inherent to pollen limitation (Burd, 1995). Relatively few studies have examined pollen limitation at various times or sites, but where this has been done significant variation has been typically found. In Burd's (1994) survey, whether a species was pollen-limited varied for 76.9% of the species for which it was measured multiple times within a season, and for 44.7% of those tested in multiple sites or years ($N=13$ and $N=38$, respectively). Ideally, the index should incorporate this variability in pollen limitation, since it defines the capacity for selective forces to influence traits governing pollen limitation. Additionally, an analysis of the ecological correlates of variance in pollen limitation could be conducted once more data are available.

Lastly, the PICs analyses depend on a number of evolutionary assumptions. In particular, they are based upon one phylogenetic hypothesis for the angiosperms. Sensitivity analyses were not conducted to test the robustness of the results to topological changes (Donoghue & Ackerly, 1996), because there is no globally parsimonious tree for the species considered. Although assumptions were also made about branch lengths and the mode of evolutionary change, the general concurrence of TIPs and PICs analyses suggests that these may not have biased our results unduly (see Ricklefs & Starck, 1996).

The relatively low predictive power provided by the traits investigated here suggests that interspecific variation in pollen limitation is also influenced by a suite of additional factors that we were unable to investigate here because of insufficient data. These include intrinsic features of individual species, such as the size and number of flowers displayed, floral longevity, ovule number, mating and pollination system, and extrinsic features, such as the specific habitat type in which the species

occurs, the demography of populations (e.g. their size and density), and local climatic conditions. To fully understand factors influencing pollen limitation, comparative experimental studies at the species and population level are required.

Experimental approaches can be used to disentangle the relative roles of intrinsic and extrinsic factors governing pollen limitation and uncover their mechanistic linkage to variation in pollen delivery. Previous intraspecific investigations have considered the effect of plant size (Hainsworth *et al.*, 1985; Dudash, 1993; Lawrence, 1993), population size (Sih & Baltus, 1987; Jennersten & Nilsson, 1993; Byers, 1995; Ågren, 1996), insularity (Spears, 1987), and seasonal and spatial variation in pollen limitation (reviewed in Burd, 1994). Most of these studies have been concerned with ecological factors, rather than with how variation in plant traits influences the likelihood of pollen limitation. Future empirical work that explicitly compares related taxa that differ in specific traits would be valuable for understanding the functional link between life-history traits and pollen limitation.

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APPENDIX

Species included in the comparative analysis that were not in Burd's (1994) compilation, listed alphabetically by family (*=new family): AMARYLLIDACEAE: two species of *Cyrtanthus*, *Haemanthus rotundifolius*, *Nerine sarniensis* (Johnson & Bond, 1997) and *Narcissus longispathus* (Herrera, 1995); ARECACEAE*: *Neodypsis decaryi* (Ratsirarson & Silander 1996) and *Calyptogyne ghiesbreghtiana* (Cunningham, 1996); CARYOPHYLLACEAE: *Silence virginica* (Dudash & Fenster, 1997); ERICACEAE: *Andromeda polifolia* and four species of *Vaccinium* (Fröberg, 1995); GENTIANACEAE: *Gentiana cruciata* (Petanidou, *et al.*, 1995); Iridaceae: *Iris lacustris* (G. Hannan, unpubl. data) and *I. versicolor* (Kron, *et al.*, 1993); LAMIACEAE: hermaphroditic individuals of *Glechoma hederacea* (Widén, 1992); LENTIBULARIACEAE*: *Utricularia vulgaris* (P. Kron & B. Husband, unpubl. data); LYTHRACEAE*: *Decodon verticillatus* (Eckert & Barrett, 1995); MELASTOMATACEAE: *Rhexia virginica* (Larson & Barrett, 1999a,b); ORCHIDACEAE: *Calypto bulbosa* (Alexandersson & Ågren, 1996), six species of *Disa* (Johnson & Bond, 1997) and *D. draconis* (Johnson & Steiner, 1997); PRIMULACEAE: legitimate pollination of *Primula sieboldii* pooled over morphs (Washitani, *et al.*, 1994) and *P. veris* (Lehtilä & Syrjänen, 1995); PROTEACEAE*: *Grevillea barklyana* (Vaughton, 1996) and *Persoonia mollis* (Krauss, 1994); ROSACEAE: *Sorbus aucuparia* (Sperens, 1996); and TRILLIACEAE: *Trillium grandiflorum* (D.M. Smith, unpubl. data).