# Pollination mode and life form strongly affect the relation between mating system and pollen to ovule ratios

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## Summary

- Pollen to ovule (P : O) ratios have been hypothesized to correlate with the degree of outcrossing and thus with the mating system of a plant. Also, P : O ratios are likely to vary with respect to pollination mode (i.e. wind pollination or animal pollination). Furthermore, constraints on the evolution of mating systems depending on life form may affect P : O ratios.
- We compiled P : O ratios and outcrossing rates for 107 angiosperm species and analyzed the relation between these traits considering pollination mode, life form and phylogenetic relatedness among species.
- In general, P : O ratios correlated significantly with outcrossing rates. However, when taking additional factors into account, the relation became ambiguous. The correlation was significantly positive in wind-pollinated species, but only marginally so in animal-pollinated species. Wind-pollinated species had higher P : O ratios than animal-pollinated taxa. In woody perennials, outcrossing was the predominant mating system and outcrossing rates did not correlate with P : O ratios. The results were not altered by accounting for phylogenetic relatedness among species.
- The results indicate that P : O ratios vary more strongly with pollination mode and life form than with the mating system.

## Introduction

Since the pioneering work of Cruden (1977), pollen to ovule (P : O) ratios have been commonly measured by plant population biologists and often have been related to the mating system, despite ongoing controversies concerning their indicative value. Cruden (1977) demonstrated that, in general, outcrossing species had higher P : O ratios than predominantly selfing species and hypothesized that P : O ratios correlate with the incidence of outcrossing because they reflect the efficiency of a pollination system. In particular, he proposed that higher P : O ratios in outcrossed species compared with autogamous species could be attributed to the lower probability of a pollen grain reaching a stigma. Thus, maximum seed set in outcrossing plants would require more pollen as a result of inefficient pollen transfer (pollination efficiency hypothesis). A number of studies verified the correlation between mating systems and P : O ratios (e.g. Plitmann & Levin, 1990; Jürgens *et al.*, 2002). However, the pollination efficiency hypothesis has been criticized because it considers the fitness of individuals only in terms of the number of seeds they produce (Charnov, 1982). Consequently, the relation between mating systems and P : O ratios has been explained within a more general theoretical framework. The sex-allocation theory regards male and female sexual function of a hermaphroditic individual as equivalent means towards fitness gain (Charnov, 1982). Analogous to the theory of local mate competition (Hamilton, 1967), it was proposed that higher inbreeding would increase competition among related pollen grains and that this, in turn, would favour the increased allocation into female function, thus decreasing P : O ratios (Charnov, 1979; Charlesworth & Charlesworth, 1981; Charnov, 1982). Although low P : O ratios might be indeed associated with high pollination efficiency, this can be interpreted, at least for species with aggregated pollen, as a correlated consequence of increased local mate competition (Harder & Johnson, 2008). Queller (1984) pointed out that under the assumption that self-fertilized seeds are half female and half male investment because of equal genetic contributions, male investment in selfing species includes not only pollen but also seed production. Further assuming proportional reproductive investment into male and female gametes as equal, a decreased pollen investment with selfing can be explained even without local mate competition among pollen grains, because male investment in selfers must be portioned among pollen and seeds. However, Cruden's hypothesis, and those derived from the sex-allocation theory, do not exclude each other when pollination efficiency is considered as one factor among others that shape the male fitness gain curve (Mione & Anderson, 1992).

Here, we focus on the possible effects of pollination mode and life form on the relation between mating system and P : O ratios. Although in general this relation is assumed to be significantly positive also for anemophilous species (Cruden, 1977), qualitative differences between pollination modes can be expected for a number of reasons.

It is often stated that P : O ratios in wind-pollinated species are higher than in zoophilous species simply because of the inefficiency of undirected pollen dispersal (e.g. Faegri & Van der Pijl, 1979; Ackerman, 2000). However, the inefficiency of wind pollination is questioned by empirical work (e.g. Honig *et al.*, 1992) and its repeated evolution from animal-pollinated ancestors (Linder, 1998; Friedman & Barrett, 2008). A combination of lower costs for individual pollen grains in wind-pollinated species and differences in gain curves expected for male and female fitness for the different pollination modes may result in higher P : O ratios in wind-pollinated species. The majority of anemophilous pollen contains less protein than zoophilous pollen (Roulston *et al.*, 2000) and thus is presumably cheaper to produce. Differences in relative sex allocation between pollination modes may arise because in biotically pollinated species many factors may impose a limit on profitable male allocation, whereas in wind-pollinated species a more linear relationship between investment and male reproductive success is expected (Lloyd, 1984). For example, animal pollen vectors may carry only a limited amount of pollen (Charnov, 1979; Harder & Thomson, 1989), whereas wind is not limited in the amount of pollen it can transport. As a result, at a certain point of relative investment, more resources allocated to the male sexual function will lead to diminishing returns in zoophilous species (Klinkhamer *et al.*, 1997). Nevertheless, studies comparing P : O ratios between wind-pollinated and zoophilous species are rare and inconsistent. Whereas Pohl (1937), in general, found that wind-pollinated species do not show higher P : O ratios than zoophilous species, Cruden (2000) found P : O ratios to be higher in wind-pollinated species.

Besides possibly higher P : O ratios in wind-pollinated species, the relation between P : O ratios and mating system may also differ from zoophilous species. For example, increased male allocation in outcrossing species relative to selfers might result in larger pollen because fertilization and competitive ability may increase with size (Barrett *et al.*, 1996). However, more resources spent on male function can be allocated into either pollen size or pollen number. Anemophilous pollen varies less in size than zoophilous pollen (Harder, 1998). Thus, for wind-pollinated species, it is likely that not size, but pollen number is varied because a strong constraint for pollen size may exist, probably because certain aerodynamical properties are favourable for wind dispersal (Whitehead, 1969) and, in particular, a larger size would reduce dispersal distances (Niklas, 1985). Hence, an increase in male allocation could be reflected in P : O ratios of wind-pollinated species directly, whereas P : O ratios could be quite uninformative about a change in relative sex allocation in zoophilous species. Thus, a given change in the outcrossing rate might not lead to equivalent changes of relative floral sex allocation in biotically and abiotically pollinated species.

Furthermore, qualitative differences between the pollination modes may arise from the fact that in wind-pollinated species the ovule number is often reduced to one (Pohl, 1929; Friedman & Barrett, 2008) and thus variation in the P : O ratios often arises solely from variation in pollen production. By contrast, in zoophilous species, in which both pollen and ovule numbers vary substantially, compensatory changes could reduce variation.

Life form may also influence the relation between mating systems and P : O ratios as a result of constraints on mating system evolution. Because plants in general do not produce a separate germ line, it has been hypothesized that long-lived, large-statured plants such as trees or shrubs accumulate relatively more somatic mutations, and thus more genetic load, than short-lived taxa (Scofield & Schultz, 2006). Therefore, the evolutionary shift from outcrossing to selfing would be impossible for large-statured plants because genetic load prevents the survival of selfed progeny (Scofield & Schultz, 2006). Indeed, it has been found that woody perennials generally self less than herbs (e.g. Barrett & Eckert, 1990). Thus, variability in P : O ratios in large-statured plants may be driven by factors other than the degree of outcrossing, simply as a result of the fact that mating system evolution is constrained to a high level of outcrossing by severe inbreeding depression.

When P : O ratios have been related to mating systems, different types of indirect mating system estimates and classifications have been used (e.g. Cruden, 1977; Preston, 1986; Plitmann & Levin, 1990; Götzenberger *et al.*, 2008). With the emergence of genetic marker systems it has become routine to measure the relative frequencies of selfing and outcrossing directly from progeny arrays (Barrett & Harder, 1996). Thus, a categorization of mating systems seems outdated and P : O ratios should be related to direct quantitative estimates of outcrossing rates. The outcrossing rate as a quantitative descriptor of the mating system has been shown to vary continuously from complete selfing to complete outcrossing, with many species practising mixed mating (Vogler & Kalisz, 2001; Goodwillie *et al.*, 2005).

In this study we investigated the relation between the mating system and floral sex allocation, represented by P : O ratio measurements, for a wide range of angiosperm species. We quantified mating systems by absolute estimates of outcrossing rates that were derived from marker-based progeny-array analyses. Although it is expected that P : O ratios and outcrossing rates are in general positively correlated, pollen vector and life form may have confounding effects on this relation, which were tested with the following hypotheses.

- The relation between outcrossing rates and P : O ratios shows qualitative differences between anemophilous and zoophilous taxa. In particular, the intercept of the regression line of P : O ratios on outcrossing rates should be higher and the slope should be steeper in wind-pollinated taxa.
- Long-lived perennials, such as trees and shrubs, are evolutionary constrained to high outcrossing rates. Hence, variability in P : O ratios among these taxa is unlikely to correlate with the mating system.

When considering a set of species, trait correlations can be the result of phenotypic convergence of different taxa undergoing a similar evolutionary response. However, among related taxa a similar evolutionary response may be also caused by phylogenetic constraints (Barrett *et al.*, 1996). Also, related species do not represent independent observations in statistical analyses (Felsenstein, 1985). Therefore, the analyses were performed with and without taking phylogenetic relatedness among species into account.

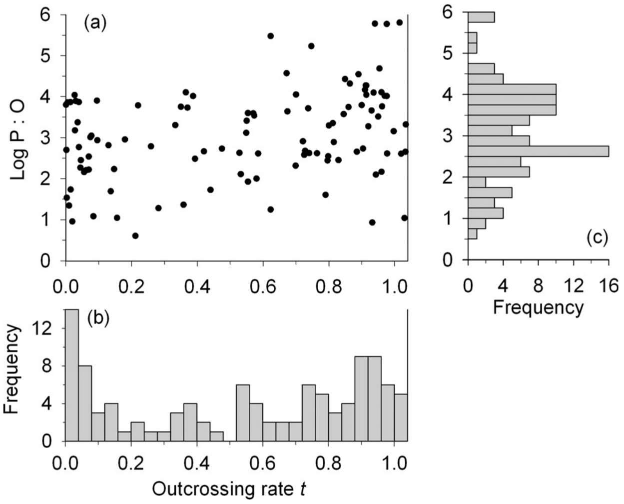

**Fig. 1** Scatterplot of pollen to ovule (P : O) ratios as a function of outcrossing rates for 107 angiosperm species (a) and the frequency distributions of outcrossing rates (untransformed, b) and P : O ratios (log transformed, c).

## Materials and Methods

P : O ratios were obtained from original papers and from our own studies. For species with known P : O ratios, outcrossing rates were compiled from the database used by Goodwillie *et al.* (2005) and from original papers, resulting in a data set of 107 species (original data and references available as Supporting Information Tables S1, S2). All outcrossing data were based on the analysis of progeny arrays using genetic markers such as allozymes or microsatellites. We did not include outcrossing rates deduced from estimates of inbreeding coefficients because these may be strongly biased by population history or by inbreeding depression (e.g. Michalski & Durka, 2007). Although the standard marker-based procedures to estimate outcrossing rates reflect the degree of outcrossing in female gametes only, the population average outcrossing rate is identical for ovules and pollen (Ross & Gregorius, 1983). If, for a species, more than one study reported P : O ratios or outcrossing rates, the median of the available data was used in the analyses. For all species, life form (herbaceous or woody) and pollination mode (anemophilous or zoophilous) were recorded. Before all analyses were carried out, P : O ratios were log transformed to meet the assumptions of standard statistical tests (Shapiro–Wilk Normality Test $W = 0.983$, $P = 0.194$). As outcrossing rates, $t$ showed a strong bimodal distribution (Fig. 1), values were logit transformed ($f[t] = \log_e(t/[1 - t])$, with $t$ values of $\geq 1$ substituted by 0.999) and residuals were deemed normal after transformation ($W = 0.981$, $P = 0.120$).

To evaluate the influence, if any, of the phylogenetic relationship among species we present results for an analysis using species traits as independent data points (cross-species analysis, A) and for an analysis considering phylogenetic relatedness among species (B). For (A), a general linear model was used to analyze the influence of P : O ratios on outcrossing rates. Additionally, the analysis included life form and pollination mode as categorical factors and all possible interactions. For (B), the nonindependence of species traits was considered by first applying a generalized least-squares approach (Grafen, 1989; Martins & Hansen, 1997), as described in Paradis (2006). Here, the phylogenetic relationships are entered into the analysis as a phylogenetic correlation structure. Two different models of character evolution were used to derive the correlation structure from the phylogeny. The first was based on the Brownian motion model and the second was based on the Ornstein–Uhlenbeck model that assumes stabilizing selection. Second, we used phylogenetically independent contrasts (PICs, Felsenstein, 1985) to account for nonindependence of data points in the comparison of the slopes and intercepts of a major axis regression of P : O ratios on outcrossing rates for the different pollination modes (discussed later).

Phylogenetic correction in many comparative analyses often relies on tree topology only because branch length information is missing. Hence, possible differences in evolutionary rates between species or clades, and thus evolutionary distances, are frequently not included in the analysis. Here, we include information on branch length by constructing a phylogenetic hypothesis of the species based on *rbcL* sequence data. In short, we compiled DNA sequences from the GenBank database. For 50 species original *rbcL* data was available, while for 47 species we used sequence information of closely related species. After alignment, a maximum likelihood tree was computed using the default settings implemented in the program 'DNAML' of the PHYLIP package version 3.6 (Felsenstein, 2004). Sequence information was missing for 10 species. These species were added as polytomies of existing branches of the respective genus (see Fig. S1 for details). The species list, trait data, GenBank accession numbers, replacement taxa and the phylogeny based on *rbcL* data are available in Tables S1 and S2, and in Fig. S1.

To evaluate whether wind and biotically pollinated taxa follow the same pattern, slopes and intercept of a standardized major axis regression of outcrossing rates on P : O ratios were tested for heterogeneity according to Warton & Weber (2002).

Hierarchical partitioning has been suggested as a solution to the possible problem of multicollinearity in one-model approaches (Mac Nally, 2000). Thus, in addition to the preceding analysis we adopted this method to identify the most likely causal factors for variation in P : O ratios. Here, independent and joint effects are estimated for each explanatory variable. These effects describe explained variation in the response variable by the explanatory variable independent of other variables and caused by intercorrelation with other variables, respectively.

All statistical analyses were performed with R version 2.7.1 (http://cran.r-project.org/) and the packages 'APE' version 2.2-2 (Paradis *et al.*, 2004), 'SMATR' version 2.1 (Warton & Ormerod, 2007) and 'HIER.PART' version 1.0-3 (Walsh & Mac Nally, 2008).

## Results

In total, data for 107 species in 38 families were included in the analysis, comprising 83 herbaceous taxa and 24 woody taxa. Of all species, 85 were animal pollinated and 22 were wind pollinated. Untransformed P : O ratios varied between 4 (*Mimulus micranthus*) and 636 594 (*Fagus sylvatica*); outcrossing rates ranged between 0.001 (*Pisum sativum*) and 1.034 (*Scrophularia nodosa*).

Pairwise comparisons of P : O ratios and outcrossing rates between pollination modes and life forms revealed that the mean P : O ratios of wind-pollinated species were significantly higher than those of animal-pollinated species (Fig. 2, Welch two-sample *t*-test, $t = 4.61$, degrees of freedom (df) = 33.7, $P < 0.001$). Also, the mean P : O ratios of woody perennials were significantly higher than those of herbaceous taxa (Fig. 2, $t = 2.29$, df = 36.7, $P = 0.014$). Outcrossing rates did not differ between wind-pollinated and animal-pollinated species (data not shown), but did differ between herbaceous species and woody species (Fig. 2, $t = 3.72$, df = 54.6, $P < 0.001$). Outcrossing rates were more variable among herbs than among woody perennials (var ($t$) = 8.66 and 4.02, respectively, $F = 0.464$, df = 23/82, $P = 0.04$).

Among species, a simple linear regression of P : O ratios on outcrossing rates was significantly positive (adjusted $R^2 = 0.06$, $P = 0.007$, Fig. 1). When considering both pollination mode and life form, the analysis using species traits as independent data points (A) and the analysis considering phylogenetic relatedness (B) gave very similar results (Table 1). In the general least-squares analyses (B), the Ornstein–Uhlenbeck model of character evolution performed significantly better than the Brownian motion model (likelihood ratio = 76.1;

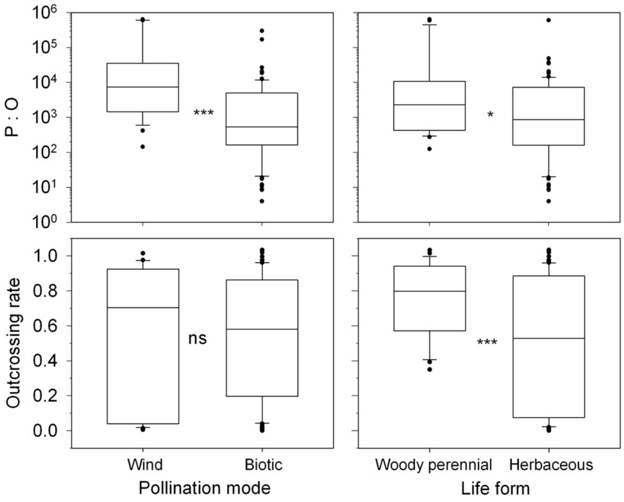

**Fig. 2** Box-and-whisker plots of pollen to ovule (P : O) ratios and outcrossing rates for the different pollination modes and life forms. Asterisks indicate significant differences among groups (***, $P < 0.001$; *, $P < 0.05$). Note that tests were carried out using log-transformed P : O ratios and logit-transformed outcrossing rates.

**Table 1** Full models analysing pollen to ovule (P : O) ratios of 107 species with species traits as independent data points (A) and considering phylogenetic relatedness among species using a maximum likelihood phylogeny based on *rbc*L sequences and the Ornstein–Uhlenbeck model of character evolution (B)

| Analysis | Independent variables/factors | *t*-value | *P* |
|---|---|---|---|
| A | outcr | 1.88 | 0.063 |
| | pollmod | 4.92 | < 0.001 |
| | lff | 2.85 | 0.005 |
| | outcr × pollmod | 0.86 | 0.389 |
| | outcr × lff | –1.59 | 0.115 |
| | pollmod × lff | –2.41 | 0.018 |
| | outcr × pollmod × lff | 2.36 | 0.020 |
| B | outcr | 2.09 | 0.040 |
| | pollmod | 4.64 | < 0.001 |
| | lff | 3.06 | 0.003 |
| | outcr × pollmod | 0.66 | 0.526 |
| | outcr × lff | –1.56 | 0.121 |
| | pollmod × lff | –2.28 | 0.025 |
| | outcr × pollmod × lff | 2.13 | 0.036 |

Models contained outcrossing rates (outcr), pollination mode (pollmod), life form (lff) and all possible interactions. Outcrossing rates were logit, P : O ratios log-transformed. The ratio of the estimate divided by the standard error (*t*-value) and the significance of the effects are given.

$P < 0.001$). Thus, only results of the former model of character evolution are considered here.

Both analyses (A and B) showed that P : O ratios varied with respect to outcrossing rates, pollination mode and life form (Table 1). Additionally, the significant highest-order interaction among outcrossing rates, pollination mode and life form indicated that the effect of outcrossing rates on P : O ratios depends on both life form and pollination mode.

In a separate linear regression analysis of P : O ratios on outcrossing rates, more variance could be explained for wind-pollinated taxa (adjusted $R^2 = 0.36$, $P = 0.002$) than for animal-pollinated taxa (adjusted $R^2 = 0.03$, $P = 0.055$). The slope of the regression did not differ between pollination modes (likelihood ratio = 0.94, $P = 0.33$), but the intercept was significantly higher in wind-pollinated species than in animal-pollinated species (Wald statistic = 23.12, $P < 0.001$). Linear regression analysis for the different life forms revealed a marginally significant relation for herbaceous species (adjusted $R^2 = 0.03$, $P = 0.051$) and no significance in the relation for woody species ($P = 0.33$). Although not significant when treated separately, depending on the pollination mode opposing trends existed for the relation between outcrossing rates and P : O ratios in woody perennials (data not shown). In wind-pollinated species the relation tended to be positive, whereas in zoophilous trees and shrubs the relation tended to be negative; however, the sample included few wind-pollinated trees ($n = 4$). For herbaceous taxa only, the results were similar to the analysis comprising all taxa (data not shown).

Correction for the phylogenetic relatedness among species by using the generalized least-squares approach did not alter the results (data not shown). Heterogeneity tests on slopes and intercepts are not possible within this approach. However, when we analyzed PICs, the slope of a standardized major axis regression forced through the origin was significantly more positive for wind-pollinated taxa than for animal-pollinated species (likelihood ratio = 16.35, $P < 0.001$).

For the cross-species data, as well as for the phylogenetically corrected data, hierarchical partitioning identified pollination mode as the strongest independent effect on P : O ratios (Fig. 3). Mating system and life form contributed less, but with similar levels to the total variance explained. All joint and independent effects estimated by hierarchical partitioning correspond to the results obtained by the preceding analyses.

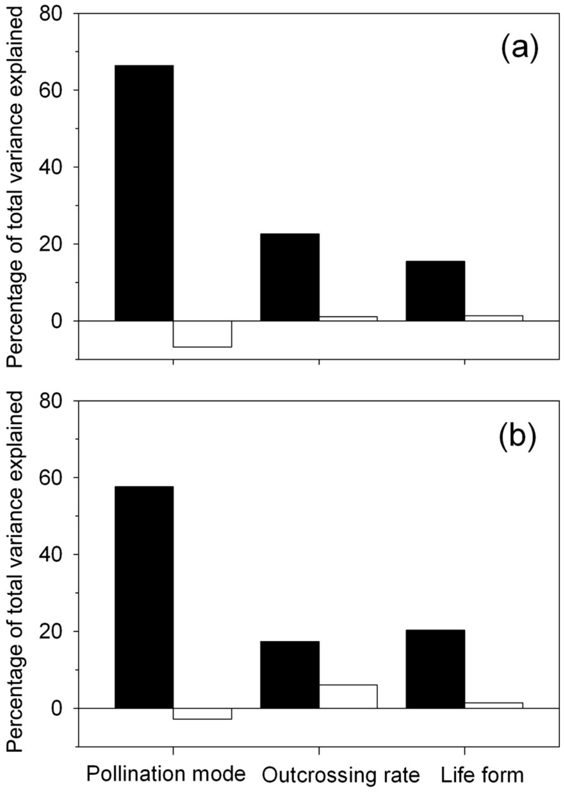

**Fig. 3** Effects of pollination mode, life form and mating system on pollen to ovule (P : O) ratios estimated by hierarchical partitioning of simple generalized least squares (a) and phylogenetic generalized least squares (b) according to analysis (A) and analysis (B), respectively (see the Materials and Methods section). Independent effects, black bars; joint effects, white bars. Note that the negative joint effect for pollination mode indicates that this factor masks the effect of other variables.

## Discussion

Although our analysis comprised only 107 species, it covered nearly the full range of P : O ratios known (1 – 3591266, Erbar & Langlotz, 2005) as well as the full range of outcrossing rates and extensive phylogenetic diversity. Still, the present results for the relation of outcrossing rates with P : O ratios should be viewed with some caution, for several reasons. Although we collected data for a wide range of angiosperms, outcrossing rates may be biased. The frequency of strictly selfing, and in particular that of completely outcrossing taxa, is possibly underestimated as a result of a high level of interest in the study of mixed mating systems (Goodwillie *et al.*, 2005; Igic & Kohn, 2006). Note that we included only species for which the outcrossing rate had been determined experimentally from progeny arrays, an effort rarely taken in species that are known to be either self-incompatible or obviously selfing. Estimates of the outcrossing rates can also differ from the primary rate of outcrossing, as inbreeding depression may occur between fertilization and sampling of the progeny array (Goodwillie *et al.*, 2005). Sources of variation in outcrossing rates also include the interaction of pollen vector and flower, environmental conditions, post-pollination mechanisms and current population characteristics. For example, in self-compatible plants, population size and density can affect the outcrossing rate (Farris & Mitton, 1984; Routley *et al.*, 1999). Both P : O ratios and outcrossing rates can show large intraspecific variability (e.g. Schoen, 1977; Affre *et al.*, 1995; Mazer & Delesalle, 1996; Sherry & Lord, 1996; Koelewijn, 2004). However, for both P : O ratios and outcrossing rates, the intraspecific variability seems relatively low when compared with the variability among species.

Our main results were not altered by correcting for phylogenetic relationships among species (Table 1, Fig. 3). Besides serving the statistical necessities, this demonstrated that our sampling was phylogenetically well balanced and the observed patterns were not the consequence of phylogenetic constraints among certain clades.

The inclusion of appropriate branch lengths is critical for appropriate Type I error when correcting for phylogenetic relatedness (Purvis *et al.*, 1994). Here, we assume that the phylogenetic hypothesis based on the *rbcL* gene leads to an improvement relative to phylogenies using unit branch lengths. However, branch lengths depend on the markers used to construct the phylogeny and thus may differ for different marker systems. A detailed analysis may be necessary to evaluate the influence of different branch lengths on the results.

The phylogenetic generalized least-squares approach allows for flexibility in the underlying microevolutionary assumptions, which was used to compare the Brownian motion model and the Ornstein–Uhlenbeck model. Götzenberger *et al.* (2008) found that the P : O ratios were very likely to evolve according to the Brownian motion model along their phylogeny used. However, we found the Ornstein–Uhlenbeck model to perform significantly better. This result seems reasonable as it is more likely to expect characters to evolve around a certain value rather than to diverge indefinitely, as assumed by the Brownian motion model.

### Mating system

Not surprisingly, this study has shown that the overall P : O ratios are indeed significantly positively correlated with outcrossing rates. However, only 6% of the variation in P : O ratios are explained by outcrossing rates and the scatter diagram (Fig. 1) and all analyses show that variation in the P : O ratios is not simply a function of the mating system. The vast number of intermediate P : O ratios (log P : O = 2–4) were associated with outcrossing rates ranging from complete selfing to complete outcrossing. This pattern is not unexpected as P : O ratios are known to vary enormously, even among species with similar mating systems (Cruden & Lyon, 1989; Cruden, 2000). For example, several outcrossing taxa are either associated with unusually low P : O ratios (Vasek & Weng, 1988; James & Knox, 1993; Ng & Corlett, 2000; Wang *et al.*, 2004; Darrault & Schlindwein, 2005) or with very high P : O ratios (Casper, 1982; Cruden, 2000). Also, inconsistencies between expectations, derived by comparing measured P : O ratios with the values given by Cruden (1977) for different mating system classes and independently derived estimates of the mating system, have been reported frequently (Ritland & Ritland, 1989; Gallardo *et al.*, 1994; Ramirez & Seres, 1994; Olesen *et al.*, 1998; Sneddon, 1999; Bosch *et al.*, 2001; Smissen & Garnock-Jones, 2002; Parachnowitsch & Elle, 2004; Chouteau *et al.*, 2006). This substantiates the need for additional factors explaining variation in P : O ratios.

### Pollination mode

The relationship between P : O ratios and mating systems became quite ambiguous when we included pollination mode and life form, suggesting a major role of these traits. We were able to show that the pollen vector influences the relation between P : O ratios and outcrossing rates. Whereas for wind-pollinated taxa the relation was quite strong, for animal-pollinated taxa it was only marginally significant. A similar pattern could be found at the family level. When looking for the two best represented families in our data set (Fabaceae, $n = 15$ and Poaceae, $n = 14$), the relation between P : O ratios and outcrossing rates was significant for only the wind-pollinated Poaceae and only when phylogenetic relatedness was considered (data not shown). This difference between pollination modes may be related to different evolutionary constraints leading also to statistical effects that have to be considered. The co-evolution of plants and their animal pollinators resulted in numerous adaptive solutions to the problem of pollen transport. By contrast, anemophily is evolutionarily connected to very similar floral morphological adaptations (Pohl, 1929; Whitehead, 1969; Friedman & Barrett, 2008). Therefore, the lack of clear correlation between P : O ratios and outcrossing rates in animal pollinated species may be a result of the variability in floral traits, which is much more pronounced than that of wind-pollinated species. For example, variation in P : O ratios depends on both pollen and ovule production. However, in contrast to zoophilous species, ovule production in wind-pollinated species is generally less variable, with only one or a few ovules per flower (Pohl, 1929; Friedman & Barrett, 2008). Indeed, when comparing P : O ratios in our data set, uniovulate, wind-pollinated Poaceae encompass only two orders of magnitude (1022–48 455), whereas multiovulate Fabaceae or Brassicaceae cover three, or even four, orders of magnitude (281–20 743 and 34–18 600, respectively). Thus, when evaluating the results, one has to keep in mind that P : O ratios are not a floral trait *per se*, but rather represent the relative number of male and female gametes. As it is a general problem of working with ratios, information about the underlying absolute values is lost.

The differences between the pollination modes are also reflected in different distributions of their outcrossing rates. Whereas abiotically pollinated species tend to be either outcrossed or selfed, the distribution of outcrossing rates is more continuous in biotic pollinated species (Goodwillie *et al.*, 2005). This may indicate a complexity in the evolution of mating systems not seen in abiotic pollination. Pollinator unpredictability could select for the capacity of both outcrossing and selfing within single flowers and thus could stabilize mixed mating systems in biotically pollinated species (Aide, 1986; Barrett & Eckert, 1990; Vogler & Kalisz, 2001).

Besides reproductive assurance, other adaptive mechanisms that can maintain mixed mating have been proposed recently (Harder *et al.*, 2008; Johnston *et al.*, 2009). For example, reproductive compensation, the production of a greater number of ovules than of mature seeds, may favour increased selfing rates despite substantial inbreeding depression (Porcher & Lande, 2005; Harder *et al.*, 2008). In wind-pollinated species, this mechanism may be less important because only a few ovules per flower are produced.

Apart from the strength of the relation, also the slopes of the regression of P : O ratios on outcrossing rates differed between pollination modes, at least when phylogenetic relationships were considered. This may indeed indicate that different sets of factors or relationships among traits exist between the different pollination modes, influencing the relation between P : O ratios and mating system. However, in the absence of a broader database (e.g. on pollen size), a discussion of these factors is entirely speculative.

As expected, the P : O ratios in wind-pollinated species were higher than in zoophilous species. This is in concordance with the results of Cruden (2000), who argued that unlike zoophilous species, the P : O ratios in wind-pollinated species are primarily affected by the relationship between pollen production and the distance between putative mates. It has also been hypothesized that resource allocation will be biased in favour of the sex that is dispersed to further distances and thus reduces sibling competition (Bulmer & Taylor, 1980; de Jong *et al.*, 2002). In wind-pollinated plants one could expect that sibling competition generated through seeds might be more intense than that of the easily dispersed pollen, and thus a more male-biased sex allocation would be expected. However, given the high frequency of uniovulate flowers in wind-pollinated species, strong competition among pollen grains cannot be precluded.

## Life form

We have demonstrated that for herbaceous species and woody perennials, different trends may exist regarding floral sex allocation and its relation to the mating system. This is concordant with the fact that severe inbreeding depression appears to be more frequent in large-statured plants rather than in small-statured plants (Husband & Schemske, 1996). Many somatic mutations per sexual generation may also be accumulated by long-lived herbaceous plants with extensive vegetative reproduction (Klekowski, 1988). As a consequence, self-incompatibility and outcrossing are the predominant mating systems in these species. Despite high inbreeding depression, relatively low outcrossing rates have been observed for some trees (e.g. Ishida, 2006). However, selfed progeny in large-statured plants is probably not adaptive but rather the consequence of adaptations for outcrossing (i.e. the result of geitonogamous selfing) (Scofield & Schultz, 2006). The P : O ratios for trees varied between 125 (*Jacaranda copaia*) and 636594 (*Fagus sylvatica*), thus covering almost the complete range of the whole data set. These results show that variation in P : O ratios, at least for trees, is affected by factors other than the mating system alone.

We found that P : O ratios were higher in woody perennials than in herbs. Gender adjustments in cosexual plants can be driven by various nonexclusive effects of plant size (Klinkhamer *et al.*, 1997). Assuming that woody perennials are larger than herbs, the differences in P : O ratios between life forms could be an effect of plant size. However, depending on a variety of factors, larger plants are not necessarily expected to be more male biased than smaller plants. For example, the sex that is dispersed more efficiently should gain increased fitness directly with increasing plant size (Klinkhamer *et al.*, 1997). The low P : O ratios for the tree *Jacaranda copaia* would thus fit to the gain from its numerous wind-dispersed seeds (Jones *et al.*, 2005). Size may also indirectly affect fitness through budget effects, assuming that larger plants have more resources to spend in reproduction than smaller plants. However, these effects are likely to depend on the pollination mode. Hence, a discussion on the effects of life form and plant size on variation in floral sex allocation has also to consider the pollination mode. The male gain curve may decelerate more strongly with increasing size in zoophilous species than in wind-pollinated species. For example, in larger zoophilous and outcrossing species exhibiting a large floral display, the chance that pollinators successively visit flowers on the same individual is increased and pollen would be lost for export (de Jong *et al.*, 1999). Hence, it may be hypothesized that in zoophilous taxa the male gain curve may decelerate more strongly than in wind-pollinated species, leading to more female-biased sex allocation with increased size in the former. However, self-compatibility in wind-pollinated species may counteract a higher fitness gain from male function with increased size, as a larger plant size will also lead to increased geitonogamous selfing (de Jong & Klinkhamer, 2005).

## Concluding remarks

Besides the mating system, P : O ratios have been related to a number of plant and pollinator traits. P : O ratios have been shown to reflect variability in seed weight (e.g. Uma Shanker & Ganeshaiah, 1984; Götzenberger *et al.*, 2006), pollen size (Gallardo *et al.*, 1994; but see Götzenberger *et al.*, 2007), stigmatic area, the pollen-bearing area of pollinators (Cruden & Miller-Ward, 1981), the size of the pollen dispersal unit (Harder & Johnson, 2008) and the effectiveness of the pollen vector (reviewed in Cruden, 2000). Although not demonstrated thoroughly, the rewarding strategy is also likely to affect P : O ratios, as plants with flowers that offer nectar as well as pollen, or only pollen as a reward, probably have higher P : O ratios than those only providing nectar for pollination services (Mione & Anderson, 1992; Cruden, 2000). Here, we also found life form and pollination mode as a source of variation in the P : O ratios. In summary, many aspects of reproduction and species biology are reflected in the P : O ratios. However, whether the P : O ratio is the consequence or a cause of other traits, or whether a significant relation is simply mediated by a covariate, often cannot be evaluated, as for the relation among pollen size, mating system and P : O ratios (Barrett *et al.*, 1996; Götzenberger *et al.*, 2007).

Interpretation of interspecific variation in P : O ratios remains a challenge that has to consider the complex associations between traits and mechanisms linked to reproduction. A comprehensive framework to interpret P : O ratios has first to consider the main effects and constraints of life form and pollination mode such as pollen vector and the type of animal–flower interaction (e.g. nectar and/or pollen reward). In a second step, other aspects of the mating system, floral traits and post-pollination processes can be linked to floral sex allocation.

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## References

**Ackerman JD. 2000.** Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. *Plant Systematics and Evolution* **222**: 167–185.

**Affre L, Thompson JD, Debussche M. 1995.** The reproductive biology of the mediterranean endemic *Cyclamen balearicum* Willk. (Primulaceae). *Botanical Journal of the Linnean Society* **118**: 309–330.

**Aide TM. 1986.** The influence of wind and animal pollination on variation in outcrossing rates. *Evolution* **40**: 434–435.

**Barrett SCH, Eckert CG. 1990.** Variation and evolution of mating systems in seed plants. In: Kawano S, ed. *Biological approaches and evolutionary trends in plants*. London, UK: Academic Press, 229–254.

**Barrett SCH, Harder LD, Worley AC. 1996.** The comparative biology of pollination and mating in flowering plants. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **351**: 1271–1280.

**Barrett SCH, Harder LD. 1996.** Ecology and evolution of plant mating. *Trends in Ecology and Evolution* **11**: 73–79.

**Bosch M, Simon J, Molero J, Blanche C. 2001.** Breeding systems in tribe Delphinieae (Ranunculaceae) in the western Mediterranean area. *Flora* **196**: 101–113.

**Bulmer MG, Taylor PD. 1980.** Dispersal and the sex ratio. *Nature* **284**: 448–449.

**Casper BB. 1982.** The efficiency of pollen transfer and rates of embryo initiation in *Cryptantha* (Boraginaceae). *Oecologia* **59**: 262–268.

**Charlesworth D, Charlesworth B. 1981.** Allocation of resources to male and female functions in hermaphrodites. *Biological Journal of the Linnean Society* **15**: 57–74.

**Charnov EL. 1979.** Simultaneous hermaphroditism and sexual selection. *Proceedings of the National Academy of Sciences, USA* **76**: 2480–2484.

**Charnov EL. 1982.** *The theory of sex allocation*. Princeton, NJ, USA: Princeton University Press.

**Chouteau M, Barabe D, Gibernau M. 2006.** A comparative study of inflorescence characters and pollen-ovule ratios among the genera *Philodendron* and *Anthurium* (Araceae). *International Journal of Plant Sciences* **167**: 817–829.

**Cruden RW. 1977.** Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* **31**: 32–46.

**Cruden RW. 2000.** Pollen grains: why so many? *Plant Systematics and Evolution* **222**: 143–165.

**Cruden RW, Lyon DL. 1989.** Facultative xenogamy: Examination of a mixed mating system. In: Bock JH, Linhart YB, eds. The evolutionary ecology of plants. Boulder, CO, USA: Westview Press, 171–207.

**Cruden RW, Miller-Ward S. 1981.** Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: an hypothesis. *Evolution* **35**: 964–974.

**Darrault RO, Schlindwein C. 2005.** Limited fruit production in *Hancomia speciosa* (Apocynaceae) and pollination by nocturnal and diurnal insects. *Biotropica* **37**: 381–388.

**de Jong TJ, Klinkhamer PGL. 2005.** *Evolutionary ecology of plant reproductive strategies*. Cambridge, UK: Cambridge University Press.

**de Jong TJ, Klinkhamer PGL, Rademaker MCJ. 1999.** How geitonogamous selfing affects sex allocation in hermaphrodite plants. *Journal of Evolutionary Biology* **12**: 166–176.

**de Jong TJ, Van Batenburg FHD, van Dijk J. 2002.** Seed sex ratio in dioecious plants depends on relative dispersal of pollen and seeds: an example using a chessboard simulation model. *Journal of Evolutionary Biology* **15**: 373–379.

**Erbar C, Langlotz M. 2005.** Pollen to ovule ratios: standard or variation – a compilation. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **126**: 71–132.

**Faegri K, Van der Pijl L. 1979.** *The principles of pollination ecology*. Oxford, UK: Pergamon Press.

**Farris MA, Mitton JB. 1984.** Population density, outcrossing rate, and heterozygote superiority in Ponderosa Pine. *Evolution* **38**: 1151–1154.

**Felsenstein J. 1985.** Phylogenies and the comparative method. *American Naturalist* **125**: 1–15.

**Felsenstein J. 2004.** PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author.

**Friedman J, Barrett SCH. 2008.** A phylogenetic analysis of the evolution of wind pollination in the angiosperms. *International Journal of Plant Sciences* **169**: 49–58.

**Gallardo R, Dominguez E, Munoz JM. 1994.** Pollen-ovule ratio, pollen size, and breeding system in *Astragalus* (Fabaceae) subgenus *Epiglottis* – a pollen and seed allocation approach. *American Journal of Botany* **81**: 1611–1619.

**Goodwillie C, Kalisz S, Eckert CG. 2005.** The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution and Systematics* **36**: 47–79.

**Götzenberger L, Durka W, Kühn I, Klotz S. 2006.** The relationship between the pollen-ovule ratio and seed size: a comparative test of a sex allocation hypothesis. *Evolutionary Ecology Research* **8**: 1101–1116.

**Götzenberger L, Durka W, Kühn I, Klotz S. 2007.** The relationship between the pollen-ovule ratio and pollen size: another comparative test of a sex allocation hypothesis. *Evolutionary Ecology Research* **9**: 1145–1161.

**Götzenberger L, Kühn I, Klotz S. 2008.** Effect of habitat disturbance and pollination type on the interspecific variation in pollen–ovule ratios. *Preslia* **80**: 423–437.

**Grafen A. 1989.** The phylogenetic regression. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **326**: 119–157.

**Hamilton WD. 1967.** Extraordinary sex ratios. *Science* **156**: 477–488.

**Harder LD. 1998.** Pollen-size comparisons among animal-pollinated angiosperms with different pollination characteristics. *Biological Journal of the Linnean Society* **64**: 513–525.

**Harder LD, Johnson SD. 2008.** Function and evolution of aggregated pollen in angiosperms. *International Journal of Plant Sciences* **169**: 59–78.

**Harder LD, Richards SA, Routley MB. 2008.** Effects of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. *Evolution* **62**: 157–172.

**Harder LD, Thomson JD. 1989.** Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* **133**: 323–344.

**Honig MA, Linder HP, Bond WJ. 1992.** Efficacy of wind pollination – pollen load size and natural microgametophyte populations in wind-pollinated *Staberoha banksii* (Restionaceae). *American Journal of Botany* **79**: 443–448.

**Husband BC, Schemske DW. 1996.** Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54–70.

**Igic B, Kohn JR. 2006.** The distribution of plant mating systems: study bias against obligately outcrossing species. *Evolution* **60**: 1098–1103.

**Ishida K. 2006.** Maintenance of inbreeding depression in a highly self-fertilizing tree, *Magnolia obovata* Thunb. *Evolutionary Ecology* **20**: 173–191.

**James EA, Knox RB. 1993.** Reproductive biology of the Australian species of the genus *Pandorea* (Bignoniaceae). *Australian Journal of Botany* **41**: 611–626.

**Johnston MO, Porcher E, Cheptou PO, Eckert CG, Elle E, Geber MA, Kalisz S, Kelly JK, Moeller DA, Vallejo-Marin M *et al.* 2009.** Correlations among fertility components can maintain mixed mating in plants. *American Naturalist* **173**: 1–11.

**Jones FA, Chen J, Weng GJ, Hubbell SP. 2005.** A genetic evaluation of seed dispersal in the neotropical tree *Jacaranda copaia* (Bignoniaceae). *American Naturalist* **166**: 543–555.

**Jürgens A, Witt T, Gottsberger G. 2002.** Pollen grain numbers, ovule numbers and pollen-ovule ratios in Caryophylloideae: correlation with breeding system, pollination, life form, style number, and sexual system. *Sexual Plant Reproduction* **14**: 279–289.

**Klekowski EJ. 1988.** Progressive cross-sterility and self-sterility associated with aging in fern clones and perhaps other plants. *Heredity* **61**: 247–253.

**Klinkhamer PGL, deJong TJ, Metz H. 1997.** Sex and size in cosexual plants. *Trends in Ecology & Evolution* **12**: 260–265.

**Koelewijn HP. 2004.** Sibling competition, size variation and frequency dependent outcrossing advantage in *Plantago coronopus*. *Evolutionary Ecology* **18**: 51–74.

**Linder HP. 1998.** Morphology and the evolution of wind pollination. In: Owens SJ, Rudall PJ, eds. *Reproductive biology in systematics, conservation and economic botany*. Kew, UK: Royal Botanic Garden, 123–135.

**Lloyd DG. 1984.** Gender allocations in outcrossing cosexual plants. In: Dirzo R, Sarukhan J, eds. *Perspectives on plant population ecology*. Sunderland, MA, USA: Sinauer, 277–300.

**Mac Nally R. 2000.** Regression and model-building in conservation biology, biogeography and ecology: the distinction between and reconciliation of 'predictive' and 'explanatory' models. *Biodiversity and Conservation* **9**: 655–671.

**Martins EP, Hansen TF. 1997.** Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist* **149**: 646–667.

**Mazer SJ, Delesalle VA. 1996.** Floral trait variation in *Spergularia marina* (Caryophyllaceae): ontogenetic, maternal family, and population effects. *Heredity* **77**: 269–281.

**Michalski SG, Durka W. 2007.** High selfing and high inbreeding depression in peripheral populations of *Juncus atratus*. *Molecular Ecology* **16**: 4715–4727.

**Mione T, Anderson GJ. 1992.** Pollen-ovule ratios and breeding system evolution in *Solanum* section *Basarthrum* (Solanaceae). *American Journal of Botany* **79**: 279–287.

**Ng SC, Corlett RT. 2000.** Comparative reproductive biology of the six species of *Rhododendron* (Ericaceae) in Hong Kong, South China. *Canadian Journal of Botany* **78**: 221–229.

**Niklas KJ. 1985.** The aerodynamics of wind pollination. *The Botanical Review* **51**: 328–386.

**Olesen JM, Forfang AS, Baez M. 1998.** Stress-induced male sterility and mixed mating in the island plant *Cedronella canariensis* (Lamiaceae). *Plant Systematics and Evolution* **212**: 159–176.

**Parachnowitsch AL, Elle E. 2004.** Variation in sex allocation and male-female trade-offs in six populations of *Collinsia parviflora* (Scrophulariaceae s.l.). *American Journal of Botany* **91**: 1200–1207.

**Paradis E. 2006.** *Analysis of phylogenetics and evolution with R*. New York, USA: Springer.

**Paradis E, Claude J, Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.

**Plitmann U, Levin DA. 1990.** Breeding systems in the Polemoniaceae. *Plant Systematics and Evolution* **170**: 205–214.

**Pohl F. 1929.** Beziehungen zwischen Pollenbeschaffenheit, Bestäubungsart und Fruchtknotenbau. *Beihefte des Botanischen Centralblatts* **46**: 247–285.

**Pohl F. 1937.** Die Pollenerzeugung der Windblütler. *Beihefte des Botanischen Centralblatts* **56**: 365–470.

**Porcher E, Lande R. 2005.** Reproductive compensation in the evolution of plant mating systems. *New Phytologist* **166**: 673–684.

**Preston RE. 1986.** Pollen-ovule ratios in the Cruciferae. *American Journal of Botany* **73**: 1732–1740.

**Purvis A, Gittleman JL, Luh HK. 1994.** Truth or consequences – effects of phylogenetic accuracy on two comparative methods. *Journal of theoretical biology* **167**: 293–300.

**Queller DC. 1984.** Pollen–ovule ratios and hermaphrodite sexual allocation strategies. *Evolution* **38** 1148–1151.

**Ramirez N, Seres A. 1994.** Plant reproductive biology of herbaceous monocots in a Venezuelan tropical cloud forest. *Plant Systematics and Evolution* **190**: 129–142.

**Ritland C, Ritland K. 1989.** Variation of sex allocation among eight taxa of the *Mimulus guttatus* species complex (Scrophulariaceae). *American Journal of Botany* **76**: 1731–1736.

**Ross MD, Gregorius HR. 1983.** Outcrossing and sex function in hermaphrodites – a resource allocation model. *American Naturalist* **121**: 204–222.

**Roulston TH, Cane JH, Buchmann SL. 2000.** What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs* **70**: 617–643.

**Routley MB, Mavraganis K, Eckert CG. 1999.** Effect of population size on the mating system in a self-compatible, autogamous plant, *Aquilegia canadensis* (Ranunculaceae). *Heredity* **82**: 518–528.

**Schoen DJ. 1977.** Morphological, phenological, and pollen-distribution evidence of autogamy and xenogamy in *Gilia achilleifolia* (Polemoniaceae). *Systematic Botany* **2**: 280–286.

**Scofield DG, Schultz ST. 2006.** Mitosis, stature and evolution of plant mating systems: low-Phi and high-Phi plants. *Proceedings of the Royal Society B-Biological Sciences* **273**: 275–282.

**Sherry RA, Lord EM. 1996.** Developmental stability in leaves of *Clarkia tembloriensis* (Onagraceae) as related to population outcrossing rates and heterozygosity. *Evolution* **50**: 80–91.

**Smissen RD, Garnock-Jones RJ. 2002.** Relationships, classification and evolution of *Scleranthus* (Caryophyllaceae) as inferred from analysis of morphological characters. *Botanical Journal of the Linnean Society* **140**: 15–29.

**Sneddon BV. 1999.** The taxonomy and breeding system of *Colobanthus squarrosus* (Caryophyllaceae). *New Zealand Journal of Botany* **37**: 195–204.

**Uma Shanker R, Ganeshaiah KN. 1984.** Does pollination efficiency shape the pollen grain to ovule ratio? *Current Science* **53**: 751–753.

**Vasek FC, Weng V. 1988.** Breeding systems of *Clarkia* Sect. *Phaeostoma* (Onagraceae). 1. Pollen-ovule ratios. *Systematic Botany* **13**: 336–350.

**Vogler DW, Kalisz S. 2001.** Sex among the flowers: the distribution of plant mating systems. *Evolution* **55**: 202–204.

**Walsh C, Mac Nally R. 2008.** hier.part: hierarchical partitioning. R package. Available at: http://cran.r-project.org/web/packages/hier.part/index.html

**Wang YQ, Zhang DX, Chen ZY. 2004.** Pollen histochemistry and pollen–ovule ratios in Zingiberaceae. *Annals of Botany* **94**: 583–591.

**Warton D, Ormerod J. 2007.** smatr: (Standardised) major axis estimation and testing routines. R package. Available at: http://web.maths.unsw.edu.au/~dwarton.

**Warton DI, Weber NC. 2002.** Common slope tests for bivariate errors-in-variables models. *Biometrical Journal* **44**: 161–174.

**Whitehead DR. 1969.** Wind pollination in angiosperms: evolutionary and environmental considerations. *Evolution* **23**: 28–35.

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Maximum likelihood phylogeny for the 107 angiosperm species used in the analysis, based on *rbcL* sequence data.

**Table S1** Species used in the analysis, their pollen to ovule (P : O) ratios, outcrossing rates (*t*), pollination syndrome (anemophily, a; biotic pollination, b), life form, *rbcL* data used for phylogenetic correction (*, original sequence data of the species available) and GenBank accession numbers

**Table S2** Reference list for P : O ratios and outcrossing rates (*t*)

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