

Correlated evolution of mating system and floral display traits in flowering plants and its implications for the distribution of mating system variation

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Summary

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- Reduced allocation to structures for pollinator attraction is predicted in selfing species. We explored the association between outcrossing and floral display in a broad sample of angiosperms. We used the demonstrated relationship to test for bias against selfing species in the outcrossing rate distribution, the shape of which has relevance for the stability of mixed mating.
- Relationships between outcrossing rate, flower size, flower number and floral display, measured as the product of flower size and number, were examined using phylogenetically independent contrasts. The distribution of floral displays among species in the outcrossing rate database was compared with that of a random sample of the same flora.
- The outcrossing rate was positively associated with the product of flower size and number; individually, components of display were less strongly related to outcrossing. Compared with a random sample, species in the outcrossing rate database showed a deficit of small floral display sizes.
- We found broad support for reduced allocation to attraction in selfing species. We suggest that covariation between mating systems and total allocation to attraction can explain the deviation from expected trade-offs between flower size and number. Our results suggest a bias against estimating outcrossing rates in the lower half of the distribution, but not specifically against highly selfing species.

Introduction

Evolutionary transitions in mating systems are known to be common in angiosperms, and wide diversity in outcrossing rates has been observed at all taxonomic levels. The evolution of mating systems is thought to be strongly associated with variation in floral traits. The observation that species

with small, inconspicuous flowers are often highly self-fertilizing dates back to the work of Muller (1883) and Darwin (1876). The pattern has been well documented in comparisons of species within genera, including *Arenaria* (Wyatt, 1984), *Collinsia* (Armbruster *et al.*, 2002), *Leptosiphon* (Goodwillie, 1999) and *Mimulus* (Ritland & Ritland, 1989), and also among populations within species (Lloyd, 1965; Lyons & Antonovics, 1991; Goodwillie & Ness, 2005; Vallejo-Marin & Barrett, 2009). In a broader survey, Cruden & Lyon (1985) found some support for this trend

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in 39 unrelated herbaceous species using the pollen : ovule ratio as an indicator of outcrossing rate (Cruden, 1977). However, the generality of this pattern across angiosperm species remains unexplored.

Theoretical treatments explain the association between outcrossing rate and attractive floral traits in terms of adaptive changes in resource allocation that accompany the evolution of the mating system. The evolution of hermaphrodite flowers has been examined in the context of sex allocation theory, in which the shapes of fitness gain curves for allocation to male and female functions influence the evolution of investment in each gender (Charnov, 1982). In general, the models predict that, as the selfing rate increases, the allocation to male function should decrease (Charlesworth & Charlesworth, 1981; Charnov, 1982; reviewed in Charlesworth & Morgan, 1991; Brunet, 1992). This is because, with higher mean selfing rates in a population, fewer ovules are available to be fertilized by outcross pollen, and the fitness gain through pollen export is reduced. Some empirical studies suggest that structures for floral attraction, such as the corolla, have a greater effect on male than on female fitness (Willson & Price, 1977; Bell, 1985). Therefore, a corollary of sex allocation theory is that selfing species should invest less in attractive structures than do outcrossing species. Models that allow separate allocation to male, female and attractive structures also predict reduced allocation to attraction with high selfing rates (Charlesworth & Charlesworth, 1987; Lloyd, 1987). Furthermore, in species practicing autonomous selfing (self-fertilization without the aid of a pollinator), selection should reduce the attraction allocation because total seed production depends less on pollinator visits at higher selfing rates. Despite the broad prediction of decreased allocation to both pollen and attraction with increased selfing, it is important to realize that the slope and shape of the decline in optimal allocation will depend on a large number of factors, including the shapes of the fertility gain curves, level of inbreeding depression and the mode of selfing (Charlesworth & Charlesworth, 1987; Lloyd, 1987). For example, when selfing requires pollinator visits, attraction remains important for both selfed and outcrossed seed production, and allocation to attraction should decline much less steeply than when pollinators are not required (Lloyd, 1987). Thus, even at high selfing rates, species requiring pollinator visits for seed production are expected to devote considerable resources to attraction. The greatest decline in attractive structures with higher selfing should be found when selfing occurs autonomously.

The causal relationships between floral display and outcrossing rate may be complex. As discussed above, reduced flower size is usually interpreted as an adaptation that optimizes resource allocation after selfing evolves. Thus, the selfing rate influences the evolution of the floral display. Alternatively, the size of the floral display might directly

affect the outcrossing rate of a plant or population. Variation in flower size has been shown to influence rates of pollinator visitation (Bell, 1985; Eckhart, 1991; Conner & Rush, 1996; Kennedy & Elle, 2008), and the pollinator visitation rate can affect the amount of outcross pollen deposited on stigmas, and thus the outcrossing rate in self-compatible plants (Brunet & Sweet, 2006). However, in this article, we focus primarily on the evolutionary effects of mating system on subsequent adaptation in the floral display.

The magnitude of total allocation to attraction is determined by both the number of flowers produced and the size of individual flowers. The partitioning of resources among flowers adds complexity to the relationship between outcrossing rate and floral display, because it affects the potential for geitonogamy, self-pollination that occurs by the transfer of pollen among flowers on the same plant. Geitonogamy can incur costs through seed and pollen discounting; that is, pollen and ovules used in geitonogamous selfing reduce the pool of gametes available for outcrossing (De-jong *et al.*, 1993; Harder *et al.*, 2004). Selection for floral traits that reduce geitonogamy can influence the optimal allocation to flower size vs number because geitonogamy reduces the male fitness advantage of a large inflorescence (Harder & Barrett, 1995; Routley & Husband, 2003). As a consequence, selection to prevent geitonogamy might yield a negative relationship between flower number and outcrossing. Furthermore, the costs of geitonogamy for male and female functions will differ for self-incompatible relative to self-compatible species, as self-incompatibility should minimize the costs of seed discounting but might exacerbate costs for males through pollen discounting (Harder & Barrett, 1995). Therefore, breeding system variation can influence the relationships between flower size, number and outcrossing rate.

Partitioning of resources to floral display varies dramatically among angiosperms, from species producing a single large flower at one extreme (e.g. *Trillium grandiflorum*) to those producing many tiny flowers at the other (e.g. *Daucus carota*). If the pool of resources for floral display is fixed, we can expect a trade-off between flower size and flower number, yielding a negative correlation between the traits. Recently, a wide survey of angiosperm species supported this prediction. Sargent *et al.* (2007) found a significant negative correlation between flower size and daily flower number in an independent contrasts analysis involving 251 species. However, a large proportion of the variation (c. 75–80%) in flower number remained unexplained by flower size in their analysis, and other tests of the trade-off hypothesis that compared species within genera, populations within species or genotypes within populations yielded mixed results, including positive relationships in some taxa (Worley & Barrett, 2000; Caruso, 2006). It has been argued that the unexpected positive correlation between

flower size and number observed within species is attributable to a variation in resource status: plants with more resources could produce both more flowers and larger flowers (Worley *et al.*, 2000). Similarly, hierarchical patterns of allocation might obscure trade-offs between flower size and number; resource allocation can be viewed as hierarchical (Delaguerie *et al.*, 1991; Dejong, 1993; Worley *et al.*, 2003), with resources partitioned at the lowest level between vegetative and reproductive functions, and reproductive resources then allocated to fruits and seeds vs attractive structures. The pool allocated to attraction, in turn, is partitioned among flowers on an inflorescence. If the total allocation to attractive traits is greater in outcrossing species, both flower size and number might be higher in outcrossing than in selfing species, yielding positive associations between the two traits among species. Thus, we predict that mating system diversity can be a source of departure from the negative correlation expected from trade-offs.

In this article, we provide the first angiosperm-wide test of the relationships between outcrossing rate and floral display traits using independent contrasts to account for the phylogenetic signal. We also explore the extent to which functional associations between outcrossing rate and total allocation to floral display counteract the trade-off-driven negative correlation between flower size and number. Overall, we have found that the variation in mating system accounts for a significant portion of the variation in floral display.

As a second major objective, we use the observed relationship between floral display size and mating systems to address a longstanding question concerning the distribution of mating system variation in higher plants and potential biases inherent in the sample of published genetic estimates of outcrossing rates (Schemske & Lande, 1985, 1986, 1987; Aide, 1986; Waller, 1986; Barrett & Eckert, 1990; Vogler & Kalisz, 2001; Goodwillie *et al.*, 2005; Igic & Kohn, 2006). Theory developed by Lande & Schemske (1985) predicts that, because selfing can purge deleterious alleles that cause inbreeding depression, populations should evolve towards one of two extremes: highly outcrossing mating systems with severe inbreeding depression or highly selfing mating systems with little inbreeding depression. Consistent with this theory, Schemske & Lande's (1985) survey of outcrossing rates in higher plants showed evidence for bimodality. However, mixed mating systems have long been argued to be common in plants (Baker, 1959; Lloyd, 1979), and recent analyses of the accumulating data have highlighted the substantial frequency of species with intermediate outcrossing rates (Vogler & Kalisz, 2001; Goodwillie *et al.*, 2005). All interpretations of the distribution, however, have acknowledged the potential for sampling biases in the species for which outcrossing rates have been estimated. In particular, it has been argued that genetic analyses are less likely to be used for outcrossing rate

estimates in species presumed to be highly outcrossing, based on the presence of self-incompatibility or dioecy, or to be highly selfing, based on floral traits. Using surveys of breeding systems for regional floras to infer their true distribution, Igic & Kohn (2006) provided evidence for a study bias against obligately outcrossing species in the database. In this article, we use floral display size as a proxy for outcrossing rate to provide the first test of the hypothesized study bias against highly selfing species.

Materials and Methods

Relationships among floral display traits and outcrossing rate

Data collection We updated the database of outcrossing rates of higher plants (Barrett & Eckert, 1990; Vogler & Kalisz, 2001; Goodwillie *et al.*, 2005) to include all studies published through 2007 in which the outcrossing rate was estimated by genotyping progeny arrays. When multiple outcrossing rate estimates were available for a species, we calculated a mean across all estimates, as has been performed in most previous analyses of the dataset. For angiosperm species in our database, we searched for flower size data in published floras and primary literature. In most species, flower size was measured as total corolla diameter, or computed as twice the petal length. For a small number of species with tubular flowers, corolla length rather than diameter is typically reported and was our metric of flower size. When a range of sizes was given, we used the midpoint. Although it would be preferable to test for associations between outcrossing rate and floral display traits at the level of individual populations, floral display data were rarely available for the specific populations used for outcrossing rate estimation. Using species' means or midpoints for each trait might have limited our ability to detect evolutionary processes occurring at the population level. Corolla diameter was measured on dried specimens at the Chicago Field Museum Herbarium (IL, USA) on 23 species for which published flower size data were not available. For all species with flower size data, we then searched for information on inflorescence size. We used the number of open flowers per inflorescence (daily flower number) as our measure, because it is positively correlated with the number of total flowers per inflorescence for several species (Harder & Cruzan, 1990; Brunet & Eckert, 1998). This is a measure of individual inflorescence size, the unit likely to be most relevant to pollinator attraction, rather than total flowers per plant. Some estimates were derived from a database of angiosperm reproductive traits compiled from the scientific literature by R. Bertin and C. M. Newman for the study of ecological correlates of dichogamy (results published in Bertin & Newman, 1993). The database was last updated in January 2001.

For species not in the Bertin database, we estimated the daily number of open flowers per inflorescence from descriptions in floras. Finally, if no other source was available, we estimated inflorescence size by inspection of photographic images of each species on a variety of botanical websites (e.g. USDA Plants Database, <http://plants.usda.gov/>), excluding horticultural and amateur websites. When possible, values reported are a mean of open flower numbers estimated from three images. Our searches resulted in flower and inflorescence size data for 154 species in the outcrossing rate database. We computed the product of flower size and flower number as an approximation of total allocation to floral display per inflorescence and took the natural logarithm of that value, hereafter referred to as 'floral display size'. The dataset is available on request to C. Goodwillie.

Data analysis A phylogenetic tree of these 154 species was obtained by pruning the Davies *et al.* (2004) angiosperm supertree using the program Phylomatic (Webb *et al.*, 2008). Phylogenetic independent contrasts (PICs) for $\log_e(\text{outcrossing rate} + 0.001)$, $\log_e(\text{flower size})$, $\log_e(\text{flower number})$ and floral display size [$\log_e(\text{flower size} \times \text{flower number})$] were obtained using the APE package in R (Paradis *et al.*, 2004). Because the tree was incompletely resolved, all statistics are the average results for 1000 random resolutions of the tree. Because of the limitations of estimating branch lengths for polytomous clades, all branch lengths were set to unit length for the PICs analyses. We used the diagnostics tool in the PDTREE module of PDAP (Midford *et al.*, 2008), implemented in Mesquite 2.6 (Maddison & Maddison, 2009), to check the assumption that the branch lengths adequately fit the tip data for all traits. The diagnostics application in PDAP tests this assumption by examining the correlation between the standardized contrasts and their standardized deviations for each trait (Garland *et al.*, 1992). Some traits required logarithmic transformation to meet the assumption of no correlation between standardized contrasts and their standardized deviations. Relationships among PICs were analyzed using the linear model function in R. Multiple regression analysis was used to test the relationship of each component of display (flower size and open flower number) to outcrossing rate holding the other constant. All linear models were forced through the origin as is standard for analyses using PICs.

Test for a bias against selfing species in the outcrossing rate distribution

Overview of methodology Using floral display size as a proxy for outcrossing rate, we tested the hypothesis that highly selfing species are undersampled in the distribution of published outcrossing rates based on genetic markers.

We focused our analyses on herbaceous species of northeastern North America because this floristic group is well represented in the outcrossing rate database and well characterized for floral traits. We first tested whether floral display size strongly predicts outcrossing rate in the subset of species in the outcrossing rate database that are herbaceous and occur in northeastern North America. We then compared the distribution of floral display sizes in this subset of species to that of an unbiased sample drawn from the same flora. We hypothesized that a bias against estimating outcrossing rates for highly selfing species would result in a deficit of species with small floral displays in the outcrossing rate database relative to a random sample.

Data collection We restricted our analysis to herbaceous, nongraminoid species (i.e. excluding species in the families Poaceae, Juncaceae and Cyperaceae) that are listed in the *Manual of the Vascular Plants of Northeastern United States and Adjacent Canada* (Gleason & Cronquist, 1991, hereafter G&C). We also eliminated several families for which the flower and inflorescence units were ambiguous and dimensions for single flowers were generally not reported (Asteraceae, Dipsacaceae, Apiaceae and Araceae), and a few small families for which flower descriptions were not given (e.g. Cannabaceae, Lemnaceae and Ruppiaceae). From this flora, we generated two datasets for comparison. (1) To create an unbiased sample of the flora, we selected every sixth species as listed in G&C that met our criteria, and we recorded the flower size reported in G&C. When, occasionally, the flower size was not given for an individual species, we replaced that species with the next in sequence. We estimated the number of open flowers per inflorescence as described above for the outcrossing database species. The resulting list of 323 species provides an unbiased sample that is proportionally representative of the taxonomic distribution of the G&C flora. For simplicity, we refer to this as the 'random' G&C dataset or sample, although, strictly speaking, the flora was evenly, rather than randomly, sampled. (2) We then searched through the outcrossing rate database for species that occurred in the G&C flora and met our criteria, yielding a list of 60 species. The two G&C samples were largely independent; only three species included in the G&C random dataset were also present in the G&C outcrossing rate dataset.

Data analysis We used linear regression of outcrossing rate on floral display to test the extent to which floral display can predict the outcrossing rate in the subset of outcrossing database species present in G&C. We reasoned that an analysis of raw data, rather than of PICs, was appropriate for this purpose, as we were testing for a predictive relationship, rather than inferring an evolutionary process. We tested for a significant difference in the shape of the distribution of floral display sizes among G&C species from the outcrossing

rate database vs the random G&C sample using a two-sample Kolmogorov–Smirnov test. To test specifically for a deficit of species with small floral displays, we found the expected first and second quartile values from the random sample and used chi-square tests to compare the observed with expected number of species in the G&C outcrossing rate sample below these values. In addition, we used the random G&C dataset to generate a distribution of mean values of floral display, and compared it with the mean value observed in the 60 species of the G&C flora present in the outcrossing database (see Results). We resampled 60 species with replacement from the random G&C dataset and calculated the mean value of floral display (R Development Core Team, 2008). This resampling was repeated 10 000 times to obtain 95% confidence intervals (CI).

Results

Relationships among floral display traits and outcrossing rate

In accordance with predictions, the correlation coefficient between contrasts for floral display size (\log_e of the product of flower diameter and open flower number) and outcrossing rate was positive and significant ($F = 39.9$, $df = 1152$, $R^2 = 0.21$, $P < 0.0001$, Fig. 1; a scatterplot of the raw data is shown in Fig. 2). PICs revealed that each individual component of display size was more weakly correlated with outcrossing rate (flower size: $F = 14.8$, $df = 1152$, $R^2 = 0.09$, $P = 0.0004$; open flower number: $F = 59.13$, $df = 1152$, $R^2 = 0.06$, $P = 0.005$, Fig. 3a,b). Multiple regression analyses revealed that each individual component of display size was significantly positively correlated with outcrossing rate contrasts when the other component was held constant (partial regression coefficients: flower size contrasts: $b_1 = 0.96$; flower number contrasts: $b_2 = 0.60$; entire model: $df = 1151$, $R^2 = 0.24$, $P < 0.001$, Fig. 3a,b). There was a correlation between flower size and number (explained below). However, the variance inflation factor for the regression between the two traits was below five, indicating the absence of strong collinearity. There was no significant interaction involving flower size and number on outcrossing rate. The independent contrasts for outcrossing rate included a large number of species' pairs for which the outcrossing rates did not differ, leading to an overrepresentation of zeroes in our outcrossing rate data and some deviation from a normal distribution. In order to account for a potential effect on the results of our statistical tests, we repeated the tests using nonparametric Spearman's rank correlations. The results of these correlations were congruent with those of the multiple regression tests, suggesting that our results are robust (results not shown). Consistent with previous studies, the relationship between flower size contrasts and flower number contrasts was negative

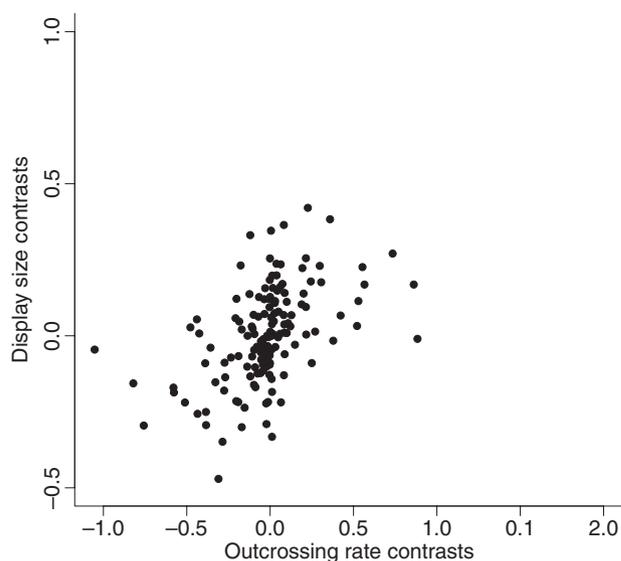


Fig. 1 Scatterplot of phylogenetically independent contrasts (PICs) for floral display size (\log_e of the product of flower size and number) and outcrossing rate.

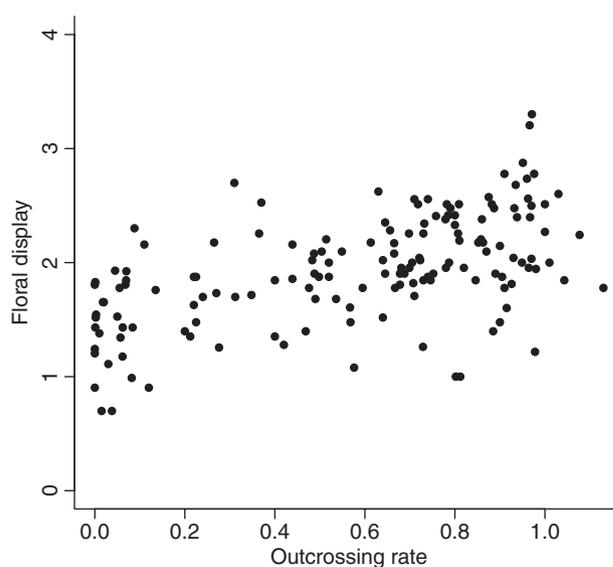


Fig. 2 Scatterplot of raw data for floral display size (\log_e of the product of flower size and number) and outcrossing rate.

($F = 27.3$, $df = 1152$, $R^2 = 0.15$, $P < 0.0001$, Fig. 4), providing support for a trade-off between these traits.

Test for a bias in the outcrossing rate database

The outcrossing rate database contained 60 herbaceous nongraminoid species that were present in the G&C flora of northeastern North America. Using only these species, the linear regression of outcrossing rate on floral display size was significant and positive ($F = 33.06$, $df = 1.58$, $P < 0.0001$, adjusted $R^2 = 0.352$, Fig. 5a). A Kolmogorov–

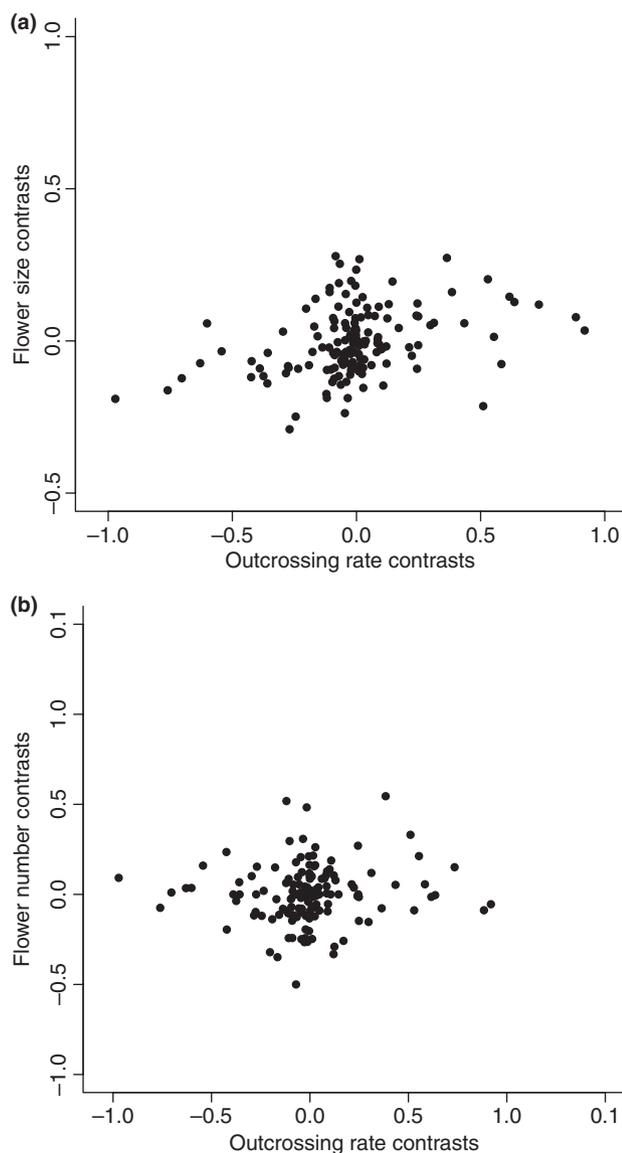


Fig. 3 (a) Scatterplot of phylogenetically independent contrasts (PICs) for flower size and outcrossing rate. (b) Scatterplot of PICs for flower number and outcrossing rate.

Smirnov goodness-of-fit test found that the distributions were significantly different from each other (Kolmogorov–Smirnov $Z = 1.839$, $P = 0.002$), although neither differed significantly from a normal distribution (Shapiro–Wilk test, random data: $W = 0.996$, $P = 0.651$; outcrossing rate data: $W = 0.99$, $P = 0.384$, Fig. 5b). The observed mean value of floral display in the outcrossing rate species that were also present in the G&C flora was significantly larger than the mean value for randomly chosen species in the G&C flora (observed mean = 1.938, random sample mean = 1.724, 95% CI = 1.593–1.856). Sixteen species in the G&C outcrossing rate dataset had floral display values that fell below the second quartile (median) of the random distribution, which was significantly fewer than the expected value of 30

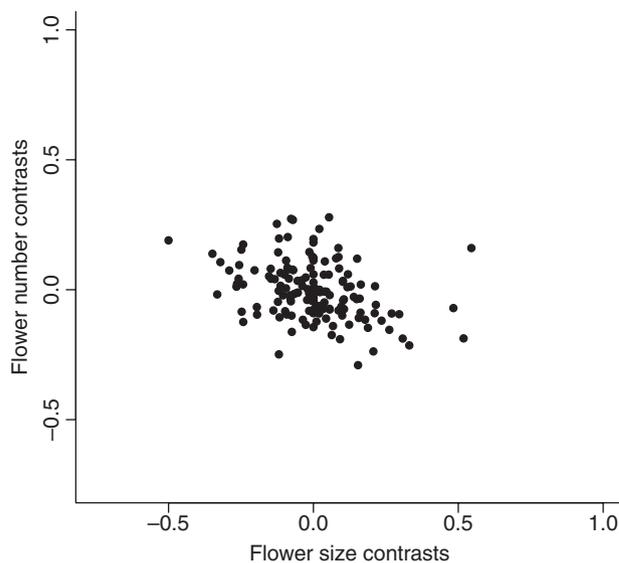


Fig. 4 Scatterplot of phylogenetically independent contrasts (PICs) for flower size and flower number.

species ($\chi^2 = 13.06$, $P < 0.001$). However, the observed number of species below the first quartile of the random sample (10) was not significantly different from the expected value of 15 ($\chi^2 = 2.22$, $P = 0.136$).

Discussion

Mating systems and floral display

A positive relationship between flower size and outcrossing rate is well documented for congeneric species or conspecific populations that vary in mating systems (Wyatt, 1984; Ritland & Ritland, 1989; Johnston & Schoen, 1996; Goodwillie, 1999; Armbruster *et al.*, 2002; Goodwillie & Ness, 2005). One strength of comparing close relatives is that other ecological and life history traits may be largely controlled for, thereby isolating the effects on floral morphology of divergent mating systems. Our analyses extended the generality of these previous results: we found positive associations between outcrossing rate and two components of floral display, as well as floral display itself, in a comparison of 154 angiosperm species with a wide taxonomic distribution. Our survey included species that ranged from small annual herbs (e.g. *Capsella bursa-pastoris*) to trees (e.g. *Magnolia stellata*) and represented a wide range of habitats and distributions. Perhaps more importantly, they use a range of pollinating vectors, which are expected to select in different ways on floral morphology (Fenster *et al.*, 2004). Given this variation, the strength of the relationship between floral display size and outcrossing rate detected here is perhaps surprising. Because we considered an absolute measure of display traits rather than a proportional measure correcting for total reproductive allocation, as is

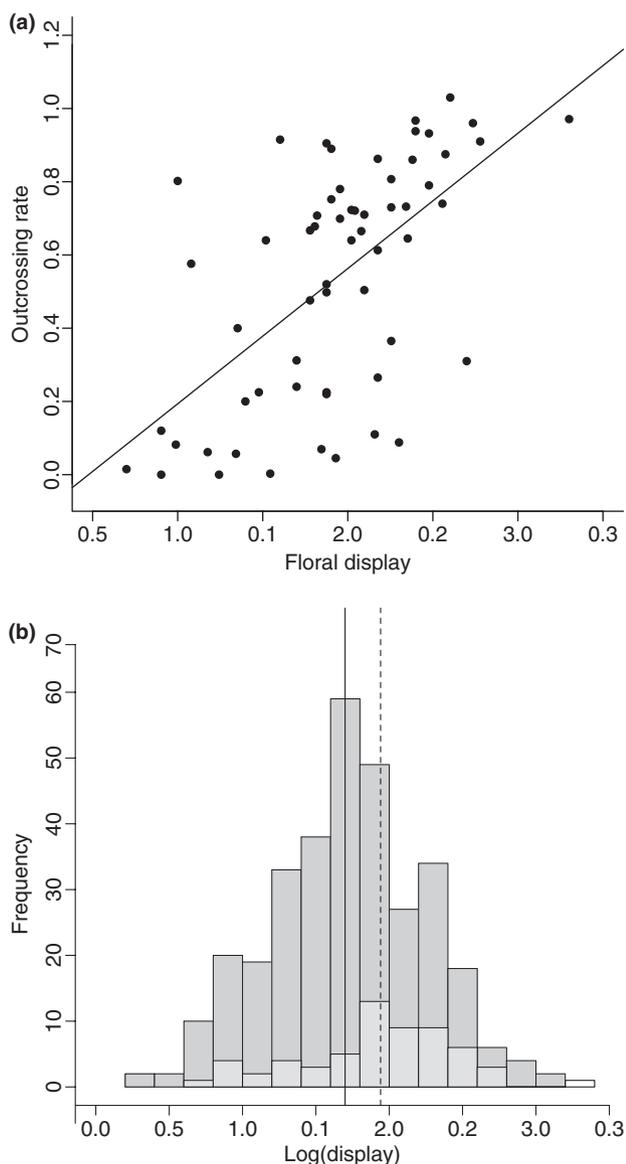


Fig. 5 (a) Scatterplot of outcrossing rate vs floral display size (\log_e of product of flower size and number) for herbaceous species in the Gleason and Cronquist (1991) (G&C) flora with regression line indicated. (b) Histogram of display size for a random sample of species from G&C (dark bars, see text for explanation of sampling protocol) and G&C species found in the outcrossing rate database (light bars). The broken line indicates the median display size for species in the outcrossing database; the full line indicates the median for species in the random sample.

typically employed in tests of allocation theory using comparisons of closely related species (e.g. Ritland & Ritland, 1989; Belaussoff & Shore, 1995), we expected that the wide variation in total resource availability would create considerable noise. Our results provide broad support for the theoretical expectation that attractive structures should be reduced in self-fertilizing species. Theory predicts reduced allocation only when selfing occurs autonomously; selfing that is facilitated by pollinators, whether within or

between flowers, requires the same structures as outcrossing (Lloyd, 1987; Brunet, 1992). Therefore, our results also suggest that autonomous selfing is a common mode of self-fertilization in our sample of angiosperms.

Evolution of flower number

To our knowledge, this is the first demonstration that the outcrossing rate is more strongly associated with floral display size (product of flower size and number) than with flower size alone. Most studies examining the effects of mating system on allocation to floral structures have focused on the size and biomass of individual flowers, rather than on the number of flowers produced. Although flower size was significantly correlated with outcrossing, we found that the product of flower number and flower size explained considerably more of the variation in outcrossing rates than flower size alone (Figs 1,3; $R^2 = 0.21$ vs $R^2 = 0.09$, respectively). This result suggests that the size of the floral display, rather than flower size alone, evolves in association with the mating system.

A positive relationship between the outcrossing rate and the product of flower size and number indicates that, consistent with sex allocation theory, total allocation to investment in attractive structures per inflorescence is lower in selfing species. Resources allocated to attraction must then be partitioned among individual flowers; if the pool of resources allocated to attraction is fixed, the product of flower size and number is expected to be invariant. By contrast, we found substantial variation among species in floral display size, here defined as the product of flower size and number. Reduced allocation to attraction in selfing species is expected to allow greater investment of reproductive resources into fruit or seed provisioning (Lloyd, 1987), which may explain, in part, the trend towards higher fruit-to-flower and seed-to-ovule ratios in selfing than in outcrossing species (Bawa & Webb, 1984; Sutherland, 1986; Charlesworth, 1989).

We considered the possibility that plant size alone, rather than floral evolution driven directly by mating system variation, might explain the observed trend. That is, large plants should have more resources available and therefore more available for attractive structures, and one might expect an association between plant size and outcrossing rate, as annual species are more likely to be small and also more often highly selfing (Barrett & Eckert, 1990; Barrett *et al.*, 1996). However, among the subset of species found in the G&C flora, plant height did not correlate with the outcrossing rate ($r = 0.21$, $P = 0.15$), suggesting that variation in plant size did not contribute substantially to the relationship between outcrossing and display size, at least in this group of species. In addition, our use of display size, rather than total display per plant, probably controlled for some of this variation.

Despite the indication from our analysis that total resources invested in attraction per inflorescence varied widely among species, we also found evidence of a flower size/number trade-off in the outcrossing rate species; a significant negative association was found in the PIC analysis (Fig. 4), as was also seen in a larger sample of angiosperms (Sargent *et al.*, 2007), supporting the idea that resources for attraction are partitioned among flowers within an inflorescence. However, in both studies, much of the variation in flower number was not explained by a variation in flower size. The analysis of species in the outcrossing rate database suggested that variation in the mating systems could explain some of the noise observed in the flower size/number trade-off. In fact, residuals of the regression of flower number on flower size showed a significant positive relationship with the outcrossing rate in a separate PIC analysis ($F = 33.7$, $df = 1152$, $R^2 = 0.18$, $P < 0.0001$). Therefore, outcrossing species tend to deviate from the negative relationship in having more flowers than expected, given their flower size. Conversely, statistical confirmation of a flower size/number trade-off in our study suggests that evolution to increase total allocation to attraction may be somewhat constrained, such that partitioning of limited resources is evident.

The mating system might also have a direct effect on the number of open flowers per inflorescence, our metric of flower number, via flower longevity. Daily flower number is determined both by the number of flowers produced and the longevity of individual flowers (Primack, 1985; Schoen & Ashman, 1995). Consequently, relationships between the mating system and daily open flower number might also arise through variation in flower longevity. For example, when flower senescence is triggered by pollination or fertilization, as has been documented in many species (reviewed in Primack, 1985), flower longevity, and therefore daily display, will be influenced by the rate of pollination (Harder & Johnson, 2005). With this scenario, species in which autonomous selfing occurs early in anthesis might have particularly short-lived flowers (Wyatt, 1984; Sato, 2002; Weber & Goodwillie, 2007), and thus a smaller number of open flowers in a display.

Our finding of a positive association between outcrossing rate and flower number contradicts predictions related to the costs of geitonogamy for both seed and pollen discounting, which should exert selection to reduce flower number in outcrossing species (Dejong *et al.*, 1993; Harder & Johnson, 2005). This is especially true in species with extensive inbreeding depression, in which geitonogamy can incur high costs of seed discounting (Dejong *et al.*, 1993). We would expect the consequences of geitonogamy to select for lower flower number in outcrossing species, rather than the positive relationship observed here. Moreover, when we consider the direct effects of floral display on the out-

crossing rate, we might expect a higher flower number to increase geitonogamy and therefore decrease the outcrossing rate in self-compatible species.

In addition, the trend towards a lower flower number in selfing species is inconsistent with theory predicting that nonlinear trade-offs, in which total resource investment can increase with the number of flowers (Sakai & Harada, 2001), should select for increased flower number in selfing species (Tomimatsu & Ohara, 2006). Although empirical support was found in a study of *Trillium*, in which partially selfing populations produced significantly more and smaller flowers than did self-incompatible populations (Tomimatsu & Ohara, 2006), our broad survey shows the opposite trend.

Evidence for a study bias against selfing species in the distribution of outcrossing rates

Our random sample of herbaceous species in a North American flora suggests a bias in the database of published outcrossing rates against species with small floral displays and, by inference, against species with outcrossing rates at the lower end of the range (Fig. 5b). One of the reasons for biased sampling might itself stem from the perceived relationship between flower size (or floral display) and outcrossing rate; that is, researchers interested in variation in mating systems assume that small-flowered or otherwise inconspicuous species will be highly selfing and thus not worth estimating mating system parameters using genetic markers. The number of selfing species in the database is also limited by the availability of genetic polymorphisms needed for marker analysis. Congruent with theoretical predictions, selfing species often contain little genetic variation within populations (Allard *et al.*, 1968; Brown, 1979; Loveless & Hamrick, 1984; Charlesworth & Charlesworth, 1995). A lack of genetic polymorphisms has prohibited outcrossing rate estimation for selfing populations in a number of studies (e.g. Glover & Barrett, 1986; Parker *et al.*, 1995; Goodwillie, 2000) and undoubtedly remains unreported in other failed attempts at marker analysis. Small-flowered species also present challenges for floral manipulations and controlled crosses. In discussing his view that predominantly selfing species benefit from occasional outcrossing, even Darwin admitted to a study bias against species with small, inconspicuous flowers: 'It has been one of the greatest oversights in my work that I did not experimentise on such flowers, owing to the difficulty of fertilising them, and to my not having seen the importance of the subject' (Darwin, 1876, p. 387).

The strength of the conclusions on a sampling bias that can be drawn from this test is limited by the scope and nature of our study. First, the relationship between outcrossing rate and display size, although significantly positive, is relatively weak. As a result, flower size is not a sufficiently accurate predictor of mating system to enable us to correct the

distribution to account for the bias, as was performed by Igic & Kohn (2006) for self-incompatible and dioecious species, which are obligately outcrossing. Second, our test is limited because it concerns only a portion of the outcrossing rate database from one floristic region. Only one such test was possible because the outcrossing rate database does not contain enough species from other distinct, nonoverlapping floras to replicate and broaden the scope of the analysis. Moreover, the distribution of outcrossing rates in gymnosperms and in grasses, which are generally wind pollinated and lack attractive floral structures, cannot be addressed using this approach. Nevertheless, it might be argued that we have underestimated the bias against more selfing species by restricting our analysis to these taxa, as an overrepresentation of certain woody, largely outcrossing taxa (e.g. *Pinus*, *Eucalyptus*) in the complete outcrossing database (Goodwillie *et al.*, 2005) is likely to be a further source of bias.

Our study complements the results of Igic & Kohn (2006), which indicate that highly outcrossing species are also undersampled. The outcrossing rate database shows a deficit of self-incompatible and dioecious species relative to their true distribution, as inferred from a number of regional floristic studies. In contrast with their study, however, the bias detected here extends to the entire lower half of the outcrossing rate distribution; in fact, the observed to expected ratio for species between the first and second quartile (7 : 15) is somewhat lower than that of species below the first quartile (10 : 15). Thus, correcting for the bias detected is not expected to produce greater bimodality, the distribution predicted by Lande & Schemske's (1985) theoretical argument for evolutionary instability of mixed mating systems. We note that there is no indication of bimodality in the distribution of floral displays of our random sample. Previous analyses have shown that mixed mating systems are considerably more common in animal-pollinated species, with bimodality more evident in wind-pollinated species (Aide, 1986; Barrett & Eckert, 1990; Vogler & Kalisz, 2001). The dataset of herbaceous, nongraminoid species used in our test is likely to be dominated by animal-pollinated taxa. Thus our results are also consistent with the idea that mixed mating is particularly frequent in species that are pollinated by animals.

In the light of Igic & Kohn's (2006) finding of a study bias against obligate outcrossers, we might also predict a deficit at the upper end of the range of floral display sizes for species in the outcrossing rate database. Contrary to this expectation, large displays appear to be somewhat overrepresented; 35% of the G&C outcrossing rate species fall within the range of display sizes corresponding to the top 25% of the random sample of species. This incongruent result is probably explained by the fact that self-incompatibility and dioecy, the breeding systems that Igic and Kohn argued are likely to be undersampled in outcrossing rate estimation, are relatively rare in herbaceous taxa. Moreover, the few dioe-

cious herbs in this flora are often wind pollinated and small flowered. Therefore, we are unlikely with this method to detect a bias against highly outcrossing species using only herbaceous species of eastern North America.

The distribution of outcrossing rates is of interest with respect not only to the stability of intermediate outcrossing, but also to the rate at which mating system transitions occur. Moreover, the relative frequency of highly selfing and highly outcrossing species has relevance to hypotheses concerning the effects of mating system on rates of speciation and extinction of evolutionary lineages (Takebayashi & Morrell, 2001; Igic & Kohn, 2006). Although the actual shape of the distribution may never be known, our analysis provides further insight and indicates the need for further study.

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