

Correlated evolution of flower size and seed number in flowering plants (monocotyledons)

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- **Background and Aims** Kin selection theory predicts that a parent may minimize deleterious effects of competition among seeds developing within ovaries by increasing the genetic relatedness of seeds within an ovary. Alternatively, the number of developing seeds could be reduced to one or a few. It has also been suggested that single or few seeded fruits may be correlated with small flowers, and multi-ovulate ovaries or many seeded fruits may be associated with large flowers with specialized pollination mechanisms. We examined the correlation between flower size and seed number in 69 families of monocotyledons to assess if correlations are significant and independent of phylogeny.
- **Methods** We first examined the effect of phylogenetic history on the evolution of these two traits, flower size and seed number, and then mapped correlations between them on the latest phylogenetic tree of monocotyledons.
- **Results** The results provide phylogenetically robust evidence of strong correlated evolution between flower size and seed number and show that correlated evolution of traits is not constrained by phylogenetic history of taxa. Moreover, the two character combinations, small flowers and a single or few seeds per fruit, and large flowers and many seeded fruits, have persisted in monocotyledons longer than other trait combinations.
- **Conclusions** The analyses support the suggestion that most angiosperms may fall into two categories, one with large flowers and many seeded fruits and the other with small flowers and single or few seeded fruits, and kin selection within ovaries may explain the observed patterns.

Key words: Kin selection, monocotyledons, flower size, seed number, evolution of correlated traits.

INTRODUCTION

Flowering plants exhibit a wide range of reproductive modes (Darwin, 1877; Stebbins, 1974; Barrett, 1992; Richards, 1997; Briggs and Walters, 2016). Correlations among reproductive traits have provided new explanations for the evolution of breeding systems (Bawa, 1980; Givinish, 1980; Felsenstein, 1985) and possibility of kin selection (Kress, 1981; Bawa, 2016), and sexual selection (Willson, 1979; Bawa, 1980) in plants. Kress (1981) noted correlations between polyads, or pollen aggregations, and multi-ovulate ovaries on the one hand, and between monads and ovaries with a single or a few ovules on the other. He invoked kin selection to explain the association between pollen aggregations with multi-ovulated ovaries, arguing that such associations increase the genetic relatedness of seeds developing within an ovary, thereby minimizing deleterious effects of competition among developing siblings.

Bawa (2016) extended the concept of kin selection to suggest that flowering plants may have followed two evolutionary trends: one that increases the genetic relatedness of seeds within multi-ovulate ovaries or many seeded fruits through evolution of specialized pollination mechanisms in large flowers, and the other a reduction in seed number to one or a few in small flowers pollinated by generalist insects or wind. Thus, he

predicted that kin selection may have generated two sets of contrasting correlated traits in flowering plants: (1) large flowers, pollen aggregations, specialist pollinators, synchronous arrival of pollen grains that interact to increase the genetic relatedness of seeds within fruits in multi-ovulate ovaries associated with the aforementioned floral traits, and (2) small flowers, a generalist mode of pollination including wind and water pollination that may interact to reduce sibling competition by decreasing seed number to one or a few within fruits.

For these two sets of correlations, critical are the correlations between: (1) small flowers and one or a few ovules per ovary or one or a few seeds per fruit, and (2) large flowers and multi-ovulate ovaries or many seeded fruits, because other correlated traits follow from these two traits.

Here, based on a broad survey of the monocotyledons (69 families), we demonstrate that an overwhelming majority of families show that large flower size is associated with multi-ovulate ovaries or multi-seeded fruits, and small flowers are associated with ovaries with a single or a few seeded fruits. We propose that most angiosperms may fall into one or the other category depending upon flower size and the associated seed number per fruit.

Further, we used modern comparative phylogenetic methods to: (1) determine if the evolution of flower size and seed

number in monocotyledons is constrained by phylogenetic relationships of taxa; (2) test the existence of correlated evolution between flower size and seed number; and (3) assess the most probable evolutionary route and directionality of flower size and seed number. Finally, we discuss these results in the context of kin selection, allometry and other explanations advanced to account for reduction in ovule or seed number in angiosperms.

We chose monocotyledons to address the above questions because they are a large and diverse group that have a widespread distribution, show variation in the traits of interest, and have an abundance of the data easily accessible in one place (Friedman and Barrett, 2009). The analyses were done at the family level because for the overwhelming majority of families, the analysed traits appear to be specific to families, with little variation among genera within families.

Our results are significant and novel in two respects. First and foremost, if such correlations hold in other groups of plants, most of the flowering plants may fall into two broad groups based on flower size and ovule and seed number. Although several authors have highlighted the correlations among flower size, mode of pollination and ovule number (Bawa, 1980, 2016; Burd et al., 2009; Friedman and Barrett, 2009, 2011), demonstration of contrasting sets of correlations among reproductive traits has been lacking. Secondly, the correlated evolution of flower size and seed number provides a possible new framework for examining the evolution of a range of reproductive traits from flower size and pollination mechanisms to seed number per fruit.

MATERIALS AND METHODS

We analysed 69 out of 74 monocotyledon families in the phylogeny of the group of Hertweck et al. (2015). We used the ultrametric tree from r8s (Sanderson, 2003) that was trimmed to one example per monocotyledon family (Hertweck et al., 2015). We used a dated phylogenetic tree because, in a dated tree, the branch length is proportional to absolute age of taxa and not to evolutionary rate, and, further, it allows a comparison of alternative scenarios of temporal divergence on phylogenetic trees. Thus, one can explicitly test the alternative models of trait evolution on a temporal scale and,

indeed, most methods for reconstructing trait evolution require a dated phylogeny (O'Meara et al., 2006; Slater et al., 2012). Data for the two traits, flower size and seed number, were collected from diverse sources including Kubitzki (1998), Watson and Dallwitz (1992), *Flora of Ecuador* (Anderson and Harling, 2000), *Flora of China* (Wu et al., 2009) and various other sites on the world wide web (Supplementary Data Table S1). The two traits were coded as binary values. Five families out of the 74 showed a lot of variation in flower size and seed number (Table 1). The comparative phylogenetic methods used have limitation of integrating variable (polymorphic) character states in the analysis, therefore the variable families were excluded further from the analysis.

Flower size

Flower size was recorded from Watson and Dallwitz (1992), Kubitzki (1998) and images from the world wide web. Kubitzki (1998) and Watson and Dallwitz (1992) specifically mention for families if the flowers are small or inconspicuous. In such cases, we noted the flowers as small, and further verified the size by examining the images in Kubitzki (1998) and Google images. The small flowers appeared to be under 10 mm along the longest dimension – in most cases much smaller. Interestingly, Vamosi et al. (2003) also use 10 mm as the cut-off point for 'small inconspicuous flowers'. We further validated our categorization of flower size by generating a random list of 35 out of the 69 families analysed. For these 35 families that included 142 genera and 642 species, we compiled information on flower size from the *Flora of Ecuador* (Anderson and Harling, 2000) and *Flora of China* (Wu et al., 2009), two of the few floras that mention flower size. *Flora of Ecuador* (Anderson and Harling, 2000) described 13 out of the 69 monocotyledon families and included 31 genera and 123 species, and the *Flora of China* (Wu et al., 2009) provided data for an additional 111 genera and 519 species for the sampled families.

For these 35 families, we sampled the first 20 genera. In most cases, however, the number of genera was much smaller than 20 and, in such cases, we sampled all the genera. From each genus, we sampled the first ten species or, if there were less

TABLE 1. *Monocotyledon families categorized according to a combination of two traits (flower size and ovule or seed number)*

| Families | Total number | Flower size and seed or ovule number |
|---|--------------|---|
| Acoraceae, Anarthriaceae, Arecaceae, Aspregeaceae, Boryaceae, Centrolepidaceae, Commelinaceae, Cymodoceaceae, Cyperaceae, Dasypogonaceae, Dioscoreaceae, Ecdiocolleaceae, Eriocaulaceae, Flagellariaceae, Hanguanaceae, Joinvilleaceae, Juncaginaceae, Lanariaceae, Pandanaceae, Poaceae, Potamogetonaceae, Restionaceae, Rhipogonaceae, Smilacaceae, Thurniaceae, Triuridaceae, Typhaceae, Zosteraceae | 28 | Small flower and single or few seeded fruits |
| Campynemataceae, Colchicaceae, Cyclanthaceae, Juncaceae, Tecophilaeaceae, Tofieldiaceae, Alstoemeriaceae, Amaryllidaceae, Blandfordiaceae, Bromeliaceae, Burmanniaceae, Butomaceae, Cannaceae, Corsiaceae, Costaceae, Doryanthaceae, Haemodoraceae, Hypoxidaceae, Iridaceae, Ixioliriaceae, Liliaceae, Lowiaceae, Mayacaceae*, Musaceae, Nartheciaceae, Orchidaceae, Petrosaviaceae, Petermanniaceae, Philesiaceae, Philydraceae, Pontederiaceae, Stemonaceae, Strelitziaceae, Velloziaceae, Xeronemataceae, Xyridaceae*, Zingiberaceae | 6 31 | Small flowers and many seeded fruits Large flower and many seeded fruits |
| Alismataceae, Heliconiaceae, Marantaceae, Asphodelaceae | 4 | Large flowers and single or few seeded fruits |
| Araceae, Asteliaceae, Hydrocharitaceae, Melanthiaceae, Rapateaceae | 5 | Could not categorize due to variation in seed number or flower size |

*Although we treated Xyridaceae and Mayacaceae as large flowered families based on Kubitzki (1998), the data from the *Flora of China* indicated ambiguity with respect to flower size. Hence we repeated the analyses treating these two families as with both small and large flowers. The results from these analyses were the same.

than ten species, we sampled all species. We compiled data on flower size for all the species for 14 small flowered families (that included 43 genera and 205 species) and 21 large flowered families (that included 99 genera and 437 species).

We plotted the distribution of flower size for 142 genera, treating genera rather than species as independent points (Supplementary Data Fig. S1a, c). For each genus, wherever there was more than one species, we took the median value. All 43 genera described as having small flowers (14 families) had flowers <10 mm in their longest axis. Of the 99 genera described as having large flowers (21 families), 23 genera had flowers <10 mm, 13 genera between 10 mm and 15 mm, and 63 genera had flowers >15 mm in their longest axis. The 23 genera with flowers <10 mm in families that we considered to have large flowers were distributed among nine large families in which an overwhelming majority of genera had large flowers (Supplementary Data Table S1). All 141 genera together showed a bimodal distribution for flower size, supporting our categorization of continuous flower size data into binary data such as small and large flower (Supplementary Data Fig. S1c).

Seed number

Seed number was classified as single or a few (≤ 4) and many (≥ 6) for our sample of 69 families. For the flora of the British Isles, Ganeshiaiah and Shaanker (1992) have shown a bimodal distribution of seed number per fruit, with most species having fruits with 1–4 seeds or many (>10 seeds) per fruit. Data for seed number – one, few or many – were directly available for 57 families from Kubitzki (1998), Watson and Dallwitz (1992), the Royal Botanical Gardens Kew and various peer-reviewed publications (sources available in Supplementary Data Table S1). These sources describe the seed number per fruit as one, few (≤ 4), many (≥ 6) and numerous (≥ 20). For the remaining 12 families for which we could not obtain seed number directly, data were inferred from the number of ovules per ovary. Of these, eight families had one ovule in one, two or three locular ovaries, and were therefore classified as having a single or a few ovules and seeds. The remaining four families were categorized as many seeded based on the description of ovule number being several or many per locule in usually three locules.

Data analysis

To establish the appropriateness of conducting a phylogenetic comparative analysis, we first evaluated the degree of phylogenetic signal in the two traits for which we wanted to test for correlated evolution (flower size and seed number). Several tests have been developed to quantify the phylogenetic signal, but there is no single method which can account for all models of evolutionary processes (Krasnov et al., 2011). Therefore, we assessed the phylogenetic signal using two alternative phylogenetic tests: the D statistic (phylogenetic dispersion) of Fritz and Purvis (2010) using the *phylo.d* function implemented in the R package *caper* (Orme et al., 2012). The D statistic is appropriate for binary traits, and D typically varies from 0 to 1. A value of 0 indicates that the trait evolves on a tree following the Brownian model (strong phylogenetic signal), and a value of

1 indicates that the trait evolves following a random model (no phylogenetic signal). D can be negative, which means that the trait evolves in a conserved manner: more conserved than predicted by the Brownian model. Additionally, we conducted a simulation (1000 permutations) to test whether an estimated D was significantly different from the predictions of a random or a Brownian motion pattern of evolution.

We further tested for phylogenetic signal in traits using Pagel's lambda (λ) with the *fitDiscrete* function in the package *geiger* in R (Harmon et al., 2008). Pagel's λ varies from 0 to 1, with a λ value of 1 indicating that traits gradually accumulate changes over time in a Brownian motion process (i.e. random change in any direction) and λ values of 0 indicating that no phylogenetic signal is present and that traits have evolved in response to selective processes. We tested for significance in the phylogenetic signal (null hypothesis of $\lambda = 0$) by 1000 randomizations of species names in phylogeny under the ARD (variable transition rate) transition model (Supplementary Data Table S2). The significance of λ was assessed with a likelihood ratio test (Pagel, 1999). The likelihood ratio test compares the likelihood of λ calculated from the true tree with the likelihood of 0. The finding of a significant phylogenetic signal in two traits (flower size and seed number) justifies the use of comparative phylogenetic analysis to test for correlated evolution of two binary traits.

We also examined the minimum number of character state transitions for individual and combined character states inferred based on parsimony using the R package *paleotree* (Bapst, 2012). We further used likelihood, Bayesian Markov chain Monte Carlo (MCMC) and stochastic mapping methods to reconstruct the ancestral history of traits and to quantify the amount of time each state spent in character, using *phytools* (Revell, 2012) and *BayesTraits* v. 3.0 (Pagel and Meade, 2006). We generated a sample of 1000 likely discrete character histories using *phytools* (Revell, 2012) and, for each tree, we counted the number of changes between character states and summed the time spent in each state along all tree edges. The character state reconstruction analysis based on parsimony was carried out using *paleotree* (Bapst, 2012). We tested for correlated evolution of the two binary characters on a phylogeny [flower size (small and large) and seed number (single or a few and many)] using two different methods: maximum likelihood (ML) and reversible jump MCMC (RJMCMC). Previously, these methods have been widely used to address the evolution of floral and other reproductive traits in angiosperms (Jabbour et al., 2008; Reinheimer et al., 2013). These methods assess the marginal likelihood of whether change in one trait preceded the evolution of another trait by fitting two models, one in which the two characters evolve independently by assuming that transition rates of each variable are independent of the state of the other (independent model), and a second more parameter-rich model in which they evolve as combined character states assuming that rates of change depend on the state of the other variable (dependent model). The transition rates between states are indicated by the parameter q_{ij} , that represents the rate of transition from state i to state j , where i is flower size and j the seed number. The ML analysis was run using two broad classes of models that differed in whether transitions between states occurred at equal rates (ERs) or whether they could vary (ARD) (Supplementary Data Table S2).

Further, within these two broader classes of models, dependent evolution of traits (dependent model) was tested using three other separate models (Supplementary Data Table S4): one in which the substitution rate of flower size depends on the state of seed number and, vice versa, one in which the rate of flower size (only) depends on the state of seed number, and finally the rate of seed number (only) being dependent on the state of flower size. These three different models differed mainly by how one character state influences the transition rate of the other character. We ran RJMCMC analysis assuming different transition rates (ARD) between states. In the case of RJMCMC, the likely transition rates between states were assessed using Z scores. The transition was considered unlikely if transition between states was assigned to zero (approximating independent models of trait evolution) in >10 % of iterations from RJMCMC, whereas those only rarely assigned to zero ($Z < 10$ %) (approximating dependent models of trait evolution) were highly likely to be evolutionary transitions.

We also determined the mean \pm s.e. of transition parameter (q -value), which indicates the strength of each transition. To account for phylogenetic uncertainty, we ran ML analysis for 10 000 iterations and RJMCMC analysis for 5 050 000 iterations with a burn-in of 50 000 iterations, and the chain was sampled every 100th iteration, creating a posterior distribution of 50 000 sample points.

Finally, we used a likelihood ratio test, $LR = 2[L(D_8) - L(I_4)]$ to ask whether a model of correlated evolution between flower size and seed number significantly better explained our data than the simpler model of independent evolution of the two traits. The significance of the likelihood ratio was tested by both χ^2 distribution (d.f. = 4) and Monte Carlo simulation. Given the traits, transition rates were calculated between four states of two traits (Fig. 2), namely families with: (1) large flowers–many seeds to large flowers–few seeds; (2) small flowers–few seeds to small flowers–many seeds; (3) large flowers–few seeds to small flowers–many seeds; and (4) large flowers–many seeds to small flowers–few seeds. Since the models are nested (i.e. the dependent model is a special case of the correlated evolution model), and the independent model has four fewer parameters because the model is constrained by setting four pairs of parameters equal (Supplementary Data Figs S4–S8: $q_{12} = q_{34}$; $q_{21} = q_{43}$; $q_{42} = q_{31}$; and $q_{13} = q_{24}$), we compared our observed likelihood ratio (twice the difference in log likelihood between the two models) with a χ^2 distribution with four degrees of freedom. Thus, the most likely parameter combination consistent with the observed traits of the taxa for the independent model yields a log-likelihood estimate of $L(I_4)$. Alternatively, the eight-parameter-dependent transition model that involves no restriction on transition rates yields a log-likelihood estimate of $L(D_8)$. Pagel's (1994) analysis of correlated evolution of traits is sensitive to root state and can potentially bias the results depending on the root state. To account for this bias and to test whether changing the root state can potentially influence the result of Pagel's (1994) analysis of correlated evolution of traits, we fixed the root state of both flower size and seed number traits to the following states: small flower–few seeds, small flower–many seeds, large flower–few seeds and large flower–many seeds. We then compared harmonic mean log-likelihood scores across the constrained models to determine which constrained model better explained the correlated

evolution of flower size and seed number, and the best model was chosen using Akaike information criterion (AIC) statistics. The function to run the Pagel (1994) analysis of correlated evolution of traits using ML and RJMCMC methods is available in R package phytools (Revell, 2012) and BayesTraits v3.0 (Pagel and Meade, 2006).

RESULTS

In our sample of 74 families, we could not characterize traits in five families because either flower size or seed number was variable within the family, preventing us from assigning a character state to the family for one or both characters (Table 1). Thirty-four families out of the 69 families analysed had small flowers and, in 28 of these 34 families, small flowers were associated with a single or a few seeded fruits, and in six families with many seeded fruits (Table 1). Out of the remaining 35 families, 31 families had large flowers associated with many seeded fruits (Table 1), and four families had a single or a few seeded fruits.

Among the five out of the 74 families excluded from the analysis, one family with large flowers and three families with small flowers had both a single or a few seeded fruits as well as many seeded fruits. The fifth family, Asteliaceae, had small and large flowers and a single or a few as well many seeded fruits.

The two alternative methods detected a significant phylogenetic signal in both flower size and seed number (Table 2), confirming that individual trait evolution is phylogenetically constrained. Ancestral reconstruction of flower size and seed number using four alternative methods (parsimony, ML, stochastic mapping and RJMCMC) showed that large flowers and many seeds were the ancestral states in monocotyledons (Supplementary Data Table S3; Supplementary Data Fig. S2). The transition rate between states of flower size (small and large) was much higher compared with seed size (one or a few and many), indicating that seed number evolves much more conservatively than flower size (Supplementary Data Fig. S3a, c). There was a biased transition rate shift towards a specific combination of character states, mainly lineages with one or a few seeds had a high transition rate towards small flowers and lineages with many seeds had a high transition towards large flowers (Supplementary Data Fig. S3b, d). Although large flower–many seeded fruits and small flower–single or few seeded fruits are found most frequently in extant families of monocots, alternative combinations (large flower–few seeded fruits, small flower–many seeded fruits) occur sporadically (Fig. 1A; Supplementary Data Fig. S2c). Moreover, the stochastic character maps based on the best-supported likelihood model (Supplementary Data Table S2) suggest that monocotyledon families have spent much more total time in the large flower–many seeded and small flower–single or few seeded states than in the other states (Fig. 1C). However, we did not recover any significant difference in total time spent in each state when each trait was considered independently (Fig. 1B). These results suggest that the probability of evolving many seeds or a single and a few seeds appears to be dependent on flower size.

Finally, two alternative methods (ML and RJMCMC) provide phylogenetically controlled and robust evidence of strong correlated evolution between flower size and seed number. The estimated transition rates between states in our independent

TABLE 2. The statistics for phylogenetic signal in traits

| | Flower size | Seed number |
|----------------------------------|--------------|--------------|
| Phylogenetic dispersion <i>D</i> | | |
| Estimated <i>D</i> | 0.331 | -0.016 |
| <i>P</i> random model | 0.093 | 0.110 |
| <i>P</i> Brownian model | 0.293 | 0.540 |
| Pagel's λ | | |
| Lambda | 0.760 | 0.382 |
| <i>P</i> -value | <0.001 | 0.042 |

The non-significant *P*-values for the *D* statistic are in bold, which means the traits are under Brownian evolution.

For Pagel's lambda, a λ value of 1 indicates that the trait gradually accumulates changes over time in a Brownian motion processes, and a λ value of 0 indicates that no phylogenetic signal is present and traits evolved in response to selective processes.

and dependent transition models are given in Fig. 2A and B. The more parameter-rich dependent model showed a better fit than the simpler independent model (Table 3; Fig. 2A, B; Supplementary Data Table S4). This suggests that flower size may influence the evolution of seed number or vice versa. In particular, we found that the rate of transition from a single or a few to many seeds was much higher in lineages with large flowers than in small flowered lineages (Fig. 2; Supplementary Data Fig. S3b). Similarly, the rate of transition to large flowers was much higher in many seeded lineages than in lineages with a single or a few seeds (Fig. 2; Supplementary Data Fig. S3b). We found similar results for alternative combinations of character states; mainly there was a bias towards transition from small to large flowers in many seeded lineages than in one or few seeded lineages, and vice versa (Fig. 2; Supplementary Fig. S3b). Overall, these biases in transition rates for flower size

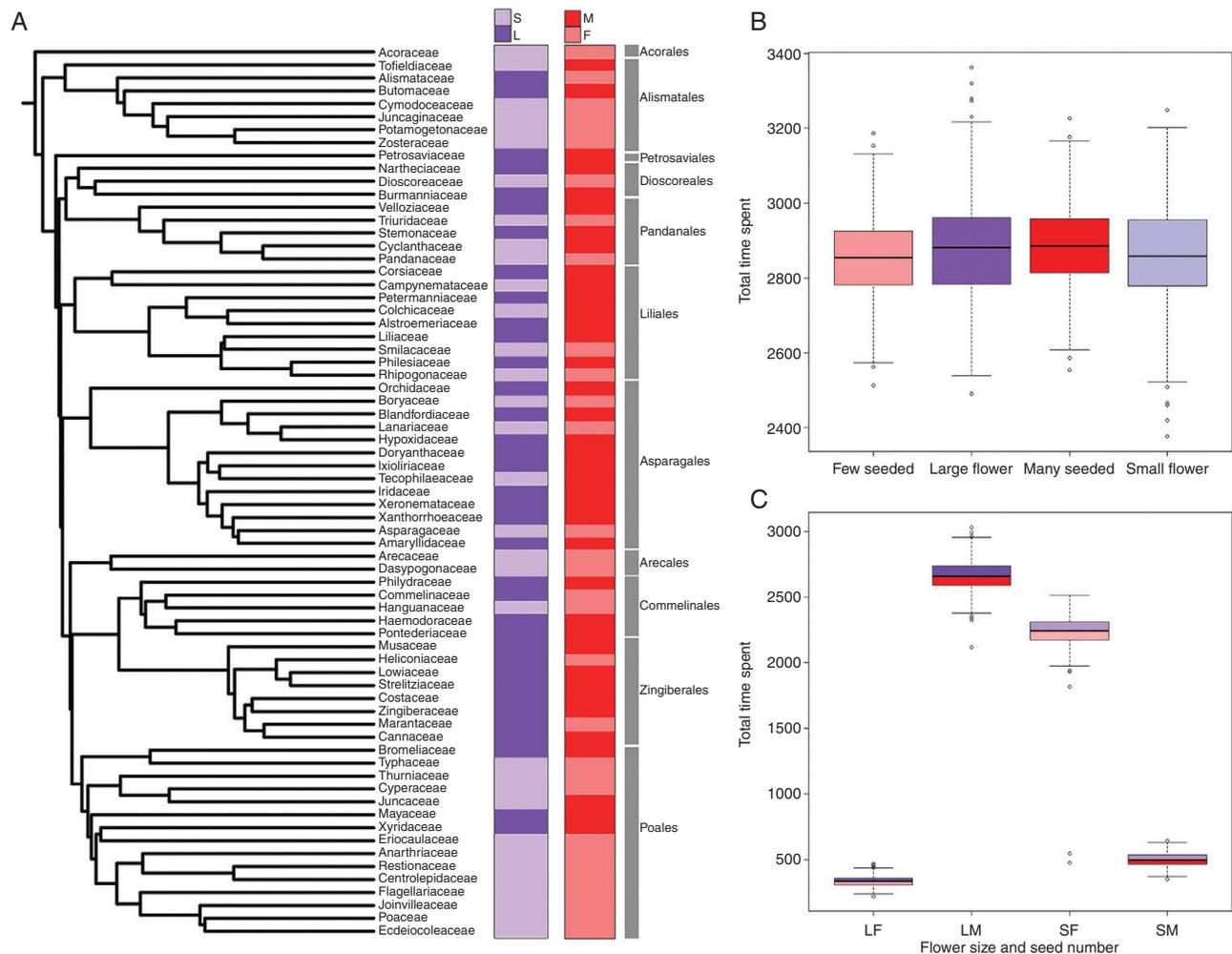


FIG. 1. (A) Phylogenetic distribution of flower size [large (L), small (S)] and seed number [many (M), few (F)] in monocot families. (B and C) Persistence time of the individual and four combined character states based on stochastic character mapping. Mean persistence time for each state represents different persistence times calculated for 1000 separate character maps. For B, the various combined character states are identified as follows: LF (large flower-few seeded), SF (small flower-few seeded), LM (large flower-many seeded) and SM (small flower-many seeded).

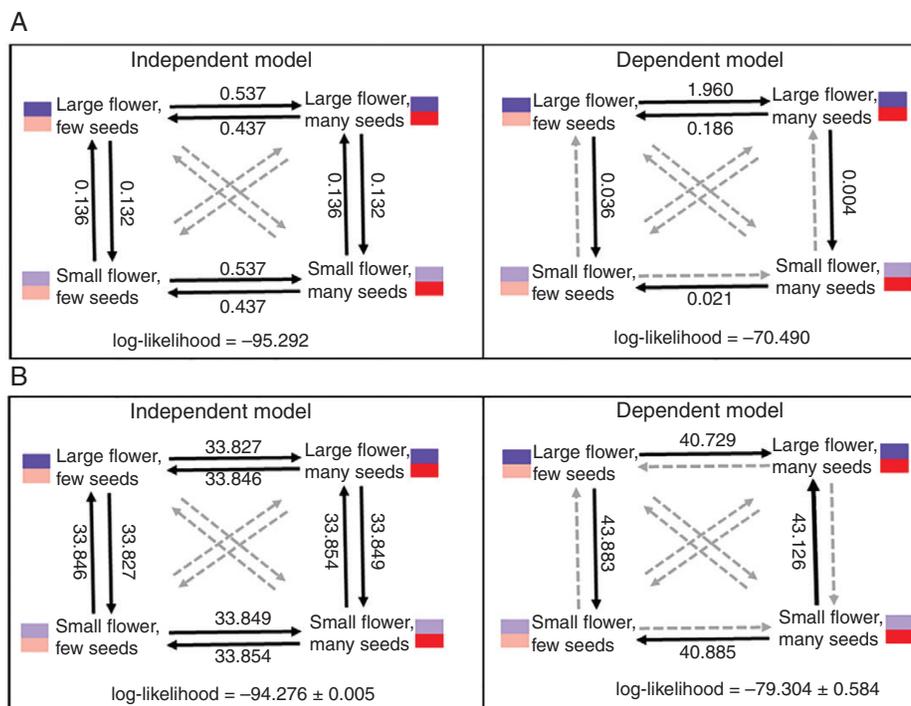


FIG. 2. Pagel's (1994) analysis for detecting 'correlated' evolution of two binary traits, i.e. flower size (large or small flowers) and seed number (many or few), gives four different combinations of characters. (A) The most likely evolutionary models (dependent and independent) based on the maximum likelihood method and (B) the most likely evolutionary models (dependent and independent) based on the RJMCMC method. Arrows represent the direction of transition and values on arrows indicate transition rates. Dashed lines indicate transition rates that are not significantly different from zero. The 'Independent model' assumes that two binary traits evolve independently from each other and, in the model, traits are constrained to evolve separately. The 'Dependent model' assumes that evolution of one binary trait influences evolution of another binary trait and, in the model, traits are constrained to evolve together.

conditioned on seed number and the converse help account for the consistent appearance of clades with large flowers and many seeds, or with small flowers and few seeds among extant lineages on the tree of monocotyledons (Fig. 1A; Supplementary Data Fig. S2c). Moreover, the significance of our results was not influenced either by character state in the root or basal node or by a variable transition rate among character states, as our analyses of alternative models of trait evolution by changing character states at the root and constraining the transition rate between character states did not change our results. This provides strong support for a dependent model of trait evolution (Table 3; Supplementary Data Table S4, Figs S4–S8).

DISCUSSION

The most spectacular result is that 59 out of 69 or 85.5 % of families could be easily assigned to two combinations of character states: small flowers with a single or a few seeds per fruit and large flowers with many seeded fruits. Five out of 74 or 6.8 % of the families had considerable variation in flower size and seed number per fruit. It is conceivable that correlations demonstrated here exist among clades within these families. For example, in one such family, Asteliaceae, bisexual flowers appear to be larger than unisexual flowers and many seeded fruits, but the two dioecious genera, *Astelia* and *Collospermum*, have small flowers and few seeded fruits.

The prevalence of species with large flowers–many seeded fruits, as well as of species with small flowers–single or few seeded fruits within monocotyledons indicates that these

character combinations are likely to constitute optimal reproductive strategies (Fig. 1A; Supplementary Data Fig. S2c). Not only are these two combinations common now, but families of monocotyledons appear to have persisted for a longer time in these states than in the other states (Fig. 1C), further confirming that these combinations of traits may represent adaptive peaks, and that acquisition of one trait in the combination increases the probability of the acquisition of the other (Figs 1C and 2). This categorization of most monocotyledons into groups with contrasting floral and seed traits is a significant result, raising the possibility that most angiosperms may fall into two groups. Furthermore, as argued below, although many authors have examined either one set of correlations or the other, no author has proposed a common framework to suggest that the contrasting sets of correlations may represent alternative evolutionary pathways.

Explanations for correlations

Stebbins (1951) was perhaps the first to propose a correlation (formally, statistical correlation can be established only with phylogenetically independent data) among reproductive traits and to suggest that certain combinations may be adaptive. However, due to unavailability of a phylogenetic data for flowering plants at that time, the trait data used by Stebbins (1951) to propose correlations may not have been phylogenetically independent. Of interest is his demonstration that few seeded carpels (fruits) are associated with flowers without a perianth or

TABLE 3. Log-likelihood scores comparing alternative models of flower size and seed number evolution

| Model and root state | Parameters | –lnL | AIC | ΔAIC | LR (independent vs. dependent model) | P-value |
|---|------------|------------------------|----------------|----------|--------------------------------------|---------|
| Maximum likelihood (ML) models | | | | | | |
| Individual character states (independent model) | | | | | | |
| Root state unchanged | 4 | –90.424 | 188.848 | 35.362 | | |
| Small flower, few seeds | 4 | –93.021 | 194.042 | 40.556 | | |
| Small flower, many seeds | 4 | –91.602 | 191.204 | 37.718 | | |
| Large flower, few seeds | 4 | –91.659 | 191.318 | 37.832 | | |
| Large flower, many seeds | 4 | –90.427 | 188.854 | 35.368 | | |
| Combined character states (dependent model) | | | | | | |
| Root state unchanged | 8 | –68.743 | 153.486 | 0 | 43.362 | <0.0001 |
| Small flower, few seeds | 8 | –71.357 | 158.714 | 5.228 | 43.328 | <0.0001 |
| Small flower, many seeds | 8 | –69.855 | 155.71 | 2.224 | 43.494 | <0.0001 |
| Large flower, few seeds | 8 | –69.742 | 155.484 | 1.998 | 43.834 | <0.0001 |
| Large flower, many seeds | 8 | –69.747 | 155.494 | 2.008 | 41.360 | <0.0001 |
| Bayesian MCMC models | | | | | | |
| Individual character states (independent model) | | | | | | |
| Root state unchanged | 4 | –94.276 (0.005) | 196.552 | 21.944 | | |
| Small flower, few seeds | 4 | –95.657 (0.001) | 199.314 | 24.706 | | |
| Small flower, many seeds | 4 | –95.673 (0.029) | 199.346 | 24.738 | | |
| Large flower, few seeds | 4 | –95.655 (0.005) | 199.31 | 24.702 | | |
| Large flower, many seeds | 4 | –95.662 (0.007) | 199.324 | 24.716 | | |
| Combined character states (dependent model) | | | | | | |
| Root state unchanged | 8 | –79.304 (0.584) | 174.608 | 0 | 29.944 | <0.0001 |
| Small flower, few seeds | 8 | –82.249 (0.664) | 180.498 | 5.89 | 26.816 | <0.0001 |
| Small flower, many seeds | 8 | –80.412 (0.580) | 176.824 | 2.216 | 30.522 | <0.0001 |
| Large flower, few seeds | 8 | –80.841 (0.532) | 177.682 | 3.074 | 29.628 | <0.0001 |
| Large flower, many seeds | 8 | –82.450 (0.771) | 180.9 | 6.292 | 26.424 | <0.0001 |

Comparisons were made between the dependent and independent model of evolution by fixing the states of flowers size (small and large flower) and seed number (few and many seeds) at the root or basal node and comparing log-likelihood scores across ML and MCMC models. For MCMC models, log-likelihood scores reflect the harmonic mean of 5.0×10^6 iterations. Values in parentheses represent \pm s.d. of the harmonic mean obtained from a burn-in of 50 000 iterations of MCMC models. The best fit model based on the Akaike information criterion (AIC) is shown in bold.

corolla, and many seeded carpels (fruits) with flowers that have a corolla. Small flowers are often without a perianth or corolla, and large flowers almost invariably have a corolla. Thus, Stebbins may have anticipated the trends observed here. It is important to note that mating system influences the evolution of many reproductive traits. Our discussion below pertains largely to strongly outcrossed or self-incompatible species.

Kin selection. Correlation between large flowers and many ovules was postulated by Bawa (2016), building on the earlier work of Kress (1981) who demonstrated a correlation between pollen assemblages including polyads and multi-ovulated ovaries. Willson (1979) suggested that evolution of specialized pollination mechanisms was a pre-condition for the evolution of multi-ovulate ovaries. Large flowers indeed are often pollinated by specialized pollinators such as birds, bats, large solitary bees and beetles (Bawa and Opler, 1975; Bawa, 1994, 2016). Bawa (2016) further suggested that specialized pollinators often transfer pollen in masses, and thus increase the genetic relatedness of seeds within fruits. He cited evidence from several studies that reveal that seeds within many seeded fruits often have a single pollen parent, though cases of mixed male parentage within fruits are known (Marshall and Ellstrand, 1986; Weller and Sakai, 1999; Teixeira and Bernasconi, 2007). Harder and Johnson (2008) while considering the association between pollen assemblages and pollinators also referred to the adaptive significance of pollen assemblages in terms of increased probability of seeds within a fruit being full sibs.

Contrary to Bawa's (2016) assertion of single paternity in many seeded fruits of several species, Pannell and Labouche (2013) state that 'Multiple paternity (is common in species) with many ovules or seeds... in self-incompatible species.' We carefully reviewed all their citations in support of their claim, and found that for some of their citations, the authors actually claim, 'Multiple paternity is rare' or 'Seeds with fruits are sired by one or few donors' (emphasis is ours). Overall, our evidence indicates that in 12 or so cases of self-incompatible and dioecious species (one) analysed so far, fruits in eight species exclusively or mostly show a single pollen donor (see Supplementary Data Box 1 for our detailed analyses and citations of papers). Furthermore, in almost all cases of multiple paternity, the authors concede the possibility of uneven contribution of pollen parents and, in most cases, the number of pollen parents does not exceed two. Thus, it is possible that a single pollen donor sires most of the seeds within fruits with many ovules and seeds. In contrast, multiple paternity might be common in species with small flowers. In the literature there are many examples of siblings actively preventing other siblings from growing (reviewed in Mock and Parker, 1997). It will be interesting to know if in such cases the interacting siblings have different pollen parents and if such interactions occur more frequently in species with relatively small flowers that may receive pollen from many parents.

Marshall and Ellstrand (1986) do show that in experimental populations of wild radish, fruits sired by multiple parents were preferentially matured, but they also show that singly sired fruits had larger seeds.

Selection for a pollen parent to increase its fitness by siring all the progeny within the ovary should also select for pollen assemblages and the ability of specialized pollinators to transfer pollen in masses may reinforce this selection. Thus, as Bawa (2016) proposed, large flowers and multi-ovulate ovaries should be correlated with a number of other traits: evolution of pollen assemblages, specialized pollinators and full sibship of seeds within a fruit. The target of selection could be both the number of seeds per fruit and the flower size. It could very well be that only large flowers can attract specialized pollinators capable of transferring pollen in masses that could increase the genetic relatedness of multiple seeds. On the other hand, seed dispersal agents may exercise a strong selection for seed number per fruit (Mckey, 1975). Selection for a single or a few seeds may be more likely in smaller than in larger flowers. Our comparative phylogenetic analyses suggest that the probability of evolving many seeds or a single and a few seeds is dependent on flower size (Fig. 2A, B). Thus, it is conceivable that the main target for selection is the co-adapted set of traits associated with flower size and seed number.

Other explanations. Cronquist (1968) first noted that small flowers tend to be wind pollinated and have uni-ovulate ovaries. In explaining the association between small flowers and single or few seeded fruits in dioecious species, Bawa (1980) suggested sexual selection and Darwin's (1877) notion of the division of labour as possible drivers for the evolution of the dioecious breeding system. Subsequently, a number of authors have explored correlates of dioecy specifically the small flowers and single or few seeded fruits (Weller and Sakai, 1999; Vamosi et al., 2003).

Uncertain pollination or pollinator limitation may also explain the reduction in number of ovules per flower (Burd et al., 2009; Friedman and Barrett, 2011). Seed number is also influenced by seed dispersal mechanisms (Bawa, 1980; Ganeshiah and Shaanker, 1992) and seed size (Venable, 1992; Paul-Victor and Turnbull, 2009). In turn seed size has a number of ecological correlates (Harper et al., 1970; Baker, 1972; Moles et al., 2005).

Similarly, several factors, including allometry and size–number trade-offs, may influence the evolution of flower size (Bell, 1985; Primack, 1987; Sargent et al., 2007; Teixido et al., 2016). Within species, small flowers may produce fewer seeds than large flowers (Delph et al., 2004; Sánchez-Lafuente and Parra, 2009); flowers produced earlier in the season may also produce more seeds than flowers later in the season (Van der Meer and Jacquemyn, 2015). It is not clear if the differences in the number of seeds in these cases are due to differences in seed set or the number of ovules produced. On the other hand, within species, others have noted a negative correlation between flower size and ovule number (Elle and Carney, 2003) or no correlation (Stanton and Young, 1994). Testing for correlations between ovary size and number of seeds within genera or families would be needed to support the notion that flower size reduction in ovule or seed number in small flowers is due to allometry. Clearly, more work on allometric relationships will be fruitful.

Conclusions

Although several factors may explain the correlated evolution of flower size and seed number, presently only kin selection provides a framework to explain the two sets of contrasting correlations (large flowers and many seeded fruits and small flowers and single or few seeded fruits) that may be widespread in angiosperms. There are, however, some caveats. First, for flower size, the critical factor is the ability of pollinators to transfer pollen in masses from a single pollen parent that could also occur in species with small flowers pollinated by specialized pollinators. Secondly, comparative methods to take into account phylogenetic relationships have been in transition during the last few decades. Although our results are robust, the methods we have used have their shortcomings (Maddison and FitzJohn, 2015). Thirdly, a confounding factor could be the proximity of small flowers, and in many cases the union of flowers or fruits resulting, respectively, in a compound inflorescence or a compound fruit. In such cases, the large inflorescences become akin to large flowers and the compound 'fruits' have many seeds.

In those cases where flowers are small and aggregated into compact inflorescences, selection may occur among fruits. Brood reduction in plants by fruit and (seed) abortion is very common (Stephenson, 1981; Bawa and Webb, 1984; Shaanker et al., 1988; Burd, 1994). Plants obviously have a wide variety of mechanisms to reduce competition among siblings within ovaries or fruits, infructescences, or larger modular units. Thus the maternal parent can exercise choice at various hierarchical levels. Moreover, all competition may not have negative impacts on fitness of the parent. There is a wealth of literature on plants and animals that suggests that abortion may increase fitness by channelling more resources to particular offspring, or developing more offspring if additional resources become available (Mock and Parker, 1997; Meyer et al., 2014)

Although the reported correlations are between flower size and ovule or seed number, other correlated traits such as assemblages of multiple pollen grains, and specialized pollination mechanisms in the case of large flowers and multi-seeded fruits (Bawa, 2016), and monads and generalist pollinators or abiotic pollination in the case of small flowers and single or few seeded fruits may have evolved in conjunction with changes in flower size and seed number (Friedman and Barrett, 2008). Further analyses may reveal associations with more traits such as floral sexuality and the breeding system. An increase in our ability simultaneously to analyse several correlated traits and the time and direction of change could provide significant insights into the drivers responsible for major transitions in reproductive traits of flowering plants (O'Meara et al., 2016).

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: details and sources of flower size and seed number trait data. Table S2: model fits for flower size and seed number considered individually and combined. Table S3: the model fit of alternative models of flower size and seed number evolution. Table S4: the model fit of alternative models of flower size and seed number evolution. Figure S1: distribution of flower size across 33

randomly selected monocotyledon families which included 119 genera and 529 species. Figure S2: ancestral reconstruction of flower size and seed number considered individually and combined based on the best likelihood model of stochastic mapping. Figure S3: schematic representation of transition rate between flower size (small and large) and seed number (one or few and many) in monocotyledons. Figure S4: evolutionary pathways and rate coefficients of the model of dependent evolution between flower size and seed number among monocotyledons without changing root state. Figure S5: evolutionary pathways and rate coefficients of the model of dependent evolution between flower size and seed number among monocotyledons by fixing states at the root or basal node to small flower and few seeds. Figure S6: evolutionary pathways and rate coefficients of the model of dependent evolution between flower size and seed number among monocotyledons by fixing states at the root or basal node to small flower and many seeds. Figure S7: evolutionary pathways and rate coefficients of the model of dependent evolution between flower size and seed number among monocotyledons by fixing states at the root or basal node to large flower and few seeds. Figure S8: evolutionary pathways and rate coefficients of the model of dependent evolution between flower size and seed number among monocotyledons by fixing states at the root or basal node to large flower and many seeds. Box 1: critical examination of evidence for polyandry of multi-seeded fruits.

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