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Evolutionary correlation of heterodichogamy and floral longevity

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Abstract Heterodichogamy, the coexistence of morphs differing in the time when the shift between male and female phases occurs, is a rare sexual dimorphism derived from synchronous dichogamy. In dichogamous populations, plants need to adjust the temporal sex role to deal with the negative frequency dependent selection imposed by population sex ratios. However, such temporal adjustment may be constrained in dichogamous species with bisexual flowers and short floral longevities (<2 days) and therefore the reciprocal floral strategy can easily invade the population, ultimately leading to heterodichogamy. We predict that heterodichogamy has evolved in species with short floral longevities. To test this prediction, we compiled data on floral longevity for 377 dichogamous and heterodichogamous species with bisexual flowers and ran a phylogenetic logistic regression between floral strategy (dichogamy/heterodichogamy) and floral longevity. The results showed that heterodichogamous species had significantly shorter floral longevities than dichogamous species. This result together with the fact that evolutionary transitions always occurred from dichogamy to heterodichogamy supports the conclusion of the existence of a significant and negative evolutionary correlation between the floral longevity and the probability to evolve heterodichogamy from dichogamy in bisexual species. We discuss that the rarity of heterodichogamy in nature may be the result of other selective pressures towards long floral longevity in dichogamous species.

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Introduction

Dichogamy is a widespread floral strategy in which pollen presentation and stigma receptivity within a plant are separated in time (Bertin and Newman 1993). Dichogamous species may have either protandrous individuals where pollen is released before stigmas become receptive, or protogynous individuals, where the opposite pattern occurs. The fitness of a dichogamous individual is strongly dependent on the time when pollen and ovules at the population level are available (Sargent et al. 2006). The sex ratio of the population could impose negative frequency-dependent selection on dichogamous plants favouring the least common sex role during specific stages of the flowering season (Brunet and Charlesworth 1995; Ishii and Harder 2012).

Under synchronous dichogamy, where shifts in the sexual phases of all flowers within an inflorescence or entire plant occur simultaneously (Lloyd and Webb 1986), the temporal sex role of an individual needs to be adjusted based on the floral sex ratio (the ratio of female- to male-phase flowers in the population) during the flowering season to maximize mating opportunities. Dichogamous plants with bisexual flowers could maximize mating opportunities by different strategies, such as intra- and inter-individual asynchronous flower presentation, modification of the length of the flowering season and sex-phase duration, or the increase in the production of a series of flowers during flowering season (Sargent et al. 2006). Although we acknowledge that all these processes may interactively work affecting floral sex ratio and mating availability, we base on theoretical analyses (Ashman and Schoen 1994; Schoen and Ashman 1995) to propose that floral longevity is one of the main mechanisms constraining the evolution of dichogamy. Floral longevity has been described as a resource allocation strategy determined by the balance between the rates of pollen removal and receipt and the costs of floral maintenance (Ashman and Schoen 1994). For dichogamy species, the flower should be maintained at least as long as required for the delayed sexual function to commence (Schoen and Ashman 1995). So, floral longevity could constrain the maximum amount of dichogamy (Schoen and Ashman 1995). Floral longevity could influence the potential sex roles of dichogamous plants with bisexual flowers through modifications in the onset of an individual flower in an inflorescence or a plant and the length of sexual phases during the flowering season, which ultimately determine pollen and ovule presentation schedules. The temporal adjustments through floral longevity could be the possible path for floral sex ratio adjustment in dichogamous species. The synchronously dichogamous hermaphroditic plants with long floral longevity can deal with the negative frequency-dependent selection by adjusting the relative length between male and female phases. However, this temporal adjustment strategy could be constrained in the dichogamous plants with very short floral longevity. Under this situation, mismatches in the floral sex ratio open an opportunity window for the invasion of mutants with the reciprocal reproductive strategy (i.e., early-flowering individuals invading late-flowering populations of dichogamous species or protandrous individuals invading populations of protogynous species). This process will ultimately result in heterodichogamous populations. So heterodichogamy could serve as one of the mechanisms to deal with population sex-ratio selection in the synchronously dichogamous hermaphroditic species with very short floral longevity.

Heterodichogamy is a rare sexual dimorphism where hermaphroditic and monoecious species, in a population express the male and female phases at different times within an individual, such that individuals in their male phase mate with those in their female phase (Renner 2001). Despite its rarity, this reproductive system is widespread across the angiosperm phylogeny, occurring in c.50 species belonging to 24 genera from 13 families of angiosperms (Teichert et al. 2011; Wang et al. 2012; Fukuhara and Tokumaru 2014; Renner 2014). Heterodichogamy is thought to have evolved from synchronous dichogamy (Renner 2001). Three types of heterodichogamy have evolved, with a similar number of species in each (Renner 2001; Pannell and Verdú 2006). In type-I heterodichogamy, all individuals in a population are uniformly dichogamous (protandrous or protogynous), but differ in the anthesis time. Then, early flowering individuals mate with late-flowering individuals. In the second and third types, protandrous and protogynous individuals of the same species co-occur and mate each other. The switch between the male and female phases occurs daily (type-II heterodichogamy) or only once during the flowering season (type-III heterodichogamy). Species belonging to types I and II usually have bisexual flowers but species belonging to type-III heterodichogamy have unisexual flowers. In this paper, we only focus on the dichogamous species with bisexual flowers because in monoecious plants, the longevity of a single flower does not involve both sex roles.

In this paper, we explore a novel explanation relating the evolution of heterodichogamy from dichogamous species with bisexual flowers by the increase of mating opportunities in dichogamous populations. We hypothesize under a phylogenetic framework that the probability of heterodichogamy to evolve from dichogamy increases in species with short floral longevity.

Materials and methods

We compiled data on floral longevity for 377 species with bisexual flowers (32 heterodichogamous species from 13 genera in 7 families and 345 dichogamous species from 203 genera in 78 families). Most of the data were obtained from the cited references in Renner (2001) and from a database kindly provided by R. Bertin (Bertin and Newman 1993). Additional data were obtained from literature searches (see references in Appendix 1).

The phylogenetic tree of the dichogamous and heterodichogamous plant species was assembled based on the supertree of the Angiosperm Phylogeny Group (Stevens 2012) with the help of the program Phylomatic, as implemented in Phylocom version 4.2 (Webb et al. 2008). The tree was subsequently calibrated with the divergence dates provided by Wikström et al. (2001) and fitted a branch length adjustment procedure that follows a birth–death evolutionary model while randomly resolves the polytomies (Kuhn et al. 2011). To do this, we first used the R20120829 reference supertree to assemble the topology of our phylogeny and subsequently resolved polytomies and adjusted branch lengths with the help of BEAST 1.5.4 (Drummond and Rambaut 2007) and the PolytomyResolver script (Kuhn et al. 2011). Chronological constraints were posed for the nodes dated by Wikström et al. (2001), and the remaining nodes were dated by BEAST with the default settings specified in the PolytomyResolver script. Markov Chain Monte Carlo (MCMC) analyses were run for 10^6 iterations and trees sampled every 10^3 iterations. A

25 % burn-in was discarded and the maximum clade credibility tree recovered with the help of the TreeAnnotator v1.5.4 software (Drummond and Rambaut 2007).

We first estimated the number of transitions from dichogamy to heterodichogamy (and vice versa) occurring in the phylogenetic tree and subsequently tested whether the probability to evolve heterodichogamy from dichogamy increases with short floral longevity. Evolutionary transitions were estimated after reconstructing through Maximum Likelihood the ancestral states of all the nodes in the phylogeny with the help of the Mesquite 3. 0 .4 software (Maddison and Maddison 2015). The evolutionary correlation between heterodichogamy and floral longevity was tested by fitting a phylogenetic logistic regression with heterodichogamy absence/presence as the binary dependent variable and floral longevity (log-transformed) as independent variable. We used the phyloglm procedure as implemented in the phylolm package for R (Ho and Ane 2014), that fits the phylogenetic logistic regression described in Ives and Garland (2010). Given the rarity of heterodichogamy in the nature, our statistical model was extremely unbalanced and contained many zeros (i.e., dichogamous state) in the dependent variable. Under this situation, most of the data may contain little information (Ives and Garland 2014). To check for the robustness of our results to such unbalance, we repeated the analysis after balancing sample size between both reproductive systems by randomly sampling the same number of dichogamous as heterodichogamous species and repeating the analysis 100 times.

Results

The frequency distribution of floral longevities of dichogamous bisexual species follows a decay pattern from short (0.4 days) to long (32 days) longevities (Fig. 1). However, most of the heterodichogamous bisexual species have flowers that last no more than 2 days. Only two exceptions to this pattern were found: *Trochodendron aralioides* and *Kingdonia uniflora* (Appendix 1).

Ancestral state reconstruction of sexual system in the phylogenetic tree show that evolutionary transition from dichogamy to heterodichogamy has occurred 11 times (four times in the Lauraceae clade, Zingiberaceae clade, *Eupomatia* clade, *Ziziphus* clade, *Anaxagora pinoides*, *Annona squamata*, *Trochodendron aralioides* and *Kingdonia uniflora* species) while only one reversion in the Zingiberaceae clade (*Curcumorpha longiflora*) has occurred.

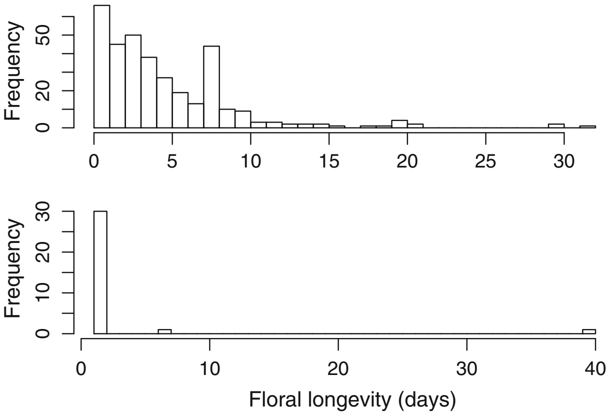

Fig. 1 Frequency distribution of floral longevity of dichogamous (*top*) and heterodichogamous bisexual species (*bottom*)

Table 1 Phylogenetic logistic regression showing the probability of heterodicogamy to evolve from dichogamy as a function of the floral longevity of the plant species

	Estimate ± Std err	z value	*p* value
Intercept	0.13 ± 0.54	0.24	0.80
Log (floral longevity)	−2.26 ± 0.83	−2.71	0.006

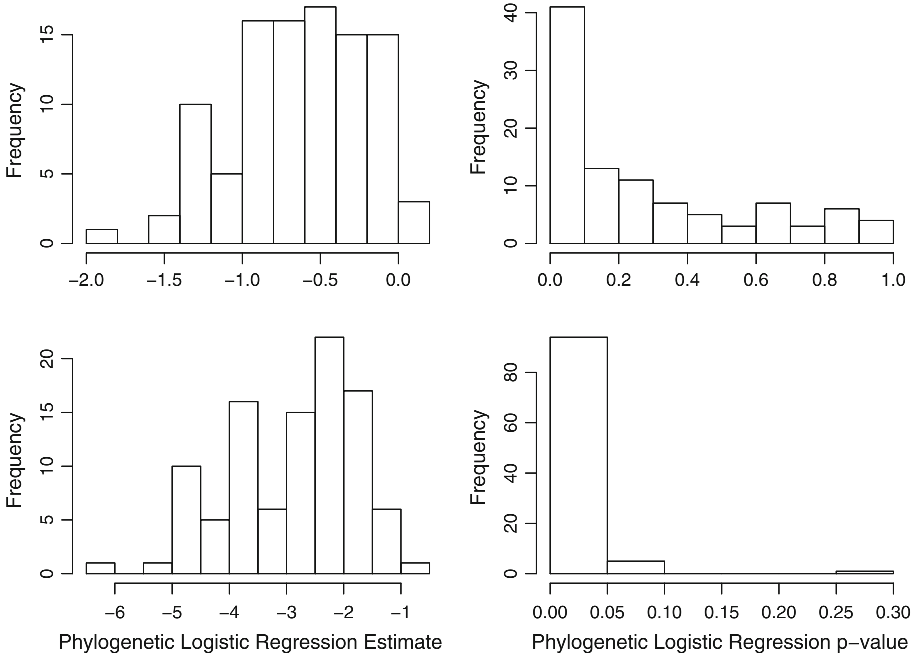

Fig. 2 Estimates and associated *p* values of the 100 Phylogenetic Logistic Regression Models constructed by randomly sampling the same number of dichogamous species as heterodichogamous. The *top panel* show the outcome of the analyses with all the heterodichogamous species in the database while analyses in the *bottom panel* exclude *Trochodendron aralioides* and *Kingdonia uniflora*

There is a significant and negative evolutionary correlation between the floral longevity and the presence of heterodicogamy (phylogenetic logistic regression in Table 1) indicating that heterodichogamy has evolved in species with short floral longevities. Negative correlations were also found in 96 % of the 100 models run with balanced sample sizes, with most of the models being statistically significant (Fig. 2; top panel). The pattern was even stronger when *Trochodendron aralioides* and *Kingdonia uniflora* were excluded from the analysis (Fig. 2; bottom panel).

Discussion

Synchronous dichogamous plants with bisexual flowers and short floral longevities could mitigate the lost mating opportunities associated with unadjusted individual to population sex ratios by producing the reciprocal floral strategy. Such strategy would allow individual flowers to increase the match between their timing of pollen (or ovule) production with that of the availability of ovules (or pollen) at the population level. Our results show that floral longevity of bisexual dichogamous species range from extremely short periods (a few hours) to 1 month. However, most of bisexual heterodichogamous species have flowers whose longevity is no longer than 2 days. This difference suggests that heterodichogamous species may have evolved from dichogamous ancestors with short floral longevities. Indeed, our phylogenetic results suggest that (1) this evolutionary path from dichogamy to heterodichogamy occurred while reversions were rare and (2) the presence of heterodichogamy is evolutionarily associated with short floral longevities. This correlation is not a statistical artifact of the huge imbalance in sample size between heterodichogamous species, which are very rare in nature, and dichogamous species, which are very common. The statistical comparison with equally sized sample sizes also reveals negative correlations between the presence of heterodichogamy and floral longevity. This pattern was even stronger after excluding *Trochodendron aralioides* and *Kingdonia uniflora*, two heterodichogamous species whose floral longevity (Chaw 1992; Wang et al. 2012) clearly departs from the expected pattern and deserve further investigation. Insect pollination may be limited at high altitudes where *K. uniflora* lives (He 2006) and each ramet produces only one (or rarely two) flowers during one flowering period in *K. uniflora* (Wang et al. 2012). Such limitations could be compensated by long floral longevities. Similary, *T. aralioides* may be compensating pollination failures occurring in habitats where the high frequency of 5–10 consecutive rainy days decreased pollinator activity and prevented adherence of pollen grains to the stigmatic surface in *T. aralioides* populations (Chaw 1992).

Dichogamous species with bisexual flowers and short floral longevities could open flowers sequentially and shift the sex phase asynchronously within an inflorescence or a plant. Thus, the sequential production of flowers associated with asynchronous sex phase shifts could provide the mechanism to deal with the negative frequency-dependent selection imposed by population sex-ratio in dichogamous species with short floral longevities. However, sequential production of flowers would not work in synchronous dichogamous species with short floral longevities. Synchronous dichogamy, where all the individuals have the same sex at the same time, produces a mismatch between pollen and ovule availability and increases population sex-ratio selection, providing thus the opportunity for the invasion of the reciprocal floral strategy. This process will ultimately result in heterodichogamous populations with 1:1 morph ratios, a typical ratio found in heterodichogamous species (Renner 2001).

Based on an evolutionary model of floral longevity including the balance between resource allocation to maintenance versus construction of repeated structures, Schoen and Ashman (1995) concluded that dichogamy favours long-lived flowers. According to theoretical predictions of this model, Gao et al. (2015) have recently shown that 21 dichogamous species have evolved longer floral longevity (averaging 3 days longer) than 16 adichogamous species in the same community. The selective pressure for dichogamous species to have long floral longevity would add evolutionary barriers for heterodichogamy, what could help to explain the rarity of this sexual system in nature (Renner 2001). In this study, we propose floral longevity as one of the possible mechanisms for the evolution of heterodichogamy. Floral longevity to constrain the evolution of heterodichogamy is necessary a very high synchrony within and among individuals of dichogamous species. However, other processes could also influence floral sex ratio and mating opportunities and therefore further research is needed to explore them. Despite the trend of heterodichogamy to evolve from species with short floral longevities, our database shows that many dichogamous species have short floral longevities, raising the question of how they reproduce if they cannot adjust their temporal adjustments through floral longevity. There could be other possible mechanisms to compensate for the negative frequency-dependent selection in dichogamous species with short floral longevity. For example, in dichogamous populations, differences in the onset of flowering among plants could decrease the lost mating opportunity, where early and late individual flowers, suffering the greatest loss of mating opportunity, only represent a small fraction of the plant's overall investment in reproduction (Sargent et al. 2006). The alternative mechanisms may also explain why heterodichogamy is so rare despite that theoretical models predict that heterodichogamous species should be quite common due to the decreased or absence of sex-ratio selection in heterodichogamous populations (Sargent et al. 2006). An interesting question is to explore possible explanations in the potential ancestors that already had short floral longevities without heterodichogamy.

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