Trait correlates and functional significance of heteranthery in flowering plants

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Summary

- Flowering plants display extraordinary diversity in the morphology of male sexual organs, yet the functional significance of this variation is not well understood. Here, we conduct a comparative analysis of floral correlates of heteranthery—the morphological and functional differentiation of anthers within flowers—among angiosperm families to identify traits associated with this condition.
- We performed a phylogenetic analysis of correlated evolution between heteranthery and several floral traits commonly reported from heterantherous taxa. In addition, we quantified the effect of phylogenetic uncertainty in the observed patterns of correlated evolution by comparing trees in which polytomous branches were randomly resolved.
- Heteranthery is reported from 12 angiosperm orders and is phylogenetically associated with the absence of floral nectaries, buzz-pollination and enantiostyly (mirror-image flowers). These associations are robust to particularities of the underlying phylogenetic hypothesis.
- Heteranthery has likely evolved as a result of pollinator-mediated selection and appears to function to reduce the conflict of relying on pollen as both food to attract pollinators and as the agent of male gamete transfer. The relative scarcity of heteranthery among angiosperm families suggests that the conditions permitting its evolution are not easily met despite the abundance of pollen-collecting bees and nectarless flowers.

Keywords: buzz-pollination, division of labour, heteranthery, phylogenetic analysis, stamen differentiation.

Introduction

Flowering plants display unrivalled diversity in the morphology of their sexual organs, particularly male structures. Variation in stamen traits is evident both among related species, between plants within populations, and also within and between flowers produced by a single individual (Darwin, 1877; Endress, 1994; D'Arcy & Keating, 1995; Barrett, 2002). Among these different levels of stamen variation, within-flower polymorphism represents a relatively uncommon but taxonomically widespread phenomenon. A particular form of this polymorphism is heteranthery involving the occurrence of more than one structurally discrete type of stamen within the same flower with contrasting functions (Müller, 1883; Vogel, 1978; Fig 1; Vallejo-Marín *et al.*, 2009; Barrett, 2010). Heteranthery occurs in diverse taxonomic groups and in a variety of forms indicating that it has most likely evolved on multiple independent occasions during the history of the flowering plants (Graham & Barrett, 1995; Jesson & Barrett, 2003).

In heterantherous species, stamen differentiation within flowers involves the shape, colour, and/or size of anthers. Most commonly, two types of anthers are distinguishable. The first is centrally located in the flower and composed of brightly coloured stamens (usually yellow) that are short in length, and which are easily manipulated by pollen-collecting visitors. The second type of anther is displaced away from the central axis of the flower, is often cryptically coloured, and the individual anthers are usually larger in size than the preceding type (Vallejo-Marín *et al.*, 2009; Barrett, 2010). Less commonly, a third type of stamens occurs resembling the centrally located anthers, although it can be slightly larger [*e.g. Solanum lumholtzianum*, Solanaceae (Whalen, 1979); *Senna* spp., Fabaceae, (Luo *et al.*, 2009)] or consists of staminodes (*e.g. Commelina* spp., Commelinaceae). Because heterantherous species are exclusively animal-pollinated (Vogel, 1978), anther variation is undoubtedly associated with various facets of the pollination process with consequences for pollen dispersal and male function.

Heteranthery is commonly associated with a suite of floral characters and particular pollinator characteristics. Heterantherous species usually lack nectar and offer pollen as the sole reward to visitors which are mainly pollen-collecting bees (Vogel, 1978; Vallejo-Marín *et al.*, 2009). Pollen dispersal in heterantherous species frequently involves buzz pollination in which pollen is released from anthers through small apical pores (poricidal anther dehiscence) as a result of vibrations of flight muscles of the wings of large bodied bees (Buchmann, 1983). Comparative analyses of monocotyledonous groups have revealed that heteranthery is commonly associated with enantiostyly [mirror-image flowers, a floral polymorphism in which the style is deflected to either the left- or right-side of a flower, with at least some anthers commonly (but not exclusively) positioned on the opposite side of the flower (see Jesson & Barrett (2003) for a review)] and aspects of perianth symmetry and floral orientation (Graham & Barrett, 1995; Jesson & Barrett, 2003). These associations strongly suggest that heteranthery represents a convergent floral syndrome that has evolved as a result of pollinator-mediated selection. However, associations between heteranthery and floral and pollination traits have not been investigated more widely in angiosperms and this is the main goal of our study.

Here, we use phylogenetic comparative methods to examine associations between heteranthery and several floral and pollination traits that have been previously observed to co-occur with this condition. We begin by identifying families in which heteranthery occurs through a literature survey and document traits commonly associated with this condition. We then specifically test for correlated evolution between heteranthery and the presence versus absence of nectaries, enantiostyly and poricidal anthers (buzz-pollination).

Materials and Methods

Data collection

We performed a literature search for families containing heterantherous species. Our primary sources included Vogel (1978), Buchmann (1983), Endress (1994; , 1996) and Jesson and Barrett (2003), and ISI Web of Science where we performed a search using the term heteran*. To record buzz-pollination, the list of poricidally-dehiscent/buzz-pollinated angiosperm families reported in Buchmann (1983) was updated and expanded using ISI Web of Science using the search terms: buzz-poll* OR buzz poll* OR poricida*. Most species with poricidal anthers are buzz-pollinated, although there are exceptions (*e.g.* Araceae, Balanophoraceae, Mayacaceae) (Buchmann, 1983). We obtained information on the presence or absence of floral nectaries from Bernardello (2007). Families containing enantiostylous taxa were obtained from Graham & Barrett (1995), Jesson & Barrett (2003) and L. K. Jesson (*pers. comm.*). For heteranthery, buzz-pollination, and enantiostyly, a family was scored as "1" (present) if it included at least some species with the trait of interest and "0" otherwise. For floral nectaries we scored families as "1", with floral nectaries, and "0", no floral nectaries, including polymorphic families in which nectaries have been lost.

Phylogeny

To determine the phylogenetic distribution of heteranthery, we used a tree of families of flowering plants available at Phylomatic (http://www.phylodiversity.net/phylomatic), which is based on the supertree by Davies *et al.* (2004). This tree combines information from multiple separate studies to create a single, large phylogenetic hypothesis. Our final tree contained 440 terminal taxa, i.e. families. We chose this particular phylogenetic hypothesis to maximize the number of taxa analysed and because this tree was the best angiosperm phylogeny available at the time of data collection. The Davies *et al.* tree differs from a recent phylogenetic hypothesis for angiosperms (APG III, Bremer *et al.*, 2009) in several ways, including the collapse of families (*e.g.* the family Cochlospermaceae is included in Bixaceae), and changes in the placement of several taxa. However, the majority of the deep nodes are similar in the two trees. Moreover, when we used the APG III phylogeny to conduct the tests of correlated evolution described below on a subset of our data (n = 377 families for the comparisons of heteranthery *vs.* poricidal anthers and heteranthery *vs.* enantiostyly; and n = 339 families for heteranthery *vs.* nectaries), we found no significant changes (results not shown) compared to our findings with the tree of Davies *et al*.. We therefore present below the results of the analysis of correlated evolution obtained using the more taxa-rich tree of Davies *et al.* Finally, to facilitate comparison with future studies, in the text we refer to families according to the taxonomic nomenclature of APG III, which can be obtained from the comprehensive list of synonymy of family names available at http://www.mobot.org/MOBOT/research/APweb/.

Data analysis on correlated evolution of traits

We conducted Pagel's test of correlated evolution (Pagel, 1994; Pagel & Meade, 2006) on the phylogenetic tree to investigate whether the evolution of heteranthery (character states: present/absent) was independent of floral characters commonly found in heterantherous species. This was carried out separately for each of three characters (buzz-pollination, floral nectaries, and enantiostyly) using the binomial classification of character states described in the previous section. Pagel's test calculates the likelihood of nested models of character evolution for pairs of characters. In the omnibus test, two models are compared. The first is a model in which the character states for both traits are allowed to change independently. The second assumes that the transition in one character depends on the state of the second character. The statistical fit of the model to the observed distribution of character states under a given phylogenetic hypothesis can be compared between nested models using a likelihood ratio test (LRT). The significance of the LRT test is obtained using a Chi-square distribution with degrees of freedom equal to the difference in parameters between the models being compared (Pagel, 1994). If the dependent model provides a significantly better fit to the data, then one can conclude that the two characters evolve in a correlated fashion.

Pagel's test of correlated evolution requires dichotomous trees with non-zero branch lengths. However, our tree included several polytomies that represent uncertainty in the phylogenetic reconstruction. To address this issue, we randomly resolved polytomies using the R-program APE (Paradis *et al.*, 2004), and created a sample of 1000 of these randomly resolved trees, in which all branch length were set to one. We then conducted Pagel's test in all 1000 trees in our sample to assess the robustness of our results to particular phylogenetic hypotheses.

Results

Taxonomic distribution of heteranthery

Heteranthery has been reported from 20 families (Endress, 1994, p. 153). We excluded some of these families from our analyses either because one set of anthers produced sterile pollen (*e.g.* Gesneriaceae, Gao *et al.*, 2006), or because we considered two taxa as part of the same family (*e.g.* Caesalpinaceae was included within Fabaceae). In the case of Liliaceae and Gentianaceae, heteranthery has been reported previously (Vogel, 1978; Endress, 1994); however, we were unable to verify these reports by finding information of the identity of heterantherous species in these families, and thus we excluded them from the present analysis. Representative species for each of the 16 families included in our analyses are provided in Table S1, together with information on floral characteristics and pollinators at the family level. The 16 families with heteranthery analyzed here belong to 12 orders, including both monocotyledons and eudicotyledons — Asparagales, Brassicales, Commelinales, Dilleniales, Ericales, Fabales, Lamiales, Malpighiales, Malvales, Myrtales, Sapindales and Solanales. The broad taxonomic distribution of families containing heterantherous taxa (Fig 2) is consistent with the hypothesis that heteranthery has had multiple origins in the angiosperms and represents a striking example of floral convergence.

The number of species in each family for which heteranthery is reported varied enormously. For example, the only report of heteranthery in the Anacardiaceae — which contains approximately 600 species in 70 genera (Zomlefer, 1994) — is for *Anacardium humile* (Vogel 1978). Other families for which heteranthery is reported in only one species include Brassicaceae, Malvaceae and Lythraceae (Table S1). In other cases, heteranthery has been documented in several species belonging to only one or a few genera. These cases include Dilleniaceae [*e.g. Dillenia, Hibbertia* (Vogel, 1978; Endress, 1997)], Lecythidaceae [*Bertholletia*, *Couroupita*, *Gustavia* (Vogel, 1978; Lloyd, 1992)], Pontederiaceae [*Heteranthera, Monochoria* (Vogel, 1978; Tang & Huang, 2007)], Solanaceae [*Solanum* (Bohs *et al.*, 2007)], Tecophilaeaceae [*Cyanella* (Dulberger & Ornduff, 1980)], , Haemodoraceae [*Dilatris*, *Schiekia*, *Haemodorum*, *Xiphidium* (Simpson, 1990; LK Jesson, unpublished data)], and Malpighiaceace [*Banisteria*, *Hiptage*, *Malpighia* (Vogel, 1978)].

In Fabaceae and Melastomataceae, heteranthery is more widespread in its distribution occurring in hundreds of species and many genera. Reports of anther dimorphism in Fabaceae include *Caesalpinia*, *Swartzia*, *Senna*, *Cassia*, *Chamaechrista*, *Crotalaria*, *Dioclea*, *Dypterix*, *Eysenhardtia*, *Mucuna*, *Ormosia*, *Platymiscium*, *Poiretia*, and *Stylosanthes* (Vogel, 1978; Dulberger, 1981; Stevens *et al.*, 2001; Laporta, 2005; Marazzi & Endress, 2008). Similarly, the Melastomataceae contain many heterantherous species in *Aciotis*, *Acisanthera*, *Adelobotrys*, *Arthrostema*, *Centradenia*, *Dissotis*, *Heterocentron*, *Melastoma*, and *Tibouchina* (Vogel, 1978; Gross, 1993; Stevens *et al.*, 2001), and in some of these taxa heteranthery is relatively common (Renner, 1989).

Family correlates of heteranthery

Table S1 documents floral characteristics and pollinators of heterantherous families. Several generalizations can be extracted from this table and from Figure 2. Families with heteranthery often exhibit other forms of within-flower polymorphism, including the presence of staminodes (present in all families but Bixaceae and Lythraceae), and heterostyly (present in Fabaceae, Lythraceae and Pontederiaceae) (Table S1). In the latter two tristylous families species also possess within flower stamen differentiation although in this case they are not functionally differentiated as in heteranthery. With a few exceptions (*e.g.* Dilleniaceae, Lecythidaceae, Malvaceae), heterantherous families tend to have few stamens and all except two families—Anacardiaceae and Brassicaceae—contain species with poricidal anther dehiscence. Nectaries occur in all but three families: Commelinaceae, Dilleniaceae, and Malpighiaceae, although heterantherous taxa most often lack nectar. With respect to floral symmetry, 10 out of 16 families with heteranthery possess slight to strongly zygomorphic perianths, at least occasionally. Finally, insects are the main pollinators of families with heteranthery, and pollen-collecting bees in particular are the most common pollinators.

Correlated Evolution of Floral Traits

Heteranthery and Poricidal Anthers. Among the 16 families containing heterantherous species included here, all but Anacardiaceae and Brassicaceae contain species with poricidal anthers. This high rate of co-occurrence of poricidal anthers and heteranthery contrasts with the lower rate of poricidal anthers in our phylogenetic sample of angiosperm families (88% *vs.* 15%, 64 poricidal families out of 440). When phylogenetic relationships among families were taken into account, we found strong support indicating that the evolution of heteranthery and poricidal anthers (buzz-pollination) are strongly associated (P <0.001; Table 1). This pattern of correlated evolution was highly significant in all of the 1000 trees included in our sample indicating that our finding is robust to the particular phylogenetic hypothesis being used.

Heteranthery and Enantiostyly. Of the 15 families with heteranthery included in our phylogenetic analysis, six contained enantiostylous species. In contrast, the incidence of enantiostyly among flowering plants as a whole is very low (<3%; 11 out of 440 families). Our analysis provided strong support for the correlated evolution of heteranthery and enantiostyly (P<0.001, Table 1); a result that was not strongly influenced by the particular phylogenetic hypothesis that was used.

Heteranthery and Nectaries. We found information on the presence versus absence of nectaries at the family level for 362 plant families. Among all families, 196 contained mostly taxa with nectaries, 156 contained taxa with and without nectaries (polymorphic), and 10 generally lacked nectaries. Of the 166 families in which nectaries have been lost, 7% (11 families) included heterantherous taxa, while heteranthery occurred in 3% (5 families) out of the 196 families in which nectaries are widespread. Tests of correlated evolution indicated that a model in which heteranthery and the absence of nectaries evolve in a correlated fashion fits the data better than one in which these two characters evolve independently (Table 1, P <0.05). The correlated evolution model provided a better fit than the independent model over our entire sample of phylogenetic trees (Table 1).

Discussion

Heteranthery is one of several types of stamen dimorphism within angiosperm flowers. It has evolved in at least 12 orders indicating independent origins and suggesting that the selective forces responsible for the evolution of heteranthery are encountered by disparate animal-pollinated taxa. The number of independent evolutionary origins of heteranthery is unknown, although it is certainly larger than the number of families in which it occurs, as heteranthery has evolved independently several times even within the same genus *e.g. Solanum* (Bohs *et al.* 2007). Our study identified several common features associated with heteranthery including the lack of floral nectaries, poricidal anthers, enantiostyly, few stamens, bee pollination, and, in some groups, weakly to strongly zygomorphic perianths. However, not surprisingly given the diverse affinities of heterantherous taxa, there are many exceptions to these patterns.

Correlated evolution

Our phylogenetic analyses revealed a strong correlation between heteranthery and poricidal anthers, lack of nectaries and enantiostyly (Table 1). Although our analyses were conducted at the family level, and in most groups heteranthery was only evident in a small proportion of species within a family, we were still able to detect patterns of correlated evolution. The fact that our analysis was sensitive enough to uncover patterns of association at the family level gives us confidence that the associations we uncovered are likely to reflect the evolution of strong functional associations. However, family-level analysis has the disadvantage that it is difficult to dissect the sequence of character state associations required to understand the assembly of the heterantherous syndrome. Knowing the order of acquisition of correlated traits is critical for understanding why heteranthery has arisen in some groups and not others.

The strong association between heteranthery and buzz-pollination seems likely to have arisen as a result of the evolution of heteranthery within buzz-pollinated clades and not vice versa (Buchmann, 1983; Vallejo-Marín *et al.*, 2009). However, it is more difficult to infer whether enantiostyly precedes or follows the evolution of heteranthery (Jesson & Barrett, 2003), or if a transition to weakly zygomorphic corollas is a pre-requisite for the evolution of heteranthery. Providing answers to these questions requires well-resolved phylogenies at the family level or below. For example, Bohs and colleagues conducted a phylogenetic analysis of the evolution of heteranthery within buzz-pollinated *Solanum* (Solanaceae) (Levin *et al.*, 2006; Bohs *et al.*, 2007); their study included the major clades of *Solanum* with more concentrated sampling in the subgenus *Leptostemonum*. The vast majority of *Solanum* species lack floral nectaries and offer pollen as the only reward to attract pollinators. The hermaphroditic, pentamerous, radially symmetric flowers of most *Solanum* species have a stereotypical morphology in which similar-sized anthers form a cone in the centre of the flower (solanoid anthers). However, some derived *Solanum* species possess heteranthery accompanied by different degrees of corolla zygomorphy. Bohs and colleagues identified up to seven independent origins of stamen dimorphism within the "spiny solanums" (Levin *et al.*, 2006) and at least one more in the Normania clade (Bohs *et al.*, 2007) . The phylogenetic distribution of heteranthery indicates that in this case buzz-pollination and lack of nectaries preceded the evolution of heteranthery, which after it originated was accompanied by changes to corolla morphology.

Convergence in function

Heteranthery represents an example of convergent evolution, but why has heteranthery evolved on multiple occasions in unrelated groups? The answer to this question requires determining the selective forces responsible for the evolution and maintenance of heteranthery. The most widely accepted explanation for the function of heteranthery posits that anther dimorphism represents the specialization of stamens into fertilizing and feeding functions (H. Müller, 1881; F. Müller, 1883). According to the "division of labour" hypothesis, the short, centrally located and brightly coloured set of anthers serves to attract and reward pollinators (feeding anthers), while the second anther or anther set of larger, cryptically-coloured, anther(s) is involved mostly in fertilization (pollinating anthers). Therefore, the division of labour hypothesis rests on two tenets: first, pollinators focus their pollen collecting efforts on feeding anthers more than on pollinating anthers; and second, pollinating anthers contribute disproportionately to fertilization (Vallejo-Marín *et al.*, 2009).

Despite the fact that the division of labour hypothesis has gained acceptance since its inception (Forbes, 1882; Darwin, 1899; Harris & Kuchs, 1902; Buchmann, 1983; Barrett, 2010), empirical confirmation of both tenets of this hypothesis has been relatively scarce and restricted to a few taxa (*e.g. Solanum*, Bowers, 1975; Vallejo-Marín et al. 2009; *Melastoma*, Luo *et al.*, 2008). Determining whether the division of labour hypothesis is a general explanation of the functional significance of heteranthery awaits empirical confirmation in other lineages.

The division of labour hypothesis predicts that heteranthery should occur in species in which pollen is the only reward for pollinators. Table S1 indicates that the main pollinators of families with heterantherous species are insects, especially bees. Our finding that heterantherous species occur in families in which nectaries are entirely absent, or have been lost in some groups, also suggests an important role for pollen as the sole floral reward. However, some heterantherous species (*e.g. Haemodorum* and *Schiekia*, Haemodoraceae) produce floral nectar. It would be interesting to determine if pollinators in these groups specialize in exploiting different rewards.

A recent theoretical investigation demonstrated that heteranthery evolves when pollinators remove more pollen than should be provided in exchange for pollination services (Vallejo-Marín *et al.*, 2009). A pre-condition for the evolution of heteranthery is therefore that pollinators act as pollen thieves. Pollen theft is a phenomenon that has only recently been recognized as an important source of selection on floral strategies (Hargreaves *et al.*, 2009). If poricidal anthers represent a mechanism to reduce the amount of pollen consumed by pollinators (Buchmann, 1983), then the evolution of heteranthery in buzz-pollinated clades may represent the escalation of male strategies that influence pollen dispensing and reduce pollen consumption. Determining the function of anther dimorphism in a broader sample of taxa will shed light on whether heteranthery indeed has evolved as a response to similar selective pressures or has multiple functions among different groups.

Why is heteranthery rare?

Heteranthery is dispersed across a wide diversity of angiosperm families, but with the exception of Fabaceae and Melastomataceae both of which contain numerous heterantherous species, it is relatively uncommon. Why is heteranthery rare given the abundance of pollen collecting bees and nectarless flowers? According to the division of labour hypothesis, if heteranthery serves to reduce the amount of pollen consumed by pollinators enabling more pollen to engage in fertilization, then heteranthery should often be selectively favoured in nectarless species. However, several factors may constrain the evolution of heteranthery. First, it is possible that pollen-consuming pollinators collect pollen that would otherwise be lost from the fertilization process (Harder & Wilson, 1998). In this scenario, excess pollen consumption may not be detrimental to plant fitness and thus there is no selection for anther specialization and dimorphism. Second, for division of labour to drive the evolution of anther dimorphism requires that changes in the placement of pollen on the pollinator's body result in differences in pollen being either consumed or reaching a stigma. If the pollinator's body cannot be successfully partitioned in this manner then heteranthery may not evolve. Pollinators of sufficient size, relative to the flower, may be required to allow for specialization of anther function. Limited availability of sites for pollen placement may constrain the ability to partition the pollinator's body among closely related species, thus disfavouring diversification through sexual specialization. Finally, anther dimorphism requires differentiation of developmental pathways and it is possible that in some groups developmental or genetic constraints may limit the capacity for organ differentiation within anther whorls. The genetic and developmental basis of floral form in heterantherous species is not well understood and this is an area that would repay future attention.

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Figure legends

Figure 1. Floral morphology and anther differentiation in two heterantherous species of *Solanum* (Solanaceae). Heteranthery has evolved multiple independent times in *Solanum*, a genus of ca. 1500 species and characterizes all species in the small Section *Androceras* illustrated here. (**a**) *S. citrullifolium*, (**b**) *S. rostratum*. The left-hand side and central panels show lateral and front views of the flowers. Notice the difference in degree of zygomorphism of the corolla in these two species. The right-hand side panels show the strong dimorphism in the size, colour and shape of anthers. PA: pollinating anther; FA: feeding anthers; s: style.

Figure 2. Phylogenetic relationships among families containing heterantherous species. Characters associated with heteranthery are shown with shaded circles for each family. For classification of character states see text. Black circles denote presence and white circles absence of the following traits: heteranthery (H), poricidal anthers (P), enantiostyly (E). In the case of nectaries (N), black circles denote presence and white circles represent either absence in the entire family or a polymorphic state, i.e. nectaries have been lost in some species. Family names and phylogenetic relationships follow APG III.

Table 1. Phylogenetic tests of correlated evolution between heteranthery and the following three traits: poricidal anthers, enantiostyly (mirror-image flowers) and nectaries. For each pair of traits two models were compared, one in which the two traits evolve independently of each other (independent model) and the other in which the transitions among characters states in one trait are dependent on the character state of the other trait (dependent model). *P*-values are shown in parenthesis and are based on a Chi-square distribution with 4 d.f. To account for uncertainty in phylogenetic reconstruction, likelihood ratios and *P*-value were calculated for each of 1000 trees representing random resolutions of polytomous branches in the original phylogeny.

Comparison	**Log likelihood independent model**	**Log likelihood dependent model**	**Likelihood ratio**	**LR range in 1000 trees sample**
Heteranthery *vs.* poricidal anthers	-224.31	-199.57	49.47 **(<0.001)**	47.59-52.60 **(<0.001)**
Heteranthery *vs.* enantiostyly	-110.23	-97.46	25.43 **(<0.001)**	25.24-27.57 **(<0.001)**
Heteranthery *vs.* nectaries	-287.96	-281.96	12.19 **(<0.05)**	11.37-13.27 **(<0.05)**