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2 **Trait correlates and functional significance of heteranthery in flowering plants**

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15 [mario.vallejo@stir.ac.uk](mailto:mario.vallejo@stir.ac.uk)16 **Word count:** Summary (200), Introduction (571), Materials and Methods (744), Results

17 (921), Discussion (1281), Number of Figures (2), Number of Tables (1), Supplementary

18 Material Table (1).

19

## 20 **Summary**

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

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

## 43 **Introduction**

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

56 In heterantherous species, stamen differentiation within flowers involves the shape,  
57 colour, and/or size of anthers. Most commonly, two types of anthers are distinguishable. The  
58 first is centrally located in the flower and composed of brightly coloured stamens (usually  
59 yellow) that are short in length, and which are easily manipulated by pollen-collecting  
60 visitors. The second type of anther is displaced away from the central axis of the flower, is  
61 often cryptically coloured, and the individual anthers are usually larger in size than the  
62 preceding type (Vallejo-Marín *et al.*, 2009; Barrett, 2010). Less commonly, a third type of  
63 stamens occurs resembling the centrally located anthers, although it can be slightly larger

64 [e.g. *Solanum lumholtzianum*, Solanaceae (Whalen, 1979); *Senna* spp., Fabaceae, (Luo *et al.*,  
65 2009)] or consists of staminodes (e.g. *Commelina* spp., Commelinaceae). Because  
66 heterantherous species are exclusively animal-pollinated (Vogel, 1978), anther variation is  
67 undoubtedly associated with various facets of the pollination process with consequences for  
68 pollen dispersal and male function.

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

85 Here, we use phylogenetic comparative methods to examine associations between  
86 heteranthery and several floral and pollination traits that have been previously observed to

87 co-occur with this condition. We begin by identifying families in which heteranthery occurs  
88 through a literature survey and document traits commonly associated with this condition. We  
89 then specifically test for correlated evolution between heteranthery and the presence versus  
90 absence of nectaries, enantiostyly and poricidal anthers (buzz-pollination).

## 91 **Materials and Methods**

### 92 **Data collection**

93 We performed a literature search for families containing heterantherous species. Our primary  
94 sources included Vogel (1978), Buchmann (1983), Endress (1994; , 1996) and Jesson and  
95 Barrett (2003), and ISI Web of Science where we performed a search using the term  
96 heteran\*. To record buzz-pollination, the list of poricidally-dehiscent/buzz-pollinated  
97 angiosperm families reported in Buchmann (1983) was updated and expanded using ISI Web  
98 of Science using the search terms: buzz-poll\* OR buzz poll\* OR poricida\*. Most species  
99 with poricidal anthers are buzz-pollinated, although there are exceptions (*e.g.* Araceae,  
100 Balanophoraceae, Mayacaceae) (Buchmann, 1983). We obtained information on the presence  
101 or absence of floral nectaries from Bernardello (2007). Families containing enantiostylous  
102 taxa were obtained from Graham & Barrett (1995), Jesson & Barrett (2003) and L. K. Jesson  
103 (*pers. comm.*). For heteranthery, buzz-pollination, and enantiostyly, a family was scored as  
104 "1" (present) if it included at least some species with the trait of interest and "0" otherwise.  
105 For floral nectaries we scored families as "1", with floral nectaries, and "0", no floral  
106 nectaries, including polymorphic families in which nectaries have been lost.

## 107 **Phylogeny**

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

## 128 **Data analysis on correlated evolution of traits**

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

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

## 151 **Results**

### 152 **Taxonomic distribution of heteranthery**

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

168 The number of species in each family for which heteranthery is reported varied  
169 enormously. For example, the only report of heteranthery in the Anacardiaceae — which  
170 contains approximately 600 species in 70 genera (Zomlefer, 1994) — is for *Anacardium*  
171 *humile* (Vogel 1978). Other families for which heteranthery is reported in only one species  
172 include Brassicaceae, Malvaceae and Lythraceae (Table S1). In other cases, heteranthery has  
173 been documented in several species belonging to only one or a few genera. These cases



174 include Dilleniaceae [*e.g.* *Dillenia*, *Hibbertia* (Vogel, 1978; Endress, 1997)], Lecythidaceae  
175 [*Bertholletia*, *Couroupita*, *Gustavia* (Vogel, 1978; Lloyd, 1992)], Pontederiaceae  
176 [*Heteranthera*, *Monochoria* (Vogel, 1978; Tang & Huang, 2007)], Solanaceae [*Solanum*  
177 (*Bohs et al.*, 2007)], Tecophilaeaceae [*Cyanella* (Dulberger & Ornduff, 1980)], ,  
178 Haemodoraceae [*Dilatris*, *Schiekia*, *Haemodorum*, *Xiphidium* (Simpson, 1990; LK Jesson,  
179 unpublished data)], and Malpighiaceae [*Banisteria*, *Hiptage*, *Malpighia* (Vogel, 1978)].

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

### 189 **Family correlates of heteranthery**

190 Table S1 documents floral characteristics and pollinators of heterantherous families. Several  
191 generalizations can be extracted from this table and from Figure 2. Families with heteranthery  
192 often exhibit other forms of within-flower polymorphism, including the presence of  
193 staminodes (present in all families but Bixaceae and Lythraceae), and heterostyly (present in  
194 Fabaceae, Lythraceae and Pontederiaceae) (Table S1). In the latter two tristylous families  
195 species also possess within flower stamen differentiation although in this case they are not  
196 functionally differentiated as in heteranthery. With a few exceptions (*e.g.* Dilleniaceae,

197 Lecythidaceae, Malvaceae), heterantherous families tend to have few stamens and all except  
198 two families—Anacardiaceae and Brassicaceae—contain species with poricidal anther  
199 dehiscence. Nectaries occur in all but three families: Commelinaceae, Dilleniaceae, and  
200 Malpighiaceae, although heterantherous taxa most often lack nectar. With respect to floral  
201 symmetry, 10 out of 16 families with heteranthery possess slight to strongly zygomorphic  
202 perianths, at least occasionally. Finally, insects are the main pollinators of families with  
203 heteranthery, and pollen-collecting bees in particular are the most common pollinators.

#### 204 **Correlated Evolution of Floral Traits**

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

215 *Heteranthery and Enantiostyly.* Of the 15 families with heteranthery included in our  
216 phylogenetic analysis, six contained enantiostylous species. In contrast, the incidence of  
217 enantiostyly among flowering plants as a whole is very low (<3%; 11 out of 440 families).  
218 Our analysis provided strong support for the correlated evolution of heteranthery and

219 enantiostyly ( $P < 0.001$ , Table 1); a result that was not strongly influenced by the particular  
220 phylogenetic hypothesis that was used.

221 *Heteranthery and Nectaries.* We found information on the presence versus absence of  
222 nectaries at the family level for 362 plant families. Among all families, 196 contained mostly  
223 taxa with nectaries, 156 contained taxa with and without nectaries (polymorphic), and 10  
224 generally lacked nectaries. Of the 166 families in which nectaries have been lost, 7% (11  
225 families) included heterantherous taxa, while heteranthery occurred in 3% (5 families) out of  
226 the 196 families in which nectaries are widespread. Tests of correlated evolution indicated  
227 that a model in which heteranthery and the absence of nectaries evolve in a correlated fashion  
228 fits the data better than one in which these two characters evolve independently (Table 1,  $P$   
229  $< 0.05$ ). The correlated evolution model provided a better fit than the independent model over  
230 our entire sample of phylogenetic trees (Table 1).

## 231 **Discussion**

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

241 surprisingly given the diverse affinities of heterantherous taxa, there are many exceptions to  
242 these patterns.

### 243 **Correlated evolution**

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

254         The strong association between heteranthery and buzz-pollination seems likely to  
255 have arisen as a result of the evolution of heteranthery within buzz-pollinated clades and not  
256 vice versa (Buchmann, 1983; Vallejo-Marín *et al.*, 2009). However, it is more difficult to  
257 infer whether enantiostyly precedes or follows the evolution of heteranthery (Jesson &  
258 Barrett, 2003), or if a transition to weakly zygomorphic corollas is a pre-requisite for the  
259 evolution of heteranthery. Providing answers to these questions requires well-resolved  
260 phylogenies at the family level or below. For example, Bohs and colleagues conducted a  
261 phylogenetic analysis of the evolution of heteranthery within buzz-pollinated *Solanum*  
262 (*Solanaceae*) (Levin *et al.*, 2006; Bohs *et al.*, 2007); their study included the major clades of  
263 *Solanum* with more concentrated sampling in the subgenus *Leptostemonum*. The vast

264 majority of *Solanum* species lack floral nectaries and offer pollen as the only reward to attract  
265 pollinators. The hermaphroditic, pentamerous, radially symmetric flowers of most *Solanum*  
266 species have a stereotypical morphology in which similar-sized anthers form a cone in the  
267 centre of the flower (solanoid anthers). However, some derived *Solanum* species possess  
268 heteranthery accompanied by different degrees of corolla zygomorphy. Bohs and colleagues  
269 identified up to seven independent origins of stamen dimorphism within the "spiny solanums"  
270 (Levin *et al.*, 2006) and at least one more in the Normania clade (Bohs *et al.*, 2007) . The  
271 phylogenetic distribution of heteranthery indicates that in this case buzz-pollination and lack  
272 of nectaries preceded the evolution of heteranthery, which after it originated was  
273 accompanied by changes to corolla morphology.

#### 274 **Convergence in function**

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

287 Despite the fact that the division of labour hypothesis has gained acceptance since its  
288 inception (Forbes, 1882; Darwin, 1899; Harris & Kuchs, 1902; Buchmann, 1983; Barrett,  
289 2010), empirical confirmation of both tenets of this hypothesis has been relatively scarce and  
290 restricted to a few taxa (*e.g.* *Solanum*, Bowers, 1975; Vallejo-Marín *et al.* 2009; *Melastoma*,  
291 Luo *et al.*, 2008). Determining whether the division of labour hypothesis is a general  
292 explanation of the functional significance of heteranthy awaits empirical confirmation in  
293 other lineages.

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

302         A recent theoretical investigation demonstrated that heteranthy evolves when  
303 pollinators remove more pollen than should be provided in exchange for pollination services  
304 (Vallejo-Marín *et al.*, 2009). A pre-condition for the evolution of heteranthy is therefore  
305 that pollinators act as pollen thieves. Pollen theft is a phenomenon that has only recently been  
306 recognized as an important source of selection on floral strategies (Hargreaves *et al.*, 2009).  
307 If poricidal anthers represent a mechanism to reduce the amount of pollen consumed by  
308 pollinators (Buchmann, 1983), then the evolution of heteranthy in buzz-pollinated clades  
309 may represent the escalation of male strategies that influence pollen dispensing and reduce

310 pollen consumption. Determining the function of anther dimorphism in a broader sample of  
311 taxa will shed light on whether heteranthery indeed has evolved as a response to similar  
312 selective pressures or has multiple functions among different groups.

### 313 **Why is heteranthery rare?**

314 Heteranthery is dispersed across a wide diversity of angiosperm families, but with the  
315 exception of Fabaceae and Melastomataceae both of which contain numerous heterantherous  
316 species, it is relatively uncommon. Why is heteranthery rare given the abundance of pollen  
317 collecting bees and nectarless flowers? According to the division of labour hypothesis, if  
318 heteranthery serves to reduce the amount of pollen consumed by pollinators enabling more  
319 pollen to engage in fertilization, then heteranthery should often be selectively favoured in  
320 nectarless species. However, several factors may constrain the evolution of heteranthery.

321 First, it is possible that pollen-consuming pollinators collect pollen that would otherwise be  
322 lost from the fertilization process (Harder & Wilson, 1998). In this scenario, excess pollen  
323 consumption may not be detrimental to plant fitness and thus there is no selection for anther  
324 specialization and dimorphism. Second, for division of labour to drive the evolution of anther  
325 dimorphism requires that changes in the placement of pollen on the pollinator's body result in  
326 differences in pollen being either consumed or reaching a stigma. If the pollinator's body  
327 cannot be successfully partitioned in this manner then heteranthery may not evolve.

328 Pollinators of sufficient size, relative to the flower, may be required to allow for  
329 specialization of anther function. Limited availability of sites for pollen placement may  
330 constrain the ability to partition the pollinator's body among closely related species, thus  
331 disavouring diversification through sexual specialization. Finally, anther dimorphism  
332 requires differentiation of developmental pathways and it is possible that in some groups

333 developmental or genetic constraints may limit the capacity for organ differentiation within  
334 anther whorls. The genetic and developmental basis of floral form in heterantherous species  
335 is not well understood and this is an area that would repay future attention.

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450

451 **Figure legends**

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

459

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

467

468

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

478

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

479