Architectural traits constrain the evolution of unisexual flowers and sexual segregation within inflorescences: an interspecific approach

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ABSTRACT

Male and female unisexual flowers have repeatedly evolved from the ancestral bisexual flowers in different lineages of flowering plants. This sex specialization in different flowers often occurs within inflorescences. We hypothesize that inflorescence architecture may impose a constraint on resource availability for late flowers, potentially leading to different optima in floral sex allocation and unisexuality. Under this hypothesis we expect that inflorescence traits increasing the difference in resource availability between early and later flowers would be phylogenetically correlated with a higher level of sexual specialization. To test this hypothesis, we performed a comparative analysis of inflorescence traits (inflorescence size, number of flowers and flower density) in the sunflower family, which displays an extraordinary variation in floral sexual specialization at the inflorescence level, i.e. hermaphroditic, gynomonoecious and monoecious species. We found that species with a complete sex separation in unisexual flowers (monoecy) had significantly denser inflorescences. Furthermore, those species arranging their flowers in denser inflorescences also showed greater differences in the size of early and late fruits, a proxy of resource variation between flowers. Our findings support the idea that floral sexual specialization and consequently sexual segregation may be the consequence of different floral sex allocation optima driven by the sequential development of flowers that results in a persistent resource decline from earlier to later flowers.

INTRODUCTION

Most angiosperms are hermaphrodite, i.e., they produce bisexual or 'perfect' flowers bearing both functional pollen grains and ovules. Combining both sexual functions in the same flower reduces direct reproduction costs, such as sharing attractive structures for pollinators (Charnov et al. 1976), and acts as an insurance against vagaries in the mating environment by allowing selfing (Wilson and Harder 2003). Thus, evolutionary biologists have wondered, since Darwin (1877), what favours non-hermaphroditic sexual systems, where flowers of different sex -male, female, or bisexual- are combined in the same or different individuals of a population. Darwin (1877) interpreted the evolution of unisexual flowers as a means of reducing selfing. Nevertheless, the simultaneous presence of self-incompatibility and unisexual flowers in many angiosperms (Bertin 1993) suggests that avoiding selfing is not the only driver of sexual specialization in flowers. Alternative explanations for the evolution of sexual specialization are the avoidance of interference between sexual functions (Charnov 1979; Willson 1979) and optimal resource allocation (Janzen 1977; Willson 1979; Lloyd 1982).

Any evolutionary explanation of sexual specialization of flowers should consider that sexes are often segregated within inflorescences due to two constraints set by inflorescence architecture. The first constraint is ontogenetic (Lee 1988; Diggle 1995, 2003): because flowers within inflorescences usually develop sequentially, early flowers usually flower and ripen fruits first and pre-empt resources for late flowers. The second constraint is strictly positional (Diggle 2003) and results in a persistent limitation of resources at certain flower positions inherent to the architecture of inflorescence axes. Both constraints provide a general proximate mechanism for sexual selection, because they can strongly influence the mating environment or resource

availability experienced by individual flowers (Brunet and Charlesworth 1995; Diggle 2003; Harder and Prusinkiewicz 2013). As resource availability may differentially affect male and female performance (Freeman et al. 1980, Lau and Stephenson 1993), this resource gradient across flowers within an inflorescence could also lead to differential sexual specialization in female or male functions at different floral positions.

On theoretical grounds, a female-biased floral allocation can be predicted in those floral positions in the inflorescence with higher resource availability, whereas a male-biased allocation will be expected in positions with less resources (Brunet and Charlesworth 1995). Intraspecific observational (Diggle 2003) and experimental evidence (Emms 1993; Diggle 1994) supports this idea, at least for plants with elongated inflorescences. For instance, *Solanum hirtum* produces inflorescences with bisexual flowers, but the late (distal) ones are labile, becoming male in resource-depleted plants (Diggle 1994). Nevertheless, it remains poorly understood whether these intraspecific plastic responses in sexual specialization and segregation within inflorescences can actually become fixed during the evolution of a lineage (Diggle 2003; Torices and Méndez 2010), giving rise to the sexual segregation within inflorescences observed in many angiosperm families. The exception is a pioneer comparative study of *Solanum* that found how plastic responses in the production of male unisexual flowers are ancestral to fixed position effects (Diggle and Miller 2013). Here, we take this comparative approach a step further, by using Asteraceae to test at a family level whether the degree of sexual specialization is phylogenetically correlated with inflorescence architectural traits which may lead to a resource decline from early to late flowers.

109 We hypothesize that three architectural traits of inflorescences, namely larger
110 size, and higher flower number and density increase resource competition among
111 flowers and likely constrain resource availability for late flowers, potentially leading to
112 male-biased floral sex allocation and unisexuality. Testing this hypothesis at an
113 interspecific level faces two challenges. First, inflorescences may differ impressively
114 across species in size, number of flowers, fruit size and flower size, even within the
115 same main inflorescence type (Leppik 1977; Burtt 1977; Wyatt 1982; Harder et al.
116 2004). Second, these traits do not necessarily covary across species, because
117 inflorescence size and flower number are under selection by several ecological drivers,
118 such as pollinators (Willson and Rathcke 1974; Thomson 1988; Andersson 1996;
119 Kirchner et al. 2005; Iwata et al. 2012), seed predators (Fenner et al. 2002), or altitude
120 and geographic ranges (Fenner et al. 2001). Consequently, the diversity of trait
121 combinations across species could obscure the detection of patterns in sexual
122 specialization. In particular, a negative covariation between flower size and number
123 (Sargent et al. 2007; Goodwillie et al. 2010; Vasconcelos and Proença 2015) could lead
124 to similar resource competition and flower density may be the more reliable indicator of
125 resource competition among flowers within an inflorescence.

126 In this study, we compared species of the sunflower family showing different
127 levels of floral sexual specialization to test whether they show different inflorescence
128 traits. We then assessed how these inflorescence traits correlate with resource
129 differences between flowers within the same inflorescence. The sunflower family
130 (Asteraceae) is a suitable model for testing this hypothesis. First, all Asteraceae share
131 the same basic inflorescence architecture, the head or capitulum (Funk et al. 2009),
132 which mainly follows a centripetal pattern in floral development and blooming (Harris
133 1995; Pozner et al. 2012). Second, three different sexual systems are common in

Asteraceae, each showing a phylogenetic gradient in sexual specialization (Torices et al. 2011): hermaphroditism, gynomonoecy and monoecy (Fig. 1). Third, a clear pattern of sexual specialization is present within inflorescences in non-hermaphroditic species. Namely, gynomonoecious species produce female flowers at the outermost positions and bisexual ones in the inner positions, while monoecious species show female flowers in the outermost positions and male flowers in inner positions (Fig. 1). Finally, there is anatomical (Alkio and Grimm 2003), physiological (Alkio et al. 2003;) and experimental evidence that architectural constraints occur within heads, and both outer flowers and positional effects can limit the available resources to late-blooming, inner flowers (Torices and Méndez 2010).

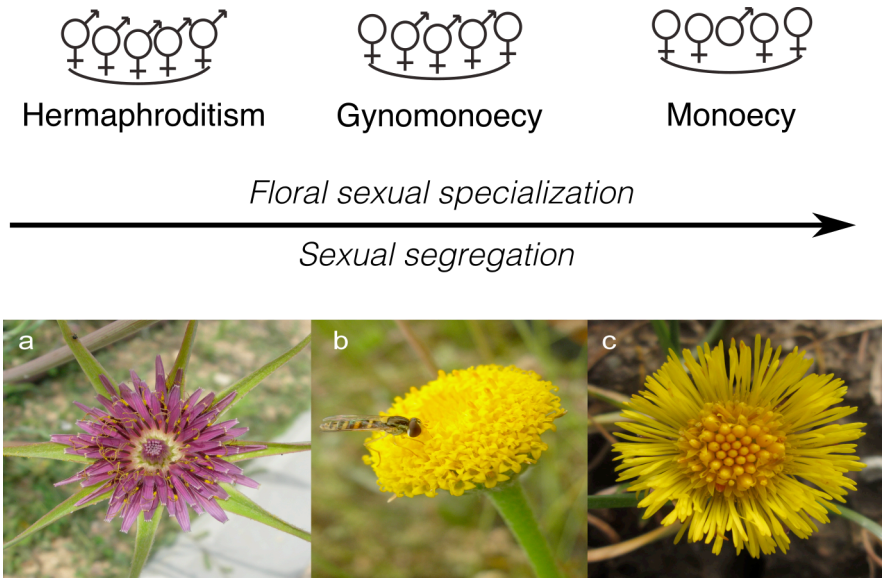


Figure 1. Sexual specialization in the Asteraceae inflorescences. Three different types of functionally hermaphroditic inflorescences and individuals can be observed in the Asteraceae: hermaphroditic, gynomonoecious and monoecious. Allocation of gametes to different flowers shows a gradient in floral sexual specialization from hermaphroditic species (only bisexual flowers) to gynomonecious species (female and bisexual flowers) and to monoecious species (female and male flowers). In Asteraceae, sexual specialization (i.e., bi- vs. unisexuality in flowers) and sexual segregation within inflorescences occur in concert. Lower panels show representative species from a) hermaphroditic heads (*Tragopogon porrifolius* L.), b) gynomonoecious heads (*Anacyclus valentinus* L.), and c) monoecious heads (*Tussilago farfara* L).

156 In this paper, we followed a comparative approach to study the role of
157 architectural constraints in the evolution of floral sexual specialization and sexual
158 segregation within inflorescences in the Asteraceae. Specifically, we assessed how
159 inflorescence traits (namely, inflorescence size, number of flowers, and flower density)
160 were associated with different levels of floral sexual specialization and sexual
161 segregation represented by hermaphroditic, gynomonoecious and monoecious species
162 (Fig. 1). In addition, we assessed how these inflorescence traits affected variation in
163 fruit size within the inflorescences, as a proxy for the resource gradient between flower
164 positions (Lee 1988; Stephenson 1992). Finally, we tested whether female flowers in an
165 inflorescence produce larger fruits than bisexual ones, as would be expected if sexual
166 specialization 'releases' female flowers from expending resources on male structures and
167 compensated the lack of the male sex function by producing larger fruits than bisexual
168 flowers.
169

170 ## MATERIAL AND METHODS

171 **Study species**

172 The Asteraceae is the largest family of angiosperms, with over 1,500 genera and
173 25,000 species, and a worldwide distribution (Funk et al. 2009). All Asteraceae share
174 the same basic inflorescence, the head or capitulum, a dense indeterminate inflorescence
175 where all the flowers are sessile and attached to a common receptacle (Fig. 1). Heads
176 represent the basic pollinator attraction unit (Burtt 1977; Leppik 1977). Different
177 degrees of sexual segregation within the heads can be observed among species of the
178 family (Fig. 1). From hermaphroditism to monoecy, a floral sexual specialization
179 occurring in individual flowers. The evolutionary transition between them occurs

180 through a gynomonoecious intermediate that bears both female and bisexual flowers
181 (Torices et al. 2011).
182 We included a total of 97 species in our study, including 44 hermaphroditic, 30
183 gynomonoecious and 23 monoecious species (Supplementary Table S1). The species
184 were studied on material from two herbaria: the Asteraceae collection of the Swedish
185 Natural History Museum Herbarium (S), and the Herbarium of the University of
186 Coimbra (COI). Herbarium sampling allowed a comprehensive sampling of Asteraceae
187 diversity, since different evolutionary lineages coming from different continents and
188 biomes were sampled.
189
190 **Inflorescence and fruit traits**
191 Three traits were measured at an inflorescence level in the 97 species: (i)
192 inflorescence size, measured as the head diameter in mm; (ii) the number of flowers per
193 inflorescence; and (iii) flower density, calculated as the ratio between the number of
194 flowers and the area of each head. Flower density was used as a measure of floral
195 aggregation and may provide a better proxy of resource competition between flowers
196 within the head than head size or number of flowers per head.
197 To minimize specimen damage, we sampled only those specimens with mature
198 fruits, i.e. fruiting heads, in which we measured inflorescence and fruit traits. Fruiting
199 heads in Asteraceae usually retain the size and structure of the inflorescence and
200 therefore they can be used to describe inflorescence traits such as size and number of
201 flowers. First, we searched those specimens belonging to the species included in the
202 phylogenetic supertrees published for this family (Funk et al., 2005; Funk, 2009).
203 Second, we selected herbarium specimens with enough mature fruiting heads and with
204 good conservation status. For each species, one specimen was selected, and at least one

205 capitulum was sampled. Following this procedure, 100 herbarium specimens were
206 sampled (78 from S and 22 from COI; Supplementary Table S1).
207 All fruiting heads were dissected to separate all fruits in their relative positions
208 within heads from the outermost to the innermost positions. When necessary, heads
209 were placed in water with a detergent for rehydration and to reduce damage to the head.
210 Data were collected for 70 species. Heads and fruits were measured using pictures taken
211 with a tripod-stabilized digital camera. More than 2,700 fruits were measured as the
212 two-dimensional projection of their outline using Image J 1.54s software (Schneider et
213 al. 2012). Although low intraspecific sample sizes may lead to increased type I error in
214 comparative studies, this effect is important only when coupled with high intraspecific
215 variation (Harmon and Losos 2005). However, when the range of taxa studied is wide,
216 variation across species is usually much greater than variation within species. In our
217 study, fruit size varied between species investigated, the largest fruits were more than
218 100-fold larger than the smallest fruits, whereas no single species showed such a degree
219 of variation in fruit size.
220
221 **Statistical analyses**
222 *Relationship between inflorescence traits and floral sexual specialization*
223 We used phylogenetic generalized least squared (PGLS) models (Freckleton et
224 al. 2002; Paradis 2012) to explore the relationship between the degree of floral sexual
225 specialization (hermaphroditism, gynomonoecy or monoecy) and inflorescence traits.
226 All models were evaluated under both an adaptive model (OU, Ornstein-Uhlenbeck
227 model) and a neutral model of evolution (BM, Brownian motion model) (Felsenstein
228 2004). PGLS were fitted using the "ape" (Paradis et al. 2004) and "geiger" packages
229 (Harmon et al. 2008) in R. The fittest model for each combination of variables was

230 selected using a likelihood ratio test comparing BM and OU models. For all fitted
231 models, the OU model had a higher goodness of fit than the BM (results not shown).
232 Therefore, we present only the results fitted under an OU model. The phylogenetic
233 relationship between the species included in the analyses was considered by using the
234 phylogenetic supertree published for the Asteraceae (Funk et al. 2005), adding a
235 calibration to include branch lengths (Torices 2010). The root of this tree was
236 previously scaled to 1 for all the analyses. Specific comparisons between
237 hermaphroditism, gynomonoecy and monoecy were explored using the marginal means,
238 using the 'lsmeans' package (Lenth 2016) in R (R Development Core Team, 2015),
239 which can be defined as a linear combination of the estimated effects from a linear
240 model.
241
242 *Relationships between fruit size, inflorescence traits and floral sexual specialization*
243 We explored how fruit size variation within inflorescences, as a proxy of the
244 resource gradient between flower positions, was related to inflorescence traits and floral
245 sexual specialization, using the meta-analytical effect size to get a standardized measure
246 of the magnitude of the difference among the size of the outer and inner fruits (fruit size
247 difference, hereafter FSD). A random-effects meta-analysis was used. Effect sizes were
248 calculated using the 'meta' package in R (Schwarzer 2015).
249 The correlation between FSD and fruit size with the degree of floral sexual
250 specialization was explored using PGLS models. FSD and fruit size were the response
251 variables, whereas the degree of floral sexual specialization was the predictor variable.
252 All models were evaluated under both an OU and a BM model (see above).
253 In addition, we assessed the allometric relationships of inflorescence traits (head
254 diameter, number of flowers and flower density) with FSD and outer and inner fruit
255 sizes. These allometric relationships were tested by fitting PGLS models. FSD and fruit

size were the response variables, and the three inflorescence traits were the predictor variables. For those significant correlations, we estimated phylogenetic reduced major axis regressions (phyloRMA) and tested whether phyloRMA slopes were significantly different from one using the 'phyl.RMA' function included in the 'phytools' package (Revell 2012). All variables were log-transformed before analysis.

Relationship between floral sexual specialization and fruit size

We explored whether unisexual flowers produced larger fruits than bisexual flowers. We assessed the effect of floral sexual specialization (female vs. bisexual flowers) using only the outer fruits because strictly female flowers are found only in these positions. In addition, given the strong effect of inflorescence traits on fruit size (see Results), we included in the model the density of flowers, to control for the size of the inflorescence and the number of flowers. We fitted two PGLS models, where fruit size was the response variable and flower sex was included as a predictor categorical variable. Flower density was included as a continuous predictor variable. The only difference between both models was the inclusion of an interaction term between the sex of the flower and the flower density. The model including an interaction did not perform better and was thus dropped. Fruit size and flower density were log-transformed. We evaluated this model under OU and BM correlation structures.

RESULTS

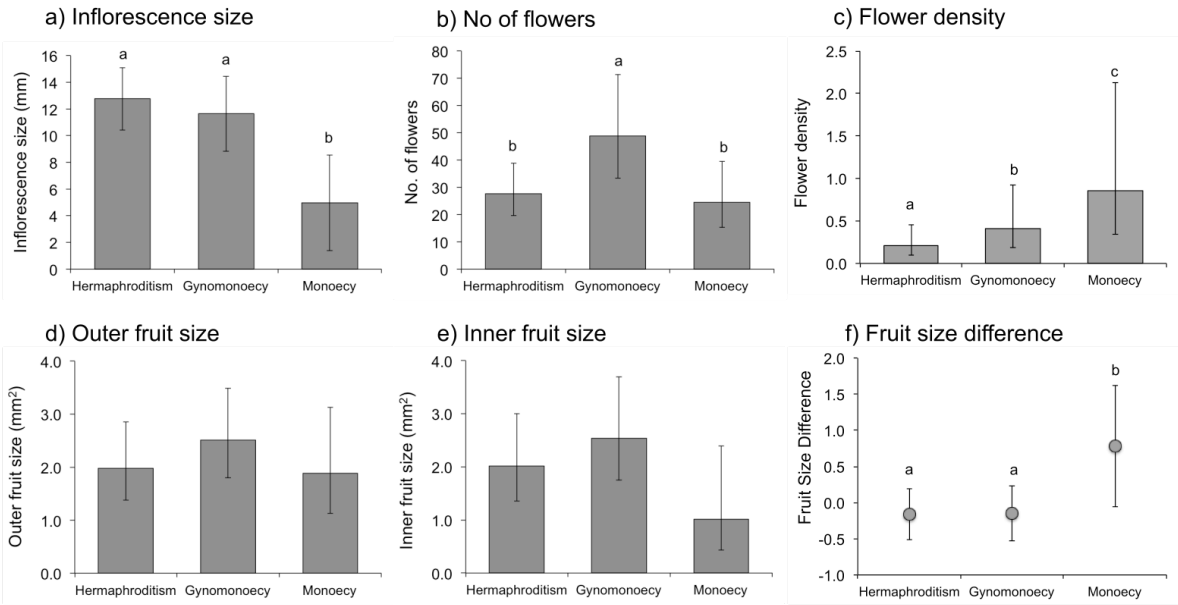
Inflorescence traits and floral sexual specialization

Inflorescence traits differed significantly among the degrees of floral sexual specialization, i.e., hermaphroditism, gynomonoecy, and monoecy (Table 1). Hermaphroditic and gynomonoecious species displayed larger inflorescences than

281 monoecious species (Fig. 2a). Gynomonoecious species had significantly more flowers
282 per inflorescence than hermaphroditic and monoecious species (Fig. 2b). Nevertheless,
283 flower density was correlated with the degree of floral sexual specialization, increasing
284 from hermaphroditic through gynomonoecious to monoecious species (Fig. 2c).

285 Inflorescence size was significantly correlated to the other two inflorescence
286 traits (Supplementary Table S2). The number of flowers increased disproportionally
287 with an increase in inflorescence size, whereas flower density disproportionally
288 decreased with an increase in inflorescence size, measured as head diameter
289 (Supplementary Table S3). Number of flowers and flower density were only marginally
290 correlated (Supplementary Table S2), and flower density increased proportionally with
291 an increased number of flowers (Supplementary Table S3).

292

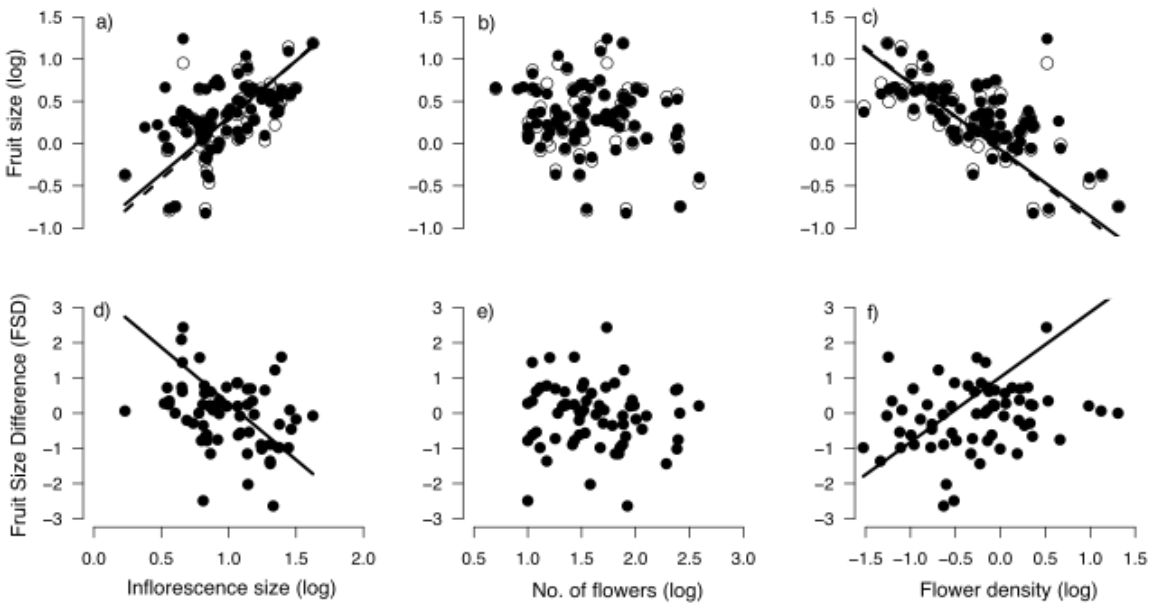


293

294 **Figure 2.** Phylogenetically controlled least-squares means (± 95% confidence interval)
295 of (a) inflorescence size (mm), (b) number of flowers, (c) flower density (no. flowers /
296 mm^2), (d) outer fruit size, (e) inner fruit size, and (f) Fruit Size Difference (FSD) for
297 different levels of sexual systems representing increasing levels of floral sexual
298 specialization within inflorescences: hermaphroditism, gynomonoecy and monoecy.
299 FSD is the standardized fruit size difference between outer and inner fruits measured as
300 the meta-analytical effect size. Means sharing the same superscript letter were not
301 significantly different at the $P < 0.05$ level.

302 **Fruit size variation within inflorescences**

303 Fruit size was not statistically different between hermaphroditic,
304 gynomonoecious and monoecious species, either for outer or inner positions (Table 1;
305 Fig. 2d,e). Two different relationships between fruit size and inflorescence traits were
306 observed, independently of position in the head. First, fruit size significantly increased
307 with an increase in inflorescence size (Fig. 3a). The phylogenetic RMA slopes were
308 significantly higher than one (outer fruits: b = 1.34, t = 3.16, d.f. = 68.9, P = 0.002;
309 inner fruits: b = 1.40, t = 3.64, d.f. = 57.4, P < 0.001) indicating a disproportionate
310 increase in fruit size with an increase in inflorescence diameter (Fig. 3a). Second, fruit
311 size decreased with an increase in flower density (Fig. 3c). The phylogenetic RMA
312 slopes were significantly < 1.0 (outer fruits: b = -0.79, t = 3.31, d.f. = 61.4, P = 0.002;
313 inner fruits: b = -0.82, t = 2.78, d.f. = 51.7, P = 0.008). Therefore, fruit size decreased at
314 a lower rate than the increase in flower density (Fig. 3c). Fruit size was not statistically
315 correlated with flower number (Fig. 3b).



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317 **Figure 3.** Phylogenetic RMA regressions between fruit size and a) inflorescence size
318 (mm), b) number of flowers, and c) flower density (no. flowers / mm^2); and between
319 FSD and d) inflorescence size (mm), e) number of flowers, and f) flower density (no.
320 flowers / mm^2). In the upper row black dots and solid lines represent outer fruits

321 whereas white dots and dashed lines indicate inner fruits. Lines represent RMA slopes.
322 Phylogenetic standard regression slopes are showed in Supplementary Table 4.
323

324 FSD, the standardized fruit size difference, significantly decreased with an
325 increase in inflorescence size (Fig. 3d; b = -3.20, t = 10.24, d.f. = 62.2, P <0.0001),
326 implying that smaller heads showed a higher difference between outer and inner fruits.
327 FSD did not show any significant relationship with the number of flowers (Fig. 3e),
328 although it significantly increased with flower density (Fig. 3f), revealing that an
329 increase in flower density was associated with a higher difference in size between outer
330 and inner fruits (b = 1.85, t = 5.28, d.f. = 61.9, P <0.0001). The degree of sexual
331 specialization did not significantly affect FSD (Table 1). However, the post-hoc
332 comparison between degrees of sexual specialization showed that monoecious species
333 had significantly larger FSD than hermaphroditic and gynomonoecious species (Fig.
334 2e). This difference was mainly mediated by the differences among sexual systems in
335 flower density, because the inclusion of flower density as a covariate removed any
336 statistical difference between monoecious species and the other two categories
337 considered (Supplementary Table S5).
338

339 **Floral sexual specialization and fruit size**

340 Fruit size decreased with increasing flower density ($F_{1,76}$ = 109.03, P < 0.0001;
341 Fig. 4). Nevertheless, female flowers produced significantly larger fruits than bisexual
342 flowers after controlling by flower density ($F_{1,76}$ = 5.87, P = 0.018, n = 78; Fig. 4).

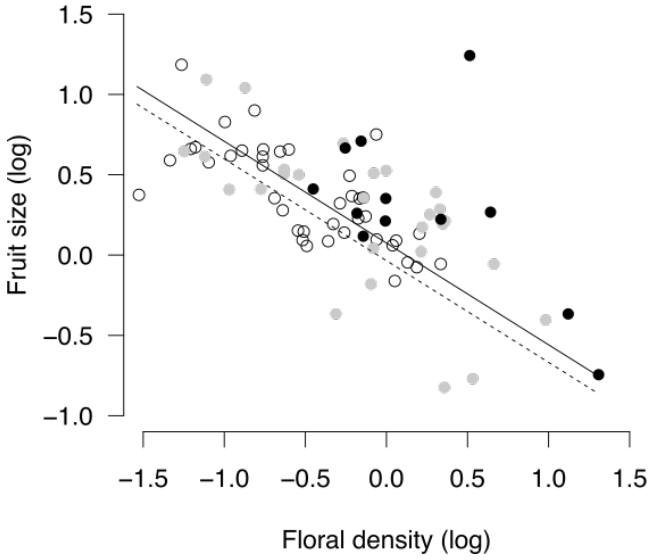

Figure 4. Relationship between fruit size produced by female (filled dots and solid line) and bisexual flowers (white dots and dashed line) and flower density. Female flowers produced by monoecious species are shown as black dots whereas those produced by gynomonoecious species are indicated by grey dots.

DISCUSSION

Our comparative study across three sexual systems in Asteraceae provided clear support for a phylogenetic association between inflorescence traits, fruit size variation within inflorescences, and the degrees of sexual specialization and segregation. In particular, we observed that monoecious species bore smaller and denser inflorescences than hermaphroditic ones, also showing the largest fruit size difference (FSD) between outer and inner fruits. These results, together with the lack of a correlation between the number of flowers and the degree of sexual specialization, support the idea that floral sexual specialization and consequently sexual segregation within inflorescences of Asteraceae might in part be the result of different sex allocation optima brought about by architecturally mediated persistent resource limitation of the inner flowers.

Although selfing and geitonogamy avoidance has been considered an important factor in the evolution of floral sex specialization and sexual segregation within

363 inflorescences (Harder et al. 2000), on its own it is unlikely to explain the pattern
364 observed in Asteraceae. The negative effects of geitonogamy are expected to be higher
365 in large inflorescences, where a higher number of flowers can lead to longer floral bouts
366 by pollinators (Harder and Barrett 1995). Thus, many-flowered inflorescences would be
367 expected to show a higher probability of exhibiting unisexual flowers than few-flowered
368 inflorescences. However, in our data set the number of flowers per inflorescence was
369 not clearly related to sexual specialization (Fig. 3b). Moreover, outer seeds should be
370 more outcrossed than inner ones, but evidence is scarce and inconclusive: two species
371 have shown higher outcrossing rate in outer flowers than inner ones (Marshall and
372 Abbott 1984, Cheptou et al. 2001), whereas two other studies have found no differences
373 (Gibson 2001, Gibson and Tomlinson 2002). Finally, the species of this family usually
374 show other mechanisms to avoid selfing such as self-incompatibility (Ferrer et al. 2007)
375 and dichogamy (both at the flower and inflorescence levels; Burtt 1977). Therefore,
376 whether limiting geitonogamy plays a key role in the evolution of sexual segregation in
377 Asteraceae requires further examination, because empirical evidence remains
378 contradictory.

379 An alternative to the selfing avoidance hypothesis is that evolutionary transitions
380 between sexual systems might have evolved as a way to optimize gamete packaging.
381 Inflorescence traits, such as the number of flowers per inflorescence and the
382 inflorescence size, are key components of gamete packaging strategies (Schoen and
383 Dubuc 1990; Fishbein and Venable 1996). Our results suggest that shifts in the so called
384 'inflorescence design', i.e. number of flowers and size of the inflorescence, can have
385 effects at the flower level too. In the Asteraceae, these shifts can have led to transitions
386 between hermaphroditism, gynomonoecy and monoecy, whereas the pollination unit
387 keeps their mating opportunities through the retention of both male and female

388 functions. For instance, the increase in flower number is usually negatively correlated
389 with flower size (Sargent et al. 2007; Goodwillie et al. 2010; Vasconcelos and Proença
390 2015). Although larger inflorescences produced both more flowers and larger fruits in
391 species in our sample, fruit size decreased with flower density. This indicates that
392 resource competition can underlay the interspecific negative relationships between the
393 number of flowers and fruit size.

394 Importantly, our study provides insights into how intraspecific size-number
395 trade-offs can translate into negative covariation between traits across species. The
396 consequences of this negative correlation between size and number at the inflorescence
397 level were not the same for all flower positions. We observed that increased flower
398 density led to decreases fruit size, especially at the innermost positions, resulting in a
399 higher FSD (fruit size difference between outer and inner fruits). Flower density might
400 thus amplify the effects of architectural constraints, which pervasively limit resources at
401 the innermost positions. Under these circumstances, theory predicts that a high resource
402 difference between flower positions can cause plants to allocate more resources to their
403 female function in flowers with more resources, and to their male function in resource-
404 depleted flowers (Brunet and Charlesworth 1995). This expectation agrees with the
405 positional pattern observed for the sex of flowers in Asteraceae inflorescences (Fig. 1),
406 where female unisexual flowers consistently appear at those earlier (or outer) floral
407 positions that generally have a higher resource supply, whereas male unisexual flowers
408 are displayed at the later (or inner) positions, which usually are the most resource-
409 limited positions (Alkio and Grimm 2003; Alkio et al. 2003; Torices and Méndez 2010).
410 Therefore, shifts in inflorescence traits modifying the density of flowers might
411 secondarily cascade to other important floral traits such as flower and fruit size.

412 Specially, flower density might have a role on the evolution of floral sex functions,
413 given its effects on fruit size and thus on the floral female performance.

414 Under the resource optimization hypothesis, floral sex specialization is expected
415 to entail an improvement in fitness compared to bisexual flowers. Previous studies
416 indicate that dioecious species have larger fruit set than cosexual species (Sutherland
417 1986) and larger seed size (Vamosi et al. 2008). While there is not a formal test on
418 dioecious species of the Asteraceae or in other groups where flowers are no longer the
419 functional unit, our results support that when the inflorescence is the main pollination
420 unit, architectural traits such as flower density may obscure direct comparisons between
421 dioecious and non-dioecious species, given its effect on fruit size. In the Asteraceae,
422 female unisexual flowers from both gynomonoecious and monoecious species had
423 significantly larger fruits than bisexual species, when the confounding effect of flower
424 density was factored out. Thus, our study provides support for an intrinsic advantage of
425 flower specialization at least in terms of female fitness.

426 Expectations of a negative covariation of traits across species assumes that
427 everything else remains equal. Vamosi et al. (2008) did not find any difference in seed
428 size between hermaphroditic and monoecious species. However it is unclear if
429 hermaphroditic and monoecious species in their dataset differed in inflorescence traits,
430 such as flower density, which could confound the effect of the floral sexual
431 specialization on seed size. Positive correlations between inflorescence parts might be
432 found if species differ in resource budget (the big house-big car effect, *sensu* Reznick et
433 al. 2000). In addition, additional selective factors on dispersal performance, which
434 usually occur at the fruit level, could indirectly drive shifts on inflorescence design. Our
435 study only revealed the evolutionary drivers in the specialization of floral sex functions
436 after taking into account other inflorescence traits that might otherwise have masked

437 those drivers. This underlies the strengh of a comparative approach for understanding
438 the mechanistic basis of the evolution of non-hermaphroditic sexual systems, in the line
439 of Diggle & Miller (2013), even when considering phylogenetic scales above the genus
440 level.
441
442 ***Conclusion***
443 Our results highlight the importance of considering architectural traits to
444 understand phenotypic diversity in modular organisms, which can have important
445 functional consequences (Herrera 2009 for a review). Architectural constraints may
446 have profound consequences in modular organisms such as plants, influencing how
447 male and female functions perform at different positions within one individual. In
448 particular, the sequential development of inflorescences and asymmetric competition
449 between early and late flowers lead to a gradient in the resource availability experienced
450 by individual flowers within the inflorescences. Thus, a separation of male and female
451 functions in different flowers might evolve not only to maximize mating patterns, but
452 also to optimize resource allocation.
453 This combination of architectural constraints and selection for optimal sex
454 allocation at the flower level could explain the pattern observed in large groups of
455 plants other than the Asteraceae. In particular, it might have an important role in the
456 evolution of floral sexual specialization of those species with inflorescences composed
457 of small flowers that work together as pollination units, such as species of the Apiaceae,
458 Dipsacaceae, some Fabaceae, or Eriocaulaceae. However, the effects of architectural
459 constraints might not be limited to those groups with very small flowers, since
460 inflorescences represent the actual unit of attraction in most angiosperms (Harder et al.
461 2004). Flowering plants usually aggregate their flowers in inflorescences, and these

inflorescences follow common development patterns. Similar comparative studies including inflorescence traits, sexual expression and fruit size variation across flowers will show us the generality of this mechanism in the evolution of unisexual flowers in Angiosperms.

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638 phenology affect pollination and fruit-set. *Am. J. Bot.* 69:585–594.
639

640 ## Tables

641 **Table 1.** Differences in inflorescence and fruit traits between inflorescences with
642 different degree of floral sexual specialization. *F* and *P* values were obtained after a
643 deviance analysis of the phylogenetic generalized linear models fitted for each
644 inflorescence trait, with the degree of floral sexual specialization within inflorescence
645 (hermaphroditism, gynomonoecy or monoecy) as the main factor. FSD is the
646 standardized fruit size difference between outer and inner fruits measured as the meta-
647 analytical effect size.

648

Inflorescence traits	*F*	d.f.	*P*
Capitulum diameter (mm)	7.27	2, 85	0.001
Number of flowers	3.65	2, 84	0.030
Flower density (no. flowers/mm^2)	6.99	2, 84	0.002
Outer fruit size (mm^2)	0.69	2, 77	0.505
Inner fruit size (mm^2)	2.01	2, 66	0.142
FSD	2.30	2, 64	0.109

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653 ## Figure Legends

654 **Figure 1. Sexual specialization in the Asteraceae inflorescences.** Three different
655 types of functionally hermaphroditic inflorescences and individuals can be observed in
656 the Asteraceae: hermaphroditic, gynomonoecious and monoecious. Allocation of
657 gametes to different flowers shows a gradient in floral sexual specialization from
658 hermaphroditic species (only bisexual flowers) to gynomonecious species (female and
659 bisexual flowers) and to monoecious species (female and male flowers). In Asteraceae,
660 sexual specialization (i.e., bi- vs. unisexuality in flowers) and sexual segregation within
661 inflorescences occur in concert. Lower panels show representative species from a)
662 hermaphroditic heads (*Tragopogon porrifolius* L.), b) gynomonoecious heads
663 (*Anacyclus valentinus* L.), and c) monoecious heads (*Tussilago farfara* L).

664

665 **Figure 2.** Phylogenetically controlled least-squares means (± 95% confidence interval)
666 of (a) inflorescence size (mm), (b) number of flowers, (c) flower density (no. flowers /
667 mm^2), (d) outer fruit size, (e) inner fruit size, and (f) Fruit Size Difference (FSD) for
668 different levels of sexual systems representing increasing levels of floral sexual
669 specialization within inflorescences: hermaphroditism, gynomonoecy and monoecy.
670 FSD is the standardized fruit size difference between outer and inner fruits measured as
671 the meta-analytical effect size. Means sharing the same superscript letter were not
672 significantly different at the $P < 0.05$ level.

673

674 **Figure 3.** Phylogenetic RMA regressions between fruit size and a) inflorescence size
675 (mm), b) number of flowers, and c) flower density (no. flowers / mm^2); and between
676 FSD and d) inflorescence size (mm), e) number of flowers, and f) flower density (no.
677 flowers / mm^2). In the upper row black dots and solid lines represent outer fruits

678 whereas white dots and dashed lines indicate inner fruits. Lines represent RMA slopes.
679 Phylogenetic standard regression slopes are showed in Supplementary Table 4.
680
681 **Figure 4.** Relationship between fruit size produced by female (filled dots and solid line)
682 and bisexual flowers (white dots and dashed line) and flower density. Female flowers
683 produced by monoecious species are shown as black dots whereas those produced by
684 gynomonoecious species are indicated by grey dots.