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3 **Fitness benefits and costs of floral advertising traits: insights from rayed and**
4 **rayless phenotypes of *Anacyclus* (Asteraceae)**

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24

25 **ABSTRACT**

26 **Premise of the study:** Ray flowers commonly observed in daisies' flowering heads are
27 a well-known example of advertising structures for enhancing pollinator attraction.

28 Despite this, ray loss has occurred in multiple lineages which still rely on pollinators,
29 suggesting that rayless phenotypes could also be adaptive for animal-pollination. Here,
30 we investigate the benefits and costs of these specialized floral advertising structures by
31 comparing rayed and rayless phenotypes in two hybridizing closely related species.

32 **Methods:** We assessed the advantages and costs of ray production in terms of floral
33 visitor's attraction, pollen limitation, and female reproductive success using the broad
34 natural variation on ray size and number at the contact zone of *A. clavatus* (rayed) and
35 *A. valentinus* (rayless). In addition, we experimentally explored the effect of rays under
36 controlled neighborhoods and the effect of ray removal on fruit production.

37 **Key results:** In sympatry, rayed phenotypes attracted significantly more visitors than
38 rayless plants, in which seed production was pollen limited. However, rayed phenotypes
39 did not show higher fruit set or seed production than rayless phenotypes. Fruit set and
40 seed production benefited from denser neighborhood displays and larger individual
41 floral displays, respectively. The removal of ray florets did not appear to enable
42 resource reallocation to fruit production.

43 **Conclusions:** Rayless heads compensated their lower visitation rate by means of a
44 higher number of flowers per head achieving similar fecundity levels to rayed plants.
45 The larger size of rayless heads might thus indicate an inflorescence-level trade-off
46 between attraction and fertility.

48 **Keywords:** Advertising-fertility trade-off; Asteraceae; Floral cost; Floral display;
49 Inflorescence; Pollinator attraction; Neighborhood floral display; Ray florets; Rayless
50 heads

51

52 **INTRODUCTION**

53 Animal pollinated plants invest a considerable amount of resources to advertise
54 their flowers and compete with other surrounding floral displays to attract pollinators.
55 Under pollen limitation, when pollination services are below the full reproductive
56 capacity of a plant, it is generally expected that plants with larger flowers are selected
57 for (Galen, 1989; Campbell, 1991; Herrera, 1993; Conner and Rush, 1996;
58 Parachnowitsch and Kessler, 2010). However, the production of larger structures for
59 attraction, such as petals, presents different types of costs. First, colorful petals
60 represent an investment of biomass and costly molecules such as pigments (Ashman
61 and Schoen, 1997; Mendéz, 2001), potentially leading to costs and trade-offs in terms
62 of fruit set, seed quality and seed germination (Andersson and Widén, 1993; Andersson,
63 1999, 2000, 2005, 2008; Castro et al., 2008). Second, larger floral displays attract
64 pollinators, but also herbivores (Knauer and Schiestl, 2017), nectar robbers (Rojas-
65 Nossa et al., 2016) and/or inefficient pollinators (Lau and Galloway, 2004; Hargreaves
66 et al., 2012; Koski et al., 2018). Finally, large floral displays frequently promote
67 successive visits within the same plant, thus promoting geitonogamous pollination and
68 ultimately reducing outcrossing, a phenomenon called “attractiveness dilemma”
69 (Klinkhamer and de Jong, 1993; Karron et al., 2009).

70 Ray flowers of the daisy family (Asteraceae) are a well-known example of
71 structures specialized in pollinator attraction. This family is easily recognized by its
72 inflorescence, the capitulum or head, which functions as a pollination unit (Burtt, 1977;
73 Lane, 1996). In many species, heads are characterized by tubular actinomorphic disc
74 florets clustered together and surrounded by a ring of florets with zygomorphic corollas,
75 the ray florets (Kim et al., 2008; Bello et al., 2013). The presence of rays has significant
76 consequences on pollination success in a number of species, mainly enhancing the

77 attractiveness of heads to pollinators (Lack, 1982; Celedón-Neghme et al., 2007;
78 Andersson, 2008), and consequently influencing the pollination success and the levels
79 of outcrossing (Marshall and Abbott, 1984; Sun and Ganders, 1990). Thus, the presence
80 of rays seems to provide an advantage for attracting pollinators, although the pollination
81 context, including pollinator abundance and individual floral display, may reduce this
82 effect (Andersson, 1996; Nielsen et al., 2002). Therefore, the efficiency of advertising
83 structures, such as rays may be context dependent.

84 Despite the observed advantages of rayed heads in attracting pollinators, rayless
85 species are frequent in Asteraceae. Several independent reversals towards rayless heads
86 have occurred in the evolution of this family, suggesting that rayless phenotypes could
87 also be adaptive for animal pollination (Bremer and Humphries, 1993; Torices and
88 Anderberg, 2009; Torices et al., 2011). Previous works have suggested that the
89 production of rays might entail a reduction of the resources available for fruit and seed
90 production (Andersson, 1999, 2008) and/or the attraction of more seed predators
91 (Fenner et al., 2002). Furthermore, as heads are usually visited by a larger number of
92 pollinators, rayed heads could attract a larger amount of less efficient pollinators than
93 rayless heads, reducing pollen transfer efficiency. Still, whether specific groups of
94 pollinators show a preference for different phenotypes (rayed *vs.* rayless) remains
95 poorly explored (but see Stuessy et al. 1986). Considering the evolutionary lability of
96 ray expression, ray polymorphisms represent a challenging model to explore the role of
97 individual and contextual components on the costs and benefits of specialized attractive
98 structures.

99 In this study, we provide an integrative approach combining observational and
100 manipulative experiments to investigate simultaneously the benefits and costs of
101 specialized floral advertising structures by comparing rayed and rayless phenotypes in

102 two closely related annual species of the genus *Anacyclus*, the rayed *A. clavatus* and the
103 rayless *A. valentinus*. These two species show a high degree of geographical overlap,
104 and several hybrid areas with pronounced variation in ray size and number (Fig. 1;
105 Humphries, 1979; Agudo, 2017). In sympatry, both rayed and rayless phenotypes
106 showed mixed ancestry precluding the attribution of rayed and rayless phenotypes to its
107 respective parental lineages (Agudo 2017). The presence of substantial phenotypic
108 variation, as commonly observed in hybrid zones, makes these areas ideal study cases to
109 evaluate the effect of floral traits on plant fitness (Hodges and Arnold, 1994; Campbell
110 et al., 1997; Campbell, 2003, 2008). Hence, using this broad natural variation in ray size
111 and number, we assessed the advantages and costs of ray production in terms of
112 pollinator attraction, pollen limitation, and female reproductive success, measured as
113 average fruit set per head and the total number of seed produced per plant. Additionally,
114 we experimentally explored the effects of rays on pollinator attraction and female
115 reproductive success by performing manipulations on ray phenotype and on the floral
116 display of neighboring congeners, i.e. the neighborhood floral display. Specifically, we
117 asked: (i) how do rays, individual floral display and surrounding neighborhood floral
118 display affect pollinator attraction?; (ii) do rayed and rayless phenotypes differ in levels
119 of pollen limitation?; (iii) does neighborhood floral display affect rayed and rayless
120 phenotypes differently?; and, (iv) do ray production and maintenance entail a cost in
121 terms of fruit production? Manipulations were performed on the entire plant, and
122 lifetime female fitness components were measured, providing estimates of the impact of
123 this floral trait on plant fitness.

124

125 **MATERIAL AND METHODS**

126 **Study species** — *Anacyclus* L. (Anthemideae, Asteraceae) exhibits exceptional variation
127 in floral morphology and sexual expression both within heads and among species which
128 arguably results from different evolutionary and hybridization events (Humphries,
129 1981). The species complex formed by *A. clavatus* (Desf.) Pers. and *A. valentinus* L.
130 (Fig. 1a, b), shows notable differences in floral morphology and, in areas where both
131 species coexist, a pronounced variation in the number and size of rays can be observed
132 (e.g. Fig. 1g, h; Bello et al. 2013). *Anacyclus clavatus* is usually found in disturbed
133 habitats, coastal beaches, open fields and roadsides, within the Circum-Mediterranean
134 Basin (Humphries, 1979). This species has gynomonoecious heads (i.e. female and
135 bisexual florets). Female florets are present at the outermost positions of the head,
136 displaying white rays (Fig. 1a), whereas yellow, bisexual disc florets with a tubular-
137 campanulate corolla are displayed in the central part of the head (Fig. 1a; Bello et al.
138 2013). *Anacyclus valentinus* is found in coastal areas in the Western Mediterranean,
139 occurring in disturbed habitats, sandy areas, lowlands, river banks, open fields and
140 roadsides (Humphries, 1979). This species also bears gynomonoecious heads, having
141 unisexual female flowers in the outermost positions of their heads, but these female
142 flowers display inconspicuous or no rays (discoid-like or rayless head, Fig. 1b,
143 Humphries 1979; Bello et al. 2013). Both species are inter-fertile and self-incompatible
144 (Humphries, 1981; Agudo, 2017), and bloom from February to July. After fertilization,
145 flowering heads from both species produce two types of achenes, i.e. one-seeded dry
146 fruits: flowers at the outermost positions, including both ray and disc outermost florets,
147 produce winged and heavier achenes, whereas the innermost disc flowers produce
148 lighter unwinged achenes (Torices et al., 2013; Afonso et al., 2014).

150 **Study sites** — This study was conducted during the spring of 2013 within the contact
151 zone of both species, around Torre del Mar (Andalusia, Spain). Three sites with a high
152 abundance of *Anacyclus* individuals were chosen, namely: i) *sympatric site* - an open
153 field, where both species grew and where intermediate phenotypes had previously been
154 observed ($36^{\circ} 43' 48.875''$ N, $4^{\circ} 6' 8.154''$ W); ii) *rayed site* - an abandoned area on the
155 edge of a road, containing only *A. clavatus* ($36^{\circ} 45' 4.186''$ N, $4^{\circ} 5' 58.289''$ W); and iii)
156 *rayless site* - an open field area next to planted palm trees, containing only *A. valentinus*
157 ($36^{\circ} 43' 50.516''$ N, $4^{\circ} 6' 4.697''$ W). Ecological conditions in these sites were similar.
158 Vegetation was characterized by several ruderal herbaceous species such as *Leontodon*
159 *longirostris* (Vill.) Mérat (Asteraceae), *Hirschfeldia incana* (L.) Lagr.-Foss. subsp.
160 *incana* (Brassicaceae), *Chrysanthemum coronarium* L. (Asteraceae), and *Echium*
161 *creticum* subsp. *granatense* (Coincy) Valdés (Boraginaceae).

162

163 **Experimental design** — We established two approaches: 1) a purely observational
164 survey and 2) an experimental manipulation of floral phenotypes. In both, we
165 investigated the benefits and costs of rayed vs. rayless phenotypes. The observational
166 survey was conducted in the *sympatric site*, where we observed plant-pollinator
167 interactions with the minimum amount of disturbance and characterized the naturally
168 occurring neighborhood floral display, i.e. the floral display of neighboring congeners.
169 In the *rayed* and *rayless sites*, we established the experimental manipulations of both
170 floral phenotypes and neighboring plants to study both phenotypes (rayed vs. rayless)
171 under two contrasting backgrounds. In addition, two complementary experiments were
172 performed at all three sites to explore the extent of pollen limitation on fruit production
173 and the potential costs of ray production in terms of fruit set and fruit size.

174 1. *Observational survey at the sympatric site* — The sympatric site was characterized by
175 extraordinary phenotypic variability in ray traits including the presence of intermediate
176 and both rayed and rayless phenotypes (Bello et al. 2013; Agudo 2017). Previous
177 surveys of the genetic structure in the contact zone between both species including this
178 site showed that both rayed and rayless phenotypes frequently had mixed ancestries in
179 sympatry (Agudo 2017). Therefore, among hybrids in this site, ray number is highly
180 variable and it is not indicative of the proportional genetic contribution from each
181 parental lineage. As floral phenotype is not useful to identify species in sympatric sites,
182 we will always refer to rayed and rayless phenotypes instead of rayed and rayless
183 species for this site. We haphazardly selected 103 plants and, to maximize the efficiency
184 of floral visitor censuses, we established 27 patches including 2 to 7 plants. Selected
185 plants were tagged and characterized based on plant and inflorescence traits and
186 neighborhood floral display. Plant traits included plant height (the distance from the
187 ground to the tallest part of the plant), plant volume (estimated as the plant's largest
188 diameter, measured parallel to the ground, multiplied by its perpendicular axis, and by
189 the plant's height) and individual floral display (the total number of blooming heads;
190 quantified every 2-3 days throughout the field season). Inflorescence traits included
191 head size (total diameter of the head, from the tip of a ray to the tip of the opposite ray),
192 disc size (diameter of the yellow central disc), ray number and ray length. In addition,
193 we quantified neighborhood floral display by counting the number of open *Anacyclus*
194 inflorescences surrounding each tagged plant within a 0.5 m radius (quantified also
195 every 2-3 days throughout the field season).

196 2. *Ray and neighborhood manipulations in rayed and rayless sites* — In the single-
197 phenotype sites, we experimentally manipulated the phenotypes and the neighborhood
198 floral display. In the *rayed site* we removed rays to create rayless plants in a set of

199 selected plants, while in the *rayless site* we added artificial rays to rayless plants (Fig.
200 1c-f).

201 At the *rayed site*, we selected 20 patches of three nearby plants, separated by ca. 1-2 m.
202 Each triplet included one rayed phenotype (control phenotype), an individual that had
203 its rays removed (experimental rayless phenotype; Fig. 1d, f) and one individual with
204 artificial rays (artificially rayed phenotype; Fig. 1c). In the experimental rayless
205 phenotypes, ray florets were completely removed with tweezers when its elongation
206 started and before its complete expansion and anthesis. Artificial rays were made with
207 white synthetic paper (polyethylene; paper commonly used to print scientific posters)
208 and were added to the heads to mimic the rayed phenotype (Fig. 1c) following a similar
209 approach successfully employed by Nielsen and colleagues (Nielsen et al. 2002). To
210 minimize variation of plant traits and neighborhood structure, we selected plants with
211 similar traits (height, dimension) and floral displays. In addition, we removed
212 inflorescence buds produced after the beginning of the experiment to keep plants
213 homogeneous during the experiment. The inclusion of an artificially rayed phenotype in
214 the *rayed site* served as a procedural control to assess the effect of artificially rayed
215 phenotypes at the *rayless site*.

216 At the *rayless site*, we selected 20 pairs of nearby plants, separated by ca. 1-2 m from
217 each other. Within each pair one individual remained as the rayless phenotype (control
218 phenotype) while the other was equipped with artificial rays (artificially rayed
219 phenotype; Fig. 1c, e). Similar plants were chosen and were kept homogeneous
220 throughout the field season as described above for the *rayed site*.

221 The neighborhood floral display was studied in all the patches of plants selected above.
222 Additionally, we removed neighboring *Anacyclus* plants in a 1 m radius in 10 additional
223 plant pairs in both sites (the artificially rayed phenotype at the rayed site was not

224 considered for this experiment) and compared solitary rayed and rayless phenotypes
225 with plants with untouched neighboring conditions.

226 *3. The effect of ray presence on pollen limitation* — To determine whether pollen
227 limitation differentially affects fruit production of rayed and rayless plants, we
228 experimentally hand-supplemented flowering heads with pollen in the *sympatric site*
229 (20 rayless and 20 rayed) and in the *rayed* and *rayless sites* (20 individuals in each). On
230 each plant, we labelled two heads at the same phenological stage: one was hand
231 pollinated with outcross pollen collected each day from at least five different plants
232 across each site (Pollen supplementation - PS); the other was used as a control (C). The
233 heads used in the experiment were selected from the central upper part of the plant to
234 avoid other confounding effects. Additionally, control heads were further compared
235 with heads from nearby unmanipulated plants and showed similar fruit set (results not
236 shown), indicating that reallocation of resources for pollen supplemented heads did not
237 significantly decrease the number of fruits produced in control heads of manipulated
238 plants (Wesselingh, 2007). Regrettably, most of the plants in the *rayless site* were eaten
239 by cattle and only four individuals ripened fruits, and therefore we did not get any
240 estimates of pollen limitation for the *rayless site*. In the *sympatric site*, three plants (two
241 rayless and one rayed) died before producing any fruit.

242 *4. The effect of ray presence on fruit production* — We assessed the cost associated to
243 develop and maintain ray structures on fruit production by means of a ray removal
244 experiment. For this, we selected 21 plants in the *rayed site*. For each plant, we selected
245 three heads in early developmental stages for the following treatments: 1) ray removal
246 and hand-supplementation with outcross pollen (RR), 2) control and hand-
247 supplementation with outcross pollen (PS), and 3) control with open pollination (C).
248 Pollen supplementation assures that fruit production was not pollen limited, whereas the

249 control left for open pollination was subjected to natural levels of pollination. At bud
250 stage, ray florets were completely removed using tweezers, and thus, they were
251 removed before disc florets developed completely. The number of fruits and fruit
252 weight were quantified in the laboratory. Since *Anacyclus* species produce two types of
253 fruits within one head (Torices et al., 2013), we tested the potential effect of ray
254 removal on the size of both types of fruits separately.

255

256 **Floral visitor censuses** — A reference collection of *Anacyclus* floral visitors was
257 gathered in a preliminary survey of pollinator assemblage within the contact zone in the
258 spring of 2012. Floral visitor observations were performed during central hours (from
259 10:30 to 18:00, GMT+1) of warm and sunny days from 30-Mar to 26-April 2013 during
260 the flowering period of both study species, throughout the three studied sites. Plant
261 patches were observed during intervals of 5 min. Observers were positioned 1 to 2 m
262 distance from the plant group and used small-range binoculars to avoid disturbing the
263 foraging activity of floral visitors to tagged plants. A floral visit was only recorded
264 when there was a direct contact between the forager and the sexual organs of the head
265 (anthers and/or stigmas). Only approaches to each individual plant were considered,
266 whereas consecutive visits within one individual plant were not considered. A total of
267 7,885 min of observation time was performed in the *sympatric site* (75-95 min/plant;
268 mean = 90 mins). A total of 4,265 min of observation time was performed in the in the
269 *rayed site* (55-65 min/plant; mean = 60 mins). A total of 3,760 min of observation time
270 was performed in the *rayless site* (50-80 min/plant; mean = 70 mins). Insect
271 identification was based on the reference collection. New pollinator taxa were collected
272 with a capture net or a vacuum container for subsequent identification. Smaller insects

273 were preserved in ethanol 70%, whereas larger ones were air-dried (Appendix S1; see
274 the Supplementary Data with this article).

275 Due to the high number and diversity of floral visitor species on *Anacyclus* plants
276 (Appendix S1), we assessed preferences of particular pollinator groups for the studied
277 plant traits. *Pollinator group* was defined as a group of pollinators which tended to
278 interact with flowers in a similar way and was established following the methodology
279 employed in Gomez *et al.* (2008). Using this method we obtained the following groups:
280 ants, beeflies, beetles, bugs (Hemiptera), butterflies, hoverflies, large bees, large flies,
281 small bees, small flies and wasps. The relative abundance of some of these groups was
282 very low (e.g. we observed only 7 plant-Hemiptera interactions throughout the whole
283 field season; Appendix S1) thus hindering the statistical analyses; therefore, we merged
284 some groups and excluded others with very few interactions, ending up with four main
285 represented groups: bees (including small and large bees), large flies, hoverflies, and
286 small flies. Bees included individuals from approximately 2-12 mm (from head to
287 abdomen), including members from the Apidae family such as *Apis mellifera*,
288 *Anthophora* sp. and *Eucera longicornis*, but also *Lasioglossum* sp. (Halictidae) and
289 some unidentified species from Megachilidae and Sphecidae. Hoverflies included
290 individuals from approximately 9-15 mm (from head to abdomen), from the Syrphidae.
291 Specifically, this included *Eristalis tenax*, *Eristalis arbustorum*, *Eupeodes* sp.,
292 *Episyrphus* sp., *Sphaerophoria* sp., *Syritta pipiens*, *Chrysotoxum* sp., and a few non-
293 identified hoverfly species. Large and small flies included members of the
294 Calliphoridae, Anthomyzidae, Tachinidae, Scathophagidae and few unidentified
295 species. Large and small flies included individuals larger or smaller than 2 mm (from
296 head to abdomen), respectively. Finally, in the *rayed site* there was very low visitation
297 and no pollinator groups were established due to statistical constraints. Detailed

298 information about floral phenotypes and morphospecies interactions are displayed in
299 Appendix S1.

300

301 ***Female reproductive success*** — We assessed two components of female reproductive
302 success: the average fruit set per head of each individual and the total number of seeds
303 produced by the plant. Fruit set refers to the proportion of viable achenes considering
304 the total number of flowers per head. After ripening, at least five flowering heads were
305 sampled from each plant. The proportion of viable and non-viable achenes was
306 investigated for all the sampled heads under a stereomicroscope. The number of achenes
307 was quantified in one quarter of the head and extrapolated for the entire head.
308 Preliminary exploration showed that estimation of seed production and fruit set in one
309 quarter of the inflorescence was highly correlated with the total value obtained from the
310 whole head ($n = 10$, $r = 0.96$, $P \leq 0.001$). The total number of seeds in those plants with
311 more than five heads was calculated by multiplying the average number of seeds per
312 head from the five collected heads with the total number of heads produced by each
313 individual plant. Relative fitness was calculated within site by dividing by the maximum
314 seed number.

315

316 ***Statistical analyses*** — Broadly, data were analyzed using general linear mixed models
317 (GLMM), using the ‘lme4’ package (Bates et al. 2014) in the R 3.0.1 software (R Core
318 Team, 2013). Before fitting any model we carefully analyzed and explored the data,
319 searching for correlation and multicollinearity among variables (Appendix S2, S3).
320 After fitting each model, we performed model validation routines plotting residuals
321 against fitted values and against each explanatory variable in the model (Zuur et al.,

322 2009), and estimating the overdispersion coefficient for Poisson models using
323 parametric bootstrapping (Harrison, 2014). We included an observation-level random
324 effect to deal with overdispersion when it was detected (Harrison, 2014). All analyses
325 included plant, either alone or together with patch. Unless otherwise noted, deviance
326 type-II tests of fixed factors were shown. In addition, differences between factor levels
327 were assessed using least square means values of the models using pairwise
328 comparisons with ‘lsmeans’ package (Lenth, 2013). Below, the statistical analyses
329 followed in each experiment are presented in detail:

330 *Observational survey at the sympatric site* — First, we assessed the effect of floral
331 phenotype (rayed vs. rayless) on floral visitors, fruit set and total seed production. These
332 three variables were fit as response variables in GLMMs wherein floral phenotype,
333 individual and neighborhood floral display were included as explanatory variables.
334 Visitation rate was modelled with a Poisson distribution and a log link function; fruit set
335 was modelled with a binomial distribution and a logit link function; and total seed
336 production using a gaussian function with identity link. Additionally, we created
337 additional models for each pollinator group: Bees, Large Flies, Hoverflies, and Small
338 flies (Tables S4, S5).

339 Second, we explored direct and indirect links between the three response variables by
340 Structural Equation Modelling (SEM) using the ‘lavaan’ package (Rosseel, 2012). We
341 fitted one model for each phenotype (rayed and rayless phenotype; Appendix S4).
342 Direct effects were calculated through direct relationships between variables. Indirect
343 effects were calculated through the multiplication of all the indirect effects between
344 variables. In both models, floral visitation, fruit set and total seed production were
345 considered as endogenous variables (i.e. response variables in these models). Ray size,
346 disc size, individual and neighborhood floral display were scaled and added as

347 exogenous variables (i.e. independent variables in these models). Because disc size did
348 not correlate with visitation rate neither significantly affected visitation rate (results not
349 shown), we did not include the direct effect of disc size on visitation rate to avoid model
350 saturation. In addition, ray number was not included in the model. Nevertheless, ray
351 number was highly correlated with ray size (Appendix S3), and when both variables,
352 number and size, were regressed against visitation rate, only ray size significantly
353 affected floral visitors (results not shown). Model fit was analyzed through Root Mean
354 Square Error of Approximation and its associated *p*-value (rayless model: $\chi^2 = 0.008$, df
355 = 1, *p* = 0.993, RMSEA = 0.000; rayed model: $\chi^2 = 1.338$, df = 1, *p* = 0.282, RMSEA =
356 0.075

357 *Ray and neighborhood manipulations in rayed and rayless sites*—We studied the effects
358 of the established manipulations on floral visitors and fruit set. For that, all models
359 included floral phenotype (rayed *vs.* rayless) and neighborhood floral display (control
360 *vs.* neighborhood removed) as explanatory variables, and further included the
361 interaction between phenotype and neighborhood display. The interaction was assessed
362 using type-III tests. As the interaction term was not statistically significant, main effects
363 were assessed by type-II tests. In addition, we tested whether artificially rayed plants
364 were efficiently simulating natural rays by assessing floral visitation rate and fruit set of
365 both rayed phenotypes from the *rayed site*.

366 *The effect of ray presence on pollen limitation*—We fitted a GLMM where fruit set was
367 modelled using a binomial distribution and a logit link function, wherein floral
368 phenotype and pollen supplementation treatment were included as explanatory
369 variables.

370 *The cost of ray presence on fruit set and weight*—We assessed the effect of ray removal
371 on fertility and fruit weight in the rayed site. Fruit set was modelled with a binomial
372 distribution, whereas fruit weight was modelled with a gaussian distribution. The
373 experimental treatment was included as the explanatory variable.

374 **RESULTS**

375 ***Observational survey at the sympatric site — Rayed vs. rayless phenotypes*** — Rayed
376 phenotypes in the sympatric site produced a higher number of heads, and thus a higher
377 individual floral display (Appendix S2). However, rayed heads had significantly smaller
378 disc diameters and fewer disc flowers per head than rayless heads (Appendix S2).
379 Consequently, the total number of disc flowers per plant did not differ between rayed
380 and rayless plants (Appendix S2).

381 The presence of rays had a significant impact on floral visitor attraction at the sympatric
382 site, so that rayed plants attracted significantly more floral visitors than rayless plants
383 (Fig. 2a, Table 1). Dipteran groups (large flies, hoverflies and small flies) drove this
384 trend, as they visited rayed plants significantly more often compared with rayless ones
385 (Appendix S6, Appendix S5). Bees visited plants with larger individual floral displays
386 more frequently, irrespectively of its floral phenotype (Appendix S5).

387 Rayed phenotypes did not show a higher probability of setting fruits nor produced more
388 seeds per plant than rayless phenotypes (Fig. 2b, c, Table 1). By contrast, as the number
389 of neighboring heads increased, i.e. the neighborhood floral display, fruit set and total
390 seed production increased for both floral phenotypes (Table 1). Higher individual floral
391 displays significantly increased total seed production in both phenotypes (Table 1).

392

393 ***Direct and indirect effects of floral traits, individual and neighborhood floral displays***
394 ***on floral visitors, and female reproductive success*** —For rayed phenotypes, ray length
395 positively affected visitation rate of floral visitors, and had a direct effect on fruit set
396 (Table 2; Appendix S4a). Nevertheless, the total effect of ray size on fruit set was not
397 significant (Table 2; Appendix S4a). Individual floral display had a significant direct

398 effect on floral visitors and total seed production (Table 2) and neighborhood floral
399 display positively influenced floral visitor's attraction and fruit set but not the total seed
400 production (Table 2). The SEM for rayed phenotypes accounts for 39%, 22% and 44%
401 of variation of floral visitors, fruit set and total seed production, respectively.

402 In rayless plants, individual floral display had a significantly positive effect on the
403 visitation rate of floral visitors, fruit set and total seed production (Table 2; Appendix
404 S4b). Disc size had a significant positive effect on fruit set and total seed production
405 (Table 2). However, neighborhood floral display did not directly or indirectly affect
406 either of the female reproductive success components (Table 2). The SEM for rayless
407 phenotypes accounts for 24%, 26% and 61% of variation of floral visitors, fruit set and
408 total seed production, respectively.

409

410 ***Ray and neighborhood manipulations in rayed and rayless sites*** — Experimental
411 removal of rays in the rayed site had no significant effects on floral visitor attraction
412 (Fig. 3a; Table 3), nonetheless ray removal significantly decreased fruit set (Fig. 3b;
413 Table 3). In the rayless site, artificially rayed plants did not receive significantly more
414 visits than control rayless individuals (Fig. 3c; Table 3); and, rayless plants had
415 significantly higher fruit set than experimental rayed ones (Fig. 3d; Table 3). Results
416 concerning the artificially rayed phenotype should be considered cautiously as we
417 observed that artificially rayed plants in the rayed site showed a lower (but only
418 marginally significant) visitation rate than control naturally occurring rayed plants
419 (Appendix S7a), and they had significantly lower fruit set (Appendix S7b) suggesting
420 that the artificial ray addition might have some negative effect on both floral visitors
421 and fruit set compared to naturally rayed plants.

422 Neighborhood removal affected visitation rate and fruit set in the rayless site (Table 3).
423 In this rayless site, those plants in which their neighboring flowering conspecifics were
424 removed received marginally fewer floral visitors and produced significantly lower fruit
425 sets than plants with control neighborhoods (Fig. 3c, d). Despite the lack of a significant
426 interaction effect between ray manipulation and neighborhood removal (Table 3),
427 solitary plants in which rays were either removed or added when they were growing in
428 the other phenotype site showed the lowest mean fruit set compared to the rest of
429 experimental groups within each site (Fig. 3b, d).

430

431 ***The effect of ray presence on pollen limitation*** — Pollen supplementation significantly
432 increased fruit set on rayless phenotypes of the *sympatric site* ($n = 36$, LRT = 9.06, $P =$
433 0.003), but not in the rayed phenotypes occurring at the same site ($n = 38$, LRT = 0.23,
434 $P = 0.634$; Fig. 4). In the *rayed site*, pollen supplementation did not significantly
435 increased fruit set compared to control open pollinated heads ($n = 42$, LRT = 1.14, $P =$
436 0.285), and we did not have sufficient data to determine the effect of pollen
437 supplementation at the rayless site.

438

439 ***The effect of ray removal on fruit production*** — Ray removal increased neither fruit set
440 nor fruit mass (Fig. 5, Appendix S8). However, outer winged fruits in control open
441 pollinated heads were heavier than in heads that were pollen-supplemented, whether or
442 not their rays were removed (Fig. 5b). This pattern was not observed for the inner
443 unwinged fruits, where no differences were observed between experimental groups (Fig.
444 5c).

445

446 **DISCUSSION**

447 This study was focused on understanding how the presence or absence of rays,
448 in combination with the individual and the neighborhood floral displays affect pollinator
449 attraction and plant reproductive success. Additionally, we assessed whether rayed and
450 rayless heads were pollen limited and explored potential costs of ray development in
451 fruit production. We observed that when the two species naturally occur in sympatry,
452 rayed phenotypes attracted significantly more floral visitors and, consequently, fruit
453 production on rayed heads was not pollen limited in contrast to rayless plants in which
454 fruit set was limited by the available pollination resources. However, rather than the ray
455 production, both larger individual floral display, and denser local neighborhood floral
456 displays were more determinant factors driving plant reproductive success. Therefore,
457 our analysis showed that the difference in attractiveness between rayed and rayless
458 phenotypes in sympatry did not result in consequences for female fecundity at the plant
459 level. Rayless plants were able to compensate the lack of rays by producing large heads
460 with many florets. Thus, our results provide insights on the fitness consequences of
461 advertising structures and on the potential inflorescence-level trade-offs associated with
462 the production of these structures.

463 Rays are effective advertising structures to attract different groups of insects.
464 The presence of rays had a generally positive effect on pollinator attraction in several
465 different study species (Lack, 1982; Marshall and Abbott, 1984; Sun and Ganders,
466 1990; Nielsen et al., 2002; Celedón-Neghme et al., 2007; Andersson, 2008). In
467 agreement with these previous studies, we observed that, in a natural hybrid site where
468 ray size showed high variation, larger rays enhanced floral visitation rate and floral
469 visitors preferred rayed phenotypes compared to rayless ones. However, our results also
470 indicated that rays might not affect all pollinator groups in the same way. *Anacyclus*

471 species were clearly generalist-pollinated, with flowers receiving visits from an
472 extensive variety of taxonomic groups, including at least 17 different families, from
473 several insect orders (Appendix S1). Within this wide diversity, we found contrasting
474 floral preferences in different pollinator groups. In this sympatric site, rays primarily
475 attracted Dipteran groups. Although it was suggested that Hymenopterans are the main
476 pollinators of Asteraceae (Lane, 1996), Dipteran pollinators, mainly hoverflies are also
477 described as important pollinators of rayed species such as *Achillea ptarmica*
478 (Andersson, 1991) and *Senecio vulgaris* (Abbott and Irwin, 1988). Moreover, a ray
479 removal experiment in *Helianthus grosseserratus*, a rayed species pollinated mostly by
480 dipteran and hymenopteran species, resulted in a reduction in Diptera visitation rates but
481 not in Hymenoptera, revealing an important role of rays in the attraction of this
482 particular group of insects (Stuessy et al., 1986). The differential effect of rays on the
483 behaviour of specific pollinator groups might thus result in different mating patterns not
484 only in single phenotype sites but also between rayed and rayless phenotypes in sites of
485 admixture.

486 The ray removal/addition experiment performed in this study focusing on two
487 closely related species, one rayed and the other rayless did not replicate the pattern
488 observed in the sympatric site. This mismatch between the visitation rates observed in
489 rayed and rayless phenotypes under natural and manipulated approaches might have
490 resulted from unsuccessful manipulations but also by a frequency dependent effect of
491 phenotype manipulations. Because manipulations were performed on sites where only
492 one floral phenotype originally occurred, either rayed or rayless, pollinators might have
493 been initially visiting these patches and choosing flowering heads driven by traits other
494 than rays. In addition, although we followed the same approach of Nielsen et al. (2002)
495 to create artificial rays, our ray addition manipulation produced unexpected negative

496 effects in terms of both pollinator attraction and fruit set compared to naturally rayed
497 plants (Appendix S7). This is most likely explained by the complexity of these
498 inflorescences (Thomas et al., 2009), including complex UV reflection (Ron et al.,
499 1977) and micro-character variation (Lane, 1996), which can, independently or
500 synergistically, influence pollinator attraction. The microstructure of the chosen paper
501 might thus not have effectively mimicked natural rays. Alternative solutions such as
502 potted plants or artificial populations where the frequency of phenotypes can be also
503 balanced should be used in future studies.

504 Despite the apparent disadvantage of rayless phenotypes compared to rayed ones
505 in attracting pollinators in sympatric sites, rayless individuals showed the same female
506 reproductive success levels than rayed plants. This outcome might result from
507 compensation by other traits. In particular, rayless heads had significantly larger disc
508 diameters and consequently more flowers per head than rayed heads (Appendix S2).
509 This larger number of flowers of rayless heads might then offset its lower visitation rate
510 resulting eventually in similar seed production when compared with the rayed
511 phenotype. The higher numbers of flowers per head has two mating consequences: an
512 increase of available ovules to be fertilized per blooming unit, and an increase of the
513 temporal availability of these ovules to be fertilized. Heads are blooming units
514 displaying several mature flowers at the same time and maturing sequentially (Harris,
515 1995; Bello et al., 2013). Single flowers are protandrous, releasing pollen grains during
516 the first day, while the stigma is exposed only at the end of the first flowering day
517 (Bello et al., 2013). Stigma remain receptive during several days until they are fertilized
518 with outcrossed pollen (Agudo, 2017). Within the same head, several rows of stigmas
519 can thus be receptive allowing a single pollinator visit to fertilize several flowers within
520 a single head. As rayless plants have more flowers per head, floral visits to rayless

521 heads might fertilize more ovules in a single visit than in rayed heads. Furthermore, a
522 longer flowering duration of rayless heads might increase the total number of visits to
523 each flower head, diminishing or cancelling the advantage conferred by higher
524 attractiveness of the rayed phenotype. Consequently, plants receiving fewer visits per
525 unit of time, such as rayless phenotypes, could still secure a sufficient amount of pollen,
526 ultimately ensuring the fertilization of the same number or even more ovules per head.

527 The difference in number of flowers per head between rayed and rayless heads
528 may also indicate a potential inflorescence-level trade-off between advertising
529 investment and head fertility. The pattern of rayless phenotypes having larger heads
530 than rayed phenotypes was evident in the hybrid population at the sympatric site, but is
531 also evident in the two pure species (Appendix S9). However, we did not observe a
532 negative correlation between the ray length and the number of disc florets in the
533 sympatric site as it might be expected under this trade-off. The study of trade-offs using
534 variation from natural populations can be misleading given the effect of variation on
535 resource acquisition which usually lead to positive correlations (Worley and Barrett,
536 2001). Thus, it is also expected that individuals growing in better microsites would have
537 more resources, potentially resulting in larger heads with both longer rays and more
538 flowers per head. Nonetheless, ray length and the number of flowers per head or disc
539 size were not significantly positively correlated either (Appendix S3). These genetic
540 trade-offs can be assessed by using controlled progenies grown under common garden
541 conditions. In fact, the usage of controlled progenies from experimental crossings
542 between rayed and rayless plants of the daisy *Senecio jacobaea* has provided some
543 evidences for this trade-off between advertising investment and head fertility
544 (Andersson 2001). In the F2 and backcrossed populations of experimental hybrids
545 between rayed and rayless plants, a principal component analysis showed that the ray

546 traits (e.g., number, size and width) and disc floret number loaded in opposite directions
547 (Andersson 2001). Additional support for this trade-off between advertising investment
548 and head fertility come from the analysis of allocation patterns in other currencies apart
549 from biomass and interspecific comparisons of flower size and number per
550 inflorescence. Phosphorous allocation to rays was negatively correlated with the
551 allocation to the female sexual organs after controlling by inflorescence size (Torices
552 and Méndez, 2014), and a negative correlation between flower size and the number of
553 flowers per inflorescence has been detected across species(Sargent et al., 2007;
554 Vasconcelos and Proen  a, 2015). Although theoretical models usually assume the
555 existence of a trade-off between advertising and primary sexual structures
556 (Charlesworth and Charlesworth, 1987; Sakai, 2013) , the evidence is still inconclusive.

557 The existence of this trade-off was not supported by the ray removal experiment
558 and our study failed to find direct costs of ray production on female reproductive
559 success (but see Andersson, 1999). Specifically, we did not find that removing rays
560 increased the number of viable fruits. This result may suggest that *Anacyclus* plants
561 might not be able to reallocate the resources from ray florets into the production of new
562 fruits once ray florets buds have been formed. Flower buds are developed sequentially,
563 so the ray flower buds are formed before than the disc flowers (Bello et al., 2013). After
564 the determination of disc flower buds, the development of the ray starts, precluding any
565 reallocation from rays to new flowers. Similar results were obtained in *Senecio*
566 *jacobaea* (Andersson, 2001), where manipulative ray removal did not lead to an
567 increase of head fertility. In *Achillea ptarmica*, ray removal had a stronger effect on
568 pollinator attraction than in fruit set (Andersson, 1991). This might suggest also that
569 pollen dispersal and siring success could be more affected than female fertility.

570 Plant reproductive success was strongly affected by the neighborhood floral
571 display rather than by the focal-plant advertising phenotype. In both naturally rayed and
572 rayless species, focal plants surrounded by a larger number of other conspecifics
573 showed higher probabilities of setting fruits. However, the effects of the surrounding
574 neighborhood floral display are very complex. Several studies have shown how
575 neighborhood conditions affect a focal plant, concluding that factors such as spatial
576 scale (Bartkowska and Johnston, 2014; Hegland, 2014), neighbour density (Makino et
577 al., 2007; Hegland, 2014), and area and/or density combined with different population
578 sizes (Williams, 2007; Dauber et al., 2010) affect pollinator visitation rates.
579 Additionally, an increase in pollinator attraction has been observed when plants are
580 surrounded by kin rather than by unrelated individuals (Torices et al., 2018). The
581 density of conspecifics may even interact with individual floral display of focal plants.
582 For example, in *Phlox hirsuta*, higher levels of conspecific density reversed the negative
583 effects of larger individual floral displays at lower densities (Ruane et al., 2014). The
584 neighborhood removal experiment herein presented showed that the removal of
585 *Anacyclus* neighborhood plants reduced plant fruit set, particularly on the less frequent
586 floral phenotype. That is, rayless and rayed solitary plants in rayed and rayless sites,
587 respectively, suffered a greater reduction in fruit number than the common floral
588 phenotype in each site when conspecific neighbors were removed (Fig. 3b, d).
589 Neighborhood floral display could have complex effects beyond mere density effects,
590 altering pollinator behavior and individuals' floral display efficiency, and ultimately
591 might also have consequences on mating patterns and genetic structure of the
592 populations.

593

594 **CONCLUSIONS**

595 In conclusion, advertising traits such as ray florets are effective structures in
596 enhancing pollinator attraction. However, plants can display different strategies to
597 assure ovule fertilization and maximize fecundity. Our study indicates that *Anacyclus*
598 plants might have two distinct strategies to get their ovules fertilized: increase pollinator
599 attraction by means of larger rays, or increase the number of reproductive units per
600 blooming head. Despite the effect of the individual floral phenotype on pollinator
601 attraction, the neighborhood floral display near each focal plant significantly determined
602 female reproductive success. The complexity of the effect of the local context,
603 accounting for many potential interactions between different phenotypes requires
604 further studies. Experimental approaches aimed to manipulate and control these factors
605 will be valuable steps towards understanding the functional consequences of intra-
606 specific interactions on mating patterns and floral evolution.

607

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622

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624 RT and IA conceived the experiment and obtained funding. JC, AAg, AAf, SC, RT
625 collected the data. JC and RT did the statistical analyses. JC and RT drafted the first
626 version of the manuscript and the remaining authors contributed to its revision.

627

628 **DATA ACCESSIBILITY**

629 Data are archived in FigShare. doi: 10.6084/m9.figshare.6839993

630

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824 **Supporting Information**

825 Additional Supporting Information may be found online in the supporting information
826 section at the end of the article.

827 Appendix S1. Number of visits of floral visitors of *Anacyclus* spp. capitula in each
828 studied site.

829 Appendix S2. Descriptive characterization of plant and inflorescence traits and
830 pollination context of *Anacyclus* individuals in the sympatric site.

831 Appendix S3. Pearson correlation coefficient of plant and inflorescence traits and
832 neighborhood structure for *Anacyclus* individuals in the sympatric site.

833 Appendix S4. Structural Equation Models for rayed plants (a) and rayless plants (b) at
834 the sympatric site.

835 Appendix S5. The effect of floral phenotypes (rayed and rayless) on specific floral
836 visitor groups at the sympatric site.

837 Appendix S6. Pollinator visits to rayed (closed dots) and rayless (open dots) phenotypes
838 in the sympatric (A), rayless (B) and rayed site (C).

839 Appendix S7. Visitation rate and fruit set of artificial rayed heads compared to control
840 rayed plants.

841 Appendix S8. The effect of ray removal on fruit production.

842 Appendix S9. Number of flowers and head diameter in allopatric populations of
843 *Anacyclus clavatus* and *A. valentinus*.

Table 1. The effect of floral phenotypes (rayed and rayless) on floral visitors, fruit set and total seed production at the sympatric site
 based on generalized linear mixed model analyses. Floral phenotype (rayed vs. rayless), individual and neighborhood floral displays are included as explanatory variables. Plant identity was included as a random variable for floral visitation rate and fruit set, whereas patch was included for total seed production. An observation-level random effects (OLRE) was included to model floral visitation rate to cope with the overdispersion of this model. Degrees of freedom (*Df*), Chi-Square (χ^2), variance and standard deviation (SD) are provided. Statistically significant differences ($P < 0.05$) are shown in bold. A sign before the χ^2 value indicates the direction of the effects for significant individual and neighborhood floral displays.

853 **Table 2. Total, direct and indirect effects of neighborhood and individual floral display and floral traits on the attraction of floral**
 854 **visitors and plant reproductive success.** Standardized direct (DE) and indirect (IE) effects and their sum (TE) were estimated by structural
 855 equation models separately for rayed and rayless plants in the sympatric site. Significant effects are shown in bold. *, $P < 0.05$; **, $P < 0.01$; ***,
 856 $P < 0.001$

857

858

| Variables | <i>Floral visitation rate</i> | | | <i>Fruit set</i> | | | <i>Total seed production</i> | | |
|-----------------------------|-------------------------------|----|----------------|------------------|-------|---------------|------------------------------|---------------|----------------|
| | DE | IE | TE | DE | IE | TE | DE | IE | TE |
| <i>Rayed plants</i> | | | | | | | | | |
| Individual floral display | 0.48*** | - | 0.48*** | -0.12 | -0.08 | -0.20 | 0.66*** | -0.07 | 0.59*** |
| Disc size | - | - | - | -0.09 | - | -0.09 | 0.18 | -0.02 | 0.16 |
| Ray size | 0.28** | - | 0.28** | 0.24* | -0.05 | 0.19 | 0.16 | 0.03 | 0.19 |
| Neighborhood floral display | 0.33** | - | 0.33** | 0.39** | -0.06 | 0.33** | -0.12 | 0.06 | -0.05 |
| Floral visitation rate | - | - | - | -0.17 | - | -0.17 | -0.05 | -0.04 | -0.09 |
| Fruit set | - | - | - | - | - | - | 0.24* | - | 0.24* |
| <i>Rayless plants</i> | | | | | | | | | |
| Individual floral display | 0.42** | - | 0.42** | 0.39** | -0.05 | 0.34** | 0.42*** | 0.23** | 0.65*** |
| Disc size | - | - | - | 0.36** | - | 0.36** | 0.30** | 0.14* | 0.44*** |
| Neighborhood floral display | -0.24 | - | -0.24 | 0.28* | 0.03 | 0.31* | 0.04 | 0.06 | 0.1 |
| Floral visitation rate | - | - | - | -0.12 | - | -0.12 | 0.23* | -0.05 | 0.18 |
| Fruit set | - | - | - | - | - | - | 0.38** | - | 0.38** |

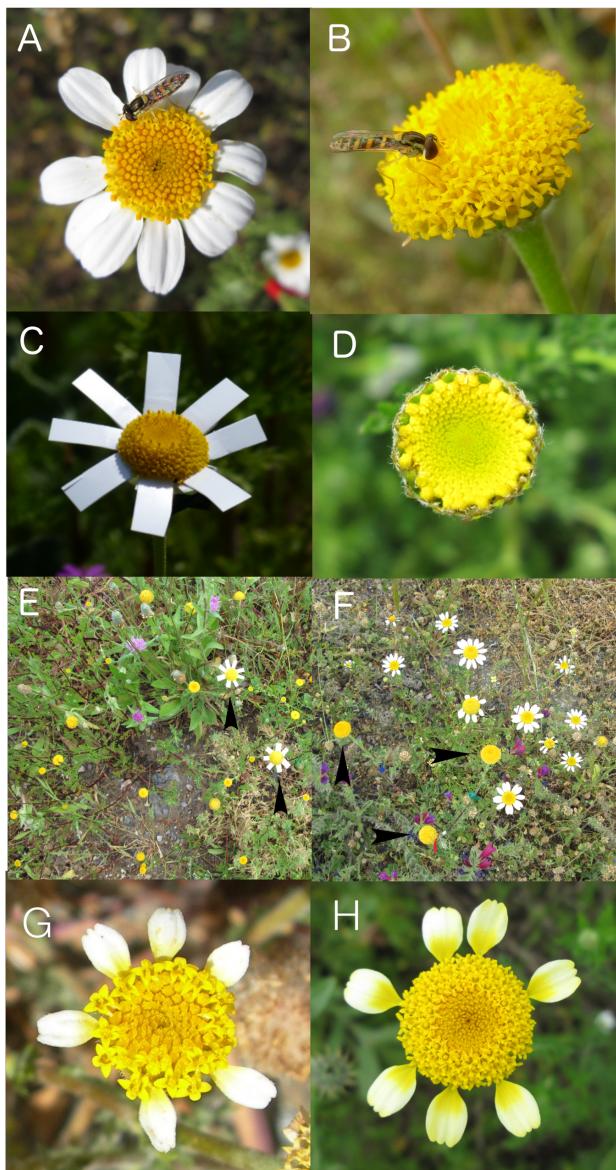
859 **Table 3. Experimental manipulation of floral phenotype and neighborhood display on floral visitors and fruit set at rayed and rayless**
 860 sites. GLMMs were fitted independently for each response variable and for size to assess the effects of floral phenotype (rayed vs. rayless),
 861 neighborhood removal (neighborhood removed vs. control) and its interaction. The interaction was assessed using type-III tests. As the
 862 interaction term was not statistically significant, main effects were assessed by type-II tests. Plant identity and patch were included as a random
 863 variable. An observation-level random effects (OLRE) was included to model floral visitation on the rayless site to cope with the overdispersion
 864 of this model. Degrees of freedom (*Df*), Chi-Square (χ^2), variance and standard deviation (SD) are provided. Statistically significant differences
 865 ($P < 0.05$) are shown in bold.
 866

| Variables | <i>Df</i> | Rayed site | | | | Rayless site | | | |
|--------------------------|-----------|------------------------|----------------------|----------------------|----------------------|------------------------|----------------------|-------------|--------------|
| | | Floral visitation rate | | Fruit set | | Floral visitation rate | | Fruit set | |
| | | χ^2 | <i>P</i> | χ^2 | <i>P</i> | χ^2 | <i>P</i> | χ^2 | <i>P</i> |
| <i>Fixed</i> | | | | | | | | | |
| Floral phenotype (F) | 1 | 0.20 | 0.651 | 9.86 | 0.002 | 0.40 | 0.527 | 5.14 | 0.023 |
| Neighborhood removal (R) | 1 | 0.49 | 0.484 | 2.39 | 0.122 | 3.61 | 0.057 | 3.90 | 0.048 |
| F x R | 1 | 0.11 | 0.742 | 1.29 | 0.256 | 0.87 | 0.352 | 0.09 | 0.763 |
| <i>Random</i> | | | | | | | | | |
| Plant (Patch) | | Variance | SD | Variance | SD | Variance | SD | Variance | SD |
| | | 0.50 | 0.71 | 0.76 | 0.87 | 0.07 | 0.27 | 1.57 | 1.25 |
| Patch | | 8.1×10^{-7} | 8.9×10^{-4} | 1.1×10^{-9} | 3.3×10^{-5} | 2.4×10^{-9} | 4.9×10^{-5} | 0 | 0 |
| OLRE | | - | - | - | - | 2.84 | 1.69 | - | - |

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869 **Figures**

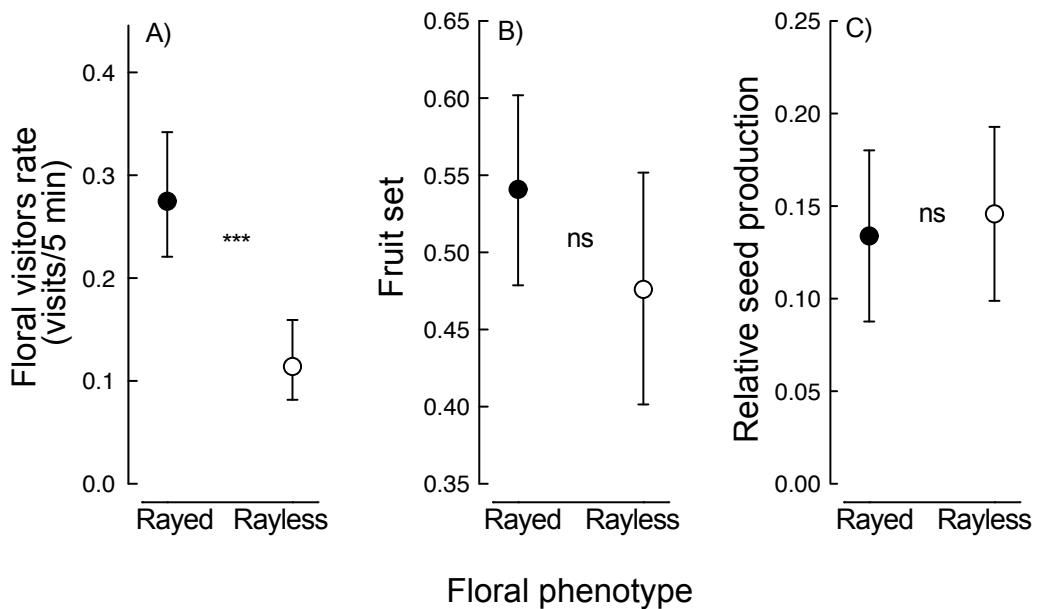


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871 **Figure 1. *Anacyclus* natural variation and manipulated set-up used in this study:**

872 A) rayed head, *A. clavatus*; B) rayless head, *A. valentinus*; C) artificially rayed head; D)
873 artificially rayless head; E) artificially rayed heads (highlighted with black arrows); F)
874 artificially rayless heads (highlighted with black arrows). G-H) intermediate phenotypes
875 observed in populations where *A. clavatus* and *A. valentinus* grow in sympatry.

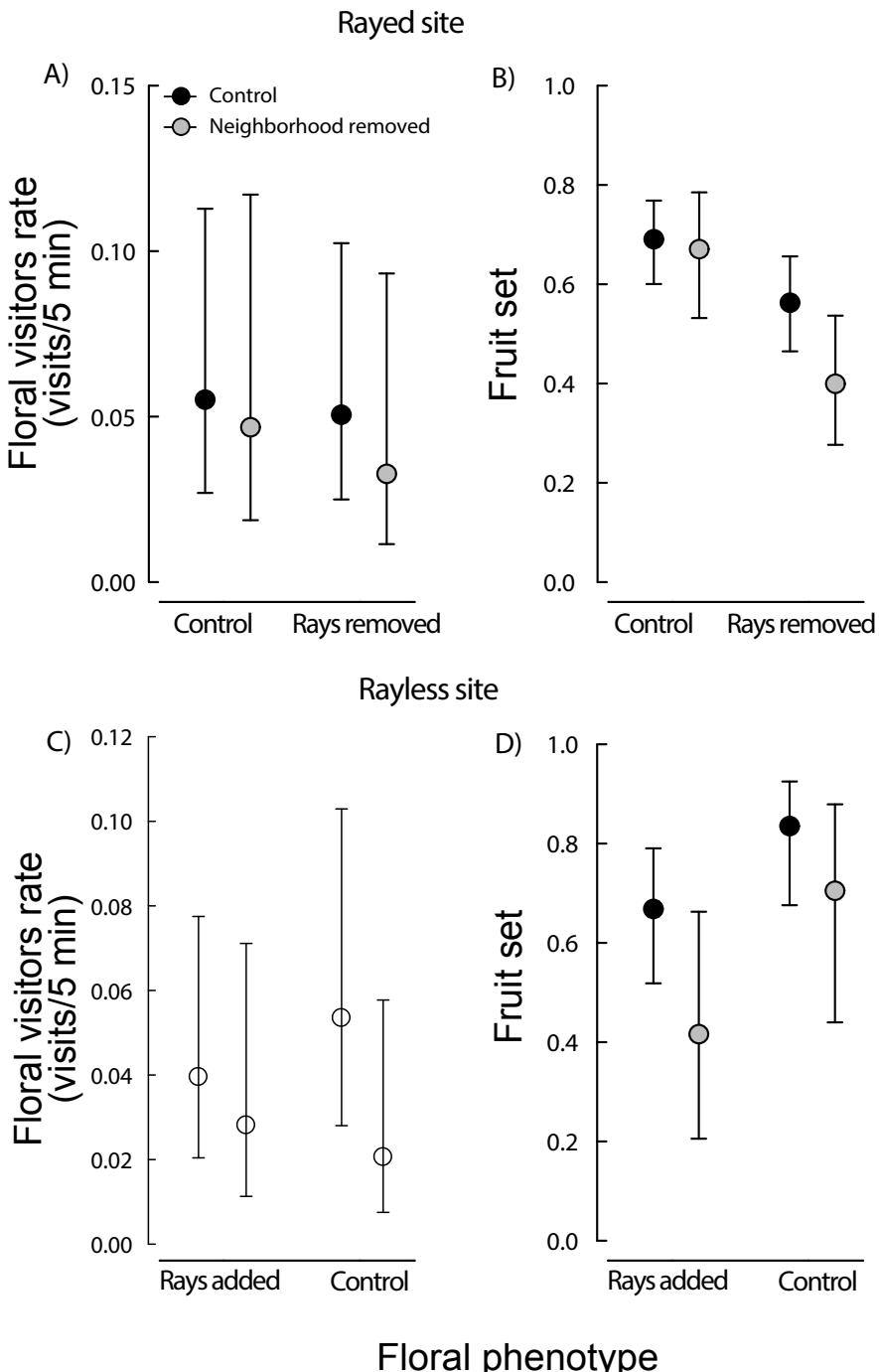
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Figure

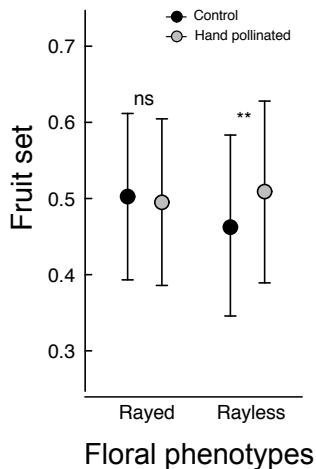
878 **2. The effect of floral phenotypes (rayed vs. rayless) on (A) floral visitation rate,**
 879 **(B) fruit set and (C) total seed production in the sympatric site.** Least square means
 880 ($\pm 95\%$ confidence intervals) of visitation rate (floral visitors per 5 min.), fruit set and
 881 total seed production. Total seed production was scaled by the highest value in the
 882 population. The details of the GLMM models are provided in Table 1. ns, non-
 883 significant at $P > 0.05$, ***, $P < 0.001$.



884

Floral phenotype

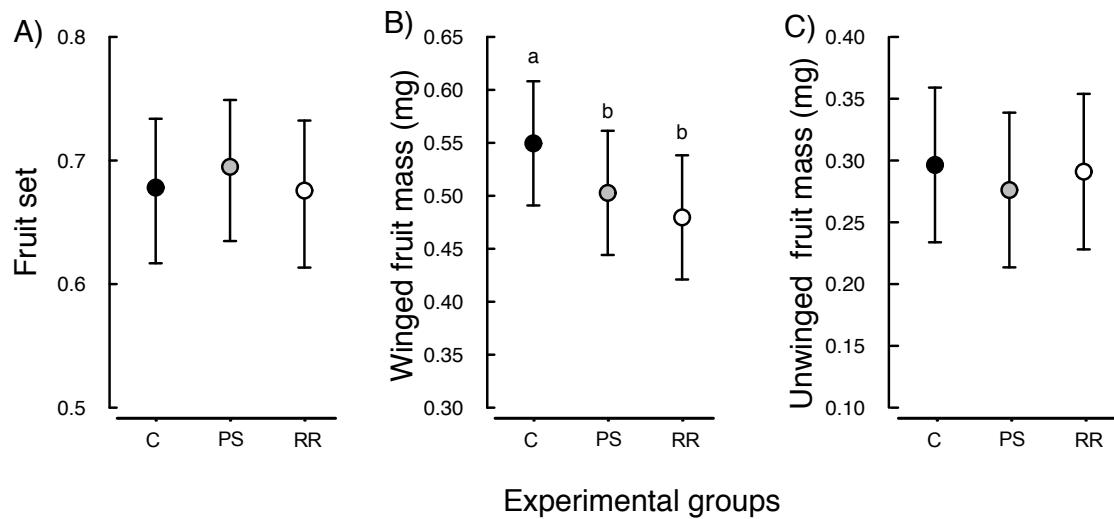
885 **Figure 3. The effect of floral phenotype and neighborhood display manipulation on**
 886 **floral visitors and fruit set.** Least square means results ($\pm 95\%$ confidence intervals) of
 887 visitation rate (A,C) and fruit set (B,D) for rayed and rayless phenotypes under control
 888 and removed neighborhoods in both: the rayed (A,B) and the rayless site (C,D). The
 889 statistical significance of each factor is indicated in Table 3.



890

891 **Figure 4. Hand supplementation with pollen to rayed and rayless plants in the**
 892 **sympatric site.** Least square means ($\pm 95\%$ confidence intervals) of fruit set in control
 893 and hand-supplemented heads in both rayed ($n = 38$, LRT = 0.23, $P = 0.634$) and rayless
 894 phenotypes ($n = 36$, LRT = 9.06, $P = 0.003$). Statistical tests were performed
 895 independently for each phenotype. ns, non-significant at $P > 0.05$, **, $P < 0.01$.

896



899 **Figure 5. The effect of ray removal on fruit set (a) and fruit weight (b, c) in the**
900 **rayed site.** Least square means ($\pm 95\%$ confidence intervals) of a) fruit set and fruit
901 weight of b) winged and c) unwinged fruits. C: control heads (closed dots); PS: pollen-
902 supplemented inflorescences (grey dots); and, RR: pollen-supplemented inflorescence
903 with rays removed (open dots). Means with different letters were significantly different
904 at $P < 0.05$. No letter is displayed in those cases where no significant differences were
905 found ($P > 0.05$). Statistical significance of each factor is indicated in Appendix S8.