

1                   **CAN SEXUAL SELECTION CAUSE DIVERGENCE IN**  
2                   **MATING SYSTEM-RELATED FLORAL TRAITS?**

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11                   Short title: LANKINEN AND STRANDH — SEXUAL SELECTION AND FLOWERS

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18        *Premise of the Research.* The wide diversity of floral traits seen among plants is shaped by  
19 neutral and selective evolutionary processes. In outcrossing species, sexual selection from  
20 competing pollen donors is expected to be important for shaping mating system-related traits  
21 but empirical evidence is scarce. In a previous evaluation of experimental evolution lines  
22 crossed with either one or two pollen donors (monogamous, M, or polyandrous, P, lines) at  
23 early floral stages in mixed-mating *Collinsia heterophylla* (Plantaginaceae), P showed  
24 enhanced pollen competitive ability and reduced maternal seed set compared to M, in  
25 accordance with sexually antagonistic evolution of pollen. Here, we asked whether the  
26 presence of sexual selection during pollen competition affect mating system-related floral  
27 traits in the same lines.

28        *Methodology.* We compared flowering start, timing of anther-stigma contact (as an  
29 indication of timing of self-pollination), timing of stigma receptivity and first seed set  
30 between M and P, and with a source line, S (starting material). The former three traits are later  
31 in outcrossers than in selfers of *Collinsia*. The latter trait was expected to be earlier in P than  
32 in M because of sexual selection for early seed siring of pollen.

33        *Pivotal Results.* Artificial polyandry for four generations resulted in later flowering start  
34 and later anther-stigma contact in P compared to M, and the latter trait was intermediate in S.  
35 Thus, P appeared more ‘outcrossing’ than M. Stigma receptivity did not differ between lines.  
36 First seed set was earlier in P than in M, as expected from sexual selection.

37        *Conclusions.* Our results from *C. heterophylla* experimental evolution lines suggest that a  
38 component of sexual selection during outcross pollination could enhance the patterns of floral  
39 divergence commonly found between outcrossers and selfers.

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## Introduction

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43 The wide floral diversity in angiosperms is considered largely to be caused by geographic  
44 variation in pollinator-mediated selection (Kay and Sargent 2009; Van Der Niet et al. 2014;  
45 Armbruster 2014). Because differences in mating system (e.g. outcrossing vs. self-  
46 fertilization, or their combination in mixed mating) is correlated with floral and  
47 developmental traits (Karron et al. 2012; Barrett 2013), selective forces influencing mating  
48 system also contribute to angiosperm floral diversity. Traits such as small flower size and  
49 reduced separation of male-female functions in space and time (herkogamy and dichogamy)  
50 are thought to directly favour selfing as reproductive assurance when pollinator visits are  
51 unpredictable (Lloyd 1979; Lloyd and Schoen 1992; Opedal 2018). These traits can also be  
52 connected to rapid maturation or reduced investment in cross-pollination in selfers (Snell and  
53 Aarssen 2005; Sicard and Lenhard 2011).

54 It has recently been argued that not only maternal outcrossing rate but also mate diversity  
55 and individual variation in mating success could contribute to mating system selection  
56 (Barrett and Harder 2017). An influence of sexual selection on mating system evolution could  
57 be important to consider for a more complete understanding of the selective forces affecting  
58 mating system, because it is expected that sexual selection should be relatively more  
59 important in outcrossing taxa than in selfing taxa (Mazer et al. 2010). For example, due to  
60 stronger sexual selection or parental conflicts in outcrossers than in selfers, previous studies  
61 have suggested that pollen competitive ability during pollen competition in the pistil (Mazer  
62 et al. 2018), pistil barriers to hybridization (Brandvain and Haig 2005) and male vs. female  
63 antagonistic influence on seed provisioning (Willi 2013) are increased in outcrossers  
64 compared to in selfers. While sexual selection can influence floral traits (Delph and Ashman

65 2006; Moore and Pannell 2011; Dai and Galloway 2013), the contribution of this selective  
66 force on floral trait divergence between outcrossers and selfers is usually not considered.

67 The mixed-mating herb *Collinsia heterophylla* belongs to a genus with extensive variation  
68 in mating system, from self-pollinating to mixed-mating species (Armbruster et al. 2002;  
69 Kalisz et al. 2012). Most outcrossing species have larger flowers, delayed selfing brought  
70 about by loss of herkogamy at late developmental stages and delayed stigma receptivity,  
71 separating timing of male and female reproductive functions (fig. 1a). These floral traits also  
72 appear to be associated with slower plant developmental rate and later flowering start (Elle et  
73 al. 2010). In *C. heterophylla*, variation in outcrossing rate is substantial and populations with  
74 higher outcrossing rates have later timing of stigma receptivity (Strandh et al. 2017). In line  
75 with the results at the genus level, delayed selfing measured as timing of anther-stigma  
76 contact was shown to be genetically correlated with timing of stigma receptivity, and tended  
77 to be genetically correlated with flowering start (Lankinen et al. 2017a).

78 The timing of stigma receptivity has been proposed to be affected by a sexual conflict in *C.*  
79 *heterophylla* (Lankinen and Kiboi 2007) (fig. 1b). A sexual conflict involves opposing  
80 selection pressures in males and females due to divergent evolutionary interests of the sexes  
81 (Parker 1979). When such conflicts occur between different loci in the two sexes (interlocus  
82 conflict), selection is expected to move trait values of one sex closer to its fitness optimum,  
83 causing a direct fitness cost in the other sex (Parker 1979; Arnqvist and Rowe 2005). Sexual  
84 selection for a trait value leading to increased reproductive success can generate sexual  
85 conflict (Kokko and Jennions 2014). The sexual conflict over timing of stigma receptivity in  
86 *C. heterophylla* involve i) pollen ability to sire seeds early to secure paternity when stigmas  
87 are partially receptive, and ii) a recipient cost of reduced early seed set (Lankinen and Kiboi  
88 2007; Madjidian et al. 2012). We recently studied the evolutionary outcome of this conflict by  
89 producing experimental evolution lines by crossing recipients at early floral development with

90 two pollen donors (polyandrous, P) or with one pollen donor (monogamous, M) (fig. 2a) for  
91 four generations (Lankinen et al. 2017b). Recipients always contributed with one offspring to  
92 the next generation, thus limiting selection on recipients compared to on pollen donors. We  
93 showed that P plants produced pollen with a higher proportion of successful crosses at early  
94 floral stages and faster tube-growth rate, and reduced seed set compared to M plants  
95 Lankinen et al. 2017b) . These results are in accordance with enhanced sexual conflict and  
96 antagonistic evolution of P pollen in response to sexual selection (Arnqvist and Rowe 2005).

97 In the current study, we continued analysing the experimental evolution lines in *C.*  
98 *heterophylla* (Lankinen et al. 2017b), asking whether the presence of sexual selection during  
99 pollen competition also affected mating system-related floral traits (fig. 2b). Because of the  
100 importance of timing of stigma receptivity in relation to both mating system and sexual  
101 conflict in *C. heterophylla*, we hypothesized that sexual selection on early pollen competitive  
102 ability could affect this trait as well as two other correlated floral traits, flowering start and  
103 timing of anther-stigma contact. We hypothesized that the P line would show later flowering  
104 start, later anther-stigma contact, and later stigma receptivity, but earlier timing of first seed  
105 set, i.e. stigma receptivity influenced by both pollen and pistil (Table 1). The prediction for  
106 later stigma receptivity is based on our previous finding of a negative correlation between  
107 male and female influence on first seed set (Hersh et al. 2015), suggesting later stigma  
108 receptivity when selecting for earlier pollen influence on stigma receptivity, given that this  
109 association is genetically determined. We also compared M and P to a source line, S,  
110 representing starting material outcrossed at late floral stages for one generation Lankinen et  
111 al. 2017b), i.e. S was not selected for early performance of pollen and pistil traits. A  
112 comparison with S could give an indication of the direction of evolutionary change of M and  
113 P from the source.

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## Materials and methods

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### *Study species and experimental evolution lines*

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120 *Collinsia heterophylla* Buist (Plantaginaceae) is a hermaphroditic, self-compatible winter-  
121 annual herb native to California (Newson 1929; Neese 1993). The species is pollinated by  
122 long-tongued, nectar-feeding bees (Armbruster et al. 2002). Flowers are zygomorphic with  
123 five-lobed corollas forming an upper and a lower lip arranged in whorls on spikes. They  
124 contain four epipetalous stamens and one single-style pistil that develop into seed capsules  
125 containing up to 20 seeds (Armbruster et al. 2002; Madjidian and Lankinen 2009). During  
126 floral development, the four anthers mature and dehisce at a rate of approximately one per day  
127 during four consecutive days (fig. 1a). The stigma becomes receptive around day 2-3 after  
128 flower opening and the style elongates, placing the stigma in contact with the mature anthers  
129 providing an opportunity for delayed selfing at about the same time as stigma receptivity  
130 (Armbruster et al. 2002; Madjidian and Lankinen 2009) (fig. 1a).

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132 Plants used in this experiment originated from seeds collected by maternal family ( $N =$   
133 200) in a large natural population in Mariposa county (situated at N 37.50196; W 120.12360)  
134 in 2008. As described in Lankinen et al. (2017b), we used this material to create an outcrossed  
135 source ( $S$ ,  $N = 177$  maternal families) population in 2010 for our experimental evolution study  
136 in 2010-2013. We performed controlled hand-pollinations at floral developmental stage four,  
137 representing day four after flower opening, i.e. when stigmas were fully receptive. Flowers  
138 were emasculated at flower opening (= stage zero). The traits investigated in Lankinen et al.  
139 (2017b) and anther-stigma contact, stigma receptivity and first seed set estimated in the  
current study (fig. 2b) were similar to previous greenhouse studies using plant material from

140 the same population (Madjidian and Lankinen 2009; Hersh et al. 2015), despite storage of  
141 seeds.

142 From S we created two evolved lines; i) monogamous line (M,  $N = 135$  maternal families)  
143 crossed with one pollen donor and ii) polyandrous line (P,  $N = 142$  maternal families) crossed  
144 with two pollen donors (fig. 2a) by conducting hand-pollinations on emasculated flowers for  
145 four generations in 2010-2012 (Lankinen et al. 2017b). The evolved lines were unreplicated.  
146 We sampled a large number of genotypes within each treatment to capture a high degree of  
147 the natural genetic variation. This will minimize the impact of genetic drift in experimental  
148 evolution (Fuller et al. 2005). To impose sexual selection on early siring success of pollen in  
149 partially receptive pistils, we conducted crosses twice per flower at early floral stages 1 and 2,  
150 i.e. day 1 and 2 after flower opening (fig. 2a). We hand-pollinated four flowers per recipient  
151 plant involving two different pollen donors (see a more detailed description in Lankinen et al.  
152 (2017b)). One of the seeds generated per plant gave rise to the next generation, thus reducing  
153 selection on the female reproductive function compared to selection on the male function. All  
154 experimental plants were raised from cold-stratified seeds and grown in an insect-free  
155 greenhouse.

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#### 158 *Estimates of floral traits among experimental lines*

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160 We assessed four floral traits of the S line as well as of the two evolved lines M and P (fig.  
161 2b). We recorded flowering start as number of days since the first plant, independent of line,  
162 started flowering ( $N$  families = 21 in S, 25 in M, 29 in P,  $N = 3-8$  plants per family). Timing  
163 of anther-stigma contact, as an indication of timing of self-pollination, was assessed by noting  
164 the floral developmental stage (= number of dehisced anthers) when the stigma was in contact

165 with the open anthers ( $N$  families = 20-22 per line,  $N = 2$  plants per family,  $N = 2$  flowers per  
166 plant and floral stage 1-4). Timing of stigma receptivity was determined in a droplet of 3%  
167 hydrogen peroxide (Kearns and Inouye 1993) in emasculated flowers at stage 1-4 (=day after  
168 flower opening) ( $N$  families = 12-17 per line,  $N = 1$  plants per family,  $N = 1-2$  flowers per  
169 plant and floral stage). Vigorous bubbling on the stigmatic surface (unharmed and pollen free  
170 tissue) suggests activity of stigmatic peroxidase, which has been shown to correlate with  
171 presence of pollen tubes in the pistil following hand-pollination in this species (Lankinen et  
172 al. 2007).

173 Timing of first seed set was calculated from one-donor hand-pollinations performed in  
174 emasculated flowers at day 1-4 after flower opening ( $N = 9$  recipients and 6 pollen donors per  
175 line,  $N = 16$  crosses per recipient involving 2 donors, and 2 flowers per stage and donor)  
176 (Lankinen et al. 2017b). Four h after the crosses the stigma and upper part of the style were  
177 removed to ensure that seed formation only occurred in flowers with stigmas receptive at the  
178 time of the cross.

179 We analyzed differences in the measured traits i) among S, M and P, or ii) M and P using  
180 ANOVAs (type III sums of squares) in SPSS (SPSS 2016). Because our main focus was to  
181 investigate M and P divergence, the latter analysis was performed when no significant  
182 differences were detected among the three lines, to evaluate differences between M and P  
183 potentially masked by inclusion of S. When more than one plant was estimated per family,  
184 we used a nested model including line (fixed) and family (random) nested within line. In other  
185 cases, we only included line. Differences among lines were determined by Tukey tests.  
186 Timing of first seed set was square-root transformed. Models were evaluated for normality  
187 and homogeneity of the residuals.

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## Results

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192 Flowering start and timing of anther-stigma contact showed divergence between M and P  
193 following four generations of experimental evolution (Table 2, M-P: flowering start,  $P =$   
194 0.034; anther-stigma contact,  $P = 0.001$ ). In accordance with our hypotheses, both traits were  
195 later in P than in M (fig. 3a,b). Our source S had earlier flowering start than the evolved lines  
196 (S-M and S-P:  $P < 0.001$ , fig. 3a), but no difference was found for timing of anther-stigma  
197 contact ( $P > 0.13$ , fig. 3b).

198 Contrary to our hypothesis, timing of stigma receptivity did not differ among all three lines  
199 (Table 2, fig. 3c) or between M and P ( $F_{1,28} = 0.026$ ,  $P = 0.87$ ). Variability was higher in P  
200 than in M ( $F$ -test;  $P = 0.037$ ,  $N = 30$ ) and in S ( $P = 0.002$ ,  $N = 26$ ), which was an unexpected  
201 result.

202 Day of first seed set following one-donor pollinations, showed, as predicted, earlier  
203 formation of seeds in P than in M (Line:  $F_{1,16} = 4.90$ ,  $P = 0.042$ , Recipient nested within line:  
204  $F_{16,54} = 0.691$ ,  $P = 0.79$ , fig. 3d). There was no significant difference among all three lines  
205 (Table 2).

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## Discussion

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210 While sexual selection has been studied in plants for several decades, this mode of selection is  
211 still not a well-integrated concept in plant evolution theory, including the theory on mating  
212 system evolution (Lankinen and Karlsson Green 2015). In the current study we investigated a  
213 potential link between sexual selection and floral traits related to the mating system in *C.*  
214 *heterophylla*, a mixed mating species belonging to a genus with extensive variation in mating

215 system and associated floral traits (Armbruster et al. 2002; Kalisz et al. 2012). To study how  
216 the presence of sexual selection impacts evolution of floral traits, we compared monogamous  
217 (M) and polyandrous (P) experimental evolution lines, both outcrossed at early floral stages  
218 (Lankinen et al. 2017b). A previous analysis of M and P showed that the presence of sexual  
219 selection in P led to higher levels of sexual conflict with increased pollen competitive ability  
220 and reduced seed set. With the help of other previous studies in *C. heterophylla* on genetic  
221 and phenotypic correlations between floral traits (Hersh et al. 2015; Lankinen et al. 2017a) we  
222 made specific predictions regarding the estimated traits flowering start, timing of anther-  
223 stigma receptivity, stigma receptivity (all three later in P vs M, Table 1) and first seed set  
224 (earlier in P vs M, Table 1).

225 In *C. heterophylla*, flowering start and timing of anther-stigma contact, the latter as an  
226 indication of timing of self pollination, showed divergence between M and P. Both traits were  
227 later in P than in M, which was in line with expectations from genetic correlations between  
228 these two traits and between timing of anther-stigma contact and stigma receptivity (Lankinen  
229 et al. 2017a, Table 1). Thus, P appeared more ‘outcrossing’ than M (Elle et al. 2010; Kalisz  
230 et al. 2012; Strandh et al. 2017). The source line (S) had earlier flowering start than both M  
231 and P. This result may imply evolution of later flowering start in both M and P, which were  
232 produced at early floral stages compared to S. We cannot, however, exclude that the earlier  
233 flowering start in S could be related to the previously found reduction in seed germination  
234 rate and number of flowers in S (Lankinen et al. 2017b), which may have resulted from longer  
235 storage of S seeds than seeds from M and P. Anther-stigma contact in S was intermediate  
236 between M and P. For this trait S was similar to that found in other greenhouse studies using  
237 plant material from the same population (Madjidian and Lankinen 2009). This suggests that  
238 divergence in anther-stigma contact was caused by P becoming later and M becoming earlier  
239 than S. We can hypothesize that not only the presence of sexual selection but also the absence

240 of sexual selection during outcrossing can impact divergence of pollen and floral traits.  
241 Interestingly, in *Clarkia xantiana* strong pollen limitation resulted in disruptive selection  
242 through female and male fitness, as reduced herkogamy and protandry increased female  
243 fitness while both large and small petal area increased male fitness (Briscoe Runquist et al.  
244 2017). It would be highly informative with more studies on the influence of sexual selection  
245 on mating system-related traits also in other study systems.

246 Timing of stigma receptivity did not differ significantly between M and P. This result was  
247 contrary to expectation (Table 1). First seed set, a proxy for stigma receptivity influenced by  
248 both pistil and pollen, was earlier in P than in M, as predicted from the previous analyses of  
249 M and P (Lankinen et al. 2017b) suggesting that P pollen was more successful at siring seeds  
250 in partially receptive pistils. This result, in combination with a detected negative relationship  
251 between male and female influence on first seed set within individual plants (Hersh et al.  
252 2015), indicated that we could expect later stigma receptivity in P. While the mean of stigma  
253 receptivity did not differ between M and P, variability of this trait was higher in P compared  
254 to in both M and S. This result was surprising. One possible explanation is that there was  
255 disruptive selection acting on this trait in P, favoring either early or late stigma receptivity. In  
256 *Drosophila melanogaster*, experimental evolution, involving disruptive selection by  
257 alternating up and down selection, increased phenotypic variation in wing shape, while  
258 fluctuating and stabilizing selection instead decreased the variation (Pélabon et al. 2010). We  
259 do not have support for disruptive selection on stigma receptivity. We could, however,  
260 hypothesize contrasting effects of direct influence of pollen (early pollen-based influence will  
261 lead to earlier receptivity, Lankinen et al. 2017b) and indirect genetic covariance (negative  
262 correlation between pollen- and pistil-based influence on stigma receptivity, Hersh et al.  
263 2015). Interestingly, several previous studies in *C. heterophylla* found that stigma receptivity

264 is more variable than anther-stigma contact (Lankinen et al. 2007; Madjidian and Lankinen  
265 2009; Hersh et al. 2015).

266 Despite that timing of stigma receptivity was not significantly later in P, we surmise that  
267 the later response in both flowering start and timing of anther-stigma contact in P was a  
268 consequence of genetic correlations among traits (Lankinen et al. 2017a). While flowering  
269 start was not significantly genetically correlated with the other two traits (Lankinen et al.  
270 2017a), it is possible that these three traits are genetically linked with plant developmental  
271 rate. Other studies indicate that selection for rapid development can be correlated with rapid  
272 flower maturation (Mazer et al. 2004; Snell and Aarssen 2005; Elle et al. 2010). However, a  
273 recent study suggested low levels of genetic covariances between herkogamy and other floral  
274 traits across 17 species representing 10 families (Opedal et al. 2017). Evolvability of  
275 herkogamy was estimated to be 9.07%, which was an order of magnitude greater than  
276 evolvabilities of the male and female organs that are components of herkogamy, and of flower  
277 size. Because these results suggest that herkogamy has a high potential to respond to natural  
278 selection, it is possible that this trait is only weakly influenced by a component of sexual  
279 selection in other study systems.

280 A weakness of our study is the lack of replication of the experimental evolution lines. This  
281 implies that we cannot fully exclude genetic drift as a cause of the results, despite following  
282 the recommendation of a large number of individuals within each line to reduce the influence  
283 of drift (Fuller et al. 2005). However, several additional experiments suggest the presence of a  
284 sexual conflict over timing of stigma receptivity in *C. heterophylla* (Lankinen and Kiboi  
285 2007; Madjidian and Lankinen 2009; Madjidian et al. 2012; Hersh et al. 2015), indicating that  
286 the reported differences between M and P in pollen competitive ability and seed set is caused  
287 by sexual selection rather than genetic drift (Lankinen et al. 2017b). Moreover, the link  
288 between floral traits is well documented both in *C. heterophylla* (Lankinen et al. 2017a) and

289 among species in *Collinsia* (Armbruster et al. 2002; Kalisz et al. 2012), further supporting a  
290 scenario of selection rather than drift.

291 In conclusion, the influence of sexual selection in *C. heterophylla* experimental evolution  
292 lines resulted in divergence of mating system-related floral traits, at least for timing of anther-  
293 stigma contact, which is related to timing of self pollination and herkogamy. P appeared more  
294 ‘outcrossing’ than M. Thus, adding a component of sexual selection during outcross  
295 pollination could enhance the patterns of floral divergence regularly seen between selfers and  
296 outcrossers (Karron *et al.*, 2012; Barrett, 2013). It would be of great interest to learn if sexual  
297 selection could impact divergence in herkogamy, or other mating system-related floral traits,  
298 also in other study systems. Such knowledge could lead to a better understanding of how  
299 multiple factors influence evolution of plant mating system and floral divergence. In line with  
300 Barrett and Harder (2017), we suggest that future studies should consider sexual selection and  
301 mate diversity in relation to plant mating system selection and divergence, and the possibility  
302 that some of the floral trait divergence we see today between outcrossers and selfers is in fact  
303 a result of sexual selection.

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411

412

**Table 1**

413

Previous results and hypotheses tested in the present study regarding floral traits estimated among M (monogamous), P (polyandrous) and S

414

(source) experimental lines in *Collinsia heterophylla*

Estimated trait	Flowering start	Timing of anther-stigma contact	Timing of stigma receptivity (pistil-based)	First seed set (pollen- and pistil-based)
Relation to mating system in <i>Collinsia</i>	Later in more outcrossing	Later in more outcrossing	Later in more outcrossing	-
Genetic correlation with other traits	anther-stigma contact; $r = 0.147, P = 0.059, df = 164^1$	stigma receptivity; $r = 0.183, P = 0.049, df = 115^1$	first seed set; $r = 0.258, P = 0.41, df = 10^2$	
Correlation pollen-based vs. pistil-based influence				$r = -0.360, P = 0.008, df = 52^2$
Hypothesis M vs. P	Later flowering start in P because prediction of later anther-stigma contact in P (as these traits are genetically correlated)	Later anther-stigma contact in P because prediction of later stigma receptivity in P (as these traits are genetically correlated)	Later stigma receptivity in P because of earlier pollen-based influence in P (as pollen and pistil components of first seed	Earlier first seed set in P because of earlier pollen-based influence in P (as the pollen component of the trait changed

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set are negatively following experimental  
correlated) evolution)

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415 Note. <sup>1</sup>Reported in Lankinen et al. (2017a), <sup>2</sup>Reported in Hersh et al. (2015)

416

417

**Table 2**

418

Analyses of variance of floral traits related to mating system among M (monogamous), P (polyandrous) and S (source) experimental lines in

419

*Collinsia heterophylla*

Source of variation	Flowering start			Anther-stigma contact			Stigma receptivity			First seed set		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Line	2,74.9	6.93	0.002	2,61.5	4.31	0.018	2,39	2.66	0.082	2,24.1	2.05	0.15
Recipient nested within line	72,354	6.33	<0.001	61,63	1.628	0.028				24,80	1.01	0.47

420 **Fig. 1** A) Floral developmental at stage 0-4 in *Collinsia heterophylla*. During floral  
421 development, the anthers dehisce and the pistil elongates. At later stages, stigma receptivity  
422 occurs and the pistil grows through its own pollen, allowing delayed selfing. Stage 0 = stage  
423 of flower opening, stage 1-4 = stage with number of dehisced anthers, respectively. Gray  
424 arrows point to the location of the stigma. B) Model of sexual conflict over timing of stigma  
425 receptivity, involving opposing selection pressures in male and female function due to their  
426 divergent evolutionary interests. Moving trait values of the male function closer to its fitness  
427 optimum (blue arrow) in *C. heterophylla* in an experimental evolution experiment, caused a  
428 direct fitness cost in the female function in terms of reduced seed set (Lankinen et al. 2017b).  
429 Photograph in A): Josefin A Madjidian.

430

431

432 **Fig. 2** Overview of experimental design and results of experimental evolution in  
433 *Collinsia heterophylla*. A) Experimental evolution lines M (monogamous, one pollen donor)  
434 and P (polyandrous, two pollen donors) were produced by controlled hand-pollinations in the  
435 greenhouse for four generations starting from S (source) line. In both M and P, four flowers  
436 per recipient were repeatedly crossed at stage 1 and 2 (unreceptive or partially receptive  
437 pistils) involving two pollen donors (gray vs. white pollen grains). In M, each flower received  
438 pollen from the same donor while in P, each flower received flowers from two different  
439 donors. B) Overview of estimated traits related to different life stages and mating system in  
440 S, M and P lines. Pollen traits (proportion early siring and pollen-tube growth rate) represent  
441 the male reproductive function and pistil traits (anther-stigma contact and stigma receptivity)  
442 represent the female reproductive function. First seed set is the result of the combined  
443 influence of male-based and female-based influence on stigma receptivity and seed set.

444 Results for traits in white boxes = reported in the current study, gray boxes = reported in  
445 Lankinen et al. (2017b).

446

447

448 **Fig. 3** Floral traits related to mating system in *Collinsia heterophylla* among M  
449 (monogamous), P (polyandrous) and S (source) experimental lines averaged over recipients  
450 and when necessary over pollen donors. A) Flowering start estimated as day from flowering  
451 of the first plant independent of line. B) Anther-stigma contact estimated as floral  
452 developmental stage (= number of dehisced anthers) when the stigma grows into the dehisced  
453 anthers. C) Stigma receptivity estimated as day after flower opening when peroxidase activity  
454 occurs. D) First seed set estimated as day after flower opening when seeds are formed  
455 following controlled hand-pollinations and subsequent pistil removal at day 1-4 after flower  
456 opening. Error bars indicate  $\pm 1$  SE. Different letters denote significant difference for tests  
457 performed among the three lines (A-B) and between M and P (D).

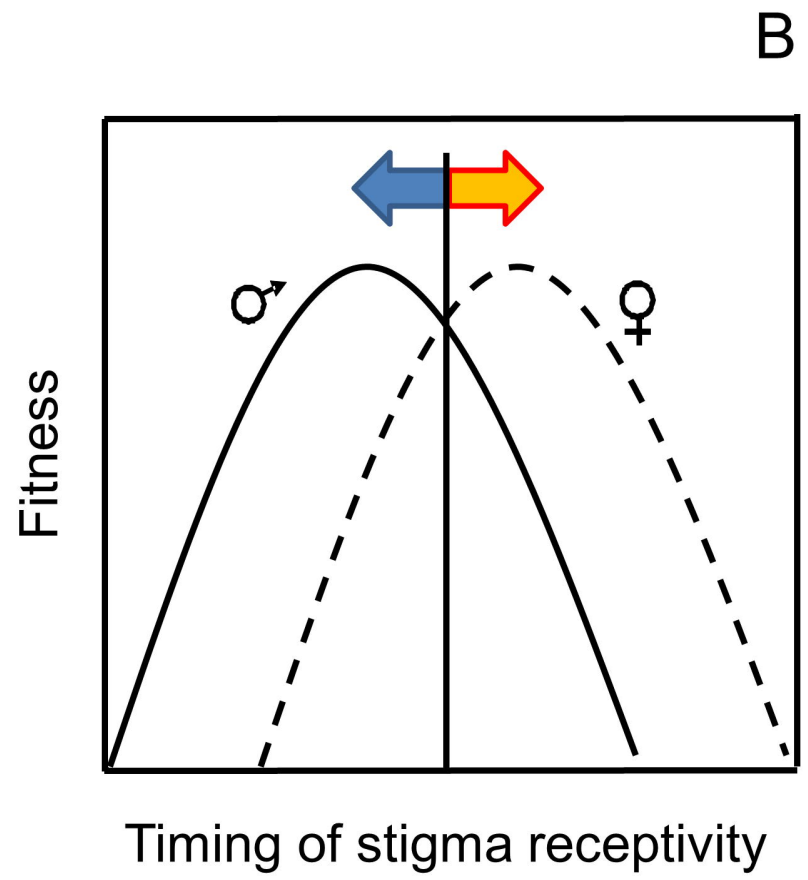
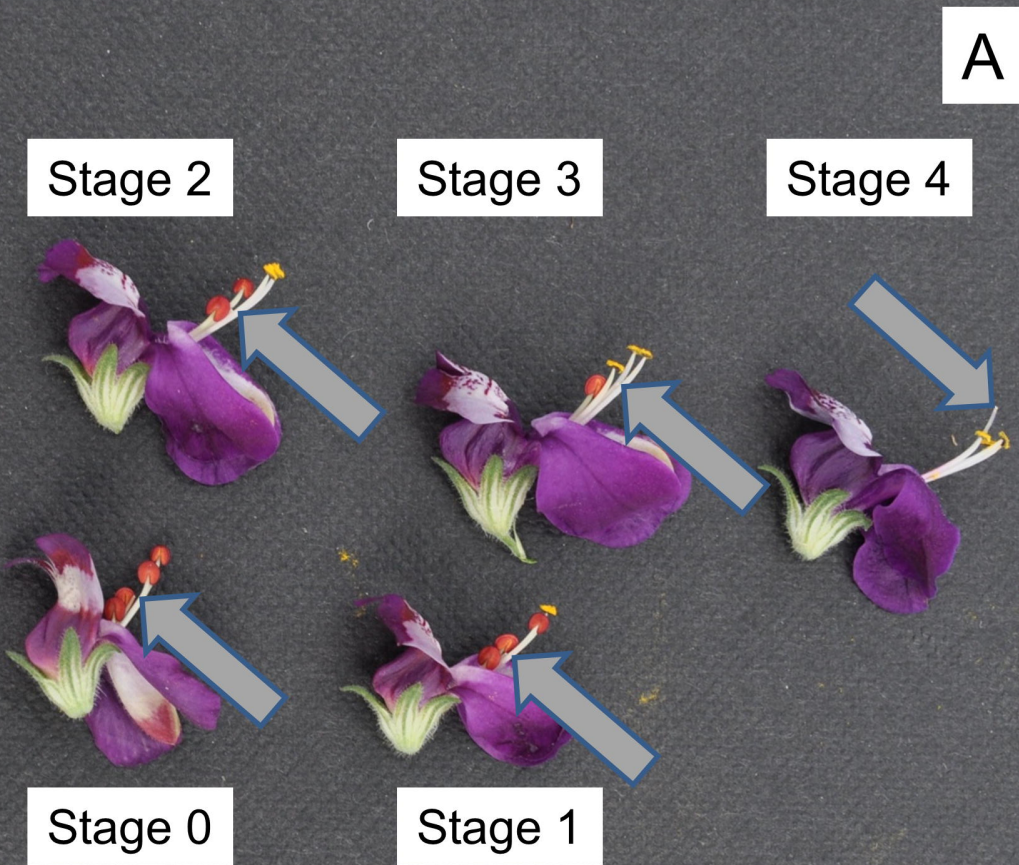
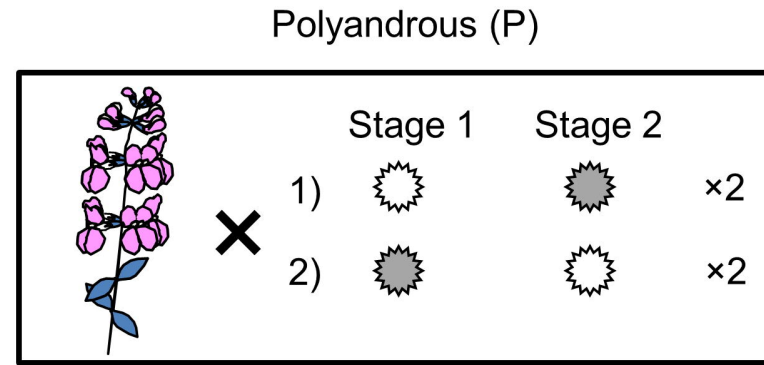
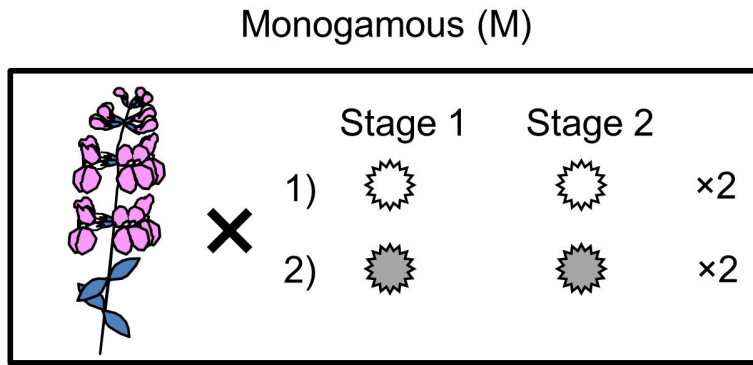


Fig.1

A



B

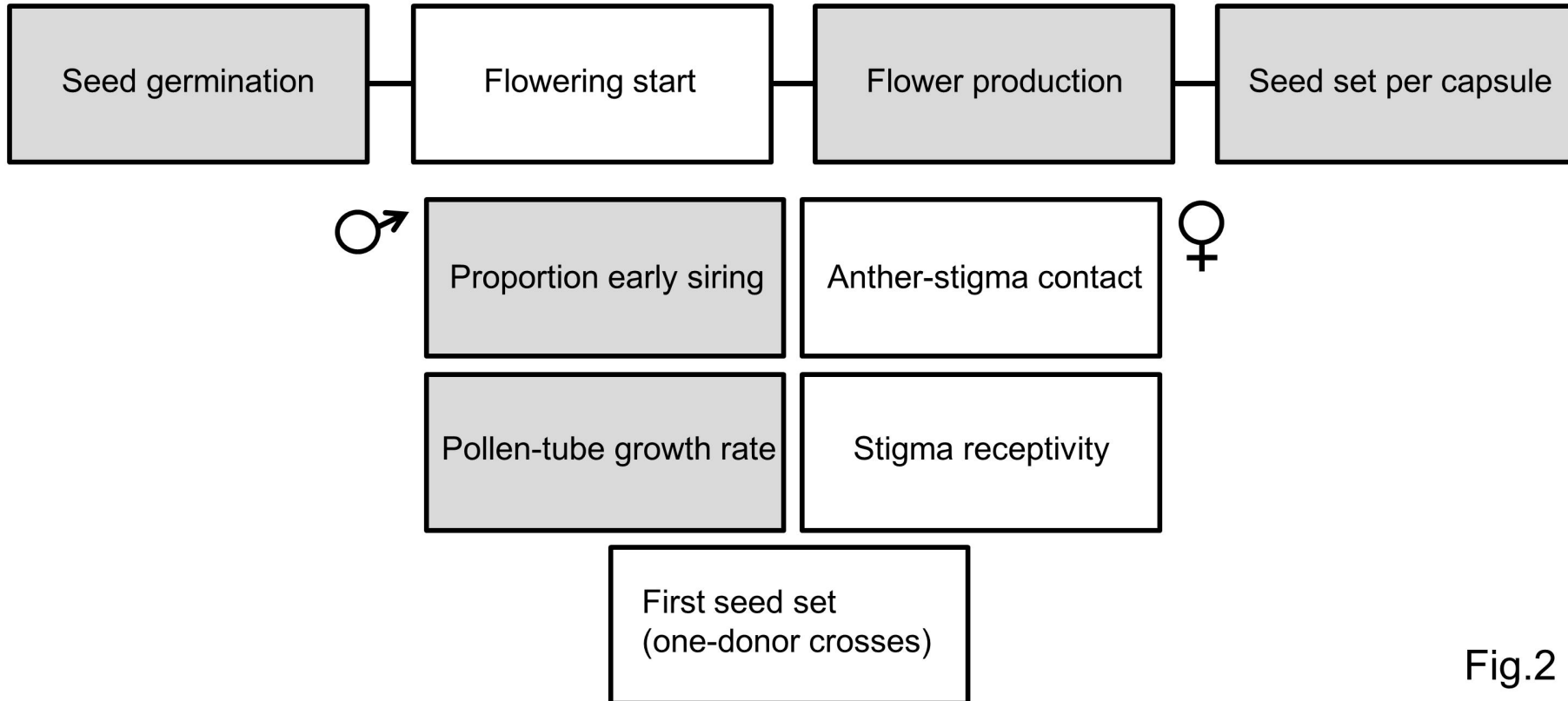


Fig.2



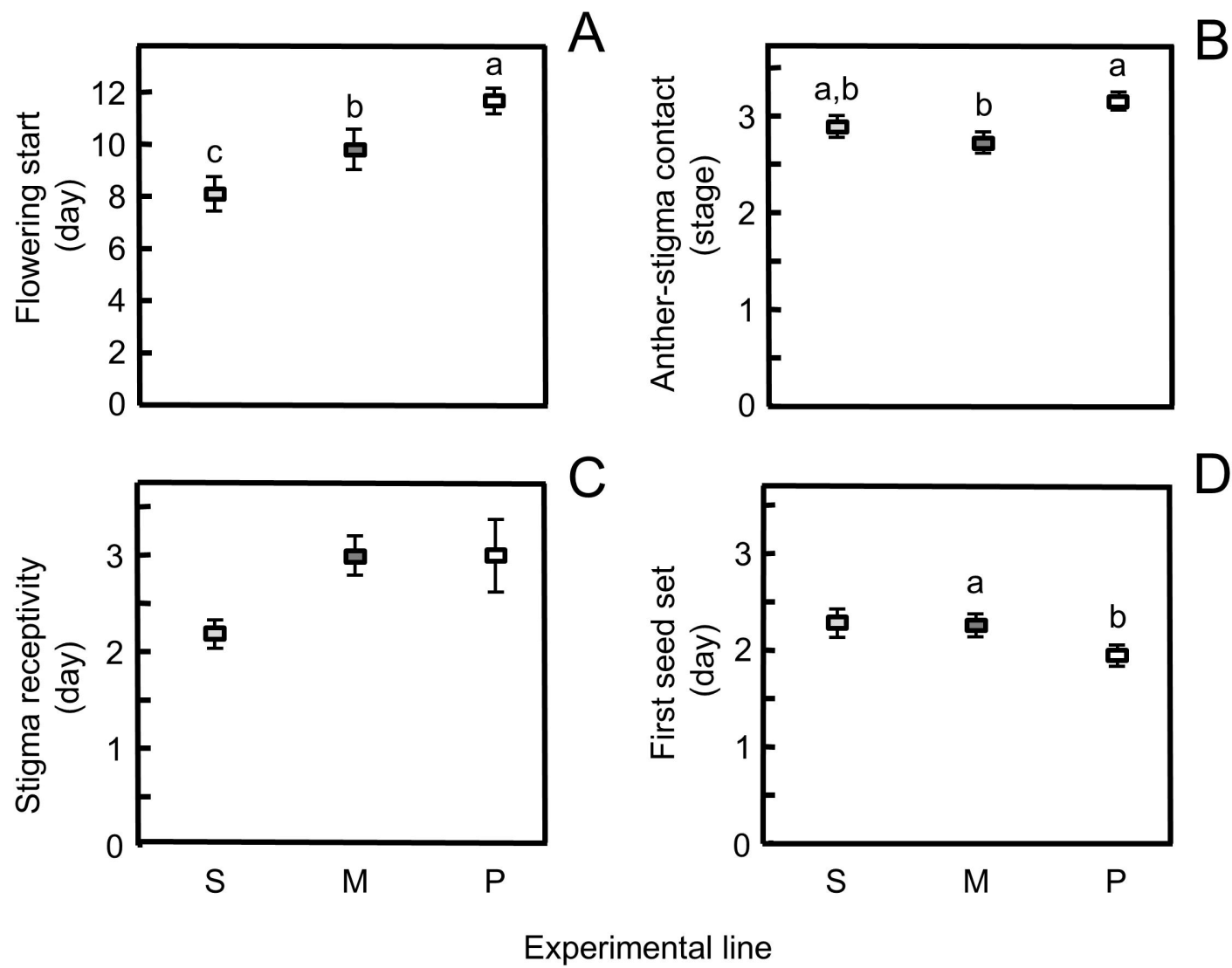


Fig.3