



HAL
open science

Rapid evolution of selfing syndrome traits in *Viola arvensis* revealed by resurrection ecology

Pierre-olivier Cheptou, Eric Imbert, Michel Thomann

► **To cite this version:**

Pierre-olivier Cheptou, Eric Imbert, Michel Thomann. Rapid evolution of selfing syndrome traits in *Viola arvensis* revealed by resurrection ecology. *American Journal of Botany*, In press, 10.1002/ajb2.16028 . hal-03808855

HAL Id: hal-03808855

<https://hal.science/hal-03808855>

Submitted on 10 Oct 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Rapid evolution of selfing syndrome traits in *Viola arvensis* revealed by
2 resurrection ecology

3

Pierre-Olivier Cheptou¹ Eric Imbert² and Michel Thomann¹

¹CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier,
EPHE, Montpellier Cedex 05, France ; ²ISEM, University of Montpellier – Montpellier, France

4 **Abstract:**

5 **Premise of the study :** As part of global change, climate warming and pollinator decline
6 are expected to affect plant phenology and plant-pollinator interactions. This paper aims
7 at characterizing rapid evolution of life history traits and floral traits over two decades in
8 the wild pansy (*Viola arvensis*), a common weed in agrosystems.

9 **Methods:** we used a resurrection ecology approach with genotypes sampled in 1991 and
10 2012 from a population in Burgundy (France). The species has a mixed mating system
11 (hereafter: mixed selfer) and presents a floral polymorphism. In order to correct for
12 maternal effects, plant traits were measured in the second generation in a common garden
13 (after a refreshing generation) to characterize plant evolution during the two decades. In
14 addition, historical population selfing rates in the 1991 and 2012 were inferred from
15 microsatellites markers through heterozygote deficiency and identity disequilibrium.

16 **Key results:** Phenotypic data revealed a significant advance in flowering date, reduced
17 flower sizes and a higher propensity of plant to set seed by autonomous selfing.
18 Moreover, we detected a change in color morph frequency with an increase of the pale
19 morph frequency. In accordance with phenotypic data, the neutral genetic data revealed in
20 increase in historical selfing rates from $s=0.68$ in 1991 to $s=0.86$ in 2012.

21 **Conclusions:** Taken together, such data suggest that the wild pansy, as mixed selfer, is
22 evolving a selfing syndrome that may be the consequence of reduced pollinator activity in
23 agrosystems.

24

25 **Key words:** resurrection ecology-selfing syndrome-pollinator decline-*Viola arvensis*-rapid
26 adaptation

27 INTRODUCTION

28 Plant-pollinator interactions are major trophic networks in ecosystems. More than 70 %
29 of angiosperms rely on insects for their reproduction. Such interactions range from
30 generalist to specialist and plant-pollinator interactions have co-evolved during the
31 radiation of angiosperms (Van der Niet et al. 2014). In hermaphroditic plants, the
32 dependence of plants on pollinators is associated with mating system traits such as showy
33 flowers, large corolla, or rewards. While fully outcrossing species require pollinators to set
34 seeds, full selfers can set seeds autonomously. Between these two extremes, mixed selfers
35 partly depend on pollinators for their sexual reproduction. Macroecological studies have
36 established that the evolution of selfing appears as a recurrent pattern in plant
37 phylogenies (Goldberg et al. 2010). While practicing selfing may appear as a rampart
38 against the risk of pollinator failure, phylogenetic patterns of evolution support the
39 hypothesis that selfing is disadvantageous in the long run (Cheptou 2019), the so called
40 “dead-end hypothesis” (Takebayashi and Morell 2001). Plant traits favoring selfing
41 encompass a variety of plant traits termed the “selfing syndrome” including
42 inconspicuous flowers and a reduced pollen–ovule ratio. The evolution of selfing
43 syndromes has been described in several genera as reported in the genus *Ipomoea*, (Duncan
44 and Rausher 2013), in *Collinsia* (Kalisz et al. 2012) or in *Clarkia* (Sicard and Lenhard,
45 2011). The authors described reduced flower size, reduced anther-stigma distance, and
46 dissolution of self-incompatibility, consistent with diverging selection among outcrossing
47 vs. selfing taxa.

48 Modern plant mating system theory has been intensively studied since the 1980's ,
49 following the publication of Lloyd's seminal work (Lloyd, 1979, Morgan and Schoen,

50 1997). Mating system theory relies on population genetics models typically adapted to
51 microevolutionary predictions. Factors governing the evolution of self-fertilisation have
52 been identified (Lloyd 1979). Among them, pollination scarcity is expected to select for
53 self-fertilisation through the reproductive assurance it confers. In contrast,
54 macroevolutionary patterns have been described empirically (Goldberg et al., 2010) but
55 theoretical arguments at this scale are mostly verbal (Stebbins, 1950; see however Glemin
56 and Ronfort, 2013).

57 Recently, plant experimental evolution has shown that plant mating system traits can
58 evolve rapidly, in fewer than ten generations. In the self-compatible species *Mimulus*
59 *guttatus*, Roels and Kelly (2011) showed that only five generations of evolution without
60 pollinators were sufficient to produce a reduction in anther-stigma distance, resulting in a
61 higher propensity for evolved plants to set seeds autonomously by selfing. Also, Gervasi
62 and Schiestl (2017) demonstrated in the fast cycling race of *Brassica rapa* that different
63 pollination treatment (Bumblebee, Syrphid, hand pollination) produced a shift in floral
64 plant traits, in particular floral scent, in nine generations. Interestingly, plant height -ie.
65 trait indirectly related to mating system- has significantly changed in the Bumblebee
66 treatment pointing out that, beyond the floral traits, adaptation to pollination
67 environment encompasses a variety of plant traits, which justifies the notion of syndrome.

68 Recent environmental changes are likely to have affected plant pollinator interaction and
69 consequently plant mating systems. Hallmann et al. (2017) reported that more than 75%
70 of insect biomass has declined during the last thirty years, potentially endangering plant
71 reproduction and plant demography (Biesmeijer et al. 2006). Moreover, for some crops,
72 yields depend on pollinators and a decrease in crops yields in the recent years has been

73 attributed to pollinator decline (Martin et al. 2019). Beyond the direct effect of pollinator
74 loss, habitat fragmentation, land use change (e.g. loss of grasslands), and global warming
75 are also likely to affect plant pollinator interactions. For instance, shifts in pollinator and
76 plant phenology (Valencia-Montoya et al. 2021) have the potential to desynchronize
77 plant-pollinator interactions. While it is difficult to identify selection pressures on plant
78 mating systems in the face of global change, there is a body of information suggesting that
79 plant-pollinator interactions may be at risk in ecosystems (Thomann et al, 2013). For
80 instance, weeds in agrosystems have experienced major declines in pollinator abundance
81 since the Second World War due to land use change and chemicals (Fried et al, 2009).
82 Such a situation is expected to generate pollen limitation for weeds dependent on
83 pollinators as a consequence of pollinator limitation and/or plant mate limitation
84 (Cheptou, 2021).

85 While plant-pollinator interactions are assumed to have played a role in angiosperms
86 radiation (Van der Niet et al. 2014), it is reasonable to hypothesize that current
87 environmental changes can lead to plant-pollinator disruption, through short-term
88 evolution. For mixed selfing plants, we may expect pollinator decline to select for higher
89 selfing (Eckert et al. 2010, Thomann et al. 2013). Detecting evolutionary changes requires
90 controlling for environmental factors affecting phenotypes. Indeed, without a control for
91 environmental changes, differences in plant traits and realised selfing rates detected in
92 longitudinal studies might result from plastic changes in response to environmental
93 differences rather than evolutionary changes. Recent resurrection ecology methodology
94 offers the possibility of filling this gap. Resurrection ecology takes advantage of dormant
95 propagules (e.g. seeds for plants) to compare old phenotypes sampled a few decades ago
96 to recent phenotypes sampled in the same population (Weider et al. 2018). The

97 comparison of old and recent genotypes in common environment allows disentangling
98 plastic and evolutionary responses and thus inferring rapid evolution of traits (Weider et
99 al. 2018).

100 In this study, we examined shifts in floral and phenological traits in the annual weed *Viola*
101 *arvensis* (Violaceae) over two decades in agrosystems in Burgundy, France. In Burgundy,
102 comparative surveys of weed communities from 1970 to 2005 have revealed that
103 entomophilous plants were declining while anemophilous and self-fertilizing plants
104 showed no temporal trends (Fried et al, 2016). Also, indirect estimates of pollination
105 services have revealed a decrease in pollination in this region (Martin et al, 2019). In
106 addition, temperature has increased in the last 50 years (WorldClim 2.1; Fick and Hijmans,
107 2017). In this context, we hypothesize (1) that flowering phenology has evolved towards
108 earlier flowering because of climate warming and (2) that increased selfing has evolved
109 because of pollinator decline. Using old and current genotypes of our studied population,
110 we first inferred realised selfing rates using microsatellites markers. Second, we measured
111 floral traits, plant phenology, and the ability of plants to produce seeds without pollinators
112 in a common garden to characterize trait shifts. Overall, our study showed that traits have
113 shifted in the directions predicted over a twenty-year period.

114 **MATERIAL AND METHODS**

115 *Studied species, sampling area and sampling population*

116 The field pansy (*Viola arvensis*, Violaceae) is an annual diploid crop weed (Clausen
117 1926) widely distributed in northern Europe. It occurs mainly in fields with C3 crops such
118 as oilseed rape (*Brassica napus*). Flowers are zygomorphic and predominantly cream-
119 colored with a yellow spot in the middle lower petal. The two upper petals may also

120 exhibit purple spots. In Burgundy (France), three different floral morphs can be found.
121 The purple morph exhibits a purple spot in the upper petals. The white morph lacks
122 purple spots, and the intermediate morph exhibits light purple spots (Figure 1). According
123 to Espeut (1996), phenotypes of the studied population refer to *Viola arvensis* subsp
124 *megalantha*. The field pansy is described as self-compatible with mixed mating system
125 (Scoppola & Lattanzi 2012) but there is no estimate of the selfing rate based on
126 molecular markers reported in the literature. The flowers produce nectar and are visited
127 by Apoids (BiolFlor database). The studied focal population of *V. arvensis* was sampled
128 near Fauverney, Côte-d'Or, Bourgundy, France (47°15'35.96"N ; 5° 9'0.84"E and
129 47°15'8.25"N ; 5° 8'34.14"E). Seeds sampled in 1991 were obtained from the Institut
130 National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Dijon.
131 Seeds were sampled from fruits mass collected on 50 mother plants. In February 2012, we
132 randomly sampled 100 seedlings in the same plot. Because the plots were several hectares
133 wide, it is possible that the recently sampled plants were located a few hundred meters
134 away from the initial sampling.

135 *Common garden experimental design*

136 In order to minimize maternal effects, a refresher generation was grown for plants
137 from the historical and recent samples, the refresher generation, were cultivated in a
138 common garden to generate seed materials in the same environment for the test
139 generation for which phenotypic traits were measured (Appendix S1; see the
140 Supplementary Data with this article). Because the recent plants were sampled as
141 seedlings, the common conditions for both samples started at seedling stage. On
142 December 2011, 1991 seed lots were placed in germination chambers in trays with
143 vermiculite. Following Baskin & Baskin (2014), germination conditions were dark-6°C

144 during 11h followed by light-15°C during 13h. Seedlings from 1991 and 2012 were placed
145 outdoor in the experimental platform “Terrains d’Experiences” platform of the Labex
146 Cemeb, Montpellier, south of France (43°38'19.97"N, 3°51'43.69"E). 140 seeds of the
147 1991 sample were sown in seed trays and 51 seeds successfully germinated (36%) but 11
148 seedlings died. Of the 100 transplanted from the field site in 2012, one seedling died.
149 Seedlings were transplanted in 0.5L pots. In spring 2012, we regenerated seeds by open
150 pollination within the 1991 and within the 2012 samples to maintain the genetic integrity
151 of each sample. After transplantation of the 1991s seedlings in 0.5L pots, all plants from
152 both samples were randomly distributed and grown in pots in common garden
153 conditions. Because we used seeds for the 1991 sample but seedlings for the 2012 sample,
154 plants from 2012 started to flower about ten days earlier than plants from 1991. 80 plants
155 from the 2012 sample successfully flowered and were naturally pollinated to produce
156 seeds for the test generation. When plants started to flower, they were pollinated within
157 population by bumblebees introduced into insect-proof greenhouses to prevent crosses
158 between the 1991 and 2012 samples.

159 For the test generation, twenty seeds were randomly picked from each of the 27 mother
160 plants for the historical sample and from 38 mother-plants for the recent sample. These
161 seeds were sown in germination chambers following the first generation germination
162 protocol. Germination occurred after ca. 20 days and germination success was high ($85 \pm$
163 2% and $74 \pm 4 \%$ for 1991 and 2012 samples respectively). All seedlings were then
164 transferred to seedling trays and placed in the greenhouse under natural photoperiod and
165 without temperature control. One month later, six randomly chosen individuals per
166 mother-plant were transferred in 0.5 L pots and grown outside in a random arrangement
167 of pots. The test generation represents thus a family design with 382 plants in total.

168 *Population genetics using microsatellites markers*

169 Plants produced from old and recent samples were genotyped using eight microsatellite
170 markers (Devresse et al, submitted). Tissue from individual plants was dried at 40°C and
171 stored in granular silica gel (Carlo Erba cobalt free). DNA was extracted with the CTAB
172 protocol (Doyle and Doyle 1987).

173 *Genetic diversity and selfing rate estimates*

174 We analyzed allelic richness and expected heterozygosity (H_e) in the two samples for the
175 height microsatellites loci to check that genetic diversity did not change over time. A
176 drastic change in allelic diversity (or H_e) would suggest either a strong bottleneck or drift
177 in natural populations (low N_e) and/or a bias caused by sampling. In addition,
178 microsatellite loci allow inference of historical selfing rates according to two methods. We
179 used the classical method based on heterozygote deficiency (F_{is}). At equilibrium, F_{is} can
180 be related to selfing rate through the formula $s = \frac{2 \cdot F_{is}}{1 + F_{is}}$. F_{is} was estimated using the R
181 package HierFstat issued from genepop software (Rousset, 2008) and confidence intervals
182 were calculated by bootstraps (9000). We also used the method developed by David et al.
183 (2007) based on identity disequilibrium. Identity disequilibrium is the relative excess in
184 double heterozygous genotypes for pairs of loci. Contrary to the methods based on F_{is} ,
185 identity disequilibrium is relatively insensitive to the non-detection of heterozygotes (null
186 alleles), which is a quite common scoring artifact in the use of molecular markers (e.g.
187 microsatellites). The Rmes software was used to estimate historical selfing rates from
188 identity disequilibrium (David et al. 2007).

189 *Phenotypic traits : phenology, floral traits and autonomous selfing*

190 Phenotypic traits were measured on the test generation. Five measures were taken in the
191 three first flowers of each individual plant: the date of anthesis, the date of senescence,

192 the maximum height, the maximum width and the floral morph (white, light purple,
193 purple spotted morphs). From these measures, we derived the following measures at the
194 scale of the individual: the date of the initiation of flowering, the mean floral longevity,
195 the mean floral height, the mean floral width and the floral color. The mean was
196 calculated as the average of the three measures. Concerning floral morph, if at least one of
197 the three flowers had a purple spot, the individual was referenced as “purple spot”; if at
198 least one of the three flowers had light diffuse purple (but no flowers had a purple spot),
199 the individual was referenced as “light purple”; if the three flowers were white, the
200 individual was referenced as “white”. Finally for each individual plant, the floral display
201 (the total number of flower simultaneously opened) was recorded at 15 days after
202 initiation of flowering, and the total flower production was recorded at 30 days after
203 initiation of flowering.

204 In addition, we estimated the ability of autonomous selfing for plants. Autonomous
205 selfing was estimated as: $A = \frac{N_A}{N_O}$ where N_A is the number of seeds from a flower under
206 autonomous selfing and N_O the number of seeds from a flower under open pollination.
207 N_O was obtained under open pollination in the experimental field, while plants were
208 placed in the insect-proof greenhouse to obtain N_A . The N_A flowers opened and senesced
209 in the greenhouse to ensure that seed production was due only to autonomous selfing.
210 Both N_A and N_O were estimated from one flower per plant, chosen at random.

211 *Statistical analysis of the phenotypic traits*

212 All reproductive traits except floral color were analyzed with a linear mixed effect models
213 (LMM, nlme R package) with sampling year (either 1991 or 2012) as a fixed effect and
214 identity of the mother-plant as a random effect. As floral color is an ordinal variable with

215 3 categories, we first considered the floral color as a binomial variable, coding “white” as
216 0 and non-white (“light purple” and “purple spot”) as 1. Secondly, we compared the non-
217 purple spotted morphs (“white” and “light purple”, coded as 0) to the “purple spot”
218 (coded as 1). For these two analyses, we used a generalized linear mixed effect model
219 (GLMM, glmmPQL R package) with a binomial distribution and fixed and random
220 effects as above. Finally, in a third analysis, we performed a quantitative analysis of color
221 by assigning score to each floral color morphs: “white” as 1, “light purple” as 2 and
222 “purple spot” as 3, and we analyzed this variable with a GLMM with a poisson
223 distribution. In this analysis, we assumed equal distances between each pair of consecutive
224 categories. Autonomous selfing was analyzed with a LMM with sampling year (either 1991
225 or 2012) as a fixed effect and mother plant as a random effect. Autonomous selfing was
226 square root transformed to improve the normality of residuals. All statistical analyses were
227 conducted using R statistical software (R Development Core Team (2012); 2.15.0
228 version).

229 **RESULTS**

230 *Genetic diversity in the 1991 and 2012 samples, and comparison between temporal and spatial F_{ST}*

231 Mean allelic richness and mean genetic diversity (H_e) at the 8 putative neutral
232 markers were not statistically different between the 1991 and the 2012 samples, and there
233 were private alleles in the samples (Table 1). Estimated F_{IS} and 95% confidence intervals
234 revealed a significantly higher F_{IS} for the 2012 sample compared with the 1991 sample,
235 which corresponds to selfing rates of 0.68 and 0.86 for the 1991 and the 2012 samples
236 respectively (Table 2). The selfing rates estimated from the analysis of multilocus
237 correlation of heterozygosity exhibited the same trend as those derived from the F_{IS} but
238 the difference between 1991 and 2012 samples was lower than the difference from the F_{IS}

239 analysis (0.71 vs 0.84), although the estimates were within the confidence intervals for the
240 Fis values (Table 2).

241 *Patterns of evolution of reproductive traits*

242 Compared with 1991 sample, plants from the 2012 sample started to flower $1.15 \pm$
243 0.51 days earlier (Figure 1A, $p < 0.05$), this pattern was conserved if we calculated the
244 mean date of flowering of the three first flowers (*not shown*). Regarding floral traits, floral
245 height and floral width were significantly smaller in the 2012 than in the 1991 sample
246 (Figure 2 B, $p < 0.001$, 1C, $p < 0.01$). Similarly, floral longevity was significantly shorter in
247 the 2012 sample than in the 1991 sample (Figure 2D, $p < 0.001$). There was a significant
248 decrease in both the frequency of colored floral morphs (“light purple” + “purple spot”
249 versus “white”), and in the frequency of purple-spotted (“purple spot” versus “white” +
250 “light purple”) in the 2012 compared with the 1991 sample (Figure 3A,B, $p < 0.001$). When
251 a numeric value was assigned to the floral color as described above, we detected
252 significantly lower color score in the 2012 sample compared with the 1991 sample (Figure
253 3 A, C, $p < 0.01$). Finally there was no difference in floral display or in total floral
254 production (Figure 2 E, F, $p > 0.05$).

255 Autonomous selfing ability was greater for the 2012 than for the 1991 samples of
256 field pansy (Figure 4, $p < 0.001$).

257

258 **Discussion**

259 In this study, we analyzed how the weed *Viola arvensis* has evolved over two decades in an
260 agrosystems. The resurrection methodology allows detection of trait shifts and minimizes
261 the effects of plasticity, and the use of microsatellites allows inference of realized selfing
262 rates in the past and present. Taken together, our findings allows us to propose an

263 evolutionary scenario for the evolution of *Viola arvensis* experiencing environmental
264 changes in agrosystems.

265 ***Resurrection ecology: potential bias***

266 While resurrection ecology appears as a powerful methodology to detect short-term
267 evolution, several potential biases have been identified (Weider et al, 2018). Indeed, there
268 is the possibility that seed preservation affects phenotypic traits, which implies that trait
269 shifts between old and recent phenotypes could be an artefact of seed storage. According
270 to Franks et al. (2019), there is the possibility that non-random seed mortality during
271 conservation modifies traits at the adult stages as measured in this study. This
272 phenomenon, named “the invisible fraction,” results from the association of seed
273 mortality with traits expressed later in the life cycle through genetic correlations. By
274 artificially aging seed of *Brassica rapa* under high temperature conservation, Franks et al.
275 (2019) have shown that flowering phenology estimates were biased by seed mortality
276 during conservation. In a mixed selfer like *V. arvensis*, a likely mechanism for differential
277 survival during seed storage is inbreeding depression (δ). If inbreeding depression affects
278 seed survival, we expect that selfing rate estimates to be biased according to the
279 relationship $s' = \frac{s(1-\delta)}{s(1-\delta)+(1-s)}$ (Barrett and Harder 1996). To account for a decrease in
280 selfing rates from 0.88 to 0.68 (our estimates) caused by inbreeding depression on seed
281 mortality during seed storage, a value of $\delta=0.7$ would be necessary. Given inbreeding
282 depression values reported for seed survival (Winn et al, 2011), it is unlikely that
283 inbreeding depression in itself is the cause of selfing rate changes. Also, according to
284 Baskin and Baskin (2014), *Viola arvensis* is highly dormant and *in situ* estimates revealed
285 high survival for dormant seeds in the soil (Pluntz et al. 2018). Moreover, genotyping was

286 performed on seedlings from natural populations in the 2012 sample but from lab-
287 germinated seedlings the 1991 sample, the latter minimizing inbreeding depression. We
288 thus expect inbreeding depression on seedling mortality to decrease the selfing rate
289 estimate in the 2012 sample (conservative with respect to our hypothesis). Overall, we
290 conclude that selfing rate changes estimated by molecular markers provide evidence for
291 higher selfing rates in 2012 compared to 1991 in natural conditions. Nevertheless, it is still
292 possible that non-random mortality (independent from inbreeding depression) have bias
293 our phenotypic results. However, the low germination rate in the 1991 sample (refreshing
294 generation) is likely higher than the germination rate in the field where the 2012 seedlings
295 of the 2012 have been sampled. If evolution is driven by non-random mortality, evolving
296 a set of trait consistent with a selfing syndrome would require very specific genetic
297 correlation (*e.g.* seed mortality linked to flower size, seed mortality linked to duration of
298 anthesis....). This interpretation is unlikely, though not it cannot be totally ruled out.

299 ***Patterns of trait evolution***

300 While the increase in selfing rates over time revealed by microsatellites may be the sole
301 result of reduced pollinator activity without evolution (plasticity), common garden allows
302 detection of trait shifts via evolution. Regarding mating system traits, we have a body of
303 evidence for ongoing evolution towards a selfing syndrome in *Viola arvensis*. The floral
304 size decrease (from 16 mm to 14.5 mm in height, from 13.5 mm to 12.5 mm in width)
305 and the reduction of floral longevity (from 5.7 days to 5.3 days) are consistent with a shift
306 towards the smaller, shorter-lived flowers characteristic of selfing species (Duncan and
307 Rausher, 2013). In addition, the floral morph frequencies have changed, with an increase
308 of the pale morphs. While we have no direct evidence of lower pollinator attractiveness of
309 the pale morph, such color patterns are in line with the evolution of a selfing syndrome.

310 The higher autonomous selfing ability in the 2012 sample compared to the 1991 sample
311 shows that the recent sample has higher ability to set seed in depauperate pollination
312 environments. The pollinator decline in Burgundy (Martin et al, 2019; Fried et al, 2016) is
313 a putative selective factor for the pattern observed.

314 Our resurrection experiment also shows a shift towards earlier flowering phenology. This
315 pattern has been reported in several studies (Franks et al, 2007; Valencia-Montoya et al,
316 2021). Climate warming and late season drought are considered to be the driving forces
317 selecting for earlier flowering (Root et al, 2003). A non-exclusive explanation for this
318 pattern may be indirect consequences of synchrony with early pollinator emergence
319 caused by warming. Overall, the shift towards earlier flowering time is consistent with
320 direct or indirect effect of climate warming.

321 ***Short-term evolution of selfing syndrome and plant-pollinator interactions in***
322 ***ecosystems.***

323 The evolution of selfing syndrome from outcrossing ancestors is a recurrent pattern
324 reported in several plant phyla on a macroevolutionary timescale (Stebbins, 1950;
325 Goldberg et al. 2010). The recognition that such a pattern can evolve on the short-term,
326 in response to environmental changes, is new in plant mating system studies. This was
327 first reported in experimental evolution studies (Gervasi and Shiestl, 2017; Roels and
328 Kelly, 2011). However, to our knowledge, the rapid evolution in nature towards a selfing
329 syndrome in twenty years reported in this study is a novel finding. Our results for *Viola*
330 *arvensis* show that current environmental changes can potentially drive evolutionary
331 change that cause plant populations to evolve as fast as those under experimental
332 evolution. Our results emphasize that the current ecological crisis generates substantial
333 selection pressures leading to rapid evolution, in contrast to the traditional view that

334 evolution was always very slow (Darwin, 1859). This study supports the idea that the
335 signature of natural selection on floral morphology and plant mating systems caused by
336 environmental changes is visible, even in a few generations.

337 The pattern of evolution observed in *Viola arvensis* has the potential to disrupt plant-
338 pollinator interactions. Assuming that pollinator decline has caused the evolution of a
339 selfing syndrome, this evolutionary change may reinforce pollinator decline and leads to a
340 positive feedback loop and ultimately to the breakdown of a major trophic relationship in
341 ecosystems (Cheptou, 2021). In this context, there is an urgent need to analyze patterns of
342 mating system evolution in floras to see if the pattern observed in *Viola arvensis* is general
343 or not. Resurrection ecology appears as a powerful methodology to analyse such patterns.
344 In Europe, the large number of seed banks (Ensconet network) has the potential to reveal
345 patterns of plant evolution in the last decades.

346 **Acknowledgments:** We warmly thank Emilie Cadet and the INRAE Dijon research
347 institute for providing ancestral seeds and location of the study population. We also thank
348 Susan Mazer, Eugenio Larios, Ann Sakai, and Stephen Weller for the invitation to submit
349 a manuscript to the special issue “*Approaches to the Study of Quantitative Fitness-Related Traits*”.

350 We thank Louis Devresse for performing the statistical analysis of Fis in the revised
351 version of the paper. Finally, we would like to thank two anonymous reviewers and
352 Stephen Weller for helpful comments.

Author Contributions: P.-O. C and E.I designed the study. M. T. performed the
experiment and lab work. MT did the statistical analysis and wrote a first version of the
results section. P.-O. C. wrote the manuscript. P.-O.C, E.I and M.T provides comments
and corrections.

Data Availability Statement: Data are available from the Dryad Digital Repository: doi: 10.5061/dryad.pvmcndp6

Additional Supporting Information may be found online in the supporting information section at the end of the article” Appendix S1: Experimental design.

Literature Cited

- 353 Barrett, S. C. H. and L. D. Harder (1996). Ecology and evolution of plant mating system. Trends in
354 Ecology & Evolution **11**(2): 73-79.
- 355 Baskin, C. and J. Baskin (2014). Seeds : Ecology, Biogeography, and, Evolution of Dormancy and
356 Germination. San diego, Academic Press.
- 357 Biesmeijer, J. C., S. P. M. Roberts, M. Reemer, R. Ohlemuller, M. Edwards, T. Peeters, A. P. Schaffers, S.
358 G. Potts, R. Kleukers, C. D. Thomas, J. Settele and W. E. Kunin (2006). Parallel declines in pollinators
359 and insect-pollinated plants in Britain and the Netherlands. Science **313**: 351-354.
- 360 Cheptou, P. O. (2019). Does the evolution of self-fertilization rescue populations or increase the risk of
361 extinction? Annals of Botany **123**(2): 337-345.
- 362 Cheptou P.O. (2021) Pollination strategies in the face of pollinator decline. Botany Letters. 168(3):316-23.
- 363 Clausen, J. 1926. Genetical and cytological investigations on *Viola tricolor* L. and *V. arvensis* Murr.
364 Hereditas 8: 1-156.
- 365 Darwin, C. 1859. On the Origin of Species by Means of Natural Selection. John Murray, London.
- 366 David, P., B. Pujol, F. Viard, V. Castella and J. Goudet (2007). Reliable selfing rate estimates from
367 imperfect population genetic data. Molecular Ecology **16**(12): 2474-2487.
- 368 Doyle, J. J. & Doyle, J. L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf
369 tissues. Phytochemistry Bulletin. 19: 11-15.
- 370 Duncan, T. M. and M. D. Rausher (2013). Evolution of the selfing syndrome in *Ipomoea*. Frontiers in
371 Plant Science **4**.
- 372 ENSCONET (2009) ENSCONET Seed Collecting Manual for Wild Species. ISBN: 978-84-692- 3926-1.
- 373 Eckert, C. G., S. Kalisz, M. A. Geber, R. Sargent, E. Elle, P. O. Cheptou, C. Goodwillie, M. O. Johnston,
374 J. K. Kelly, D. A. Moeller, E. Porcher, R. H. Ree, M. Vallejo-Marin and A. A. Winn (2010). Plant mating
375 systems in a changing world. Trends in Ecology & Evolution **25**(1): 35-43.
- 376 Espeut, M. 1996. Confirmation of the presence of *Viola arvensis* J.A. Murray subsp. *megalantha* Nauenb.
377 Bulletin mensuel de la Société linnéenne de Lyon 65: 296-304.
- 378 Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global
379 land areas. International Journal of Climatology. 37:4302– 4315
- 380 Franks SJ, Sim S, Weis AE. Rapid evolution of flowering time by an annual plant in response to a climate
381 fluctuation. Proceedings of the National Academy of Sciences of the United States of America.
382 2007;104(4):1278-82.
- 383 Franks, S. J., M. R. Sekor, S. Davey and A. E. Weis (2019). Artificial seed aging reveals the invisible
384 fraction: Implications for evolution experiments using the resurrection approach. Evolutionary Ecology
385 **33**(6): 811-824.
- 386 Fried G, Petit S, Dessaint F & Reboud X (2009) Arable weed decline in Northern France: crop edges as
387 refugia for weed conservation? *Biology and Conservation* **142**, 238– 243.

388 Fried G, Dessaint F, Reboud X. Local and regional changes in taxonomic and functional diversity of
389 arable weed communities in Burgundy (France) between the 1970s and the 2000s. *Botany Letters*.
390 2016;163(4):359-71.

391 Gervasi, D. D. L. and F. P. Schiestl (2017). Real-time divergent evolution in plants driven by pollinators.
392 *Nature Communications* **8**.

393 Glemin, S. and J. Ronfort (2013). Adaptation and maladaptation in selfing and outcrossing species: new
394 mutations versus standing variation. *Evolution* **67**(1): 225-240.

395 Goldberg, E. E., K. J.R., R. Lande, K. Robertson, S. A. Smith and B. Igic (2010). Species selection
396 maintains self-incompatibility. *Science* **330**: 493-495.

397 Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, et al. More than 75 percent decline
398 over 27 years in total flying insect biomass in protected areas. *Plos One*. 2017;12(10).

399 Kalisz S, Randle A, Chaiffetz D, Faigeles M, Butera A, Beight C. Dichogamy correlates with outcrossing
400 rate and defines the selfing syndrome in the mixed-mating genus *Collinsia*. *Annals of Botany*.
401 2012;109(3):571-82.
402

403 Lloyd, D. G. (1979). Some reproductive factors affecting the selection of self-fertilization in plants.
404 *American Naturalist* **113**(1): 67-79.

405 Morgan MT, Schoen DJ. The role of theory in an emerging new plant reproductive biology. *Trends in*
406 *Ecology and Evolution*; 1997;12(6):231-4.
407

408 Martin, G., C. Fontaine, F. Accatino and E. Porcher (2019). New indices for rapid assessment of
409 pollination services based on crop yield data: France as a case study. *Ecological Indicators* **101**: 355-363.

410 Pluntz, M., S. Le Coz, N. Peyrard, R. Pradel, R. Choquet and P.-O. Cheptou (2018). A general method for
411 estimating seed dormancy and colonisation in annual plants from the observation of existing flora.
412 *Ecology Letters* **21**(9): 1311-1318.

413 R Development Core Team (2012). R: A language and environment for statistical computing. R
414 foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL [http://www.R-](http://www.R-project.org/)
415 [project.org/](http://www.R-project.org/). Roels, S. A. B. and J. K. Kelly (2011). Rapid evolution caused by pollinator loss in *Mimulus*
416 *guttatus*. *Evolution* **65**(9): 2541-2552.

417 Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C. & Pounds, J. A. 2003. Fingerprints
418 of global warming on wild animals and plants. *Nature* **421**: 57-60

419 Rousset F. GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows
420 and Linux. *Molecular Ecology Resources*. 2008;8(1):103-6.

421 Scoppola, A. & Lattanzi, E. 2012. *Viola* section *Melanium* (Violaceae) in Italy. New data on morphology
422 of *Viola* tricolor-Group. *Webbia* **67**: 47-64.

423 Sicard A, Lenhard M. The selfing syndrome: a model for studying the genetic and evolutionary basis of
424 morphological adaptation in plants. *Annals of Botany*. 2011;107(9):1433-43.

425 Stebbins, G. L. (1950). *Variation and evolution in plants*. New York, USA., Columbia University Press.

426 Takebayashi, N. and P. L. Morell (2001). Is self-fertilization an evolutionary dead end? Revisiting an old
427 hypothesis with genetic theories and macroevolutionary approach. *American Journal of Botany* **88**(7):
428 1143-1150.

429 Thomann, M., E. Imbert, C. Devaux and P. O. Cheptou (2013). Flowering plants under global pollinator
430 decline. *Trends in Plant Science* **18**(7): 353-359.

431 Valencia-Montoya, W. A., E. Flaven, J. Pouzadoux, E. Imbert and P.-O. Cheptou (2021). Rapid divergent
432 evolution of an annual plant across a latitudinal gradient revealed by seed resurrection. *Evolution* **75**(11):
433 2759-2772.

- 434 Van der Niet, T., R. Peakall and S. D. Johnson (2014). Pollinator-driven ecological speciation in plants:
 435 new evidence and future perspectives. *Annals of Botany* **113**(2): 199-211.
- 436 Weider, L. J., P. D. Jeyasingh and D. Frisch (2018). Evolutionary aspects of resurrection ecology: Progress,
 437 scope, and applications-An overview. *Evolutionary Applications* **11**(1): 3-10.
- 438
- 439

Tables

- 440 Table 1. Genetic richness and genetic diversity (He) in the temporal samples of *V. arvensis*.
 441 Private alleles in 1991 and 2012 samples are shown in brackets.
- 442

locus	Allelic richness (private alleles)			Genetic diversity (He)	
	1991	2012	Total	1991	2011
Varv03	7 (1)	8 (2)	9	0,74	0,77
Varv06	5	6 (1)	6	0,64	0,71
Varv08	2	4 (2)	4	0,24	0,47
Varv09	2 (1)	2 (1)	3	0,40	0,14
Varv10	8 (2)	6	8	0,83	0,81
Varv15	2	3 (1)	3	0,07	0,14
Varv37	3 (1)	2	3	0,32	0,26
Varv42	2	2	2	0,51	0,18
moy \pm ES	3,87 \pm 0,87	4,12 \pm 0,81	4,75 \pm 0,92	0,47 \pm 0,09	0,43 \pm 0,10

443

444

445 Table 2. Selfing rates estimated by hierFstat (Fis [95% CI] in italic) and by RMES (\pm SE)
446 in the 1991 and the 2012 samples of *Viola arvensis*.

447

448

Sample age	Selfing rate in natural conditions	
	Genepop	RMES
1991	0.68	0,71 \pm 0,07
	<i>0,51 [0.36-0.67]</i>	
2012	0.86	0,84 \pm 0,04
	<i>0,76 [0.67-0.85]</i>	

449

450

451

452 **Figure legends.**

Figure 1. Floral morphs for *Viola arvensis* subsp *megalantha* in the study population.

453 **Figure 2.** Shifts in phenotypic traits between 1991 and 2012 in *Viola arvensis* estimated

454 from historical and recent samples. Estimates \pm SE are adjusted to account for the

455 random effect. Significance level: n.s., $P > 0.1$; †, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, P

456 < 0.001 .

457

458 **Figure 3.** Change in floral morph frequencies between 1991 and 2012 in *Viola arvensis*

459 estimated from historical and recent samples. (A) spineplot of the row data for the floral

460 morphs : white for white morph, light gray for light purple morph and dark gray for

461 purple spot morph (see Figure 1). (B) statistical comparison of floral morph frequencies.

462 (C) Numerical score for historical and recent population : 1, 2, 3 encoded for white, light

463 purple and purple spot morphs respectively. The horizontal full bar and the horizontal

464 dotted bars represent the estimated score of each sample with its \pm SE, respectively.

465 Significance level: n.s., $P > 0.1$; †, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

466

467 **Figure 4.** Shift in autonomous selfing between 1991 and 2012 in *Viola arvensis* estimated

468 from historical and recent samples. Autonomous selfing ability is estimated from the ratio

469 (square root transformed in the statistical analysis) between seed set in the absence of

470 pollinators and seed set under natural pollination. Back-transformed estimates \pm SE are

471 presented.

472

473

474

475 **Fig1**

476

Scale :1 cm



White
morph



Light purple
morph



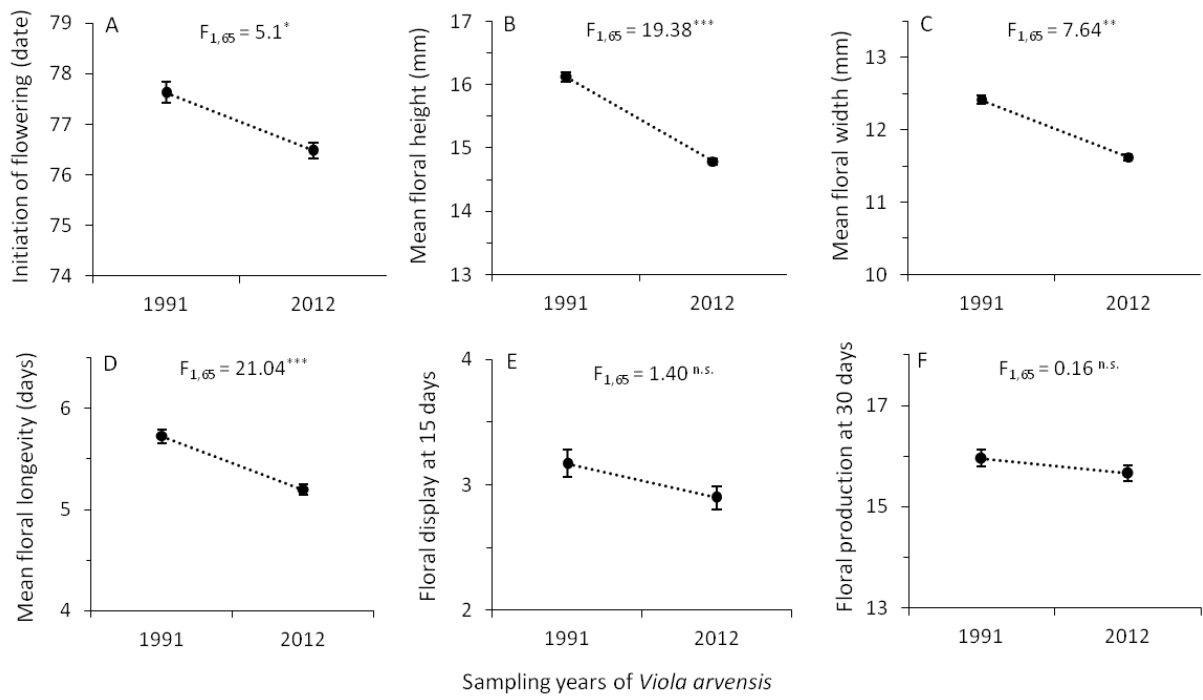
Purple spotted
morph

477

478

479

480 **Fig2**

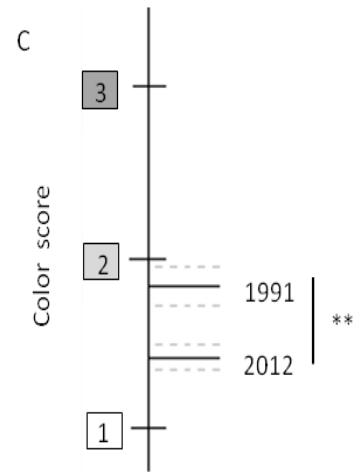
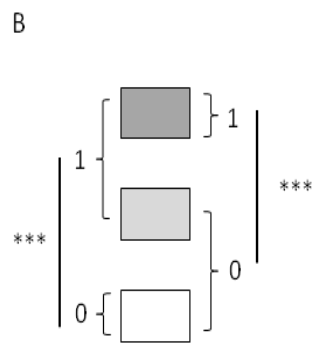
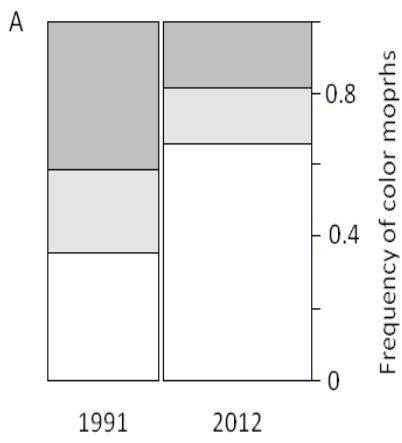


481

482

483

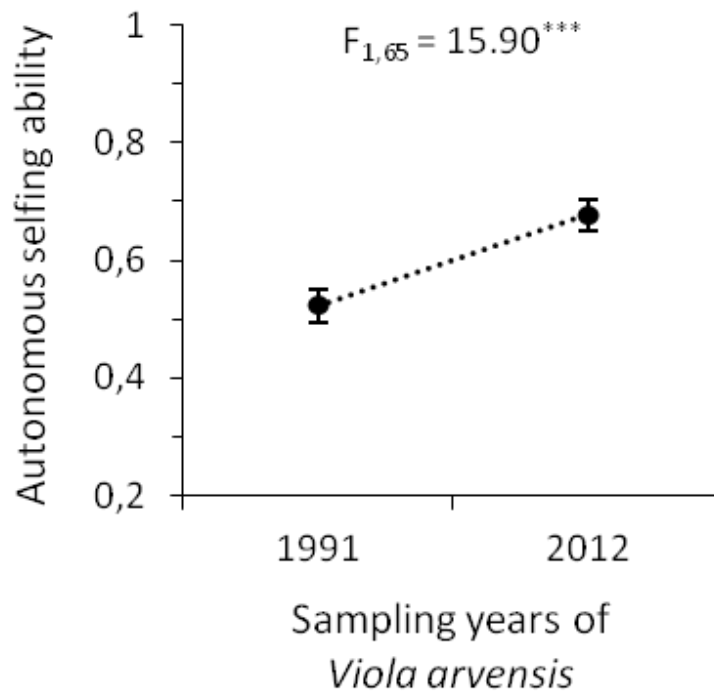
484 **Fig3**



485

486

487 Fig 4



488