



HAL
open science

Phenotypic plasticity, population structure and adaptation in a young weed species with a worldwide distribution

A. Cornille, M. Tiret, A. Salcedo, H.R. Huang, M. Orsucci, P. Milesi, D. Kryvokhyzha, K. Holm, X.J. Ge, J.R. Stinchcombe, et al.

► **To cite this version:**

A. Cornille, M. Tiret, A. Salcedo, H.R. Huang, M. Orsucci, et al.. Phenotypic plasticity, population structure and adaptation in a young weed species with a worldwide distribution. 2020. hal-03081389

HAL Id: hal-03081389

<https://hal.science/hal-03081389>

Preprint submitted on 6 Jan 2021

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 **Phenotypic plasticity, population structure and adaptation in a young weed species**
2 **with a worldwide distribution.**

3

4 Cornille, A.^{1,2¶*}, Tiret, M.^{1*}, Salcedo, A.^{3*}, Huang, H.R.^{4,5*}, Orsucci, M.⁶, Milesi, P.^{1,7},
5 Kryvokhyzha, D.¹, Holm, K.¹, Ge, X.J.^{4,5}, Stinchcombe, J.R.³, Glémin, S.^{1,8¶}, Wright,
6 S.I.^{3¶}, Lascoux, M.^{1¶}.

7

8 1. Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala
9 University, Uppsala, Sweden

10 2. Génétique Quantitative et Évolution - Le Moulon, AgroParisTech, CNRS, INRAE,
11 Université Paris-Saclay, Gif-sur-Yvette, France

12 3. Department of Ecology and Evolutionary Biology, University of Toronto, Toronto,
13 Canada

14 4. Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South
15 China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

16 5. Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of
17 Sciences, Guangzhou 510650, China

18 6. Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala
19 Sweden

20 7. Science for life laboratory, Uppsala, Sweden

21 8. UMR CNRS 6553 ECOBIO, Université de Rennes I, Rennes Cedex, France

22

23 Corresponding author: amandine.cornille@inrae.fr, +33 1 69 33 23 52.

24 ¶: co-directed the work; *: contributed equally

25

26 .

27 **Summary**

28

29 The colonization success of a species depends on phenotypic plasticity, adaptive
30 potential and population structure. Assessing their relative contributions during a
31 colonization process is challenging, and a large-scale experiment had yet to be done. In
32 this study, we attempted to tease apart their effects on the fitness of one of the most
33 common plant on Earth, the shepherd's purse (*Capsella bursa-pastoris*), a self-fertilizing
34 and allopolyploid weed, with a worldwide distribution. The overarching goal is to
35 eventually understand how the shepherd's purse extensive distribution range was
36 established so rapidly. To do so, we carried out three common gardens, located in Europe,
37 Asia and North America, and measured several life-history traits on field-collected
38 accessions belonging to three distinct genetic clusters (Middle East, Europe, and Asia).
39 Our experiment showed that (i) the success of *C. bursa-pastoris* is mainly due to its high
40 degree of phenotypic plasticity; and (ii), genetic cluster effect reflected a classic pattern
41 observed in core vs marginal populations, with the Middle Eastern cluster (putative core
42 population) outperforming the European and Asian clusters. This study therefore
43 revealed, in a model species, different relative contributions of plasticity and adaptation
44 to fitness, depending on the population and the time since colonization occurred.

45

46

47 **Keywords**

48 **Adaptation, *Capsella bursa-pastoris*, common garden, Finlay-Wilkinson regression,**
49 **fitness components, phenotypic plasticity, population structure.**

50

51

52 Introduction

53 Some species have tiny natural ranges, others very large ones. In the latter, the
54 current size was sometimes reached surprisingly fast something that might have left a
55 strong footprint on the current pattern of genetic and phenotypic variation. In the
56 present study we will focus on such a case, namely the shepherd's purse, *Capsella*
57 *bursa-pastoris* (L.) Medik. (*Brassicaceae*), one of the most common plants on Earth
58 (Coquillat, 1951). Since plants are sessile organisms, the expansion speed mainly
59 depends on their ability to disperse efficiently and establish themselves successfully in
60 new environments. Sometimes the environment will be quite similar to the one in
61 which the parental plants were growing, sometimes it will not. In this latter case,
62 establishment success in the short term may depend on phenotypic plasticity and in the
63 long run on the capacity to adapt to local conditions. Phenotypic plasticity is defined
64 here as the ability of a given genotype to produce different phenotypes in different
65 environments (Bradshaw, 1965; Grenier *et al.* 2016 and references therein), and
66 adaptation as the natural selection process by which a population progressively
67 increases its fitness in a given environment (Linhart & Grant, 1996; Chevin *et al.* 2010
68 and references therein). These two evolutionary mechanisms are not mutually
69 exclusive, so that each successful expansion will likely correspond to a particular
70 combination of both. In any case, when species succeed to extend their distributions to
71 a worldwide scale, granted that they hence face strongly different environmental
72 conditions, signatures of both adaptation and phenotypic plasticity are likely to be
73 observed. We can conjecture that phenotypic plasticity might be more important in
74 newly established populations and adaptation in older ones. Such predictions are
75 fraught with difficulties, since the age of populations is generally hard to estimate and
76 patterns of phenotypic plasticity may vary depending on the time since colonization.
77 Indeed, theoretical models indicate that while adaptation to a new extreme
78 environment may lead to a transient increase in plasticity, this is followed by a second
79 period of genetic assimilation which, on the contrary is associated to decreased
80 plasticity (Lande, 2015). These complex dynamics could explain why different studies
81 on phenotypic plasticity of colonizing species led to divergent conclusion: some recent
82 studies concluded that phenotypic plasticity could play a role during colonization of
83 new environments (e.g., Daehler, 2003) while others did not (e.g., Davidson *et al.*
84 2011, Godoy *et al.* 2011). Likewise, while evolutionary pressures underlying
85 adaptation in populations at equilibrium are fairly well documented, the literature is
86 more limited for recent and marginal populations that are usually characterized by non-

87 equilibrium demographics (Excoffier *et al.*, 2009; Gilbert *et al.*, 2017). As illustrated
88 by Willi *et al.* (2018) determining the respective role of phenotypic plasticity and
89 adaptation during colonization can be challenging.

90 Two prominent properties of plants that might influence a plant's adaptive potential
91 and its phenotypic plasticity are ploidy level and mating system. Both have their own
92 costs and benefits, though arising on a different timescale. Allopolyploidy and its evolu-
93 tionary success can appear paradoxical since its birth will also be accompanied by nu-
94 merous early challenges (Yant & Bomblies, 2015; Pelé *et al.*, 2018). These challenges are
95 first encountered during the initial hybridization event between two divergent genomes,
96 implying, among other things, potential changes of gene expression patterns (Bomblies *et*
97 *al.*, 2016). On the other hand, the increase in chromosome number in polyploids creates
98 genetic redundancy, which potentially allows pattern of expression to diverge, and the
99 evolution of new functions as well as tissue-specific expression of different gene copies
100 (Buggs *et al.*, 2010, 2011). This greater genomic flexibility could lie behind the increased
101 trait plasticity of tetraploids in heterogeneous environments, as was recently observed in
102 strawberries (Wei *et al.*, 2019). As to mating system, the shift from out-crossing to self-
103 fertilization (a.k.a. selfing) confers “reproductive assurance” when the number of mates
104 is limited. Reproductive assurance is expected to favor colonization of new environments
105 as a few individuals can establish a new population (Baker *et al.* 1965; Pannell *et al.*,
106 2015). The benefit of self-fertilization might, however, be short-lived because the lack of
107 genetic mixing is predicted to limit adaptation and to lead to the genome-wide accumula-
108 tion of deleterious mutations (Heller & Smith, 1978; Hollister *et al.*, 2015, Glémin *et al.*,
109 2019). In agreement with these predictions, selfing species tend to have larger ecological
110 ranges (Grossenbacher *et al.*, 2015) but decreasing niche breadth with time (Park *et al.*,
111 2018) compared to their outcrossing congeners, aligned with the “evolutionary dead end”
112 hypothesis (Stebbins 1957; Takeyabashi & Morell, 2001 and references therein). This
113 phenomenon is even more pronounced during a colonization process (Slatkin &
114 Excoffier, 2012; Gonzalez-Martinez *et al.*, 2017 and references therein).

115 *C. bursa-pastoris* is a successful worldwide self-fertilizing colonizer of recent allopol-
116 yloid origin that arose some 100,000 years ago from the hybridization between the self-
117 fertilizing, *C. orientalis* (Fauché & Chaub.) Boiss. and the out-crossing, *C. grandiflora*
118 (Klokov) (Douglas *et al.*, 2015). As opposed to its two parents, which are restricted to
119 specific areas (from Central Asia to eastern Europe for *C. orientalis* and mountains of
120 northwest Greece and Albania for *C. grandiflora*), *C. bursa-pastoris* has an almost
121 worldwide distribution. The rapid expansion of the shepherd's purse did not prevent the

122 emergence of three distinct genetic clusters: Asia (ASI), Middle East and northern Africa
123 (ME), and Europe and the Russian Far East (EUR) (Cornille *et al.*, 2016). Demographic
124 inferences showed that these three clusters are the result from a range expansion that
125 started either from the Middle East or Europe (the starting point is not known with cer-
126 tainty), and was followed by a consecutive colonization event towards Asia. This recent
127 worldwide spread was, in some cases, likely associated to human migrations, as for in-
128 stance the spread to eastern Siberia of western European accessions (Cornille *et al.*,
129 2016), or of southern European and Middle Eastern accessions to North America (Hurka
130 & Neuffer, 1997; Cornille *et al.*, 2016).

131 Given conflicting predictions, it remains challenging to explain the apparent ecologi-
132 cal success of species, such as *C. bursa-pastoris*, that recently expanded their range.
133 Moreover, adopting a “reciprocal transplant” framework to address this question would
134 be hard to implement as it would require numerous transfers to capture the genetic diver-
135 sity and the environmental range of *C. bursa-pastoris*. Instead, in order to investigate the
136 joint role of phenotypic plasticity and adaptation in the colonization success of *C. bursa-*
137 *pastoris*, we implemented an experimental design with three large common gardens lo-
138 cated in three contrasted environments. Firstly, in order to reflect the diversity of envi-
139 ronmental conditions that *C. bursa-pastoris* faced during its range expansion, we in-
140 stalled two common gardens at extreme latitudes in Eurasia, one in East Asia and one in
141 Northern Europe, and a third one in North America, which lies outside of the native range
142 of the species. Secondly, to capture the demographic history of *C. bursa-pastoris*, we
143 used a comprehensive sampling of populations from Europe, Asia, North Africa, the
144 Middle East and North America. And thirdly, to be able to characterize phenotypic varia-
145 tion and accession’s performances, we measured several life-history and phenological
146 traits, some of which are main fitness components. This provided us with a solid frame-
147 work to: (i) measure the responsiveness of individual accessions to different environ-
148 ments (i.e., assess their phenotypic plasticity) by comparing the performance of each ac-
149 cession across the three common gardens; (ii) estimate the genetic cluster effect associat-
150 ed to past demographic history by comparing the performances of the three genetic clus-
151 ters across the common gardens; and (iii) assess the variation in responsiveness among
152 accessions of a same cluster using Finlay-Wilkinson regressions (Finlay & Wilkinson,
153 1963). Evidence of the role of adaptation in the colonization process would be provided
154 by analyzing all the results together and notably by estimating the genotype (genetic clus-
155 ter) by environment (common garden) interactions. We will discuss these results in the
156 light of *C. bursa-pastoris* colonization history and its accumulated expansion load.

158 **Materials and Methods**

159 **Common gardens**

160 **Localization.** One common garden was located at Uppsala (59°51'N, 17°38'E,
161 Sweden), the second one at Guangzhou (23°08'N, 113°16'E, China), and the third one at
162 Toronto (43°39'N, 79°23'W, Canada). To account for the sensitivity of *C. bursa-pastoris*
163 to extreme environmental conditions and to guarantee intermediate temperatures,
164 experiments were started at different periods of the year: at Uppsala, the experiment
165 started in early May 2014 for 139 days; at Guangzhou, in early November 2014 for 193
166 days; and at Toronto, in early June 2014 for 118 days.

167 **Environmental conditions.** At Uppsala and Guangzhou, environmental conditions
168 were monitored daily at ground level using temperature and humidity sensors (TGP-
169 4017[®], Tinytag[™]). At Toronto, temperature and humidity were extracted from a public
170 database (<https://www.timeanddate.com>). For each location, day length was obtained
171 with the R package *geosphere* (function *daylength*; Hijmans, 2019).

172

173 **Plant materials**

174 **Sampling.** We used a collection of 267 accessions from 65 sites distributed across
175 Europe, Asia, North Africa, the Middle East and North America (for more details, see
176 Cornille *et al.*, 2016, Kryvokhyzha *et al.*, 2016, Kryvokhyzha *et al.* 2019b). In Toronto,
177 due to limited space, we had to subsample this collection down to 160 accessions, and
178 were chosen to share less than 97% of identity-by-state (for further details, see Salcedo,
179 2015), to maximize the genetic diversity.

180 **Genotypes.** Genotyping-by-sequencing (GBS) data for the 267 accessions acquired by
181 Cornille *et al.*, (2016, <https://datadryad.org/stash/dataset/doi:10.5061/dryad.71f99>) were
182 used to assign accessions to different clusters (Asia, Middle East or Europe). First, we ran
183 a Multi-Dimensional Scaling analysis (function *cmdscale* from the base R package *stats*)
184 on the GBS data that gave the five first principal components on which we ran an
185 unsupervised k-means algorithm with $K = 3$ (function *kmeans* from the base R package
186 *stats*). We then tested its robustness by using a random forest approach (function
187 *randomForest* from the R package *randomForest*; Liaw & Wiener, 2002), with a
188 bootstrap (1000 iterations) of 20% of the dataset (80% being used as a training set). Only

189 accessions that were assigned more than 95% of the iterations to the same cluster were
190 kept for further analyses (Table S1 and Figure S1).

191 **Seeds preparation.** Before the establishment of the common garden experiment, and
192 to limit maternal effects, seeds collected from the field were first sown and grown under
193 controlled conditions in growth chambers (55% moisture, 22°C, 12h:12h light:darkness
194 cycles). Their offspring (seeds) were then used to establish the common gardens. About
195 20 seeds per accession were surface-sterilized the same day and germinated in Petri
196 dishes, with MS medium and agar (see protocol in Kryvokhyzha *et al.*, 2016). Petri
197 dishes were then stratified for seven days at 4°C in the dark to promote germination.
198 After this cold treatment, Petri dishes were placed in a greenhouse with no additional
199 light or heating, in order to protect seeds from rainfall and to facilitate acclimation to
200 outdoor conditions. Petri dishes were randomized over tables and moved every day to
201 avoid micro-environmental effects, and they were left in the greenhouse until seedlings
202 reached a four-leaf stage.

203 **Transplantation.** At Uppsala and Guangzhou, once a seedling reached the four-leaf
204 stage, it was thinned to one per pot (7 cm x 7 cm) containing mud (basic soil mixed with
205 water). The pots were left seven days in the greenhouse (watered automatically twice a
206 week) and then they were pierced on the bottom and placed outside in the common
207 garden. The pots were dispatched into six blocks (1m x 3.2m, grids of 9 x 30) arranged at
208 2 m spacing and containing basic soil. Each block contained exactly one replicate per
209 accession, so that we ended up with six replicates per accession in total. An accession
210 was included in the common garden experiment even if less than six seedlings
211 germinated. In such case, and to keep the same individual density in each block,
212 seedlings from another accession from the same sampling site were planted. At Toronto,
213 after reaching the four-leaf stage, seedlings were directly transplanted from Petri dishes
214 to planting beds (13 cm x 33 cm) containing Promix soil (thoroughly wet). These beds
215 contained exactly one replicate per accession, and we used the same workaround when
216 germination failed. The beds were covered with shade cloth for two days after planting.
217 At each location, apart from the initial shading and watering, we did not provide any
218 support to the seedlings. The experiment lasted until the senescence of the last plant, *i.e.*,
219 when the last plant dried up but had not yet shed its fruits.

220 **Subdividing datasets.** We considered three approaches for studying the 267
221 accessions: for exploratory analyses, we kept the “whole dataset”, accepting potential
222 unbalance; this dataset was thereafter referred to as the whole dataset. Since the

223 interaction effect between common garden and genetic cluster was significant (see
224 Supplementary materials), following the recommendation of Crawley (2012), we chose
225 to analyze each common garden separately; this dataset was thereafter referred to as the
226 “common garden dataset”. Finally, in order to mimic a reciprocal transplant experiment
227 and test for signature of local adaptation, we considered a sub-dataset made only of
228 accessions from Sweden and South China (12 accessions in total, sampled close to
229 Uppsala or Guangzhou), and excluding their phenotype measurement in Toronto; this
230 dataset was thereafter referred to as the “local adaptation dataset”.

231

232 **Statistical analyses**

233 **Phenotypes.** After senescence, we recorded for each plant the *height of the highest*
234 *inflorescence* (in dm), the *number of basal inflorescences*, and the *number of fruits along*
235 *a section of 1 dm* in the middle of the main inflorescence. In order to compare the overall
236 performances, we considered the total number of fruits per individual as a proxy of
237 fitness (denoted w), that were measured differently in the common gardens: at Uppsala
238 and Guangzhou, we considered a composite index of fitness, computed as the product of
239 the aforementioned three traits (*number of basal inflorescences*, *height* and *number of*
240 *fruits along a section of 1 dm*). At Toronto, the total number of fruits was directly
241 measured for each individual.

242 Three phenological traits were also measured, *bolting time* (*i.e.*, time until
243 differentiation of the bud from vegetative parts indicating the initiation of the
244 reproductive period), *flowering time* (*i.e.*, time until the appearance of the first opened
245 flower) and *senescence time* (*i.e.*, time until the drying state of the plant). In addition,
246 *flowering time span* was computed as being the inter-event time between *flowering time*
247 and *senescence time*. At flowering time, we also recorded the rosette leaf number and the
248 maximum diameter of the rosette. Finally, at Uppsala and Guangzhou, germination time
249 was also measured.

250 **Statistical modeling.** For the statistical analyses, we fitted the following generalized
251 linear mixed model to the data:

$$252 \quad \text{Fitness}_{ijkl} \sim \mu + \text{cluster}_i + \text{block}_j + \text{accession}_k + e_{ijkl} \quad [1]$$

253 where μ is the overall mean, ‘cluster’ is the fixed effects of the genetic cluster, ‘block’
254 and ‘accession’ are uncorrelated random effects (‘accession’ being nested into ‘cluster’),
255 and ‘e’ is the residual. We used a log link function and the residual distribution was fitted

256 to a Negative Binomial distribution (function *glmer.nb* of R package *lme4*; see Bates *et*
257 *al.*, 2015). This model was thereafter referred to as the “GLMM model”. For
258 phenological traits, we fitted the GLMM model to the four inter-event durations (between
259 sowing, germination, bolting, flowering, and senescence), square-rooted, with the starting
260 date of each time span as an additional explaining variable, and with the residual fitted to
261 a normal distribution (function *lmer* of R package *lme4*). Statistical significance of the
262 cluster effect was assessed with a type II Wald chi-square test, and the difference between
263 genetic clusters was assessed with Tukey’s HSD test.

264 **Finlay-Wilkinson regression (FWR).** We further investigated the specificity of each
265 genetic cluster by performing a Finlay-Wilkinson regression (Finlay & Wilkinson, 1963),
266 enabling us to characterize individual genotype responses for a trait across different
267 environments. Following the formulation of Lynch & Walsh (1998), a FWR for one
268 accession can be expressed as follows:

$$269 \text{Trait}_{ij} \sim \mu + (1 + b_j) \mu_j + e_{ij} \quad [2]$$

270 where ‘Trait’ is the focal trait (fitness or phenological traits), *i* is the block, *j* is the
271 common garden, μ_j is the average value of the trait at the common garden *j*, b_j is the
272 regression factor of μ_j and e_{ij} is the residual which is assumed to be normally distributed.
273 We performed as many regressions as the number of accessions. This model was
274 thereafter referred to as the FWR model.

275 The intercept (μ) can be interpreted as the accession’s performance in a poor
276 environment, and the slope (b_j) can be viewed as the accession’s relative responsiveness
277 to environmental changes (the steeper the slope, the more responsive the accession;
278 Lynch & Walsh, 1998). By definition, across the whole dataset, we expect a null average
279 intercept ($\mu = 0$) and a unit average slope ($b_j = 0$). Each genetic cluster can, however,
280 have its own specificity and deviate from the global trend. We used FWR to assess
281 whether a genetic cluster was homogeneous, *i.e.*, with a trend followed by every
282 individual belonging to this cluster, or heterogeneous and actually driven by some
283 “outliers”. For intercept (μ) and slope (b), the statistical significance of difference in
284 average or variance between genetic clusters was assessed with a pairwise Welch’s *t*-test
285 or a Fisher’s *F*-test, respectively.

286 Results

287 Statistical interaction between “common garden” and “genetic cluster” was significant
288 for most traits (Table 1 and Supplementary materials), therefore we focused our analyses
289 on the common garden dataset with the GLMM model; the use of the whole dataset was
290 restricted to the description of phenotypic variation (Table 1 and Figures 1 to 3). Due to
291 experimental issues (*e.g.*, failed germination, climatic events), common garden
292 experiments ended up with an unbalanced block design: the whole dataset was left as is,
293 but we shrank the common garden dataset to balance it, ending up with 186 accessions in
294 Uppsala, 86 in Guangzhou and 116 in Toronto (Table S1). Also, due to these issues, the
295 number of accessions common to all three common gardens in the common garden
296 dataset was reduced to a total of 34 accessions (12 Asian, 9 European and 13 Middle
297 Eastern): we therefore fitted the FWR model on the (unbalanced) whole dataset filtered
298 for accessions for which a fitness score was available in all three common gardens,
299 ending up with a total of 114 accessions (46 Asian, 46 European and 22 Middle Eastern).

300 At the time of the experiment (2014), environmental conditions (in terms of
301 temperature, humidity and day length) strongly differed among the common gardens
302 (Figure 1). In Uppsala, accessions grew in rather cold and wet climatic conditions ($15.2 \pm$
303 5.1°C , $76.8 \pm 14.1\%$); in Toronto they grew under a relatively warm and dry climate
304 ($20.7 \pm 3.1^\circ\text{C}$, $71.2 \pm 8.9\%$); finally, in Guangzhou they grew in a warm and humid
305 weather ($19.3 \pm 5.0^\circ\text{C}$, $81.1 \pm 11.4\%$). The photoperiod and the magnitude of its variation
306 during the experiment also strongly differed among common gardens, day length being
307 the longest in Uppsala ($16.9\text{h} \pm 1.7\text{h}$, ranging from 13h to 18.8h), the shortest in
308 Guangzhou ($11.9\text{h} \pm 1.0\text{h}$, ranging from 10.7h to 13.5h), and intermediate in Toronto
309 ($14.3 \pm 1.1\text{h}$, ranging from 12h to 15.4h). For further details, see Tables S2 to S4 and
310 Figures S2 to S5.

311

312 Environmental variation as the main explanatory factor of the 313 phenotypic variation of *Capsella bursa-pastoris*

314 Most of the phenotypic variation was explained by the common garden, with a strong
315 environmental effect (Figure 1, Table 1).

316 Accessions showed contrasted phenotypes among common gardens (Figures 2 and
317 S6), highlighting their phenotypic plasticity. In Toronto, accessions tended to be more
318 “bushy” with relatively more branches and more rosette leaves, whereas in Guangzhou,

319 accessions tended to be relatively taller. In Uppsala, accessions were globally smaller and
320 performed poorly for most phenotypic traits. The common garden effect was even more
321 pronounced on phenology than on life history traits ($\chi^2_{pheno} = 1051 \pm 557.5$; $\chi^2_{life\ history\ traits}$
322 $= 293.3 \pm 111.7$; all $p < 0.001$; Table 1). In particular, *bolting time*, *flowering time* and
323 *senescence time* were longer in Guangzhou (respectively 61.3 ± 13 days, 71.5 ± 13.1
324 days, 138 ± 9.5 days) than in Toronto (28.0 ± 4.8 days, 31.9 ± 7.5 days, 79.8 ± 9.5 days)
325 and Uppsala (30.9 ± 6.1 days, 36.4 ± 5.6 days, 85.3 ± 7.6 days).

326 More importantly, environmental effect on fitness was significant ($\chi^2 = 128.5$, $df = 2$, p
327 < 0.001 , type II Wald chi-square test; Table 1): fitness was much lower on average in
328 Uppsala ($w = 224 \pm 178$) than in Guangzhou ($w = 918 \pm 753$) and Toronto ($w = 1161 \pm$
329 788). Toronto presented the most favorable conditions in terms of temperature and day
330 length, two factors *C. bursa-pastoris* is known to be particularly sensitive to.

331

332 **Genetic effect associated to the past demographic history** 333 **significantly explained variation within a common garden**

334 Genetic effect comes second and explains the phenotypic variation within each
335 common garden (Figures 1 and 2).

336 **Phenotypes.** Within each common garden, except for the number of primary
337 branches, phenotypic variation showed a strong genetic effect (Table 2). The cluster
338 effect for the number of primary branches was not significant in Uppsala, and strongly
339 significant ($p < 0.01$) in Guangzhou and Toronto. Surprisingly, the cluster effect for
340 rosette characteristics (number of leaves and diameter) was much more significant than
341 that for height of the main inflorescence or number of primary branches (Table 2). On
342 average, the size and the number of leaves of the European accessions were much higher
343 (respectively 188.6 ± 55.5 mm and 40.8 ± 30.4) than those of the Middle Eastern
344 accessions (respectively 172 ± 54.8 mm and 19.3 ± 8.4) and of the Asian accessions
345 (121.0 ± 56.5 mm and 19.5 ± 11.7).

346 **Phenology.** Phenological variation also showed a strong and significant cluster effect
347 (Table 2). The Asian cluster exhibited a late germination, early bolting, early flowering
348 and late senescence, therefore a long flowering time span. In contrast, the European
349 cluster germinated early, but bolted, flowered and withered late (Table S5). European
350 accessions' late flowering partly explains their larger rosette, since they had more time
351 for vegetative growth. Accessions from the Middle-East genetic cluster did not follow a

352 global trend (Table S5), deploying again a certain amount of plasticity in their
353 phenological response.

354 For *bolting time*, mean intercept in the FWR model was significantly lower for the
355 Middle Eastern than for the Asian and the European clusters (all $p < 0.05$, Welch's t -test;
356 Table S6): in an environment pushing *C. bursa-pastoris* to bolt early on average, ME
357 bolted even earlier.

358 **Fitness.** Variation in fitness also showed a strong and significant genetic effect (Figure
359 1 and Table 2). The Middle Eastern cluster globally outperformed the other clusters, with
360 an overall mean performance of $w = 946 \pm 916$, compared to the Asian ($w = 565 \pm 613$)
361 and the European ($w = 652 \pm 685$) genetic clusters. At each common garden, its
362 performance is either significantly higher than another cluster, or not significantly smaller
363 than another one (Table 3). The Middle Eastern cluster deployed a significantly higher
364 phenotypic variance during this experiment than the other clusters (all $p < 0.001$, Fisher's
365 F-test; Table S7), and also showed a significantly higher responsiveness (slope in the
366 FWR model; all $p < 0.01$, Welch's t -test; Table 4), corroborating the hypothesis of a
367 higher plasticity of the Middle Eastern cluster.

368

369 **The Middle Eastern accessions had a more homogeneous** 370 **response to environmental variation than Asian or European** 371 **accessions**

372 **Phenology.** The Middle-Eastern cluster showed, according to the FWR model, a lower
373 variance in their phenological behavior: the variance of the slope for *flowering time*,
374 *flowering time span* and *bolting time* was significantly smaller than that of the Asian and
375 the European accessions (all $p < 0.001$, Fisher F-test; Table S6), therefore suggesting that
376 the Asian and European clusters are far more diverse than the Middle Eastern one.

377 **Fitness.** Variance of the intercept in the FWR model was too large compared to its
378 mean value to be interpreted, given the coefficients of variation of 27.4 for the Asian, 6.3
379 for the European and 7.7 for the Middle Eastern (Table 4) clusters. However, the slope
380 was more discriminant: the Middle Eastern cluster had a significantly higher (all $p <$
381 0.01 , Welch's t -test; Table 4) responsiveness (1.27 ± 0.36) than the Asian (0.91 ± 0.47)
382 and the European (0.96 ± 0.44) clusters. Slope variance of the Middle-Eastern cluster was
383 smaller than for the two other clusters, although not significantly (Table S6).

384 **Linking fitness and phenology.** Surprisingly, Pearson's correlation between different
385 FWRs was weak (absolute values < 0.2 if putting ME aside; Table 5), suggesting that
386 phenology explained only a small fraction of the variance of fitness. However, for the
387 Middle-Eastern cluster, Pearson's correlation between *fitness* and *flowering time* or
388 between *fitness* and *flowering time span* was equal to $r = 0.30$ and $r = 0.50$ respectively,
389 suggesting that accessions that were plastic for part of the phenology were likely also
390 plastic for the number of fruits.

391

392 **Hints of signature of local adaptation when mimicking a** 393 **reciprocal transplant**

394 In the presence of local adaptation, we expect a higher fitness of accessions in their
395 own geographical range. In our case, we expected a higher relative fitness of the
396 European accessions in Uppsala, and a higher relative fitness of the Asian accessions in
397 Guangzhou.

398 **With the common garden dataset.** Whereas the Middle Eastern cluster globally
399 outperformed the other clusters, the European cluster ($w = 252 \pm 216$) significantly
400 outperformed the Asian cluster ($w = 203 \pm 153$) in Uppsala ($z = 2.65$, $p < 0.05$, Tukey's
401 HSD test; Table 3). However, although a trend of the Asian cluster ($w = 852 \pm 634$)
402 outperforming the European cluster ($w = 697 \pm 604$) in Guangzhou can be seen, this
403 difference was not significant ($z = -1.83$, $p = 0.16$, Tukey's HSD test; Table 3).

404 **With the local adaptation dataset.** The pattern of local adaptation was actually even
405 less clear when considering the local adaptation dataset: the performances of the South
406 Chinese and Swedish accessions did not differ significantly. Indeed, in Guangzhou,
407 though the performance of Chinese accessions ($w = 926 \pm 598$) tended to be higher than
408 that of Swedish accessions ($w = 524 \pm 533$), the difference was not significant ($z = -$
409 1.379 , $p = 0.17$, Tukey's HSD test); likewise, in Uppsala, difference in terms of
410 performance between Chinese accessions ($w = 208 \pm 130$) and Swedish accessions ($w =$
411 210 ± 193) was not significant ($z = -0.752$, $p = 0.45$, Tukey's HSD test). One accession
412 from Sweden outperformed all Chinese accessions though, suggesting that the lack of a
413 significant signature of local adaptation might be caused by the limited number of
414 accessions sampled ($N_{EUR} = 3$ and $N_{ASI} = 9$).

415

416 **Discussion**

417 Overall, the location of the common gardens explained a large part of the phenotypic
418 variation of *C. bursa-pastoris*, suggesting that the underlying mechanism of its success is
419 probably a high degree of phenotypic plasticity. To a lesser extent, genetic clusters
420 explained phenotypic variance within common gardens. Assuming that populations in the
421 colonization front gradually undergo a shift of their prior phenotypic plasticity to
422 adaptation to local conditions, *C. bursa-pastoris* seems to be in this very transient state.
423 As a matter of fact, the hypothesis of the presence of a transient state in invasive species
424 could explain most of the discrepancies in the literature about the prevalence of either
425 phenotypic plasticity or adaptation. Indeed, if the colonization event is recent, it is
426 possible, like in our study or in VanWallendael *et al.* (2018), that signatures of local
427 adaptation are not yet observable (but see Godoy *et al.*, 2011; Ducatez *et al.*, 2016).

428

429 **The overall success of the Middle Eastern accessions might** 430 **obscure the scale of local adaptation**

431 *C. bursa-pastoris* is a self-fertilizing species that underwent a relatively recent
432 worldwide expansion, and has a low genetic variation that could, by itself, explain the
433 lack of local adaptation signature in the present study. *C. bursa-pastoris* and its diploid
434 relatives are eminently ruderal and even a casual observation of their habitats highlights
435 the importance of very local factors for their establishment, such as soil disturbance
436 (Orsucci *et al.*, *in press*). Nonetheless *C. bursa-pastoris* is genetically structured across
437 its worldwide distribution (Cornille *et al.*, 2016), and therefore, to account for this
438 structure, we needed to implement a large-scale experiment with a large number of
439 accessions. Thus, perhaps more than in other species, geographical scale is crucial in
440 studies of adaptation in the *Capsella* genus and what is observed at a given scale may not
441 apply at another one, as exemplified by Neuffer (1996).

442 To the best of our knowledge, however, there has not been a general study of the local
443 ecological niches of the shepherd's purse. Our own observations and the few studies
444 available, in particular the study by Caullet (2011) that exhaustively studied populations
445 of *C. bursa-pastoris* and *C. rubella* across a 4 km² agricultural landscape of Central
446 France over two years, indicate that *C. bursa-pastoris* is actually restricted to highly
447 disturbed environments (hedges of fields, for instance). Within such environments it does
448 not seem to be confined to specific types of soil though (Aksoy *et al.*, 1998; Neuffer *et*
449 *al.*, 2018). The large spectrum of climatic environments covered by the accessions

450 included in our experiment also suggests that insufficient sampling is unlikely to explain
451 the lack of a strong local adaptation pattern.

452 As shown above, though the effect of either environment or genetic background seems
453 clear, the interaction between genetics and environment is complex. When considering
454 all common gardens and all clusters at once, there is no clear evidence of local
455 adaptation, mainly because the Middle Eastern cluster outperformed the other clusters
456 everywhere. The Middle Eastern cluster is less variable for the traits considered here, and
457 is consistently better and more responsive to variation in environmental conditions than
458 those from the two other clusters. One of the possible explanations of this complex
459 pattern, obscuring a potential signature of local adaptation, is that there is no or few local
460 adaptation yet: an absence of local adaptation coupled with a pronounced plasticity have
461 already been observed in other invasive species at the colonization front, such as in
462 *Reynoutria japonica* (VanWallerdael *et al.*, 2018) or in clonally reproducing
463 macrophytes (*Egeria densa*, *Elodea canadensis* and *Lagarosiphon major*: Riis *et al.*,
464 2010).

465 It is also worth pointing out that the absence of evident GxE effect may be due to the
466 fact that we did not consider the whole life cycle. Recently, Orsucci *et al.* (2020)
467 emphasized that studying only one component of fitness could be insufficient and
468 potentially misleading if interpreted as fitness, since they do not account for possible
469 trade-offs between fitness components. Germination rate of the mother plant was not
470 measured here, and is the only part of the life cycle that was controlled in a growth
471 chamber. Accounting for the germination rate might change the ranking among clusters,
472 as shown in Orsucci *et al.* (2020), we can thus only say that the Middle Eastern cluster
473 outperformed others in terms of vegetative growth and fruit production, but whether it
474 also does in term of fitness remains to be tested.

475

476 **A species with a worldwide distribution might present a complex** 477 **genetic structure and local adaptation pattern**

478 The absence of local adaptation signature could also be explained by the marginality
479 of our common gardens in deference to the species distribution described by Hurka *et al.*
480 (2012). Indeed, Uppsala is close to the northern boundary of the distribution of European
481 accessions; Guangzhou, likewise, is close to the southern boundary of the distribution of
482 Asian accessions; and Toronto is even outside the natural species distribution.
483 Environmental conditions can be extreme in Uppsala and Guangzhou compared to other

484 locations of the same continent. The extreme localization of our common gardens could
485 thus have hidden the importance of local adaptation among clusters, as discussed in Klisz
486 *et al.* (2019). We attempted to mimic a reciprocal transplant experiment by only
487 considering Swedish and South Chinese accessions at Uppsala and Guangzhou.
488 However, the results were mixed, with no apparent signature of local adaptation (i.e., no
489 apparent genetic cluster effect). To rigorously address this problem in further studies, it
490 will be necessary to either (i) increase the amount of actual local accessions, so that the
491 common gardens are less marginal relative to the set of accessions sampled, e.g., more
492 Swedish and South Chinese accessions; or (ii) consider several common gardens in a
493 smaller geographical scale, e.g., two or more common gardens in Europe.

494 The absence of evident G x E effect for all of the clusters leads to question about the
495 geographical scale at which local adaptation occurs. Although the populations we
496 considered are at the right genetic scale in the sense that our samples do capture the
497 major genetic clusters in Eurasia (Cornille *et al.*, 2016; Kryvokhyzha *et al.*, 2019a), local
498 adaptation might occur at a much more restricted geographical scale, as pointed out
499 above. First, *C. bursa-pastoris* is a ruderal species and probably a human commensal too,
500 as suggested by its distribution, and therefore it grows primarily, if not uniquely, in very
501 specific, disturbed environments, that will share common characteristics under different
502 latitudes. Second, *C. bursa-pastoris* is mainly self-fertilizing which implies that gene
503 flow occurs through seeds rather than pollen, thus slowing down its scattering. The
504 hypothesis of adaptation to micro-environment rather than macro-environment is also
505 supported by the fact that phenology is mostly influenced by environmental variation,
506 and only secondly by genetics.

507 The apparent lack of local adaptation observed in the present study at the phenotypic
508 level can be related to the results obtained by Kryvokhyzha *et al.* (2016). Using RNA-
509 Seq data from 24 accessions originating from the three clusters, Kryvokhyzha and co-
510 authors showed that the genes that were significantly differentially expressed between the
511 three clusters, were no longer so once one had corrected the data for the genetic
512 relationship between the three clusters. The differentiation could then have been mostly
513 neutral or both neutral and adaptive in which case demographic history and adaptive
514 differentiation were highly correlated and correcting for the first one would remove the
515 effect of the latter. The fitness results obtained here would support at least a significant
516 contribution of neutral processes. As in the RNA-Seq experiment, the present data does
517 not rule the presence of significant local adaptation at a more local scale. However, even

518 at that scale local adaptation does not seem to be pronounced in *C. bursa-pastoris*
519 (Caullet, 2011; Huang *et al.*, 2018).

520 It is therefore likely that *C. bursa-pastoris* was able to cope with diverse environments
521 primarily through phenotypic plasticity. Caullet (2011) also found that plasticity best
522 explained fitness across soil niches when they planted both *C. bursa-pastoris* and *C.*
523 *rubella* in four different disturbed environments where both species are naturally found:
524 along paths, hedges of the paths, or within a transition zone between paths and fields and
525 inside the internal hedge of the fields. The transplant environment did not significantly
526 affect the fitness of *C. bursa-pastoris*. As a whole, as suggested by Caullet (2011),
527 phenotypic plasticity was higher in *C. bursa-pastoris* than in *C. rubella* but there was no
528 evidence of strong local adaptation in *C. bursa-pastoris*. Another possible explanation for
529 the absence of signature of local adaptation is that *C. bursa-pastoris* failed to adapt
530 locally due to its high genetic load. Indeed, *C. bursa-pastoris* is a selfer, with limited
531 genetic diversity and it might have faced a drift barrier and lacked the initial effective
532 population size needed to express local adaptation.

533

534 **Phenological traits, between plasticity and adaptation**

535 Controlling the timing of phenology to cope with biotic and abiotic stresses is the
536 response adopted by many plant populations, especially those with an annual life cycle.
537 Adaptation is one of the possible evolutionary responses, as seen in temperate crop
538 species (e.g., wheat, barley or pea) with distinct vernalization strategies in different
539 varieties (Roux *et al.*, 2006). As to *C. bursa-pastoris*, in agreement with previous studies
540 (Neuffer & Hurka 1986; Neuffer, 1990), our experiment revealed that its phenological
541 response was mainly through phenotypic plasticity, with a rather equivalent
542 responsiveness among genetic clusters. Although Neuffer & Hurka (1986) evoked a
543 “general purpose genotype” (a term borrowed from Baker, 1965) for germination
544 strategy, we could not observe this pattern in our study, as germination seemed as plastic
545 as other phenological traits.

546 Within each common garden, accessions from the Asian cluster flowered earlier than
547 accessions from the other clusters, a phenological characteristic that might be an
548 adaptation to Asia: (i) as a response to avoid biotic competition (Orsucci *et al.*, 2020),
549 thus compensating being in the colonization front and its consequent genetic load; or (ii)
550 as a response to a rather unpredictable environment such as Asia (e.g., typhoon,
551 monsoon), as observed in *Arabidopsis thaliana* (Simpson & Dean, 2002; Roux *et al.*,
552 2006), that is a phylogenetically close species (Beilstein *et al.*, 2006). It can also be a side

553 consequence of a higher sensibility to photoperiod, as observed in tropical species such
554 as rice (*Oryza sativa*) and maize (*Zea mays*) since the photoperiod is the main cue to the
555 alternation of dry and humid seasons in these climates (Roux *et al.*, 2006). According to
556 this last hypothesis, early flowering reflects a different sensitivity to photoperiod, not an
557 advantage of early flowering *per se*. In addition, several studies showed that, in several
558 species such as *Arabidopsis thaliana* (Le Corre *et al.*, 2002; Komeda, 2004) or *Triticum*
559 *monococcum* (Yan *et al.*, 2003), late flowering seems to be the ancestral character: if it is
560 also the case for *C. bursa-pastoris*, it is probable that the “early flowering” phenotype
561 recently evolved during *C. bursa-pastoris* range expansion in Asia.

562 Our study also showed that the European cluster flowered later than the other clusters,
563 causing a longer time span for vegetative growth, and partly explaining the significantly
564 larger rosette. In the Alpine accessions of *C. bursa-pastoris*, overwintering in the rosette
565 state is frequently observed (Neuffer, 1990): a large rosette could be an adaptation
566 towards harsh winter conditions in Europe, though accessions with large rosette were not
567 specifically from the Alps. In our study, correlation between the rosette size and the
568 flowering time was of $r = 0.52$ (see also Figure 2). It is not surprising to see an
569 association between flowering trends and rosette characteristics, as it has been shown
570 previously that QTLs associated with these two traits are closely linked in *C. bursa-*
571 *pastoris* (Linde *et al.*, 2001); likewise, in *Arabidopsis thaliana*, rosette leaf number has
572 been shown to be sensitive to stimuli of flowering, such as shading (Cookson & Granier,
573 2006) or length of photoperiod (Lewandowska *et al.*, 2017).

574

575 **Conclusion**

576 Despite the large scale of the current study, involving three common gardens on as
577 many continents and a large number of accessions representative of the natural range of
578 the shepherd’s purse, we only detected phenotypic plasticity and adaptation signatures,
579 but not of local adaptation. Does this indicate that local adaptation did not contribute to
580 the rapid colonization of *C. bursa-pastoris*? It is probably too early to conclude, but the
581 present study suggests that understanding local adaptation in *C. bursa-pastoris* and other
582 ruderal species that went through a rapid range expansion would likely require a
583 combination of more targeted reciprocal transfer experiments as well as more small scale
584 experiments.

585

586 **Acknowledgements**

587 We thank the Swedish Research Council and the Erik Philip Sörensen Foundation (to
588 ML), Uppsala University and EMBO Short-Term Fellowship fund (to AC), and the
589 H2020 European consortium B4Est (to MT) for funding. This research was also funded
590 by the National Natural Science Foundation of China (grant No. 31870353, to HRH). AS
591 was supported by an NSERC CGS-M and an Ontario Graduate Scholarship. We thank
592 Julia Dankanich, Josefine Stångberg, Johanna Nyström, Sara Kurland and Uriel Gélín for
593 field assistance in Uppsala; Jean-Tristan Brandenburg for R scripting support; Lin-Lin
594 Wu, Zhi-Bing Xie, Qiu-Ling Guan, Gui-Yu Lin for field assistance in Guangzhou; Viktor
595 Mollov and Niroshini Epitawalage for field assistance in Toronto; and finally, we also
596 thanks Benoit Pujol, Benoit Facon, Denis Faure for a their comments on the first
597 versions on the manuscript.

598

599 **Author Contribution**

600 AC, SG, SW and MT planned and designed the common gardens. AC, SG, SW and
601 ML directed the work. AC, AS, HH, DK, and KH performed experiments (installing the
602 common garden and phenotyping every accessions). AC, MT, MO and PM analyzed
603 datasets. AC and MT wrote the article, with thorough help from MO and PM. AC, MT,
604 AS and HH contributed equally.

605

606

607 **Data availability**

608 The data that support the findings of this study are openly available in [repository name
609 e.g “figshare”] at [http://doi.org/\[doi\]](http://doi.org/[doi]), reference number [reference number].

610 **References**

611

612 Aksoy A., Dixon J. M. and Hale W. H. (1998), '*Capsella bursa-pastoris* (L.) Medikus
613 (*Thlaspi bursa-pastoris* L., *Bursa bursa-pastoris* (L.) Shull, *Bursa pastoris* (L.) Weber)',
614 *Journal of Ecology*, 86 (1), 171-186.

615

616 Baker H. G., Stebbins G. L. et al. (1965), genetics of colonizing species, proceedings, in
617 'International Union of Biological Sciences Symposia on General Biology 1964:
618 Asilomar, Calif.),' *Academic Press*.

619

620 Beilstein M. A., Al-Shehbaz I. A. and Kellogg E. A. (2006), 'Brassicaceae phylogeny
621 and trichome evolution', *American journal of botany*, 93 (4), 607-619.

622

623 Bomblies K., Jones G., Franklin C., Zickler D. and Kleckner N. (2016), 'The challenge
624 of evolving stable polyploidy: could an increase in "crossover interference distance" play
625 a central role?', *Chromosoma*, 125 (2), 287-300.

626

627 Bradshaw A. D. (1965), 'Evolutionary significance of phenotypic plasticity in plants',
628 *Advances in genetics*, 13 (1), 115-155.

629

630 Buggs R. J., Elliott N. M., Zhang L., Koh J., Viccini L. F., Soltis D. E. et al. (2010),
631 'Tissue-specific silencing of homoeologs in natural populations of the recent
632 allopolyploid *tragopogon mirus*', *New Phytologist*, 186 (1), 175-183.

633

634 Buggs R. J., Zhang L., Miles N., Tate J. A., Gao L., Wei W. et al. (2011),
635 'Transcriptomic shock generates evolutionary novelty in a newly formed, natural
636 allopolyploid plant', *Current Biology*, 21 (7), 551-556.

637

638 Caultet C. (2011), Dispersion et adaptation des capselles: *Capsella rubella* et *Capsella*
639 *bursa-pastoris* dans un agroécosystème, PhD thesis, Université de Bourgogne.

640

641 Chevin L.-M., Lande R. and Mace G. M. (2010), 'Adaptation, plasticity, and extinction
642 in a changing environment: towards a predictive theory', *PLoS Biol*, 8 (4), e1000357.

643

- 644 Cookson S. J. and Granier C. (2006), 'A dynamic analysis of the shade-induced plasticity
645 in *Arabidopsis thaliana* rosette leaf development reveals new components of the shade-
646 adaptative response', *Annals of botany*, 97 (3), 443-452.
647
- 648 Coquillat M. (1951), 'Sur les plantes les plus communes à la surface du globe',
649 Publications de la Societe Linneenne de Lyon 20 (7), 165-170.
650
- 651 Cornille A., Salcedo A., Kryvokhyzha D., Glémin S., Holm K., Wright S. et al. (2016),
652 'Genomic signature of successful colonization of Eurasia by the allopolyploid shepherd's
653 purse (*Capsella bursa-pastoris*)', *Molecular ecology*, 25 (2), 616-629.
654
- 655 Crawley M. J. (2012), The R book , John Wiley & Sons.
656
- 657 Daehler, C.C. (2003). 'Performance comparisons of co-occurring native and alien
658 invasive plants: implication for conservation and restoration', *Annual Review of Ecology,*
659 *Evolution, and Systematics*, 34 (1), 183-211.
660
- 661 Davidson A. M., Jennions M. and Nicotra A. B. (2011), 'Do invasive species show
662 higher phenotypic plasticity than native species and, if so, is it adaptive? a meta-analysis',
663 *Ecology letters*, 14 (4), 419-431.
664
- 665 Douglas G. M., Gos G., Steige K. A., Salcedo A., Holm K., Josephs E. B. et al. (2015),
666 'Hybrid origins and the earliest stages of diploidization in the highly successful recent
667 polyploid *Capsella bursa-pastoris*', *Proceedings of the National Academy of Sciences*,
668 112 (9), 2806-2811.
669
- 670 Ducatez S., Crossland M. and Shine R. (2016), 'Differences in developmental strategies
671 between long-settled and invasion-front populations of the cane toad in Australia',
672 *Journal of evolutionary biology*, 29 (2), 335-343.
673
- 674 Excoffier L., Foll M. and Petit R. J. (2009), 'Genetic consequences of range expansions',
675 *Annual Review of Ecology, Evolution, and Systematics*, 40 , 481-501.
676
- 677 Finlay K. and Wilkinson G. (1963), 'The analysis of adaptation in a plant-breeding
678 programme', *Australian journal of agricultural research*, 14 (6), 742-754.

679

680 Fisher R. (1930), 'The genetical theory of natural selection oxford university press',
681 *London, UK.*

682

683 Gilbert K. J., Sharp N. P., Angert A. L., Conte G. L., Draghi J. A., Guillaume F. et al.
684 (2017), 'Local adaptation interacts with expansion load during range expansion:
685 maladaptation reduces expansion load', *The American Naturalist*, 189 (4), 368-380.

686

687 Glémin S., François C. M. and Galtier N. (2019), Genome evolution in outcrossing vs.
688 selfing vs. asexual species, in 'Evolutionary Genomics', *Springer*, pp. 331-369.

689

690 Godoy O., Valladares F. and Castro-Díez P. (2011), 'Multispecies comparison reveals
691 that invasive and native plants differ in their traits but not in their plasticity', *Functional*
692 *Ecology*, 25 (6), 1248-1259.

693

694 González-Martínez S. C., Ridout K. and Pannell J. R. (2017), 'Range expansion
695 compromises adaptive evolution in an outcrossing plant', *Current Biology*, 27 (16), 2544-
696 2551.

697

698 Grenier S., Barre P. and Litrico I. (2016), 'Phenotypic plasticity and selection:
699 nonexclusive mechanisms of adaptation', *Scientifica*, 2016 .

700

701 Grossenbacher D., Briscoe Runquist R., Goldberg E. E. and Brandvain Y. (2015),
702 'Geographic range size is predicted by plant mating system', *Ecology letters*, 18 (7), 706-
703 713.

704

705 Heller R. and Smith J. M. (1978), 'Does muller's ratchet work with selfing?', *Genetics*
706 *Research*, 32 (3), 289-293.

707

708 Hijmans R. J. (2019), geosphere: Spherical Trigonometry. R package version 1.5-10.

709

710 Hollister J. D., Greiner S., Wang W., Wang J., Zhang Y., Wong G. K.-S. et al. (2015),
711 'Recurrent loss of sex is associated with accumulation of deleterious mutations in
712 oenothera', *Molecular Biology and Evolution*, 32 (4), 896-905.

713

- 714 Huang H.-R., Liu J.-J., Xu Y., Lascoux M., Ge X.-J. and Wright S. I. (2018),
715 `Homeologue- specific expression divergence in the recently formed tetraploid *Capsella*
716 *bursa-pastoris* (Brassicaceae)', *New Phytologist*, 220 (2), 624-635.
717
- 718 Hurka H., Friesen N., German D. A., Franzke A. and Neuffer B. (2012), ``Missing link'
719 species *Capsella orientalis* (Brassicaceae) and *Capsella thracica* elucidate evolution of
720 model plant genus *Capsella*', *Molecular Ecology*, 21 (5), 1223-1238.
721
- 722 Hurka H. and Neuffer B. (1997), `Evolutionary processes in the genus *Capsella*
723 (Brassicaceae)', *Plant Systematics and Evolution*, 206 (1), 295-316.
724
- 725 Klisz M., Buras A., Sass-Klaassen U., Puchaaka R., Koprowski M. and Ukalska J.
726 (2019), `Limitations at the limit? diminishing of genetic effects in norway spruce
727 provenance trials', *Frontiers in plant science*, 10 , 306.
728
- 729 Komeda Y. (2004), `Genetic regulation of time to flower in *Arabidopsis thaliana*', *Annu.*
730 *Rev. Plant Biol.* 55 , 521-535.
731
- 732 Kryvokhyzha D., Holm K., Chen J., Cornille A., Glémin S., Wright S. I. et al. (2016),
733 `The influence of population structure on gene expression and flowering time variation in
734 the ubiquitous weed *Capsella bursa-pastoris* (Brassicaceae)', *Molecular Ecology*, 25 (5),
735 1106-1121.
736
- 737 Kryvokhyzha D., Milesi P., Duan T., Orsucci M., Wright S. I., Glémin S. et al. (2019a),
738 `Towards the new normal: Transcriptomic convergence and genomic legacy of the two
739 subgenomes of an allopolyploid weed (*Capsella bursa-pastoris*)', *PLoS genetics*, 15 (5),
740 e1008131.
741
- 742 Kryvokhyzha D., Salcedo A., Eriksson M. C., Duan T., Tawari N., Chen J. et al. (2019b),
743 `Parental legacy, demography, and admixture influenced the evolution of the two
744 subgenomes of the tetraploid *Capsella bursa-pastoris* (Brassicaceae)', *PLoS genetics*, 15
745 (2), e1007949.
746
- 747 Lande R. (2015), `Evolution of phenotypic plasticity in colonizing species', *Molecular*
748 *ecology*, 24 (9), 2038-2045.

749

750 Le Corre V., Roux F. and Reboud X. (2002), 'DNA polymorphism at the frigida gene in
751 *Arabidopsis thaliana*: extensive nonsynonymous variation is consistent with local
752 selection for flowering time', *Molecular biology and evolution*, 19 (8), 1261-1271.

753

754 Lewandowska-Sabat A. M., Fjellheim S., Olsen J. E. and Rognli O. A. (2017), 'Local
755 populations of *Arabidopsis thaliana* show clear relationship between photoperiodic
756 sensitivity of flowering time and altitude', *Frontiers in plant science*, 8 , 1046.

757

758 Liaw A. and Wiener M. (2002), 'Classification and regression by randomforest', *R news*,
759 2 (3), 18-22.

760

761 Linde M., Diel S. and Neuffer B. (2001), 'Flowering ecotypes of *Capsella bursa-pastoris*
762 (L.) Medik.(Brassicaceae) analysed by a cosegregation of phenotypic characters (QTL)
763 and molecular markers', *Annals of Botany*, 87 (1), 91-99.

764

765 Linhart, Y.B., & Grant, M.C. (1996). 'Evolutionary significance of local genetic
766 differentiation in plants.', *Annual Review of Ecology and Systematics*, 27(1), 237-277.

767

768 Lynch M. and Walsh B. (1998), *Genetics and Analysis of Quantitative Traits*, Vol. 1,
769 Sinauer Associates, Sunderland, MA.

770

771 Neuffer B. (1990), 'Ecotype differentiation in *Capsella*', *Vegetatio*, 89 (2), 165-171.

772

773 Neuffer B. (1996), 'RAPD analyses in colonial and ancestral populations of *Capsella*
774 *bursa-pastoris* (L.) med. (Brassicaceae)', *Biochemical Systematics and Ecology*, 24 (5),
775 393-403.

776

777 Neuffer B. and Hurka H. (1986), 'Variation of development time until flowering in
778 natural populations of *Capsella bursa-pastoris* (Cruciferae)', *Plant Systematics and*
779 *Evolution*, 152 (3), 277-296.

780

781 Neuffer B., Wesse C., Voss I. and Scheibe R. (2018), 'The role of ecotypic variation in
782 driving worldwide colonization by a cosmopolitan plant', *AoB PLANTS*, 10 (1), ply005.

783

784 Orsucci M., Milesi P., Hansen J., Girodolle J., Glémin S. and Lascoux M. (2020), 'Shift
785 in ecological strategy helps marginal populations of shepherd's purse (*Capsella bursa-*
786 *pastoris*) to overcome a high genetic load', *Proceedings of the Royal Society B:*
787 *Biological Sciences*, 287 (1927), 20200463.
788

789 Pannell J. R., Auld J. R., Brandvain Y., Burd M., Busch J. W., Cheptou P.-O. et al.
790 (2015), 'The scope of baker's law', *New Phytologist*, 208 (3), 656-667.
791

792 Park D. S., Ellison A. M. and Davis C. C. (2018), 'Mating system does not predict niche
793 breath', *Global Ecology and Biogeography*, 27 (7), 804-813.
794

795 Peischl S., Dupanloup I., Kirkpatrick M. and Excoffier L. (2013), 'On the accumulation
796 of deleterious mutations during range expansions', *Molecular Ecology*, 22 (24), 5972-
797 5982.
798

799 Peischl S. and Excoffier L. (2015), 'Expansion load: recessive mutations and the role of
800 standing genetic variation', *Molecular Ecology*, 24 (9), 2084-2094.
801

802 Peischl S., Kirkpatrick M. and Excoffier L. (2015), 'Expansion load and the evolutionary
803 dynamics of a species range', *The American Naturalist* 185 (4), E81-E93. PMID:
804 25811091.
805

806 Pelé A., Rousseau-Gueutin M. and Chèvre A.-M. (2018), 'Speciation success of
807 polyploid plants
808 closely relates to the regulation of meiotic recombination', *Frontiers in Plant Science*, 9 ,
809 907.
810

811 R Core Team (2020), R: A Language and Environment for Statistical Computing , R
812 Foundation
813 for Statistical Computing, Vienna, Austria.
814

815 Riis T., Lambertini C., Olesen B., Clayton J. S., Brix H. and Sorrell B. K. (2010),
816 'Invasion strategies in clonal aquatic plants: are phenotypic differences caused by
817 phenotypic plasticity or local adaptation?', *Annals of Botany*, 106 (5), 813-822.
818

- 819 Roux F., Touzet P., Cuguen J. and Le Corre V. (2006), 'How to be early flowering: an
820 evolu-tionary perspective', *Trends in plant science*, 11 (8), 375-381.
821
- 822 Salcedo A. (2015), 'Genomic signals of adaptation in the allotetraploid weed capsella
823 bursa-pastoris', Master thesis, University of Toronto.
824
- 825 Simpson G. G. and Dean C. (2002), 'Arabidopsis, the rosetta stone of flowering time?',
826 *Science*, 296 (5566), 285-289.
827
- 828 Slatkin M. and Excoffier L. (2012), 'Serial founder effects during range expansion: a
829 spatial analog of genetic drift', *Genetics*, 191 (1), 171-181.
830
- 831 Stebbins L. G. (1957), 'Self fertilization and population variability in the higher plants',
832 *The American Naturalist*, 91 (861), 337-354.
833
- 834 Takebayashi N. and Morrell P. L. (2001), 'Is self-fertilization an evolutionary dead end?
835 revisiting an old hypothesis with genetic theories and a macroevolutionary approach',
836 *American Journal of Botany*, 88 (7), 1143-1150.
837
- 838 VanWallendael A., Hamann E. and Franks S. J. (2018), 'Evidence for plasticity, but not
839 local adaptation, in invasive japanese knotweed (*reynoutria japonica*) in north america',
840 *Evolutionary Ecology*, 32 (4), 395-410.
841
- 842 Wei N., Cronn R., Liston A. and Ashman T.-L. (2019), 'Functional trait divergence and
843 trait plasticity confer polyploid advantage in heterogeneous environments', *New*
844 *Phytologist*, 221 (4), 2286-2297.
845
- 846 Willi Y. (2019), 'The relevance of mutation load for species range limits', *American*
847 *journal of botany*, 106 (6), 757-759.
848
- 849 Willi Y., Fracassetti M., Zoller S. and Van Buskirk J. (2018), 'Accumulation of
850 mutational load at the edges of a species range', *Molecular biology and evolution*, 35 (4),
851 781-791.
852

853 Yan L., Loukoianov A., Tranquilli G., Helguera M., Fahima T. and Dubcovsky J. (2003),
854 `Positional cloning of the wheat vernalization gene VRN1', *Proceedings of the National*
855 *Academy of Sciences* 100 (10), 6263-6268.
856
857 Yang X., Lascoux M. and Glémin S. (2018), `Variation in competitive ability with
858 mating system, ploidy and range expansion in four capsella species'.
859
860 Yant L. and Bomblies K. (2015), `Genome management and mismanagement-cell-level
861 opportunities and challenges of whole-genome duplication', *Genes & development*, 29
862 (23), 2405-2419
863
864
865

866 **Tables**

867 **Table 1.** Analysis of variance of model [S1] (see Supplementary materials). Statistics
 868 (χ^2), degree of freedom (df) and p-values of the type II Wald chi-square test. Only
 869 variables that were measured in all the common gardens are kept. FP: time between
 870 bolting and flowering; SP: time between flowering and senescence. The distributions of
 871 the residual are normal distributions. Significance levels are : $p^{***} < 0.001$; $p^{**} < 0.01$;
 872 $p^* < 0.05$; $p^{n.s.} > 0.05$. Since germination time was not monitored in Toronto, analysis of
 873 variance was not assessed for germination time and the inter event between bolting time
 874 and germination time.

	Genetic cluster		Common garden		Genetic cluster x common garden	
	χ^2	df	χ^2	df	χ^2	df
Fitness (Nb. fruits)	67.1***	2	128.5***	2	135.1***	4
Height	109.9***	2	248.9***	2	122.0***	4
Nb. primary branches	3.2 ^{n.s.}	2	420.8***	2	194.7***	4
Nb. rosette leaves	227.5***	2	358.7***	2	517.5***	4
Rosette diameter	88.8***	2	309.4***	2	45.4***	4
FP	66.2***	2	656.8***	2	2.2 ^{n.s.}	4
SP	10.4***	2	1445.2***	2	6.1 ^{n.s.}	4

875

876 **Table 2.** Analysis of variance of the GLMM model. Statistics (χ^2), degree of freedom (df)
 877 and p-values of the type II Wald chi-square test. GP: time between sowing and
 878 germination; BP: time between germination and bolting; FP: time between bolting and
 879 flowering; SP: time between flowering and senescence. The distributions of the residual
 880 are : negative binomial (NB), or Normal (N). Significance levels are : $p^{***} < 0.001$; p^{**}
 881 < 0.01 ; $p^* < 0.05$; $p^{n.s.} > 0.05$. Since germination time was not monitored in Toronto,
 882 analysis of variance was not assessed in Toronto for GP and BP.

Trait	Genetic cluster effect						Residual
	Uppsala		Guangzhou		Toronto		
	χ^2	df	χ^2	df	χ^2	df	
Fitness	8.30*	2	34.29***	2	8.16*	2	NB
Height	81.4***	2	38.4***	2	40.8***	2	N
Nb. primary branches	1.8 ^{n.s.}	2	11.5**	2	11.4**	2	N

Nb. rosette leaves	127.1***	2	52.0***	2	90.6***	2	N
Rosette diameter	99.3***	2	33.8***	2	24.2***	2	N
GP	123.8***	2	181.6***	2	NA	2	N
BP	60.2***	2	21.3***	2	NA	2	N
FP	14.1***	2	37.4***	2	31.5***	2	N
SP	10.1**	2	1.9 ^{n.s.}	2	0.1 ^{n.s.}	2	N

883

884 **Table 3.** Estimate of genetic cluster effect in the GLMM model, in log-scale at each
 885 common garden (Uppsala, Guangzhou, Toronto). Estimates of EUR and ME effects are
 886 relative to ASI effect; Tukey contrast analysis of fitness in the GLMM model: statistics
 887 and p-values of the Tukey's HSD test. Significance levels are : $p^{***} < 0.001$; $p^{**} < 0.01$;
 888 $p^* < 0.05$; $p^{n.s.} > 0.05$.

Cluster	Uppsala			Guangzhou			Toronto		
	Estimate	Tukey's HSD		Estimate	Tukey's HSD		Estimate	Tukey's HSD	
		EUR	ME		EUR	ME		EUR	ME
ASI	4.56	-2.6*	-1.8 ^{n.s.}	6.12	1.8 ^{n.s.}	-4.5***	6.13	-1.4 ^{n.s.}	-2.8*
EUR	+0.21	-	0.3 ^{n.s.}	-0.20	-	-5.6***	+0.14	-	-1.8 ^{n.s.}
ME	+0.18	-	-	+0.50	-	-	+0.37	-	-

889

890 **Table 4.** Mean and standard deviation of responsiveness (slope) and performance in poor
 891 environment (intercept) of fitness (the FWR model), for each genetic cluster (ASI, EUR,
 892 and ME); pairwise Welch's *t*-test between mean values of genetic clusters: statistics (T),
 893 degree of freedom (Df) and p-values. Significance levels are : $p^{***} < 0.001$; $p^{**} < 0.01$;
 894 $p^* < 0.05$; $p^{n.s.} > 0.05$.

Cluster	Intercept						Slope					
	Mean ± SD	Welch's <i>t</i> -test				Mean + SD	Welch's <i>t</i> -test					
		EUR		ME			EUR		ME			
		T	Df	T	Df		T	Df	T	Df		
ASI	6.70 ± 183.5	0.94 ^{n.s.}	90.0	-0.38 ^{n.s.}	40.4	0.91 ± 0.47	-0.5 ^{n.s.}	90.0	-3.5**	53.2		

EUR	-29.92 ± 189.0	-	-	$-1.1^{n.s.}$	41.5	0.96 ± 0.44	-	-	-3.1^{**}	50.4
ME	25.47 ± 188.6	-	-	-	-	1.27 ± 0.36	-	-	-	-

895

896 **Table 5.** Correlation between different Finlay-Wilkinson regressions (slope and intercept)

897 on fitness and flowering time (FT), or flowering duration (SP).

Cluster	Intercept		Slope	
	FT	SP	FT	SP
ASI	0.15	0.03	0.06	-0.07
EUR	-0.10	0.09	-0.19	0.10
ME	-0.15	0.14	-0.50	0.30

898

899

900 **Figures**

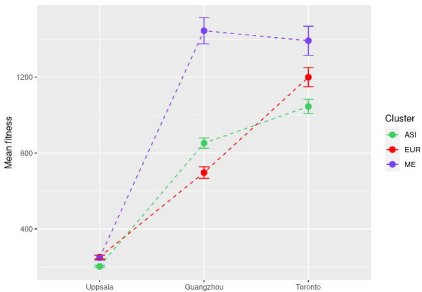
901

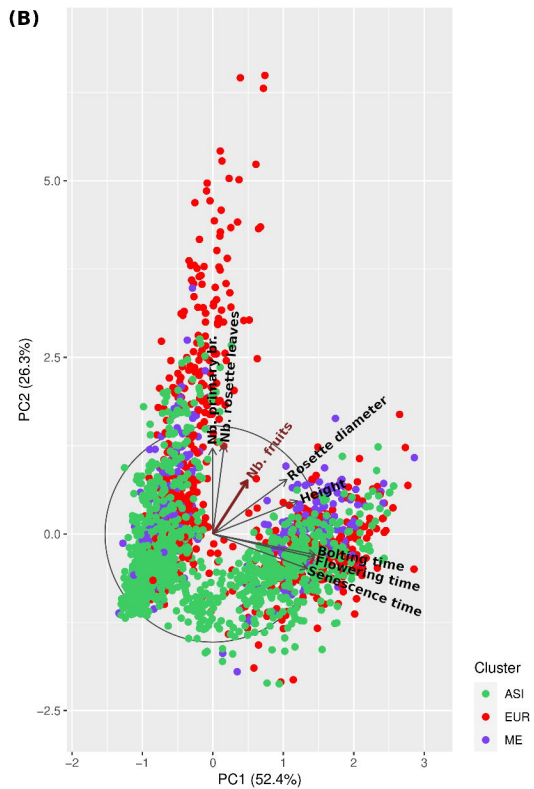
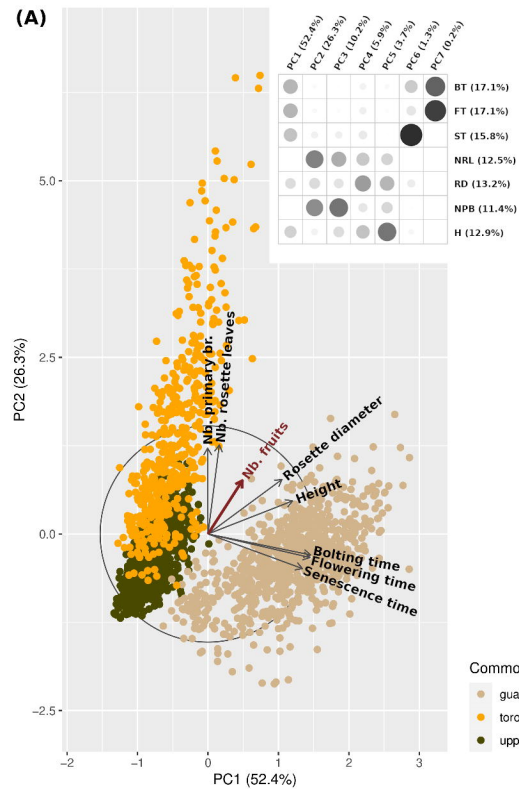
902 **Figure 1.** Interaction plot of fitness in *C. bursa-pastoris* between the three common
903 gardens (Uppsala, Guangzhou and Toronto, on the x-axis) and the genetic clusters (ASI
904 in green, EUR in red, and ME in blue), using the whole dataset. Error bars correspond to
905 standard errors.

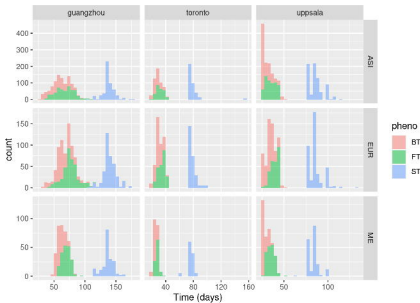
906 **Figure 2.** Principal Component Analysis of the phenotypes of accessions of *C. bursa-*
907 *pastoris*: bolting time (BT), flowering time (FT), senescence time (ST), number of rosette
908 leaves (NRL), rosette diameter (RD), height of the main inflorescence (H), and the
909 number of primary branches (NPB), using the whole dataset (267 accessions). The
910 number of fruits (the fitness) was added as a supplementary variable. PC1 captured
911 52.4% of the variance, and PC2 captured 26.3%. The box on the top right corner of (a)
912 highlights the correlation of each variable to the corresponding axis: the darker and
913 bigger the circle, the higher the correlation (in brackets, the percentage of contribution of
914 each variable to the first two PCs). Both (a) and (b) are the same PCA, only the color
915 label changes. (a) PCA labeled according to the common gardens (Uppsala in brown,
916 Toronto in orange, and Guangzhou in beige): common gardens appeared to be clearly
917 delimiting three groups. (b) PCA labeled according to the genetic clusters (ASI in green,
918 EUR in red, and ME in blue): genetic clusters did not appear to be clearly separating the
919 dataset.

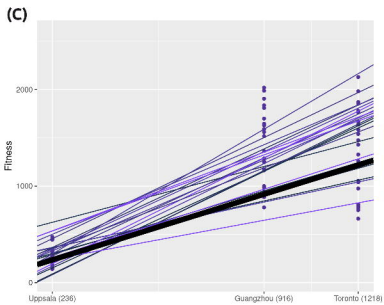
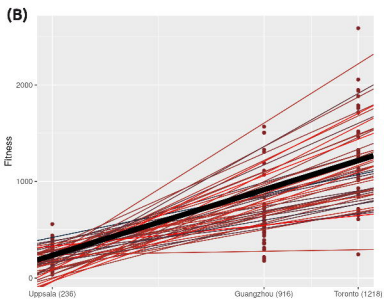
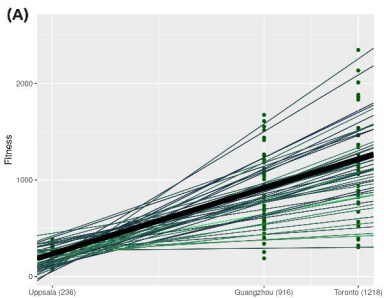
920 **Figure 3.** Histograms of the phenological traits of *C. bursa-pastoris* (bolting time as BT,
921 flowering time as FT, and senescence time as ST) according to the common garden and
922 genetic cluster, using the whole dataset. Germination time was not plotted here for
923 consistency with Toronto.

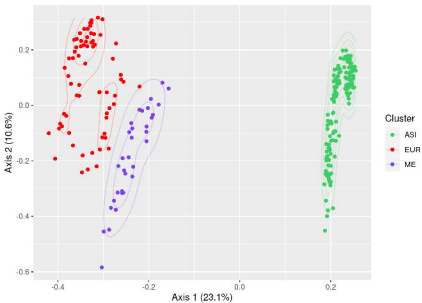
924 **Figure 4.** Finlay-Wilkinson regressions of fitness (the FWR model) of *C. bursa-pastoris*.
925 On the x-axis, the mean population fitness at each common garden; on the y-axis the
926 mean fitness of each accession. Each dot is an accession at a specific common garden,
927 and each line is a FWR for one accession. The bold black line is the population average
928 FWR. (A) FWR of accessions belonging to the cluster ASI (green); (B) FWR of acces-
929 sions belonging to the cluster EUR (red); (C) FWR of accessions belonging to the cluster
930 ME (blue).

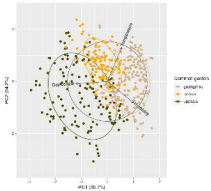


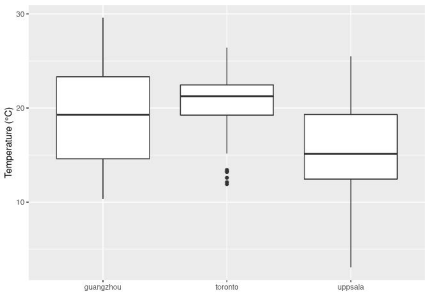


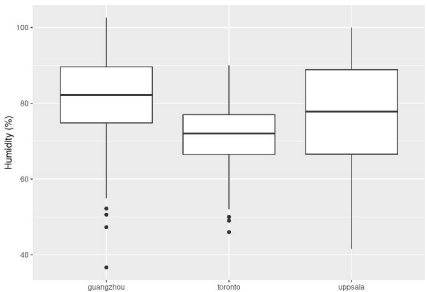


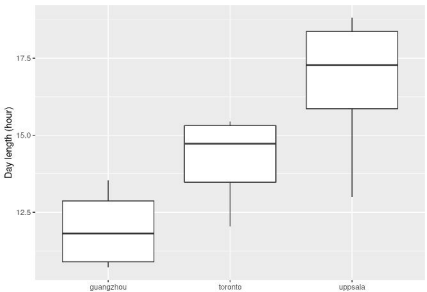


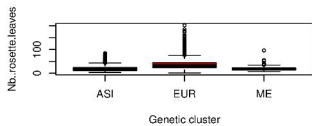
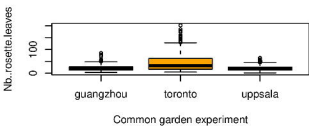
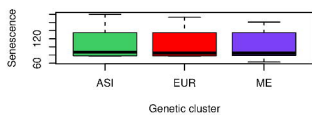
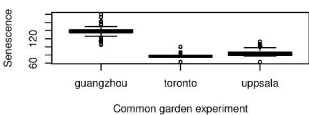
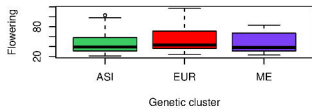
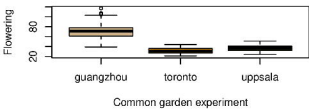
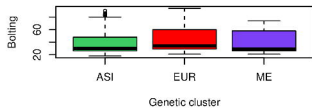




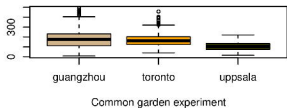




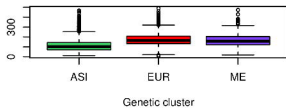




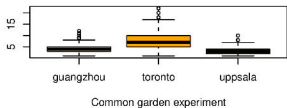
Diameter rosette



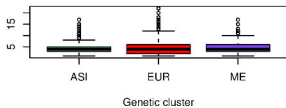
Diameter rosette



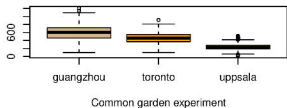
Nb..pr..br.



Nb..pr..br.



Height



Height

