The relative role of plasticity and demographic history in
Capsella bursa-pastoris: a common garden experiment in
Asia and Europe

Amandine Cornille1,2,¶, Mathieu Tiret1,§, Adriana Salcedo3,4, Huirun R. Huang4,5,¶, Marion Orsucci6, Pascal Milesi1,7, Dmytro Kryvokhyzha1, Karl Holm1, Xue-Jun Ge4,5, John R. Stinchcombe3, Sylvain Glémin1,8,¶, Stephen I. Wright3,¶ and Martin Lascoux1,7,¶

1Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, 75236 Uppsala, Sweden
2Université Paris Saclay, INRAE, CNRS, AgroParisTech, GGE - Le Moulon, 91190 Gif-sur-Yvette, France
3Department of Ecology and Evolutionary Biology, University of Toronto, M5S 3B2 Toronto, ON, Canada
4Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
5Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of Sciences, Guangzhou 510650, China
6Department of Plant Biology, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden
7Science for Life Laboratory, 752 37 Uppsala, Sweden
8UMR CNRS 6553 ECOBIO, Université de Rennes I, 35042 Rennes Cedex, France

*Corresponding author’s e-mail address: amandine.cornille@cnrs.fr
¶These authors co-directed equally the work.
§These authors contributed equally to the work.

Abstract
The colonization success of a species depends on the interplay between its phenotypic plasticity, adaptive potential and demographic history. Assessing their relative contributions during the different phases of a species range expansion is challenging, and requires large-scale experiments. Here, we investigated the relative contributions of plasticity, performance and demographic history to the worldwide expansion of the shepherd’s purse, Capsella bursa-pastoris. We installed two large common gardens of the shepherd’s purse, a young, self-fertilizing, allopolyploid weed with a worldwide distribution. One common garden was located in Europe, the other in Asia. We used accessions from three distinct genetic clusters (Middle East, Europe and Asia) that reflect the demographic history of the species. Several life-history traits were measured. To explain the phenotypic variation between and within genetic clusters, we analysed the effects of (i) the genetic clusters, (ii) the phenotypic plasticity and its association to fitness and (iii) the distance in terms of bioclimatic variables between the sampling site of an accession and the common garden, i.e. the environmental distance. Our experiment showed that (i) the performance of C. bursa-pastoris is closely related to its high phenotypic plasticity; (ii) within a common garden, genetic cluster was a main determinant of phenotypic differences; and (iii) at the scale of the experiment, the effect of environmental distance to the common garden could not be distinguished from that of genetic clusters. Phenotypic plasticity and demographic history both play important role at different stages of range expansion. The success of the worldwide expansion of C. bursa-pastoris was undoubtedly influenced by its strong phenotypic plasticity.

Keywords: Capsella bursa-pastoris; common garden; demographic history; environmental distance; fitness components; phenotypic plasticity.

Introduction
Range expansion can leave a strong footprint on current patterns of genetic and phenotypic variation (Excoffier and Ray 2008; Excoffier et al. 2009). The factors influencing the speed of range expansion and range limits, and their impacts on genetic and phenotypic variation are still being debated (Angert et al. 2020). Current and future threats to biodiversity, including invasive species outbreaks and their consequences for ecosystem health and services, have made this question more relevant than ever.

The speed of range expansion of sessile organisms generally depends on reproductive capacity, seed dispersal efficiency and the species’ ability to establish itself successfully in new environments (Sheth et al. 2020). Establishment success may depend on phenotypic plasticity in the short term, and on adaptive capacity, in the long term (Lande 2009; Hendry...
2016), especially if the new environment and that of the parents differ in terms of abiotic (e.g. temperature, light, photoperiod, soil composition) and biotic conditions (e.g. neighbouring plants, local microbiomes, pathogens, pollinator communities). Phenotypic plasticity is defined here as the ability of a given genotype to produce different phenotypes in different environments (Bradshaw 1965; Grenier et al. 2016 and references therein), and genetic adaptation as evolution through natural selection whereby the average fitness of a population gradually increases in a given environment (Linhart and Grant 1996; Chevin et al. 2010 and references therein). A successful expansion will correspond to a particular combination of these two evolutionary mechanisms, which are not mutually exclusive (Hendry 2016). Species with a worldwide distribution inevitably face a large array of environments and signatures of both phenotypic plasticity and adaptation are likely to be observed (Lande 2013). Investigating plasticity in newly established populations is fraught with difficulties, since the age of populations is generally hard to estimate and patterns of phenotypic plasticity may vary depending on the time since colonization. Indeed, theoretical models indicate that while adaptation to a new extreme environment may lead to a transient increase in phenotypic plasticity, this can be followed by a second period of genetic assimilation, which, perhaps unexpectedly, is associated with a decrease in plasticity (Lande 2015). These complex dynamics could explain why different studies on phenotypic plasticity of colonizing species led to divergent conclusions: during the colonization of new environments, high mean phenotypic plasticity has been under- (e.g. Daehler 2003) or over-represented (e.g. Davidson et al. 2011; Godoy et al. 2017). Likewise, while evolutionary forces underlying adaptation in populations at equilibrium are fairly well documented, the literature is more limited for recent and marginal populations that are usually characterized by non-equilibrium demographics. In such populations, random genetic drift can play a larger part and obscure the effect of adaptive forces, making the patterns of genetic variation harder to interpret (Excoffier et al. 2009; Gilbert et al. 2017), as illustrated in Mercurialis annua (González-Martínez et al. 2017) or in Arabidopsis lyrata (Willi et al. 2018). In summary, as illustrated in the review of Hendry (2016), estimating the relative role of phenotypic plasticity and past adaptation during colonization remains a very open question.

Phenotypic plasticity and demographic history, and their interplay, have probably played a key role in the worldwide success of shepherd’s purse, Capsella bursa-pastoris (Brassicaceae). The shepherd’s purse is a self-fertilizing colonizer of recent allopolyploid origin. The shepherd’s purse arose some 100 000 years ago from hybridization between the diploid self-fertilizing Capsella orientalis and the outcrossing Capsella grandiflora (Douglas et al. 2015). Autogamy and allopolyploidy could partly explain why C. bursa-pastoris has an almost worldwide distribution while its two parents are restricted to specific areas: from Central Asia to eastern Europe for C. orientalis, and only the mountains of north-west Greece and Albania for C. grandiflora (Hurka et al. 2012). The shepherd’s purse is genetically structured in three distinct clusters: eastern Asia (ASI), Middle East and northern Africa (ME) and Europe and the Russian Far East (EUR) (Cornille et al. 2016). Wesse et al. (2021) found a similar clustering, where the Middle Eastern and northern African cluster corresponds to their Mediterranean lineage and the European and the Russian Far East to their temperate lineage. Demographic inferences showed that the three clusters (ASI, EUR, ME) resulted from a range expansion that started either from the Middle East or Europe (the starting point is not known with certainty), and was followed by a subsequent colonization event towards Asia. This recent worldwide spread was, in some cases, likely associated with human migrations, as for instance the spread to eastern Siberia of western European accessions (Cornille et al. 2016), or of southern European and Middle Eastern accessions to North America (Hurka and Neuffer 1997; Cornille et al. 2016). The shift from outcrossing to self-fertilization (a.k.a. selfing) confers ‘reproductive assurance’, which is expected to facilitate colonization of new environments as only one or a few individuals are required to establish a new population (Baker et al. 1965; Pannell et al. 2015). The benefit of self-fertilization might be short-lived, however, as the lack of genetic diversity and effective recombination is expected to limit adaptation and to lead to a genome-wide accumulation of deleterious mutations (a.k.a. genetic load; Heller and Smith 1978; Hollister et al. 2015; Glémin et al. 2019). The Asian cluster of C. bursa-pastoris shows such a genetic load (Kryvokhyzha et al. 2019a). In agreement with these predictions, selfing species tend to have larger ecological ranges (Grossenbacher et al. 2015) but decreasing niche breadth over time (Park et al. 2018) compared to their outcrossing congeners, aligned with the ‘evolutionary dead end’ hypothesis (Stebbins 1957; Takebayashi and Morrell 2001 and references therein). The phenomenon of a decreased niche breadth is even more pronounced during a colonization process (e.g. Slatkin and Excoffier 2012; González-Martínez et al. 2017).

Hence, understanding the causes of ecological success of a species requires the estimation of the respective roles of phenotypic plasticity and demographic history during its range expansion, and C. bursa-pastoris is a perfect model species for this. To that end, we implemented an experiment with two large common gardens located in two contrasting environments (i.e. environments with differing day length, temperature, moisture, soil and plant community). First, to capture the diversity of environmental conditions that C. bursa-pastoris faced during its range expansion, we installed two common gardens at extreme latitudes in Eurasia, one in East Asia and one in Northern Europe (Fig. 1A). Second, to capture the demographic history of C. bursa-pastoris, we used a comprehensive sample of populations from Europe, Asia, North Africa and the Middle East (Fig. 1B and C).

Materials and Methods

Plant materials

Sampling. We used a collection of 232 accessions—offspring of a single self-fertilizing mother plant—from 59
sites distributed across Europe, Asia, North Africa and the Middle East (Fig. 1A; see Supporting Information—Fig. S1). These accessions were previously described in Cornille et al. (2016) (see also Kryvokhyzha et al. 2016; Kryvokhyzha et al. 2019b). The collection was constituted to represent the global population structure of C. bursa-pastoris which is divided in at least three main genetic clusters (Cornille et al. 2016): the European (EUR), Middle Eastern (ME) and Asian (ASI). Sixty-six accessions come from EUR, 27 from ME and 139 from ASI [see Supporting Information]. Each accession was represented by four to six progenies in each common garden, so that the study included in total 2403 plants, 1312 from ASI, 324 from EUR and 39 from ME in each common garden: for 139 days from May to September 2014 in Uppsala, and for 193 days from November 2014 to May 2015 in Guangzhou. Throughout the experimental period, day length becomes longer in Uppsala, and shorter in Guangzhou.

Environmental data. Environmental conditions were monitored daily at ground level using temperature and humidity sensors (TGP-4017®, Gemini Data Loggers; Chichester, West Sussex, UK). The overall environmental conditions were reported in Supporting Information—Fig. S2 and Tables S1 and S2. In Uppsala, climatic conditions were rather cold and wet (15.2 ± 5.1 °C, 76.8 ± 14.1 %), while in Guangzhou accessions experienced warm and humid weather (19.3 ± 5.0 °C, 81.1 ± 11.4 %).

Day length was obtained with the package geosphere (function daylength; Hijmans et al. 2015) in the R environment for statistical computing (R-3.6.3; R Core Team 2020). The photoperiod was longer in Uppsala (16.9 ± 1.7 h, ranging from 13 to 18.8 h) than in Guangzhou (11.9 ± 1.0 h, ranging from 10.7 to 13.5 h). Climate data were downloaded from WorldClim database (19 bioclimatic variables, 2.5 arc-minute resolution, from 1960 to 2000; Fick et al. 2017).

Experiment protocol
Seed preparation. In both common gardens, to reduce maternal effects, seeds collected from the field were first sown...
and grown under controlled conditions in growth chambers (55% moisture, 22 °C, 12h:12h light:darkness cycles). Their progenies were then used to establish the common gardens. More specifically, about 20 seeds per accession were surface-sterilized the same day and germinated in Petri dishes (one per accession), with Murashige and Skoog (MS) medium and agar (see protocol in Kryvokhyzha et al. 2016). Petri dishes were then all stratified for 7 days at 4 °C in the dark to promote germination (avoiding differential dormancy release; see Neuffer and Hurka 1988), and then placed in a greenhouse with no additional light or heating, in order to avoid the impact of micro-environmental effects and were left in the greenhouse until seedlings reached a four-leaf stage.

**Transplantation.** In both common gardens, once seedlings reached a four-leaf stage, they were thinned to one per pot (7 cm × 7 cm) containing standard plant nursery soil mixed with water (at Uppsala: yrkesplantjord, Wexthuset, Enhörna, Sweden; at Guangzhou, Jiffy substrates; Zwijndrecht, The Netherlands). The pots were randomly placed in the greenhouse, and left 7 days. During the 7 days, the pots were watered automatically twice a week, and randomly moved every day to avoid the impact of micro-environmental variation in the greenhouse. After 7 days, the pots were pierced on the bottom and placed outside in the common garden, so that the plants were using the soil from Uppsala or Guangzhou regardless of the initial nursery soil. The pots were dispatched into six blocks (1 m × 3.2 m, grids of 9 × 30) arranged at 2 m spacing and containing standard soil. Each block contained one replicate per accession, with a total of six replicates per accession. When less than six seedlings from an accession germinated, seedlings from another accession of the same sampling site complemented the block, in order to keep a uniform individual density. Apart from the initial shading and watering, no additional support was provided to the seedlings. The experiment lasted until the senescence of the last plant, i.e., when the last plant dried up but had not yet shed its fruits.

**Statistical analyses**

**Effect of the genetic cluster.** The common gardens were first analysed with the following mixed model:

\[ Y_{ijklm} \sim \mu + c_i + s_j + B_k + A_l + e_{ijklm} \]  

(1)

where ‘\(Y_{ijklm}\)’ is a phenotype of the individual \(m\) from the accession \(l\) of the genetic cluster \(i\) in the block \(k\) of the common garden \(j\); ‘\(\mu\)’ is the overall mean, ‘\(c_i\)’ is the fixed effect of the \(i\)th genetic cluster, ‘\(s_j\)’ is the fixed effect of the \(j\)th common garden (Uppsala or Guangzhou), ‘\(B_k\)’ and ‘\(A_l\)’ are the uncorrelated random effects of the \(k\)th blocks and the \(l\)th accession, respectively (the accession’s effect is nested in the cluster’s effect) and ‘\(e_{ijklm}\)’ is the residual. The residual was fitted to different distributions according to the lowest Akaike information criterion and depending on the trait (Table 1). Model fitting relied on the R package glmer (function lmer and glmer.nb; Bates et al. 2015). In order to account for the difference in sample sizes of the genetic clusters, additional analyses were also carried out after downsampling. Unless otherwise specified, the results can be assumed to be robust to uneven sample size (for further details, see Supporting Information). To remove the unbalance in block design that was caused by experimental issues (e.g., failed germinations, climatic events), the block design was balanced before each statistical analysis by downsampling the data set down to four repetitions per accession per

**Table 1. Effects of the genetic cluster (i.e. Middle East, Asia or Europe) on each trait in each common garden (Uppsala or Guangzhou), with an analysis of variance of model (1), in Capsella bursa-pastoris.** Mean and standard deviation (SD), statistics (\(\chi^2\)) and df of the type II Wald chi-square test. GP: time between sowing and germination; BP: time between germination and bolting; FP: time between bolting and flowering; SP: time between flowering and senescence. The distributions of the residual are: negative binomial (NB), normal (N) or normal with the starting date as an additional variable (N*). Significant values are bolded. Significance levels are: ***\(P < 0.001\); **\(P < 0.01\); *\(P < 0.05\); n.s. \(P > 0.05\).

| Trait                | Genetic cluster effect | Residual |
|----------------------|------------------------|----------|
|                      | Uppsala                | Guangzhou |          |
|                      | Mean ± SD \(\chi^2\) df| Mean ± SD \(\chi^2\) df |          |
| No. of fruits        | 224 ± 174              | 1069 ± 808 | 40.61*** 2   |
| Height (in cm)       | 23.3 ± 8.6             | 60.1 ± 19.0  | 36.21*** 2   |
| No. of fruits over 10 cm | 31.0 ± 14.1           | 37.3 ± 13.9  | 17.5*** 2    |
| No. of primary branches | 2.91 ± 1.47           | 4.12 ± 1.91  | 11.11*** 2   |
| No. of secondary inflorescences | 5.78 ± 5.00 | 3.87 ± 1.82  | 1.14n.s. 2  |
| No. of rosette leaves | 20.7 ± 10.3            | 21.9 ± 10.8  | 44.31*** 2   |
| Rosette diameter (in cm) | 10.6 ± 4.3            | 18.9 ± 8.9   | 24.15*** 2   |
| Germination (in days) | 3.94 ± 3.55            | 1.23 ± 1.32  | 138.83*** 2  |
| Bolting (in days)    | 30.6 ± 5.7             | 61.7 ± 12.1  | 8.93* 2     |
| BP (in days)         | 27.0 ± 5.5             | 60.4 ± 12.2  | 145.14*** 2  |
| Flowering (in days)  | 35.8 ± 5.5             | 72.0 ± 12.5  | 17.94*** 2   |
| FP (in days)         | 7.39 ± 3.1             | 10.4 ± 3.0   | 20.18*** 2   |
| Senescence (in days) | 84.0 ± 6.4             | 139 ± 11     | 8.72* 2     |
| SP (in days)         | 48.8 ± 8.3             | 65.6 ± 13.6  | 11.29** 2    |

Significant values are bolded. Significance levels are: ***\(P < 0.001\); **\(P < 0.01\); *\(P < 0.05\); n.s. \(P > 0.05\).
common analyses were run within common garden to focus on the effect of genetic clusters.

For the four inter-event phenological periods, the starting date of each time span was included in the model as an additional fixed effect. Statistical significance of the genetic cluster effect was assessed with a type II Wald chi-square test, and the difference between genetic clusters was assessed with Tukey’s HSD (honestly significant difference) tests (for expected values; using the function glibt of the package multcomp; Hothorn et al. 2008) and Fisher’s F-tests (for variances; using the function var.test of the package base stats).

Effect of environmental distance. In a worldwide scale experiment, what matters most for phenotypic variation is the environmental differentiation rather than the geographical distance. Following the approach of Lovell et al. (2021), we considered the ‘environmental distance’, defined as the distance between a sampling site and the common garden in terms of bioclimatic variables. The distance, reported in Supporting Information—Table S3, was computed on the principal component analysis (PCA) of the 19 bioclimatic variables, truncated to the first 10 principal components, explaining 94.21% of the total variance (Fig. 1B; see also an alternative in Samis et al. 2019). To investigate the effect of environmental distance, the following linear mixed model was fitted to the data:

\[ Y_{ijkl} \sim \mu + d_k + c_i + B_j + A_k + e_{ijkl} \]  

where ‘d’ is the fixed effect of the environmental distance of the kth accession between its sampling site and the common garden, and the other terms are as described above for model (1). An additional statistical model was introduced to investigate the sole effect of environmental distance, i.e. excluding the genetic cluster effect, and was formulated as follows:

\[ Y_{ijkl} \sim \mu + d_k + B_j + A_k + e_{ijkl} \]  

where the terms are as described above for models (1) and (2). Statistical significance of d in models (2) and (3) was assessed using an analysis of deviance (type II Wald chi-square test). Additional analyses were run within a genetic cluster to focus on the effect of environments.

Partial leverage values in models (2) and (3) were particularly high in Guangzhou for accessions sampled in north-eastern China (Harbin) and north-western China (Xining; see Supporting Information—Fig. S3), despite the robustness of the genetic clustering (Cornille et al. 2016; Fig. 1C; see Supporting Information). To remove an artificially large environmental distance despite the genetic proximity, these populations were excluded from models (2) and (3), excluding 93 plants across common gardens (9 accessions), and leaving 2310 plants in total (223 accessions).

Measure of phenotypic plasticity. In order to quantify phenotypic plasticity, we modelled each accession as follows:

\[ Y_{ijkl} \sim s_j + e_{ijkl} \]  

where the terms are as described above in model (1). For each accession, we estimated the site effect of Guangzhou and the site effect of Uppsala, and studied the plasticity through the difference between these estimated site effects. In order to study the magnitude of plasticity—not its direction—we considered the absolute value, hereafter denoted P-score. Statistical significance of the P-score was assessed with a Student’s t-test.

When assessing the association between the P-score and a phenotype, we used the following model:

\[ Y_{ijkl} \sim \mu + P_i + e_{ijkl} \]  

where \( P_i \) is the P-score of the \( i \)th accession estimated in model (4), and the other terms are as defined in model (1). We studied model (5) under different conditions: considering phenotypes across common gardens, within Uppsala, or within Guangzhou. Statistical significance of the P-score in model (5) was assessed with a Fisher’s F-tests.

Results

Between common gardens, environmental variation was the main explanatory factor of phenotypic variation

The phenotypes of C. bursa-pastoris are mostly explained by the environments [see Supporting Information—Fig. S4], as the site effect in model (1) was significant for all phenotypes (apart from the number of rosette leaves and FP) at an adjusted Bonferroni cut-off of 0.0036 (all \( P < 0.001 \)). The PCA of the phenotypes also supported this trend (Fig. 2A), as the location—Uppsala or Guangzhou—was strongly correlated with the first component PC1 (explaining 41.2% of the variance). Accordingly, the measure of phenotypic plasticity—the P-score—was significant at the same adjusted Bonferroni cut-off for every phenotype (all \( t > 3.79, df = 1, P < 0.0013 \)). The environment was much more decisive than genetic differentiation in explaining phenotypic differences, as the genetic cluster effect explained significantly less than the site effect in model (1) for most of the traits [see Supporting Information—Table S4], and as the PCA revealed the genetic differentiation only from the third component [see Supporting Information—Fig. S5].

As a consequence of this strong environmental effect, accessions were markedly different in the two common gardens. In Uppsala, accessions performed poorly for most morphological phenotypes: the performance was lower (Table 1) for traits positively correlated to the number of fruits—a proxy of fitness—such as height (Pearson \( r = 0.63, P < 0.001 \)), number of fruits over 10 cm (\( P = 0.65, P < 0.001 \)) and number of primary branches (\( P = 0.66, P < 0.001 \)). Similarly, the phenotype which is much less correlated to the number of fruits (\( P < 0.16, P < 0.001 \)) shifted significantly between Uppsala and Guangzhou [see Supporting Information—Fig. S6], with bolting time, flowering time and senescence time being longer in Guangzhou (respectively, 61.7 ± 12.1 days, 72.0 ± 12.5 days and 139 ± 11 days) than in Uppsala (30.6 ± 5.7 days, 35.8 ± 5.7 days and 84.0 ± 6.4 days). Since C. bursa-pastoris is a facultative long-day species (i.e. flowering is inhibited by short day length; Hurka et al. 1976), flowering was, as expected, inhibited during a longer period in Guangzhou than in Uppsala (Cepulis et al. 2005).

Accessions with the highest plasticity in some morphological traits were the most successful in terms of number of
fruits—a proxy of fitness, since the $P$-scores of height, number of fruits over 10 cm and number of primary branches were significantly associated with the number of fruits in model (5) ($F > 17.81$, $df_1 = 1$, $df_2 = 67$, $P < 0.001$; see Supporting Information—Table S5). The significant associations were due to the most plastic accessions having more fruits in Guangzhou but not in Uppsala: $P$-scores in model (5) were significant at the adjusted Bonferroni cut-off of 0.0036 in Guangzhou for height, number of fruits over 10 cm ($F > 22.24$, $df_1 = 1$, $df_2 = 67$, $P < 0.001$), but significant for none of the phenotypes in Uppsala ($F < 8.20$, $df_1 = 1$, $df_2 = 39–67$, $P > 0.006$). In Uppsala, compared to Guangzhou, the most plastic accessions in terms of number of fruits had smaller height (model (5), $P_i = -0.02$, $P = 0.67$), number of fruits over 10 cm (model (5), $P_i = -0.44$, $P < 0.001$) and number of primary branches (model (5), $P_i = -0.22$, $P = 0.007$). In other words, plasticity in some morphological traits was beneficial in terms of number of fruits only in Guangzhou. In Uppsala, plasticity only affected morphology, but not the number of fruits. None of the associations between the number of fruits and the $P$-scores were significantly specific to a genetic cluster (Fig. 3), apart from $P$-score of height in ASI and ME ($F > 19.28$, $df_1 = 1$, $df_2 = 18–31$, $P < 0.001$; see Supporting Information—Table S5).

Within each common garden, most of the phenotypic variation is explained by the genetic clusters
Within a common garden, each accession experienced the same environment, so that only genetics and the interaction
between genetics and environments (G x E) can explain the variation among accessions. Statistical analyses of model (1) within each common garden suggested a genetic origin of the phenotypic variation (Table 1) as the genetic cluster effect was, at a Bonferroni adjusted cut-off of 0.0018 (28 comparisons: 14 phenotypes in two common gardens), significant for 9 traits out of 14 in Uppsala (average $\chi^2 = 41.4$, df = 2), and 9 out of 14 in Guangzhou (average $\chi^2 = 37.6$, df = 2). Genetic cluster had a significant effect in both common gardens for most of the traits ($\chi^2 > 7.16$, df = 2, $P < 0.05$; Table 1). For a handful of traits, genetic cluster had a significant effect in only one common garden: not significant in Uppsala for the number of primary branches and FP ($\chi^2 = 0.64$, df = 2, $P = 0.73$), while significant in Guangzhou ($\chi^2 = 11.11$, df = 2, $P < 0.01$); not significant in Guangzhou for the number of secondary inflorescences ($\chi^2 = 1.14$, df = 2, $P = 0.57$), while significant in Uppsala ($\chi^2 = 18.80$, df = 2, $P < 0.001$). Across the common gardens, the relation of phenotypes between the genetic clusters remained stable between ASI and EUR, and stable for some traits (number of rosette leaves, germination, flowering) with ME (Fig. 4). For the number of fruits, Middle Eastern accessions were significantly higher (Tukey’s HSD > 4.54, $P < 0.05$) than the other clusters in Guangzhou (Tukey’s HSD < –3.30, $P < 0.01$). The Middle Eastern accessions had significantly larger rosettes than Asian accessions in both common gardens (Tukey’s HSD > 3.74, $P < 0.001$).

Phenological variation was also strongly determined by the genetic cluster of the accession in Uppsala (average $\chi^2 = 32.6$, df = 2) and in Guangzhou (average $\chi^2 = 50.1$, df = 2). The Asian cluster exhibited late germination, early bolting, early flowering and late senescence, and therefore a long flowering duration. In contrast, the European cluster germinated early, but bolted, flowered and withered late. European accessions’ late flowering (but not all; see Neuffer 2011) partly explains their larger rosette, since they had more time for vegetative growth. Accessions from the Middle Eastern cluster did not follow any particular trend, showing a certain amount of plasticity in their phenological response. Pairwise ranking of phenological traits was consistent among common gardens (Table 2).

When focusing on the total number of fruits, the European cluster significantly outperformed the Asian cluster in

Figure 3. Phenotypic plasticity of Capsella bursa-pastoris. The mean number of fruits across common gardens (y-axis) as a function of the P-score (x-axis). Each dot represents one accession. Genetic clusters: ASI; red; EUR; green; ME; blue. (A) Number of rosette leaves. (B) Rosette diameter (in cm). (C) Height (in cm). (D) Germination time (in days). (E) Flowering time (in days). (F) Senescence time (in days). For colour figure refer online version.
Uppsala (Tukey’s HSD = 2.39, P < 0.05), although this trend was no longer significant (P = 0.38) after downsampling. In Guangzhou the Middle Eastern cluster outperformed both the Asian and the European clusters (Tukey’s HSD > 4.54, P < 0.001). Results suggested that the interplay between common garden and genetic cluster for the total number

Table 2. Differences in phenotypes among Capsella bursa-pastoris genetic clusters in each common garden (Uppsala and Guangzhou) in model (1). Statistics of the Tukey contrast analysis. Significant values are bolded. Significance levels are: ***P < 0.001; **P < 0.01; *P < 0.05; n.s. P > 0.05.

| Traits                  | Uppsala | Guangzhou |
|-------------------------|---------|-----------|
| No. of fruits           | EUR-ASI | ME-ASI    | ME-EUR | EUR-ASI | ME-ASI | ME-EUR |
|                         | 2.39*   | 1.76**    | -0.05** | -2.75* | 4.54*** | 6.34*** |
| Height                  | 8.69*** | 3.70**    | -2.47*  | 2.63*  | 5.99*** | 3.53**  |
| No. of fruits over 10 cm| -1.58** | 2.13**    | 3.07**  | -2.83* | 2.02**  | 4.10*** |
| No. of primary branches | 0.49**  | -0.49**   | -0.78** | -1.71* | 2.01**  | 3.33**  |
| No. of secondary inflorescences | 3.55** | 3.34** | 0.64** | 0.86*  | 0.91**  | 0.17*   |
| No. of rosette leaves   | 11.34***| 0.11**    | -8.44***| 6.64***| 1.92**  | -3.30** |
| Rosette diameter        | 9.22*** | 6.06**    | -1.74** | 4.29** | 3.74*** | 0.25**  |
| Germination             | -4.83***| -5.60***  | -1.95** | -9.97***| -9.27***| -1.53** |
| Bolting                 | 4.10*** | -1.89**   | -4.63***| 2.93*  | 0.48**  | -1.73** |
| BP                      | 8.64*** | 7.21***   | 0.56**  | 10.32***| 9.41*** | 1.29**  |
| Flowering               | 4.71*** | 0.12**    | -3.46** | 4.11***| 0.51**  | -2.68** |
| FP                      | -0.65*  | 0.80*     | 1.17**  | 3.81** | -0.90** | -3.87** |
| Senescence              | -1.24** | -3.15**   | -2.11** | 1.06*  | -2.22** | -2.95** |
| SP                      | -4.54** | -0.34**   | 3.15**  | -3.29**| -0.43** | 2.00**  |

Figure 4. Phenotypic variation among genetic clusters and common gardens for Capsella bursa-pastoris. Box plots of the most significant phenotypes in C. bursa-pastoris, in each common garden, for each cluster (ASI: red; EUR: green; ME: blue). For each trait and each common garden, block design was balanced as described in Supporting Information. (A) Number of rosette leaves. (B) Rosette diameter (in cm). (C) Total number of fruits. (D) Germination time (in days). (E) Flowering time (in days). (F) Senescence time (in days). For colour figure refer online version.
of fruits was due to the height and the number of primary branches; the Middle Eastern accessions were significantly smaller than the European accessions in Uppsala (Tukey’s HSD = −2.47, P < 0.05), and significantly taller in Guangzhou (Tukey’s HSD = 3.53, P < 0.01); the Middle Eastern accessions did not have significantly more primary branches than European accessions in Uppsala (Tukey’s HSD = −0.78, P = 0.71), while they did in Guangzhou (Tukey’s HSD = 3.33, P < 0.01). On the other hand, some patterns were consistent across common gardens: Asian accessions were significantly smaller than European and the Middle Eastern clusters in both common gardens (Tukey’s HSD > 3.70, P < 0.01), and likewise, the Middle Eastern cluster had significantly more flowers over a segment of 10 cm than the European cluster in both common gardens (Tukey’s HSD > 3.07, P < 0.01). However, Asian accessions were no longer significantly smaller in Guangzhou than the European accessions (P = 0.25) after downsampling. Variance of number of fruits was also higher for the Middle Eastern cluster than that of other clusters (Fisher’s F > 0.80, P < 0.001; see Supporting Information—Table S6), corroborating the hypothesis of higher plasticity of the Middle Eastern cluster, knowing that ME was not more genetically differentiated than the European and Asian clusters (see Supporting Information—Fig. S1; see also Cornille et al. 2016).

Environmental distance was mostly confounded with genetic clusters

When accounting for the effect of both genetic cluster and environmental distance between the sampling site and the common garden (model [2]), statistical analyses suggested a strong genetic cluster effect and an overall weak environmental distance effect: at a Bonferroni adjusted cut-off of 0.0018 (28 comparisons), the genetic cluster effect was significant for 17 out of 28 phenotypes, whereas the environmental distance was significant for none of the 28 phenotypes (though significant for a few phenotypes without the Bonferroni correction; Table 3). In addition, downsampling did not change the trend [see Supporting Information]. Environmental distance was probably confounded with the effect of genetic clusters, as the genetic cluster effect was significant for 18 out of 28 when excluding environmental distance (model (1), Table 1), and the effect of environmental distance was significant for 3 out of 28 phenotypes when excluding genetic cluster (model (3); see Supporting Information—Table S7). When analysing within a genetic cluster, the effect of environmental distance was negative for most of the traits (e.g. the closer the sampling site is to the common garden, the more performant the accession is), although very few were significant [see Supporting Information—Table S8].

The constraining environment of Uppsala (lower performance on average for most of the traits) compared to Guangzhou probably exacerbated the effect of environmental distance [see Supporting Information—Figs. S7, S8 and S9], as it was significant for two phenotypes in Uppsala, but significant for only one in Guangzhou (Table 3). In Uppsala, accessions from distant places had the lowest number of rosette leaves ($\chi^2 = 4.73$, df = 1, P < 0.05) and the shortest lifespan ($\chi^2 = 6.52$, df = 1, P < 0.05).

Discussion

Capsella bursa-pastoris is a tetraploid and self-fertilizing species that expanded worldwide relatively recently (Douglas et al. 2015) and populations can today be grouped into at least three main genetic clusters (Cornille et al. 2016). We investigated the effect of the genetic clusters in a large-scale experiment with a large number of accessions (223) across two common gardens installed in contrasting environments (Sweden and Eastern China). Our experiment shows that (i)
the success of *C. bursa-pastoris* is closely related to its high degree of phenotypic plasticity, (ii) genetic variation had a strong effect on phenotypic variation within a common garden and (iii) the effect of environmental distance was confounded by genetic clusters, impeding a proper assessment of the role of local adaptation.

The shepherd's purse is a highly plastic ruderal weed
As stated above, *C. bursa-pastoris* is a species with a worldwide distribution, thus *a priori* facing very contrasted environments, which is expected to favour high phenotypic plasticity for traits associated to fitness (Hendry 2016). In agreement with this expectation and previous studies (Aksoy et al. 1998; Caulet 2011; Neuffer 2011; Neuffer et al. 2018), our experiment detected phenotypic plasticity for all traits (Fig. 2); all accessions were able to survive in both common gardens, and all showed strong differences in phenotypes between the two common gardens. Though its dissemination is often associated to human activities (Cornille et al. 2016), the establishment ability and the worldwide success of *C. bursa-pastoris* were undoubtedly, and at least partly, related to its strong plasticity that is not so common (Palacio-Lopez et al. 2015). Phenotypic plasticity of certain morphological traits was related to individual performance (Fig. 3; see Supporting Information—Table S5), where higher plasticity was only beneficial in a richer environment (Guangzhou), but had no effect in poorer environment (Uppsala).

The flexible mating system of *C. bursa-pastoris*, predominantly a selfing species but with an outcrossing rate up to 10% under field conditions (Hurka et al. 1989), probably explains the strong establishment ability. Indeed, controlling the timing of phenology to cope with biotic and abiotic stresses is a response adopted by many plant populations, especially those with an annual life cycle (e.g. Davis et al. 2003; Leverett et al. 2018). Our experiment clearly showed that phenotypic plasticity of *C. bursa-pastoris* allows a contrasted phenological response, supporting the views of Anderson et al. (2012) in *Boechera stricta* (Brassicaceae), or Neuffer and Hurka (1986) that evoked a ‘general purpose genotype’ (a term borrowed from Baker 1965). However, within each common garden, European accessions flowered later than those from other clusters, indicating also a strong genetic determinism. The late flowering causes a longer time span for vegetative growth, and partly explains the significantly larger rosette. An association between flowering trends and rosette characteristics is not surprising. We indeed observed a strong correlation between the rosette size and the flowering time in our study \((r = 0.52)\). Linde et al. (2001) showed that quantitative trait loci (QTLs) associated with these two traits are closely linked in *C. bursa-pastoris*, and likewise, in *Arabidopsis thaliana*, rosette leaf number has been shown to be sensitive to stimuli of flowering, such as shading (Cookson and Granier 2006) or length of photoperiod (Lewandowska-Sabat et al. 2017). The late-flowering phenotype might not be constitutive of all European accessions though, since within small regions and along altitudinal clines in the Alps, highly differing ecotypes from early flowering in the valley to late-flowering types in higher elevations (or even not flowering within one vegetation period) have been recorded (Neuffer and Hurka 1986), which then was again observed along clines in North America (Neuffer and Hurka 1999).

Our study also showed that accessions from the Asian cluster flowered earlier than those from other clusters. Several studies showed that in many species late flowering appears to be the ancestral state (e.g. *A. thaliana*, Le et al. 2002; Komeda 2004; *Triticum monococcum*, Yan et al. 2003). It if was also the case for *C. bursa-pastoris*, it is probable that the ‘early-flowering’ phenotype recently evolved during *C. bursa-pastoris* range expansion in Asia. Early flowering in Asia can be (i) a response to avoid biotic competition (Orsucci et al. 2020), thus compensating the higher genetic load commonly reported in colonization fronts (Excoffier et al. 2009); (ii) a response to rather unpredictable environments such as those recorded in Asia (e.g. typhoon, monsoon), as observed in *A. thaliana* (Simpson and Dean 2002; Roux et al. 2006); or (iii) a side consequence of a higher sensitivity to photoperiod, as observed in tropical species such as rice (*Oryza sativa*) and maize (*Zea mays*), which is the main cue to the alternation of dry and humid seasons in these climates (Roux et al. 2006).

A strong genetic cluster effect impeding the observation of environmental differentiation
In addition to significant phenotypic plasticity, a clear difference among genetic clusters was observed for almost all traits. We tried to assess whether genetic clustering could be associated with environmental distance, but did not succeed due to confounding between these two factors (see also Samis et al. 2019). While our common garden experiment is adequate to study global trend in plasticity and adaptation among genetic clusters, the geographical scale considered here is probably too large to observe local adaptation that may take place at a smaller geographical scale. When investigating within a genetic cluster and at a smaller scale, strong adaptive differentiation can be observed in *A. thaliana* (Ågren and Schemske 2012), a weedy species phylogenetically close to *C. bursa-pastoris* (Beilstein et al. 2006). Undoubtedly, local adaptation plays a central role in the establishment success of *C. bursa-pastoris*, but was not detectable at the scale of our study. Alternatively, following the theory that populations shift from phenotypic plasticity at the start of their establishment towards adaptation to local conditions once established (Lande 2015), local adaptation may not have had enough time to be established. An absence of local adaptation coupled with a pronounced plasticity has already been observed in invasive species at the colonization front, such as in *Reynoutria japonica* (VanWallendael et al. 2018) or in clonally reproducing macrophytes (*Egeria densa*, *Elodea canadensis* and *Lagarosiphon major*: Riis et al. 2010). The absence of local adaptation might also explain the surprisingly low number of genetic clusters given the extensive distribution of *C. bursa-pastoris*.

The absence of signature of environmental differentiation might also be explained by pure neutral demographic dynamics, at least for the Asian cluster. Quite consistently, the Asian cluster had a lower performance compared to the other clusters, which might be due to its high genetic load (Cornille et al. 2016; Kryvokhyzha et al. 2019a, b). The Asian cluster is most certainly a marginal population, and has undergone an accumulation of deleterious mutations, which is common in colonization fronts (Excoffier et al. 2009). Although the long-term establishment success can be favoured by high genetic diversity (Hovick and Whitney 2019), a higher genetic load does not necessarily imply a lower performance in the
short term (Orsucci et al. 2020), and further investigations are needed to account for possible trade-offs between fitness components, especially since germination and seedling stage selection were removed.

**Conclusion**

The large-scale of our experiment, involving two common gardens and a large number of accessions representative of the natural range of the shepherd’s purse, allowed us to detect that a relatively high level of phenotypic plasticity is essential for a successful rapid range expansion. For the particular case of *Capsella bursa-pastoris*, its success appears to be strongly associated to its high phenotypic plasticity and its flexible mating system. Does this indicate that local adaptation does not contribute to a rapid colonization? It is probably too early to conclude, but the present study suggests that understanding local adaptation, in *Capsella bursa-pastoris* or other ruderal species, that went through a rapid range expansion would likely require a combination of more targeted reciprocal transplant experiments as well as experiments at smaller geographical scale.

**Supporting Information**

The following additional information is available in the online version of this article—

**Text S1.** Updating the genetic clustering, balancing the block design, the effect of downsampling, part of genetic differentiation vs environmental variation, environmental analyses with bioclimatic variables.

**Table S1.** Average values of temperature, humidity and length of photoperiod at Uppsala for each month in *Capsella bursa-pastoris*.

**Table S2.** Average values of temperature, humidity and length of photoperiod at Guangzhou for each month in *Capsella bursa-pastoris*.

**Table S3.** Environmental distance from each sampling site to a common garden.

**Table S4.** Ratio of variance explained by the genetic clusters and the common garden in *Capsella bursa-pastoris*, in terms of sum of squares (model [1]), noted R above.

**Table S5.** Significance of the link between phenotypic plasticity (P-score) and the number of fruits, as described in model (5).

**Table S6.** Variance ratio of the number of fruits for different genetic clusters (ASI, EUR and ME): statistics and degree of freedom (df) of the Fisher’s F-test.

**Table S7.** Effects of the environmental distance on each trait in *Capsella bursa-pastoris* in each common garden (Uppsala or Guangzhou), with an analysis of variance of model (3).

**Table S8.** Effect of environmental distance within genetic clusters (ASI, EUR, ME) of *Capsella bursa-pastoris* in each common garden (Uppsala or Guangzhou), with the model (3).

**Figure S1.** Multi-dimensional scaling of the genotypes (first axis explained 23.47% of the variance, and the second explained 15.74%).

**Figure S2.** Principal component analysis (PCA) of the environmental data: day length, temperature and humidity.

**Figure S3.** Partial leverage per sampling site for the number of fruits in Guangzhou in model (2).

**Figure S4.** Reaction norm of the number of fruits in Uppsala and Guangzhou.

**Figure S5.** Principal Component Analysis of Figure 2, with PCA 3 and 4, showing the genetic differentiation.

**Figure S6.** Histograms of the phenological traits of *Capsella bursa-pastoris* (germination time in red, bolting time in green, flowering time in blue, and senescence time in purple) according to the common garden and the genetic cluster.

**Figure S7.** Box plots of the number of fruits per environmental distance (discretized in intervals), per common gardens, and categorized per genetic cluster: red for ASI, green for EUR, and blue for ME.

**Figure S8.** Table of significance of the model (S1) for each phenotype and for each bioclimatic variable, in Uppsała.

**Figure S9.** Table of significance of the model (S1) for each phenotype and for each bioclimatic variable, in Guangzhou.

**Sources of Funding**

This work was supported by the Swedish Research Council and the Erik Philip Sörensen Foundation (to M.L.); Uppsala University and EMBO Short-Term Fellowship fund (to A.C.); the H2020 European consortium B4EST (to M.T.); National Natural Science Foundation of China (grant no. 31870353, to H.R.H.); and NSERC CGS-M and an Ontario Graduate Scholarship (to A.S.).

**Conflict of Interest**

None declared.

**Acknowledgements**

We thank Julia Dankanich, Josefine Stångberg, Johanna Nyström, Sara Kurland and Uriel Gélín for field assistance in Uppsala; Jean-Tristan Brandenburg for R scripting support; Lin-Lin Wu, Zhi-Bing Xie, Qiu-Ling Guan, Gui-Yu Lin for field assistance in Guangzhou; and Benoit Pujol, Benoit Facon, Denis Faure for their comments on the first versions of the manuscript. The computations and data handling were enabled by resources provided by the Swedish National Infrastructure for Computing (SNIC) at UppMax partially funded by the Swedish Research Council through grant agreement no. 2018-05973.

**Contributions by the Authors**

A.C., S.G., S.I.W. and M.L. planned and designed the common gardens. A.C., S.G., S.I.W. and M.L. directed the work. A.C., A.S., H.R.H., D.K. and K.H. performed experiments (installing the common garden and phenotyping every accession). A.C., M.T., M.O. and P.M. analysed the data. A.C., A.S., H.R.H. and M.L. wrote the article, with help from all authors and particularly from M.O. and P.M. A.C., M.T., A.S. and H.R.H. contributed equally.

**Data Availability**

Data and scripts are available from this link: https://orgemia.inra.fr/amandine.cornille/capsella_aobp.

**Literature Cited**

Ågren J, Schemske DW. 2012. Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. *New Phytologist* 194:1112–1122.
Anderson JT, Inouye DW, McKinney AM, Colautti RI, Mitchell-Olds T. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proceedings of the Royal Society B: Biological Sciences 279:3843–3852.

Angert AL, Bontrager MG, Ågren J. 2020. What do we really know about adaptation at range edges? Annual Review of Ecology, Evolution, and Systematics 51:341–361.

Aksosy A, Dixon JM, Hale WH. 1998. Biological flora of the British Isles. Capsella bursa-pastoris (L.), Medikus (Thlaspi bursa-pastoris L., Bursa bursa-pastoris (L.) Shull, Bursa pastoris (L.) Weber). Journal of Ecology 86:171–186.

Baker HG. 1965. Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL, eds. The genetics of colonizing species. New York, NY: Academic press; 147–172.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Beilstein MA, Al–Shehbaz IA, Kellogg EA. 2006. Brassicaceae phylogeny and trichome evolution. American Journal of Botany 93:607–619.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13:115–155.

Cauller C. 2011. Dispersion et adaptation des capsules: Capsella ru bella et Capsella bursa-pastoris dans un agroécosystème. PhD Thesis, University of Bourgogne, France.

Ceplitis AL, Su Y, Lascoux M. 2005. Bayesian inference of evolutionary history from chloroplast microsatellites in the cosmopolitan weed Capsella bursa-pastoris (Brassicaceae). Molecular Ecology 14:4221–4233.

Chevin LM, Lande R, Mace GM. 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS Biology 8:e1000357.

Cookson SJ, Granier C. 2006. A dynamic analysis of the shade-induced trichome evolution in plants. Advances in Genetics 50:346–363.

Cornille A, Salcedo A, Kryvokhyzha D, Glémín S, Holm K, Wright SI, Lascoux M. 2016. Genomic signature of successful colonization of Eurasia by the allopolyploid shepherd’s purse (Capsella bursa-pastoris). Molecular Ecology 25:616–629.

Daehler CC. 2003. Performance comparisons of co-occurring native and alien invasive plants: implication for conservation and restoration. Annual Review of Ecology, Evolution, and Systematics 34:183–211.

Davidson AM, Jennions M, Nicotra AB. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology Letters 14:419–431.

Davis MB, Shaw RG, Etterson JR. 2005. Evolutionary responses to changing climate. Ecology 86:1704–1714.

Douglas GM, Gos G, Steiga KA, Salcedo A, Holm K, Josephs EB, Arunkumar R, Ågren JA, Hazzouri KM, Wang W, Platts AE. 2015. Hybrid origins and the earliest stages of diploidization in the highly successful recent polyploid Capsella bursa-pastoris. Proceedings of the National Academy of Sciences of the United States of America 112:2806–2811.

Excoffier L, Foll M, Petit RJ. 2009. Genetic consequences of range expansions. Annual Review of Ecology, Evolution, and Systematics 40:481–501.

Excoffier L, Ray N. 2008. Surfing during population expansions promotes genetic revolutions and structuration. Trends in Ecology & Evolution 23:347–351.

Fick SE, Hijmans RJ. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Internal Journal of Climatology 37:4302–4315.

Gilbert KJ, Sharp NP, Angert AL, Conte GL, Draghi JA, Guillaume F, Hargreaves AL, Matthey-Doret R, Whitlock MC. 2017. Local adaptation interacts with expansion load during range expansion: maladaptation reduces expansion load. The American Naturalist 189:368–380.

Glémín S, François CM, Galtier N. 2019. Genome evolution in outcrossing vs. selfing vs. asexual species. In: Anisimova M, ed. Evolutionary genomics. Methods in molecular biology. New York, NY: Humana; 331–369.

Godoy O, Saldana A, Fuentes N, Valladares F, Gianoli E. 2011. Forests are not immune to plant invasions: phenotypic plasticity and local adaptation allow Prunella vulgaris to colonize a temperate evergreen rainforest. Biological Invasions. 13:1615–1623.

González-Martínez SC, Ridout K, Pannell JR. 2017. Range expansion compromises adaptive evolution in an outcrossing plant. Current Biology 27:2544–2551.

Greiner S, Barre P, Lirico I. 2016. Phenotypic plasticity and selection: nonexclusive mechanisms of adaptation. Scientifica 2016:1–9.

Grossenbacher D, Briscoe Runquist RD, Goldberg EE, Brandvain Y. 2015. Geographic range size is predicted by plant mating system. Ecology Letters 18:706–713.

Hendry AP. 2016. Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. Journal of Heredity 107:25–41.

Heller R, Smith JM. 1978. Does Muller’s ratchet work with selfing? Genetics Research 32:289–293.

Hijmans RJ, Williams E, Vennes C. 2015. Geosphere: spherical trigonometry. R package version 1.5-10. https://cran.r-project.org/package=geosphere. Accessed 20 May 2014.

Hollister JD, Greiner S, Wang W, Wang J, Zhang Y, Wong GK, Wright SI, Johnson MT. 2015. Recurrent loss of sex is associated with accumulation of deleterious mutations in Oenothera. Molecular Biology and Evolution 32:896–905.

Holhorn T, Breit F, Westfall P. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346–363.

Hovick SM, Whitney KD. 2019. Propagule pressure and genetic diversity enhance colonization by a ruderal species: a multi-generation field experiment. Ecological Monographs 89:e01368.

Hurka H, Freundner S, Brown AH, Plant Holt U. 1989. Aspartate aminotransferase isozymes in the genus Capsella (Brassicaceae): subcellular location, gene duplication, and polymorphism. Biochemical Genetics 27:77–90.

Hurka H, Friesen N, German DA, Franzke A, Neuffer B. 2012. ‘Missing link’ species Capsella orientalis (Brassicaceae) and Capsella bhracica elucidate evolution of model plant genus Capsella. Molecular Ecology 21:1223–1238.

Hurka H, Krauss R, Reiner T, Wohrmann K. 1976. Das Blühenverhalten von Capsella bursa-pastoris (Brassicaceae). Plant Systematics and Evolution 125:87–95.

Hurka H, Neuffer B. 1997. Evolutionary processes in the genus Capsella (Brassicaceae). Plant Systematics and Evolution 206:295–316.

Komedà Y. 2004. Genetic regulation of time to flower in Arabidopsis thaliana. Annual Review of Plant Biology 55:521–535.

Kryvokhyzha D, Holm K, Chen J, Cornille A, Glémín S, Wright SI, Lagercrantz U, Lascoux M. 2016. The influence of population structure on gene expression and flowering time variation in the ubiquitous weed Capsella bursa-pastoris (Brassicaceae). Molecular Ecology 25:1106–1121.

Kryvokhyzha D, Miledi P, Duan T, Orsucci M, Wright SI, Glémín S, Lascoux M. 2019a. Towards the new normal: transcriptomic convergence and genomic legacy from chloroplast microsatellites in the cosmopolitan weed Capsella bursa-pastoris (Brassicaceae). Molecular Ecology 28:295–316.

Lande R. 2009. Adaptation to an extraordinary environment by evolution of genetic plasticity and local adaptation. Journal of Evolutionary Biology 22:1435–1446.

Lande R. 2015. Evolution of phenotypic plasticity in colonizing species. Molecular Ecology 24:2038–2045.
Le CV, Roux F, Reboud X. 2002. DNA polymorphism at the frigida gene in Arabidopsis thaliana: extensive nonsynonymous variation is consistent with local selection for flowering time. Molecular Biology and Evolution 19:1261–1271.

Leverett LD, Schiedel GF, Donohue K. 2018. The fitness benefits of germinating later than neighbors. American Journal of Botany 105:20–30.

Lewandowska-Sabat AM, Fjellheim S, Olsen JE, Rognli OA. 2017. Local populations of Arabidopsis thaliana show clear relationship between photoperiodic sensitivity of flowering time and altitude. Frontiers in Plant Science 8:1046.

Linde M, Die S, Neuffer B. 2001. Flowering ecotypes of Capsella bursa-pastoris (L.) Medik. (Brassicaceae) analysed by a cosegregation of phenotypic characters (QTL) and molecular markers. Annals of Botany 87:91–99.

Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics 27:237–277.

Lovell JT, MacQueen AH, Mamidi S, Bonnete J, Jenkins J, Napier JD, Sreedasyam A, Healey A, Session A, Shu S, Barry K, Bonos S, Boston L, Daum C, Deshpande S, Ewing A, Grabowski PP, Haque T, Harrison M, Jiang J, Kudrna D, Lipzen A, Pendergast TH IV, Plott C, Qi P, Sasaki CA, Shakirov EV, Sims D, Sharma M, Sharma R, Stewart A, Singan VR, Tang Y, Thibiviller S, Webber J, Weng X, Williams M, Wu GA, Yoshinaga Y, Zane M, Zhang L, Zhang J, Behrman KD, Boe AR, Fay PA, Fritschi FB, Jastrow JD, Lloyd-Reilly J, Martinez-Reyna JM, Matamala R, Mitchell RB, Rosquette FM Jr, Ronald P, Saha M, Tobias CM, Udvardi M, Wing RA, Wu Y, Bartley LE, Casler M, Devos KM, Lowry DB, Rotkhasr DS, Grimwood J, Juenger TE, Schmutz J. 2021. Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. Nature 590:438–444.

Neuffer B. 2011. Native range variation in Capsella bursa-pastoris (Brassicaceae) along a 2500 km latitudinal transect. Flora-Morphology, Distribution, Functional Ecology of Plants 206:107–119.

Neuffer B, Hurka H. 1986. Variation of development time until flowering in natural populations of Capsella bursa-pastoris (Cruciferae). Plant Systematics and Evolution 152:277–296.

Neuffer B, Hurka H. 1988. Germination behaviour in populations of Capsella bursa-pastoris (Cruciferae). Plant Systematics and Evolution 161:35–47.

Neuffer B, Hurka H. 1999. Colonization history and introduction dynamics of Capsella bursa-pastoris (Brassicaceae) in North America: isozymes and quantitative traits. Molecular Ecology 8:1667–1681.

Neuffer B, Wesse C, Voss J, Scheibe R. 2018. The role of ecotypic variation in driving worldwide colonization by a cosmopolitan plant. AoB PLANTS 10:ply005; doi:10.1093/aobpla/ply005.

Orsucci M, Milei P, Hansen J, Girodolle J, Glémé S, Lascoux M. 2020. Shift in ecological strategy helps marginal populations of shepherd's purse (Capsella bursa-pastoris) to overcome a high genetic load. Proceedings of the Royal Society B: Biological Sciences 287:20200463.

Palacio-López K, Beckage B, Scheiner S, Molofsky J. 2015. The ubiquity of phenotypic plasticity in plants: a synthesis. Ecology and Evolution 5:3389–3400.

Pannell JR, Auld JR, Brandvain Y, Burd M, Busch JW, Cheptou PO, Conner JK, Goldberg EE, Grant AG, Grossenbacher DL, Hovick SM, Ijic B, Kalsz S, Petanidou T, Randle AM, Rubio de Casas R, Pauw A, Vamosi JC, Winn AA. 2015. The scope of Baker's law. New Phytologist 208:656–667.

Park DS, Ellison AM, Davis CC. 2018. Mating system does not predict niche breath. Global Ecology and Biogeography 27:804–813.

R Core Team. 2020. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Roux F, Touzet P, Cuguen J, Le Corre V. 2006. How to be early flowering: an evolutionary perspective. Trends in Plant Science 11:375–381.

Salisbury EJ. 1963. Intermittent germination of Capsella. Nature 199:1303–1304.

Samis KE, Stinchcombe JR, Murren CJ. 2019. Population climatic history predicts phenotypic responses in novel environments for Arabidopsis thaliana in North America. American Journal of Botany 106:1068–1080.

Sheh SN, Morueta-Holme N, Angert AL. 2020. Determinants of geographic range size in plants. New Phytologist 226:650–665.

Simpson GG, Dean C. 2002. Arabidopsis, the rosetta stone of flowering time? Science 296:285–289.

Slatkin M, Excoffier L. 2012. Serial founder effects during range expansion: a spatial analog of genetic drift. Genetics 191:171–181.

Stebbings LG. 1957. Self-fertilization and population variability in the higher plants. The American Naturalist 91:337–354.

Takebayashi N, Morrell PL. 2001. Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. American Journal of Botany 88:1143–1150.

VanWallsdauel A, Hamann E, Franks SJ. 2018. Evidence for plasticity, but not local adaptation, in invasive Japanese knotweed (Reynoutria japonica) in North America. Evolutionary Ecology 32:395–410.

Weske C, Welk E, Hurka H, Neuffer B. 2021. Geographical pattern of genetic diversity in Capsella bursa-pastoris (Brassicaceae)—a global perspective. Ecology and Evolution 11:199–213.

Willi Y, Fracassetti M, Zoller S, Van Buskirk J. 2018. Accumulation of mutational load at the edges of a species range. Molecular Biology and Evolution 35:781–791.

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J. 2003. Positional cloning of the wheat vernalization gene VRN1. Proceedings of the National Academy of Sciences of the United States of America 100:6263–6268.