Changing environments and genetic variation: natural variation in inbreeding does not compromise short-term physiological responses

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ABSTRACT

Selfing plant lineages are surprisingly widespread and successful in a broad range of environments, despite showing reduced genetic diversity, which is predicted to reduce their long-term evolutionary potential. However, appropriate short-term plastic responses to new environmental conditions might not require high levels of standing genetic variation. In this study, we tested whether mating system variation among populations, and associated changes in genetic variability, affected short-term responses to environmental challenges. We compared relative fitness and metabolome profiles of naturally outbreeding (genetically diverse) and inbreeding (genetically depauperate) populations of a perennial plant, *Arabidopsis lyrata*, under constant growth chamber conditions and an outdoor common garden environment outside its native range. We found no effect of inbreeding on survival, flowering phenology or short-term physiological responses. Specifically, naturally occurring inbreeding had no significant effects on the plasticity of metabolome profiles, using either multivariate approaches or analysis of variation in individual metabolites, with inbreeding populations showing similar physiological responses to outbreeding populations over time in both growing environments. We conclude that low genetic diversity in naturally inbred populations may not always compromise fitness or short-term physiological capacity to respond to environmental change, which could help to explain the global success of selfing mating strategies.

Key words: *Arabidopsis lyrata*, inbreeding, selfing, genetic variation, metabolomics, plasticity

BACKGROUND

Genetically informed conservation management programmes often assume that adaptive potential is limited by the amount of additive genetic variation maintained in a population [1]. Inbreeding is predicted to compromise long-term evolutionary potential through several mechanisms: the erosion of genetic variation, the reduced efficacy of selection [e.g. 2], and inbreeding depression due to both the increased phenotypic expression of deleterious recessive mutations [3, 4] and the loss of heterozygote advantage following increased homozygosity [5]. Selfing (inbred) lineages are thus predicted to show reduced long-term potential to adapt to environmental change and higher extinction rates than related but more genetically variable self-incompatible (outcrossed) lineages [2]. However, self-fertilising plant species are often geographically widespread and even invasive [6, 7], and selfing can be advantageous when reproducing in a new environment where conspecifics are scarce [8], suggesting that high levels of genetic diversity may not be required for appropriate responses to new environments.

One explanation for this pattern is that neutral genetic variation may not always predict adaptive genetic variation and therefore the evolutionary potential of a population [9]. Supporting this, even highly endangered species sometimes show adaptation despite extremely low levels of genome-wide variation [reviewed in 1]. Purging of genetic load in highly inbred lineages can also reduce the impacts of inbreeding depression at the population level [4, 10]. However, few studies have directly tested the effects of the resulting low additive genetic variation on short-term plastic responses to new environments.

Experimental laboratory studies using artificially-induced inbreeding suggest that the negative effects of inbreeding on trait plasticity may be most apparent under stressful environments [11]. For example, inbred families or experimental lines show reduced survival under extreme temperature stress [12], reduced tolerance to herbivores [13], and reduced induction of anti-herbivore defence traits [14, 15]. Recent work on the molecular

basis of inbreeding effects has revealed altered gene expression patterns associated with artificially inbred lines, as well as interactive effects of environmental stress and inbreeding on both gene expression [16] and particular metabolites [17]. However, other studies suggest that there is no general relationship between stress intensity and inbreeding depression; specific types of stressors and environmental novelty may instead increase phenotypic variability and therefore inbreeding depression [18]. Furthermore, in other experiments, the effects of inbreeding on trait plasticity were either not observed [19], or were not consistent across inbred families or traits [13, 20]. So, even experimental inbreeding in outcrossing species, when the effects of inbreeding depression should be greatest, might not compromise trait and physiological plasticity. To date though, few studies have examined the consequences for trait plasticity of natural mating system variation within species. We know far less about how populations with a sufficiently long history of inbreeding to purge deleterious recessive mutations will be able to adapt to changing environmental conditions.

To address this, we tested how natural mating system variation in the perennial herb *Arabidopsis lyrata* impacts short-term physiological responses to abrupt environmental change. *Arabidopsis lyrata* is distributed across the Northern hemisphere and although exclusively outcrossing in Europe (subspecies *A. l. petraea*), shows extensive variation in mating system around the Great Lakes region in North America (subspecies *A. l. lyrata*) [21-23]. North American populations show significantly reduced genetic diversity compared to European populations, suggestive of a historical bottleneck [24, 25], but heterozygosity is further reduced in inbred compared to outcrossed populations within North America [23, 26]. Patterns of population genetic structure suggest that the loss of self-incompatibility occurred multiple times during several independent postglacial colonisations of the Great Lakes region [23], but the lack of substantial changes in floral morphology predicted to be associated with the evolution of a selfing phenotype [27] suggests that these transitions were very recent. Instead, variation in floral morphology

was often better explained by postglacial genetic structure (and associated genetic drift) than mating system [27], suggesting that population-level factors such as phylogeographic history and broad environmental gradients, may be important for explaining trait variation in this species.

Strong inbreeding depression in growth and germination-related traits, as well as altered patterns of gene expression under stable environmental conditions, has been observed for experimentally-inbred European populations [28-30]. By contrast, both outcrossing and inbreeding populations from North America show more subtle fitness reductions in response to experimental inbreeding when grown in a stable environment [30] or outdoor common garden environments [31, 32], suggesting some purging of the genetic load. Even when challenged by herbivores, inbreeding depression in defence traits was low for populations of either mating system [33, 34]. Previous physiological studies in *A. l. petraea* have revealed variation in metabolite profiles and cold tolerance responses among and within populations from different geographic regions [35-37], although metabolomic divergence was mostly independent of population genetic structure [36]. Yet, these analyses were restricted to European outcrossing populations, so the effects of natural variation in inbreeding on physiology remain untested. North American *A. l. lyrata* is therefore a good model to assess the impacts of inbreeding-associated loss of genetic diversity on short-term plastic responses to environmental change, without the potentially overwhelming effects of strong differences in inbreeding depression.

The purpose of this study was to test whether naturally inbred populations show reduced fitness and altered physiological responses in a common garden environment relative to outbred populations. The common garden environment was situated outside the native range of *A. l. lyrata* and therefore provided growing conditions that differed from those naturally experienced. Specifically, we asked: 1) Is inbreeding associated with reduced fitness compared to outcrossing populations when individuals are transplanted to the common garden environment? 2) Is fitness-related trait variation better explained by

population latitude and/or population phylogeographic history than by history of inbreeding? 3) Is there a change in the metabolome over time when plants are transplanted to a naturally variable environment compared to those kept constant environmental conditions? 4) Does inbreeding alter the direction or magnitude of physiological plasticity over time or across environments?

MATERIALS AND METHODS

Seed sampling and plant origins

We sampled seeds from eight outcrossing and five inbreeding populations (Fig 1; Table S1; supplementary methods), classified based on a combination of previously estimated outcrossing rates (t_m) using progeny arrays based on microsatellite markers (inbreeding t_m <0.5), and proportion of self-compatible individuals (reflecting the potential for inbreeding; inbreeding > 0.5) taken from [23], as well as observed heterozygosity (H_o; reflecting actual history of inbreeding; inbreeding H_o <0.03) estimated using Restriction Associated DNA sequencing (Table S2; [26]). One population TSSA showed intermediate outcrossing rates, but similar heterozygosity to outcrossing populations, hence was categorised as outcrossing.

Previous STRUCTURE analysis of multi-locus microsatellite data for an extensive sampling across the Great Lakes region classified populations (that we label with –I or –O to indicate inbreeding and outcrossing) into five genetic groups [23], which were largely consistent with geographic distribution (Fig. 1a): A) IND-O, SAK-O, SBD-O; B) TC-I, TSS-O, TSSA-O, MAN-O; C) RON-I, PTP-I, PCR-O, PIN-O; D) LPT-I; and E) KTT-I. Most populations occurred on sand dunes along lakefronts, except for TC-I (growing on limestone on cliff edges), TSSA-O (limestone alvar site close to sand dunes), and KTT-I (the only population not on a lakefront, found in an isolated oak woodland sandflat).

Measuring growth, survival and reproduction in the common garden

To compare relative fitness of outbred and inbred *A. l. lyrata*, we established a common garden at the University of Glasgow Scottish Centre for Ecology and the Natural Environment (SCENE) on Loch Lomond (56.1289°N, 4.6129° W). The summer months in this part of Scotland tend to be relatively cool and wet, and winter months milder, than the corresponding times of the year around the North American Great lakes, so we expected this common garden to represent a novel environment for *A. l. lyrata.* Seeds were germinated from 20 maternal families per population and transplanted to the common garden in their 40-cell trays on 21st September 2012 (see supplementary methods). For the three largest populations (IND-O, PIN-O and RON-I) we used 40 families to obtain more precise estimates of fitness, and seeds from only 14 maternal plants were available for one inbred population (PTP-I). Four blocks were set up, with each block containing 80 individuals (one individual from each of 5 families per population, or 10 for the three largest populations) systematically distributed across four 40-cell trays. The position of each population in a block was randomised across the four blocks (see Fig S1). To explore population divergence in seedling growth rates, circular rosette area before transplant (7 weeks after germination) was estimated from two perpendicular measurements of rosette diameter. The proportion of plants with at least one open flower was then recorded once a week in the spring from 23rd April till 4th June 2013 (when all but two plants had flowered). The proportion of plants surviving overwinter was recorded in late spring over two years (28th May 2013 and 21st May 2014).

Measuring metabolomic responses to growing environment, time and population inbreeding history

To determine whether population inbreeding history affected short-term physiological responses under contrasting environmental conditions, we compared metabolomic profiles over time for three outcrossing (PCR-O, PIN-O, and TSS-O) and three inbreeding (LPT-I, RON-I, TC-I) populations when transplanted outdoors to the

158 common garden, or when kept under controlled growth chamber conditions (16h: 8h,
159 20°C:16°C, light: dark cycle). The common garden experiment for metabolomics samples
160 was established on 17th June 2013 and constituted one new experimental block
161 independent of the larger common garden study. For each mating type, two geographically
162 close populations (on the same lakefront) and one distant population were selected (see Fig
163 1). The two "distant" populations" (TSS-O and TC-I) are proximally located to one
164 another on the Bruce Peninsula. We grew seedlings from five maternal families per
165 population under controlled growth cabinet conditions for six weeks. Then, in August 2013
166 one seedling from each mother was either transplanted to 6cm deep trays containing F2+S
167 compost under the same growth chamber conditions, or transplanted outdoors to the
168 common garden environment.
169 To examine changes in the metabolome over time, two similar-sized leaves were
170 sampled from the rosette of each individual at three time points: 1) before transplanting
171 (4th August 2013); 2) ~24h after transplantation to their respective environments to test for
172 'transplant shock' effects (7th August 2013); and 3) 1 month after transplantation (5th
173 September 2013), to give plants time to respond to the growing environments. We were
174 unable to measure fresh leaf mass in the field, so instead compared similar-sized leaves,
175 making the assumption that changes in leaf mass would not strongly alter the relative
176 amounts of different metabolites (at least independent of broader responses to different
177 growing environments). Leaf samples were immediately frozen in liquid nitrogen,
178 transported on dry ice and stored until use at -70°C. Plants showing heavy damage by
179 herbivores or heavy pathogen infections were excluded from the metabolomics analysis
180 (see supplementary methods).
181 Seedlings from three maternal families per population were selected for metabolite
182 screening, resulting in nine samples each from inbreeding and outcrossing populations per
183 treatment per timepoint (108 samples in total). Samples were extracted in a chloroform:
184 methanol: water (1:3:1 ratio) mix (see supplementary methods for details) and analysed

using LC-MS. Briefly, 10µL of each sample was introduced to a liquid chromatography system (UltiMate 3000 RSLC, Thermo, UK) and separated on a 4.6 mm x 150 mm ZIC-pHILIC analytical column with a 2 mm x 20 mm guard column. The eluents were A: water with 20mM ammonium carbonate and B: acetonitrile. The gradient ran from 20% A, 80% B to 80% A, 20% B in 15min with a wash at 95% A for 3min followed by equilibration at 20% A for 8min. Metabolites were detected using an Orbitrap Exactive (Thermofisher, UK) instrument in positive/negative switching mode at resolution 50,000 with a m/z scan range of 70-1400. In total, 108 samples, plus a sample of pooled individual extractions for quality control, were run in a randomised order interspersed with twelve blank extraction buffer samples. No extraction internal standards were used, because our analysis focused on relative quantitation among treatments rather than absolute quantitation. We follow additional published guidelines to avoid detector sensitivity differences and drift over time among sample batches [38]. Data were annotated using a bespoke bioinformatics pipeline (mzMatch, IDEOM and PiMP) developed at Glasgow Polyomics [39-41], which resulted in a final dataset of 936 metabolites, of which 106 metabolites were confidently identified through comparison to a panel of standards (supporting methods). Raw peak heights for each putative compound in each sample were corrected by subtracting the average of the twelve blank readings for that compound; these corrected peak heights were used for subsequent analyses.

Statistical analyses

For the four response variables (rosette area, proportion plants flowering and survivorship in each year) we used generalised linear mixed effects models (GLMMs) from the R package *lme4* to test for the fixed effects of inbreeding history, genetic structure and population latitude, whilst controlling for the random effects of experimental block and population. Rosette area before transplant (in 2012) was used as a covariate in models for proportion flowering and survival in 2013. To explore whether adaptation to

broad environmental gradients varied with mating system, we also tested for an interaction between inbreeding history and latitude. History of inbreeding was modelled using either mating system class ("inbreeding" or "outcrossing", a categorical predictor) or observed heterozygosity (*Ho*, a continuous predictor). *Ho* consistently separates mating system groups across study years, whereas estimates of outcrossing rates (t_m) and the proportion of self-compatible plants can vary across years (Mable, unpublished data). As these predictors are correlated, we first fitted two full models using either mating system class or *Ho*: (i) response ~ mating system + genetic cluster + latitude + latitude:mating system + (1|population) + (1|block); (ii) response ~ *Ho* + genetic cluster + latitude + latitude:*Ho* + (1|population) + (1|block)

We selected the model with the lowest AIC and then performed backwards model selection, removing non-significant factors and comparing nested models using likelihood ratio tests. We assumed a Gaussian error distribution for rosette area and binomial error for the three binary response variables of flowering status and survivorship (in 2013 and 2014). Gaussian models were fitted using maximum likelihood, and we assessed Gaussian model fit by examining plots of residuals against fitted values and quantile-quantile plots.

We also separately tested for a genetic cluster-by-inbreeding interaction for two well-sampled clusters containing both outcrossing and inbreeding populations: MAN-O, TSS-O, TSSA-O vs TC-I (cluster B), and PCR-O, PIN-O vs RON-I, PTP-I (cluster C). Model simplification proceeded as described above, although the starting model was different: response ~ inbreeding history + genetic cluster + genetic cluster:inbreeding history + (1|block) + (1|population)

For the metabolomics data, we first conducted Principal Components Analysis using the R function *prcomp* (with variables scaled to have unit variance) to visualise changes in the metabolome with respect to experimental growing condition, time point and population inbreeding history. As peak height data for some metabolites was non-normally distributed, we also compared our PCA results to those of an unconstrained, distance-based

239 Non-metric Dimensional Scaling (NMDS) approach implemented using the R package
240 *vegan*. To assess physiological plasticity, we compared the magnitude and direction of
241 metabolome shifts in response to the two different growing environments, and plotted the
242 difference in values of the first five principal components (PCs) for related individuals
243 (same maternal family) growing in the different environments at time point 3. We
244 predicted that individuals from genetically-depauperate inbreeding populations would
245 show a reduced magnitude of change in each PC relative to those from outcrossing
246 populations. We tested the fixed effect of inbreeding history on the magnitude of change
247 for each of the first five PCs.
248 Metabolite diversity was estimated with a set of diversity measures that have been
249 developed to estimate the relative importance of differences in the abundance of species in
250 a community [42]. We estimated both 'metabolite richness' (the number of metabolites)
251 and 'abundance-corrected diversity' (reduced emphasis on low abundance metabolites;
252 details in supplementary methods). We used LMMs with Gaussian error family to fit the
253 following model: diversity ~ time*environment*mating system class + (1|population).
254 Model fit and simplification was examined as described above for fitness-related data.
255 To explore whether inbreeding history explained variation in individual
256 metabolites, we used LMMs (with Gaussian error) to model variation in corrected peak
257 heights for each of the 936 metabolites separately. For each growing environment
258 separately, we tested the significance of the effects of time, inbreeding history and their
259 interaction, whilst accounting for the random effect of population. Given that time points 1
260 and 2 showed similar multivariate metabolomic patterns, we focused on data from time
261 points 1 and 3. Due to the large number of metabolites involved, we did not assess model
262 fit, but corrected for multiple testing using the Benjamini-Hochberg procedure for
263 restricting the false discovery rate to 5%. We also estimated log2-fold change in
264 confidently identified metabolites among treatments, identifying those that were on
265 average >1-fold higher or lower in the common garden samples relative to the growth

chamber samples at time point 3, but which showed no difference (<1-fold changes) at time point 1 (when all plants were in the growth chamber).

RESULTS

Natural population inbreeding does not reduce survival or alter phenology in a novel common garden environment

Rosette size before transplant, as a proxy for relative growth rate, did not significantly vary with population inbreeding history (Table 1). However, divergence in rosette size between inbreeding populations (see Fig S2) resulted in a significant interaction between population latitude and inbreeding history, and a significant effect of genetic structure (Table 1). Specifically, rosette size tended to increase with latitude for inbreeding, but not outcrossing populations (Fig 2a). Analysis of data from two well-sampled genetic clusters (TSSA-O, MAN-O, TSS-O, TC-I vs PCR-O, PIN-O, RON-I, PTP-I) also revealed a significant interaction between genetic structure and inbreeding history (Table S4a), driven by the large rosette size of the inbreeding TC in genetic cluster B (Table S4b).

Of the 310 transplanted individuals, 251 (79.0%) survived the first winter, with no significant effect of either inbreeding history, latitude or rosette size at the time of transplant on survival (Fig 2b; Table 1). Genetic structure significantly affected first year survival, with individuals from genetic cluster C (PCR, PIN, RON and PTP) and E (KTT) showing higher rates of survival than other clusters (Table 1; Table S3). Only 34 individuals (11.0%) survived to spring 2014, and again no effect of inbreeding history was observed (Table 1). Over the second winter inbreeding populations showed both the highest (PTP-I) and lowest (LPT-I, TC-I, KTT-I) rates of survival, driving a significant interaction of latitude with inbreeding history (Fig 2c) and a significant effect of genetic cluster (Table 1; Table S3). However, this effect of genetic cluster disappeared when only the two well-sampled genetic clusters were analysed (Table S4a).

293 Within a 20-day time period in May 2013, 88% of plants flowered, with no
294 phenological differences between inbreeding and outcrossing populations. However, there
295 were clear population effects, with individuals from SBD-O flowering earliest and those
296 from KTT-I flowering latest (Fig 1). On 10th May 2013, when just over 50% of plants were
297 flowering, there was no effect of inbreeding history or rosette size on the likelihood of
298 flowering (Table 1), but we observed a significant interaction between inbreeding history
299 and latitude, as well as an effect of genetic structure; specifically, the proportion of plants
300 flowering increased with latitude for outcrossing populations, but not inbreeding
301 populations (Fig 2d). For the two well-sampled genetic clusters, a genetic cluster-by-
302 inbreeding history interaction was observed (Table S4a), with inbreeding TC-I flowering
303 later than outcrossing populations in cluster B, but inbreeding PTP-I and RON-I flowering
304 faster than outcrossing populations in cluster C (Table S4b).
305
306 ***Physiological responses to novel environments are driven by time and experimental***
307 ***treatments, with limited effects of inbreeding.***
308 The first five principal components (PCs) extracted from all compounds explained
309 50.1% variation in the metabolome. Variation in these five PCs (and the two NMDS axes)
310 were mostly explained by the interacting effects of growing environment and time, rather
311 than inbreeding history (Table S5A, B). Plotting PC1 (19.9% variance) against PC2
312 (13.1%) showed clear evidence for divergence in metabolite profiles at time point 3
313 compared to the earlier time points (Fig 3a), particularly in the outdoor common garden, a
314 pattern that was also supported by the NMDS analysis (Figure S4a). In both the PCA and
315 NMDS analysis we observed no separation of samples from inbreeding and outcrossing
316 populations.
317 The absence of marked divergence between time points 1 and 2 suggests minimal
318 transplant shock, and that sampling leaves three days earlier did not significantly alter the
319 metabolome. There was some evidence for population divergence in metabolomic profiles

at time point 1, with LPT-I differing from other populations (Fig S3a). However, genetically and geographically distinct TC-I and TSS-O showed similar metabolic profiles to other populations at both time points (Fig S3a,b).

Despite a strong metabolomic shift over time in the experiment, there was no evidence for altered physiological plasticity at time point 3 in inbreeding compared to outcrossing populations. The direction and magnitude of change in PC1 was mostly consistent across families and independent of population inbreeding history (Fig 3b-c). Supporting this, PC2 to PC5, and the two NMDS axes, also showed no significant effect of inbreeding history on metabolomic plasticity (Fig S5a-h; Fig S4b-e).

The diversity of metabolites changed significantly over time in the outdoor common garden environment, but mostly independent of population inbreeding history. Specifically, metabolite richness showed a significant time*treatment interaction ($P < 0.0001$; Fig S6a), with fewer metabolites detected at time point 3 in the outdoor common garden than the growth chamber. By contrast, abundance-corrected diversity showed a significant time*treatment and mating system*treatment interaction (combined model significance: $P < 0.0001$). This was driven by a greater number of abundant compounds at time point 3 in the outdoor common garden, and a tendency for inbred individuals to show an elevated number of abundant compounds relative to outcrossed populations at all time points in the growth chamber, but not in the common garden (Fig S6b).

In both growing environments, time-by-inbreeding history interactions, or effects of inbreeding history alone, were not significant for any metabolites following multiple testing correction (Table 2). By contrast, time since transplant had significant effects on 36.0% of metabolites in the growth chamber and notably 1.6x more (59.2%) metabolites changed over time in the outdoor common garden.

Of 106 confidently identified compounds, 28 were >1-fold higher and 18 were >1-fold lower in the outdoor common garden samples relative to the growth chamber samples at time point 3 (Table S6). Compounds that showed the strongest fold-changes included

the vitamin ascorbate (also annotated as D-Glucuronolactone), several members of the TCA cycle (S-malate and citrate) and several phosphorylated compounds associated with glycolysis and the pentose phosphate pathway (phosphoenolpyruvate, D-glucose/D-Fructose 6-phosphate and D-ribose 5-phosphate). By contrast, 12 of 18 compounds that showed the greatest decrease in the common garden samples were amino acids or amino acid derivatives.

DISCUSSION

In this study, we found that inbreeding and outcrossing populations of *A. l. lyrata* sampled from multiple genetic lineages show similar fitness and short-term physiological responses when exposed to a new environment. Specifically, individuals from inbreeding (genetically depauperate) populations showed similar growth rates, survival rates and flowering phenology to individuals from outbreeding (genetically diverse) populations in a common garden environment outside their native range. Instead, population genetic structure and environmental gradients associated with population latitude, consistently explained more variation in fitness-related traits. Furthermore, by assessing variation in metabolome profiles over time, we found that population inbreeding history had little impact on physiological responses to the novel environment.

Rates of survival in the common garden were not significantly explained by population inbreeding history in either study year, even when survival was much lower over the second winter compared to the first. Such low survival may reflect root degradation under the relatively mild winter conditions observed in 2013 (Fig S7); conditions that are rarely encountered in the native range of *A. l. lyrata* around the North American Great Lakes. We found only a weak effect of genetic structure on first year survival, but second year survival was explained by genetic structure and an interaction between inbreeding history and latitude (a proxy for climatic conditions). Specifically, survival rates declined with increasing latitude for inbreeding populations but not

374 outcrossing populations, though this significant interaction likely resulted from one
375 inbreeding population (PTP-I) showing much higher rates of survival in 2014 than other
376 inbreeding populations. Interestingly, geographically proximate populations in the same
377 genetic cluster around Lake Erie also varied in rates of survival, suggesting that
378 phylogeographic history explains some, but not all of the population variation in
379 overwinter survival. Instead, other population-level factors such as maternal effects (we
380 used field-collected seeds) or random genetic drift have likely contributed to the observed
381 variation in survival.

382 A shift to selfing (and associated inbreeding) might also be associated with altered
383 selection on flowering traits [43], which could impact responses to new environmental
384 conditions. On the other hand, population-level, but not inbreeding effects, have been
385 observed for several flowering traits (flower size and corolla length) in *A. l. lyrata* sampled
386 from the same geographic region [27]. Based on flowering time data from one season, we
387 observed no overall effect of inbreeding history on time to first flowering, but again found
388 effects of genetic structure and population latitude in interaction with inbreeding history.
389 For outcrossing populations, the proportion of plants flowering at a single time point was
390 higher for populations from higher latitudes, consistent with a faster transition to flowering
391 with a more contracted growing season. Supporting this, field observations suggest
392 flowering is delayed by approximately one month for plants at the highest latitudes on
393 Georgian Bay relative to those growing on the shores of Lake Erie (BM, personal
394 observation). Of the inbreeding populations, three from separate genetic clusters (TC-I,
395 LPT-I and KTT-I) flowered more slowly in the common garden than the genetically
396 clustered populations RON-I and PTP-I. Such a pattern might partly reflect local
397 phenological adaptation (for example KTT-I occupies a distinct oak woodland sand flat
398 habitat away from a lakefront), but could also result from stochastic fixing of phenotypic
399 variation during the postglacial colonisation of the Great Lakes by different genetic
400 lineages [23]. Interestingly, the probability of specific alleles fixing during colonisation is

predicted to be higher for inbreeding than outcrossing populations, which might also explain the high levels of variation in flowering phenology and rosette size observed among inbreeding populations. Nevertheless, a greater sampling of both inbreeding and outcrossing populations would be necessary to thoroughly test this prediction.

Interestingly, other common garden experiments using *A. l. lyrata*, have found mating system effects on survival and reproductive traits in individual study years, but no cumulative effect over multiple years in common gardens within [31] or outside the native range [32]. It is possible that growing plants in a novel environment to which all populations are maladapted could hide the effects of inbreeding depression (as predicted by a theoretical study: [44]); alternatively, enhanced levels of phenotypic variation in a novel environment leads to increased inbreeding depression [18]. Nevertheless, our data on flowering phenology, and survival suggest that reduced genetic variation due to inbreeding is not a consistent driver of variation in relative fitness in this species.

Using untargeted metabolomics, we found little evidence that inbreeding alters physiological responses to a novel common garden environment. Effects of inbreeding were absent for the first two principal components, which explained 32.9% of variation in the metabolome and were instead strongly influenced by interactions between growing environment and time since transplantation. There was some metabolomic clustering by population at timepoint 1 (in the growth chamber), but the clusters were less distinct by timepoint 3 in the outdoor environment, suggesting that similarly strong plastic physiological responses in outcrossing and inbreeding populations overwhelmed minor effects of physiological divergence with respect to source habitats. The weak effects of population and genetic background on metabolomic fingerprints observed in our study are similar to patterns seen for genetically distinct European populations [36].

We also found no significant effects of mating system, but strong effects of time since transplantation, on variation in amounts of individual metabolites in both growing environments. Such a result contrasts with previously described effects of experimental

inbreeding on important biosynthetic pathways related to specific stressors, such as anti-herbivore defence induction [14, 15]. One explanation for the absence of mating system effects in our study is that the common garden environment was not stressful enough to detect inbreeding effects on stress-related metabolic processes [45]. However, experimental evidence using *A. l. lyrata* from these same populations also suggested no consistent negative effect of inbreeding on resistance to the pathogen *Albugo candida* [46], or defence induction by herbivores [33]. The similarity of responses of individuals from inbreeding and outcrossing populations in our study suggests that the reduced heterozygosity resulting from multiple generations of selfing may not have compromised physiological plasticity. Alternatively, given that the inbreeding populations have persisted following postglacial expansion into the Great Lakes region [23], selection could have already removed those individuals with the greatest inbreeding load.

When metabolite diversity was estimated with reduced emphasis on low abundance metabolites, inbred populations showed elevated metabolic diversity in the benign growth chamber environment, but not the common garden. Interestingly, experimentally inbred progeny from two self-incompatible *A. l. petraea* populations grown in a controlled environment also showed elevated expression of stress and photosynthesis related genes relative to outbred progeny [28]. Although we do not have evidence that elevated metabolite diversity negatively affects plant fitness, these results could emphasise the importance of the environmental context when considering inbreeding depression [11, 12].

Plants grown in our outdoor common garden were exposed to potential abiotic stressors, which are known to significantly alter the leaf metabolome [47, 48]. The observed changes in confidently-identified metabolites in our experiment are consistent with plants in the common garden responding to increased light intensity and levels of radiation; the common garden samples showed elevated levels of the vitamin ascorbate, a compound associated with UV-B tolerance [49], as well as elevated levels of compounds linked to glycolysis and the TCA cycle, suggesting elevated rates of photosynthesis [49].

By contrast, the reduced levels of these metabolites and elevated levels of amino acids in growth chamber samples could reflect the higher growth rates of plants under controlled growth chamber conditions [e.g. 50](see photos in Fig S8). Additional controlled experiments would therefore be necessary to understand the adaptive nature of these divergent metabolomic responses to different growing environments. Given the observed leaf reddening in the common garden (Fig S8) that suggests abiotic stress, future assays should target metabolites, such as anthocyanins, known to play a role in stress adaptation and defence. Nevertheless, the use of untargeted metabolomics clearly offers promise for better understanding the different molecular pathways activated under novel environments or by particular stressors, as well as the impacts of genome-wide diversity on metabolite diversity and plasticity. Together, these results offer new insights into the importance of intraspecific patterns of genetic variation for tolerating changing environmental conditions.

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AUTHOR CONTRIBUTIONS

JB and BKM designed the experiment. JB conducted the experiments. JB, CAC, RD, KB and BKM analysed the metabolite data and JB analysed the field data. JB and BKM wrote the manuscript and all authors contributed to revisions.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.w0vt4b8m8. Raw metabolomics datafiles have been deposited to the EMBL-EBI MetaboLights database (DOI: 10.1093/nar/gks1004. PubMed PMID: 23109552) with the identifier MTBLS883, and can be accessed at: https://www.ebi.ac.uk/metabolights/MTBLS883.

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TABLES

Table 1: Statistics detailing the evidence for effects of population latitude, neutral genetic structure, inbreeding history and the interaction between latitude and inbreeding history on growth rates, survival (in 2013 and 2014) and flowering phenology.

Explanatory factors [a]	Rosette area [c]	Survival (2013) [c]	Proportion plants flowering [c]	Survival (2014) [c]
Inbreeding*Latitude [b]	**LR= 12.5, *P*= 0.0004**	LR= 0.474, *P*= 0.491	**LR= 17.9, *P*<0.0001**	**LR= 14.9, *P*= 0.0001**
Genetic structure	**LR = 20.0, *P*= 0.0005**	**LR= 10.0, *P*= 0.040**	**LR= 27.7, *P*<0.0001**	**LR= 18.9, *P*= 0.0008**
Inbreeding [b]	LR= 2.68, *P*= 0.101	LR= 0.019, *P*= 0.891	LR= 1.87, *P*= 0.171	LR= 1.76, *P*= 0.185
Latitude	LR= 2.34, *P*= 0.126	LR= 0.014, *P*= 0.906	LR= 0.269, *P*= 0.604	LR= 0.197, *P*= 0.657
Rosette area	*Not included*	LR= 0.033, *P*= 0.857	LR= 3.16, *P*= 0.075	*Not included*

[a] Explanatory factors included in the full model: Genetic structure + Latitude + Inbreeding + Inbreeding:Latitude + Rosette area
[b] Inbreeding history represents either mating system class (outcrossing vs inbreeding; used for flowering in 2013 and survival in 2014) or observed heterozygosity (H_o; used for rosette area and survival in 2013), depending on which produced the full model with the lowest AIC.
[c] Likelihood ratio statistics (LR) and p-values for the removal of this explanatory factor from the model. Degrees of freedom for the model comparison test are as follows: Genetic structure *df* = 4; all other factors *df* = 1. Bold text indicates those factors included in the minimal adequate (best-fitting) model.

Table 2: The number of metabolites showing significant interactions between time (points 1 vs 3) and inbreeding history in each growing environment.

	Growth chamber		Common garden	
Factors tested	N P < 0.05 [a]	N (FDR 5%) [a]	N P < 0.05 [a]	N (FDR 5%) [a]
inbreeding*time	85	0	42	0
inbreeding	77	0	63	0
time	405	337	586	554

[a] number of 936 metabolites significant at P < 0.05, or with a false-discovery rate of 5%

FIGURE LEGENDS

Figure 1: Proportion of plants flowering per inbreeding and outcrossing population over an 8-week period. Colours represent the five genetic structure groups identified by [23], with several interesting populations labelled. The arrow on the x-axis indicates the timepoint at which the proportion plants flowering was statistically compared (Fig 2d). Inset map: the eight outcrossing and five inbreeding populations of *Arabidopsis l. lyrata* sampled for this study. The six populations used for metabolomics analysis are indicated by *. Photo credit: Peter Hoebe.

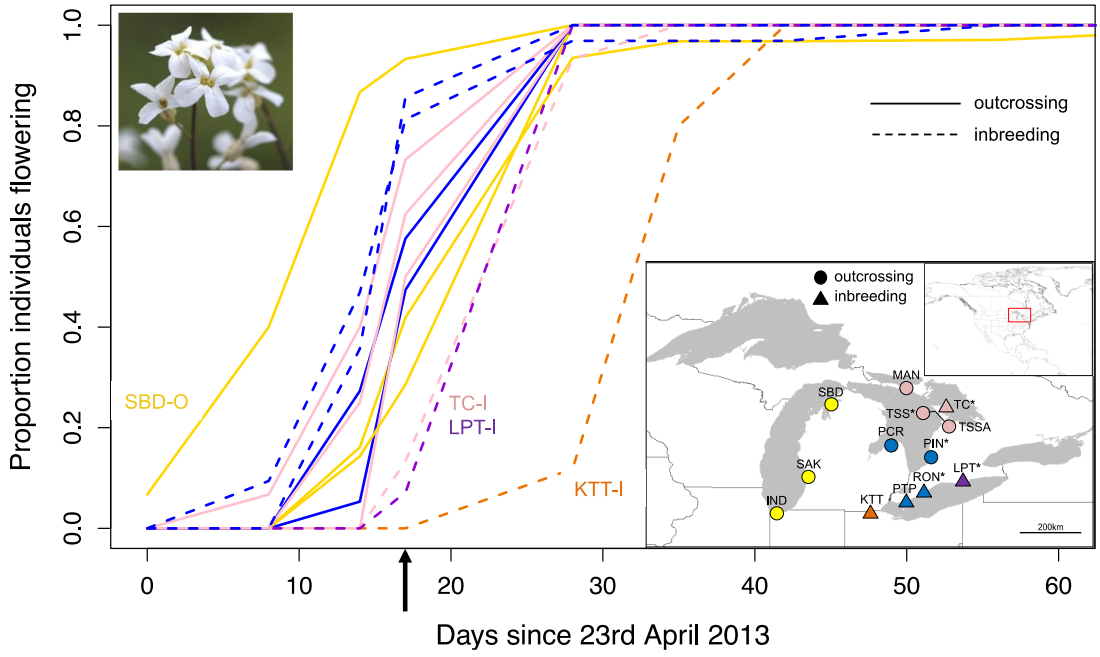

Figure 2: Regression plots illustrating the interaction between four fitness-related traits and latitude for individuals from outcrossing (dark grey) and inbreeding (light grey) populations: (a) mean rosette area per population just before transplant; (b) proportion of plants alive per population in spring 2013; (c) proportion of plants alive per population in spring 2014; (d) proportion of plants flowering per population at an early season timepoint (10^{th} May 2013). Lines indicate predictions of linear models to help visualise trends. Where relevant, the significance of the interaction between inbreeding history and latitude is given.

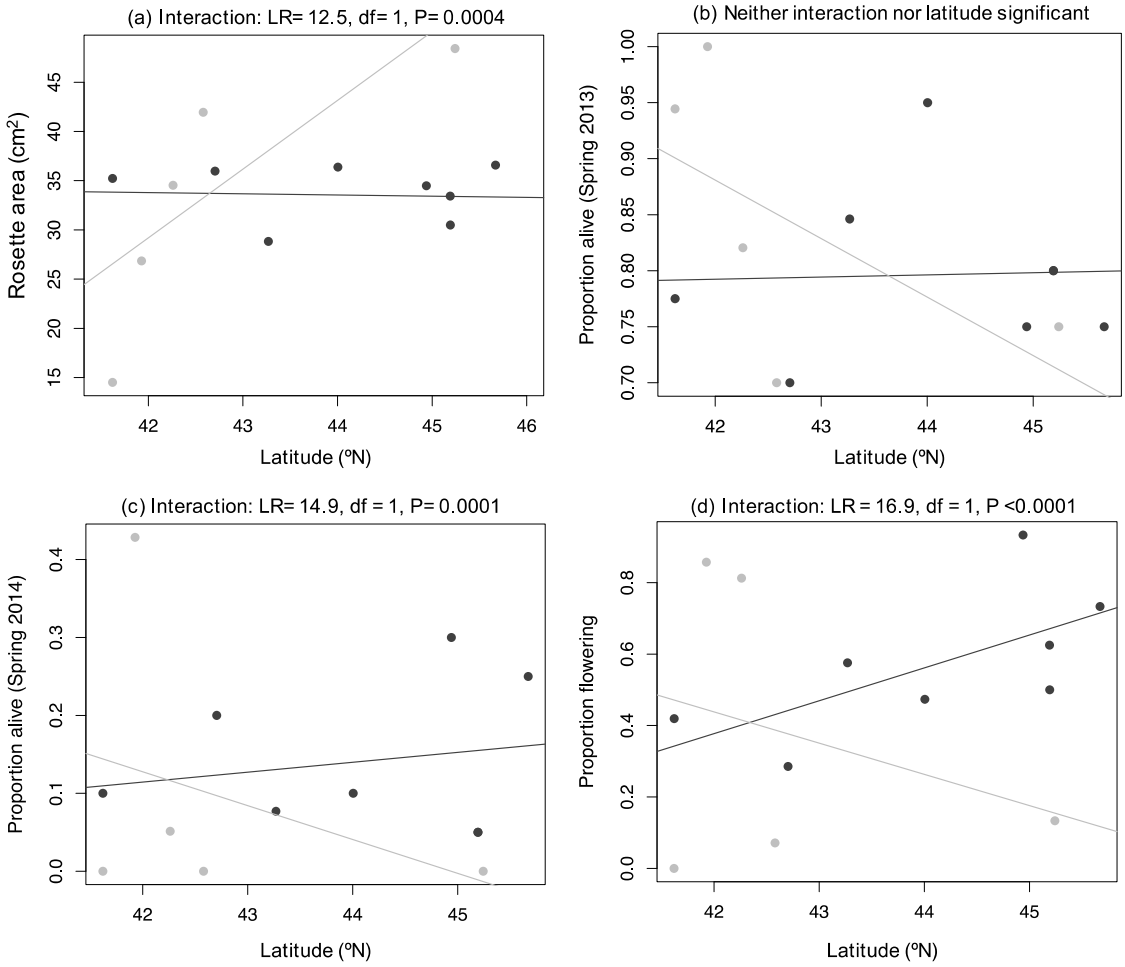

Figure 3: Metabolome variation and plasticity in *Arabidopsis l. lyrata* with respect to inbreeding history, time and growing environment. (a) plot of principal components 1 and 2 with respect to population inbreeding history (open symbols= inbreeding; filled = outcrossing), time and growing environment. Each level of the growing environment*inbreeding history*timepoint interaction is represented by nine individuals (108 in total). (b) changes in values of PC1 for each individual at time point 3 for the two environments, with lines joining related individuals from the same family. (c) boxplot representing the change in PC1 (magnitude of plasticity) between growing environments at time point 3 for individuals grouped by population inbreeding status. FigS5a-h gives similar plots for PC2-5.