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

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Total word count (main text, including acknowledgements) = 8732

Running heading: Natural inbreeding does not alter physiological plasticity

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 long-term evolutionary potential. However, short-term capacity to respond appropriately to new conditions might not require high levels of standing genetic variation. The purpose of this study was to directly test whether mating system variation and its associated changes in genetic variability in natural populations affected responses to short-term environmental challenges.
- We compared relative fitness and metabolome profiles of naturally outbreeding (genetically diverse) and inbreeding (genetically depauperate) populations of a long-lived perennial plant, *Arabidopsis lyrata*, under constant growth chamber conditions and an outdoor common garden environment outside its native range.
- We found no effect of mating system on survival or reproductive output, although several phenological traits showed different associations with latitude for outcrossing and inbreeding populations. Natural inbreeding had no effect on the plasticity of physiological responses, using either multivariate approaches or analysis of variation in individual metabolites. Moreover, while both growing environment and time significantly affected the relative abundance of individual metabolites, inbreeding populations responded similarly to outbreeding populations, suggesting adaptation to the outdoor environment, regardless of mating system.
- We conclude that low genetic diversity in naturally inbred populations may not compromise fitness or short-term capacity for appropriate physiological responses to environmental change. The absence of natural costs of inbreeding could help to explain the global success of clonal or asexual mating strategies for adapting to a wide range of environments.

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

INTRODUCTION

Genetically informed conservation management programmes often assume that adaptive potential is limited by the amount of additive genetic variation maintained in a population (O'Brien, 1994; Hoffmann, Sgro, and Kristensen, 2017). However, many geographically widespread and invasive plant species are self-fertilising or asexually reproducing and the evolutionary transition from an outcrossing to a selfing mating system has occurred frequently (e.g. Igic, Lande, and Kohn, 2008; Razanajatovo et al., 2016). Such shifts are associated with increased genome-wide homozygosity, as well as reduced efficacy of purifying and positive selection (e.g. Wright, Kalisz, and Slotte, 2013). Inbreeding is thus thought to compromise long-term evolutionary potential through erosion of genetic variation and increase the risk of extinction through inbreeding depression, defined as the exposure of deleterious recessive mutations through increased homozygosity (Charlesworth and Charlesworth, 1987; Keller and Waller, 2002). Consistent with this, selfing lineages are predicted to show higher extinction rates than related self-incompatible lineages (Goldberg et al., 2010). Together, these lines of evidence support the view that the long-term potential to adapt to environmental change will be compromised in low diversity selfing (inbred) lineages, compared to higher diversity outcrossed lineages (Bijlsma and Loeschcke, 2012; Wright, Kalisz, and Slotte, 2013).

However, multiple lines of evidence suggest that low levels of genetic variation associated with selfing may not affect the ability of lineages to respond appropriately to shorter-term environmental change. Firstly, many highly self-fertilising or asexually reproducing plants (modes of reproduction that reduce genetic variation) show broad distributions, and are able to invade and colonise new locations (Barrett, Colautti, and Eckert, 2008; van Kleunen et al., 2008; Razanajatovo et al., 2016). There are multiple examples of self-fertilising species rapidly colonising and adapting to new environments (Colautti et al., 2017; Willoughby et al., 2018), despite starting with low levels of genetic diversity. Secondly, low levels of neutral genetic variation may not always equate to low

levels of adaptive genetic variation, and therefore may be a poor proxy for the evolutionary potential of a population (Whitlock, 2014). The loss of individual selfed or clonal lineages with a high genetic load can also reduce the impacts of inbreeding depression at the population-level (Keller and Waller, 2002; Hedrick and Garcia-Dorado, 2016). Finally, selfing has obvious advantages when reproducing in a new environment where conspecifics are scarce (Eckert et al., 2010), but few studies have directly tested the effects of the resulting low additive genetic variation on short-term physiological plasticity.

Experimental laboratory studies suggests that the negative effects of inbreeding on trait plasticity may be strongest under stressful environments (Bijlsma and Loeschcke, 2012). For example, artificially inbred families or experimental lines show reduced survival under extreme temperature stress (Kristensen et al., 2008), reduced tolerance to herbivores (Ivey, Carr, and Eubanks, 2004), and reduced induction of anti-predator or anti-herbivore defense traits (Auld and Relyea, 2010; Campbell, Thaler, and Kessler, 2012; Kariyat et al., 2012; Campbell et al., 2014; Swillen, Vanoverbeke, and De Meester, 2015). However, in other experiments, the effects of inbreeding on trait plasticity were either not observed (Schlichtling and Levin, 1986), or varied among traits and were not consistent across inbred families (Ivey, Carr, and Eubanks, 2004; Schou, Kristensen, and Loeschcke, 2015). So, even in a context of experimental inbreeding in normally outcrossing species (when inbreeding depression should be high), short-term responses might not always be compromised by reduced heterozygosity or diversity in inbred lineages.

Recent work on the molecular basis of inbreeding effects on plasticity has revealed altered gene expression patterns associated with artificially inbred lines, as well as interactive effects of environmental stress and inbreeding on gene expression (Kristensen et al., 2010; Paige, 2010). Inbred *Drosophila* lines exposed to temperature stress showed increased variance in metabolite profiles relative to outcrossed lines, as well as consistent effects of inbreeding on particular metabolites (Pedersen et al., 2008). Similarly, experimentally inbred plant families (*Solanum carolinense)* showed reduced expression of

anti-herbivore defensive metabolites (Campbell, Thaler, and Kessler, 2012). Thus, it seems clear that an initial shift from outcrossing to inbreeding could compromise appropriate metabolic responses to stressors through inbreeding depression. However, most studies have compared the consequences of artificially-induced inbreeding, or mating system variation between species, rather than 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, despite reduced levels of genetic variation compared to their outcrossing relatives.

To test how natural mating system variation within a species impacts physiological responses to abrupt environmental change we used *Arabidopsis lyrata*, a predominantly outcrossing perennial herb that shows natural variation in mating system around the Great Lakes region in North America (Mable et al., 2005; Mable and Adam, 2007; Foxe et al., 2010). Inbreeding populations, in which the majority of individuals are capable of self-fertilisation, show significantly reduced heterozygosity and genetic diversity relative to outcrossing populations (Foxe et al., 2010; Buckley et al., 2016), although they show only minor changes in floral morphology consistent with the evolution of a selfing phenotype (Carleial, van Kleunen, and Stift, 2017b). Populations around the Great Lakes occupy several distinct habitats (rocky alvar and sand dune) and are distributed across a broad latitudinal gradient, characterised by variation in climate and the length of the growing period, so are also an interesting system in which to study local adaptation and its interaction with mating system. Patterns of population genetic structure suggest that the loss of self-incompatibility arose multiple times during several independent postglacial colonisations of the North American Great Lakes region (Hoebe, 2009; Foxe et al., 2010).

Experimentally-induced inbreeding in outcrossing *A. lyrata* populations from Europe has been found to result in strong inbreeding depression in growth and germination-related traits, as well as changes to constitutive patterns of gene expression under stable environmental conditions (Sletvold et al., 2013; Stift et al., 2013; Menzel et

al., 2015). In contrast, experimentally-inbred North American outcrossing populations show more subtle fitness reductions (Stift et al., 2013) and show suprisingly few differences compared to geographically proximate inbreeding populations. While naturally inbreeding populations from North America showed significantly reduced germination rates compared to closely-related outcrossing populations, no inbreeding depression was found for seedling growth rates or induced defense responses when challenged with herbivores for either mating system type (Joschinski, van Kleunen, and Stift, 2015; Carleial, van Kleunen, and Stift, 2017a). Moreover, inbreeding load has been found to be low and not substantially different in naturally inbreeding and outcrossing populations from North America, although the former show a greater increase in fitness when crossed to other populations (Willi, 2013). Similarly, in common garden experiments using experimental crosses between populations, heterosis was found to be higher in inbred compared to outcrossed populations, but magnitudes of inbreeding depression were similar regardless of mating system (Oakley, Spoelhof, and Schemske, 2015). North American populations of *A. lyrata* show a substantial reduction in genetic diversity compared to European populations, suggestive of a historical bottleneck (Ross-Ibarra et al., 2008; Mattila et al., 2017), but also that there has been some purging of the genetic load in both outcrossing and inbreeding populations (Stift et al., 2013; Willi, 2013). *Arabidopsis lyrata* is therefore a good model to assess the impacts on adaptive potential of loss of genetic diversity within a species caused by inbreeding without being overwhelmed by large differences in inbreeding depression in relation to mating system. Previous physiological studies in *A. lyrata* have revealed extensive variation in metabolite profiles among populations from different geographic regions (Davey et al., 2008; Kunin et al., 2009), as well as divergence in cold tolerance responses among regions (Davey, Woodward, and Quick, 2008), but these analyses were restricted to outcrossing populations from Europe. An important gap in our knowledge is thus whether natural variation in levels of

inbreeding and genetic diversity affects short-term physiological responses to environmental change.

The purpose of this study was to test whether naturally inbred populations show reduced fitness and altered physiological responses in a common garden environment when compared to outbred populations. The common garden environment was situated outside the native range of *A. 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 there a change in the metabolome over time when plants are transplanted to a naturally variable environment compared to those that are kept under constant environmental conditions? 3) Does inbreeding alter the direction or magnitude of physiological plasticity over time or across environments?

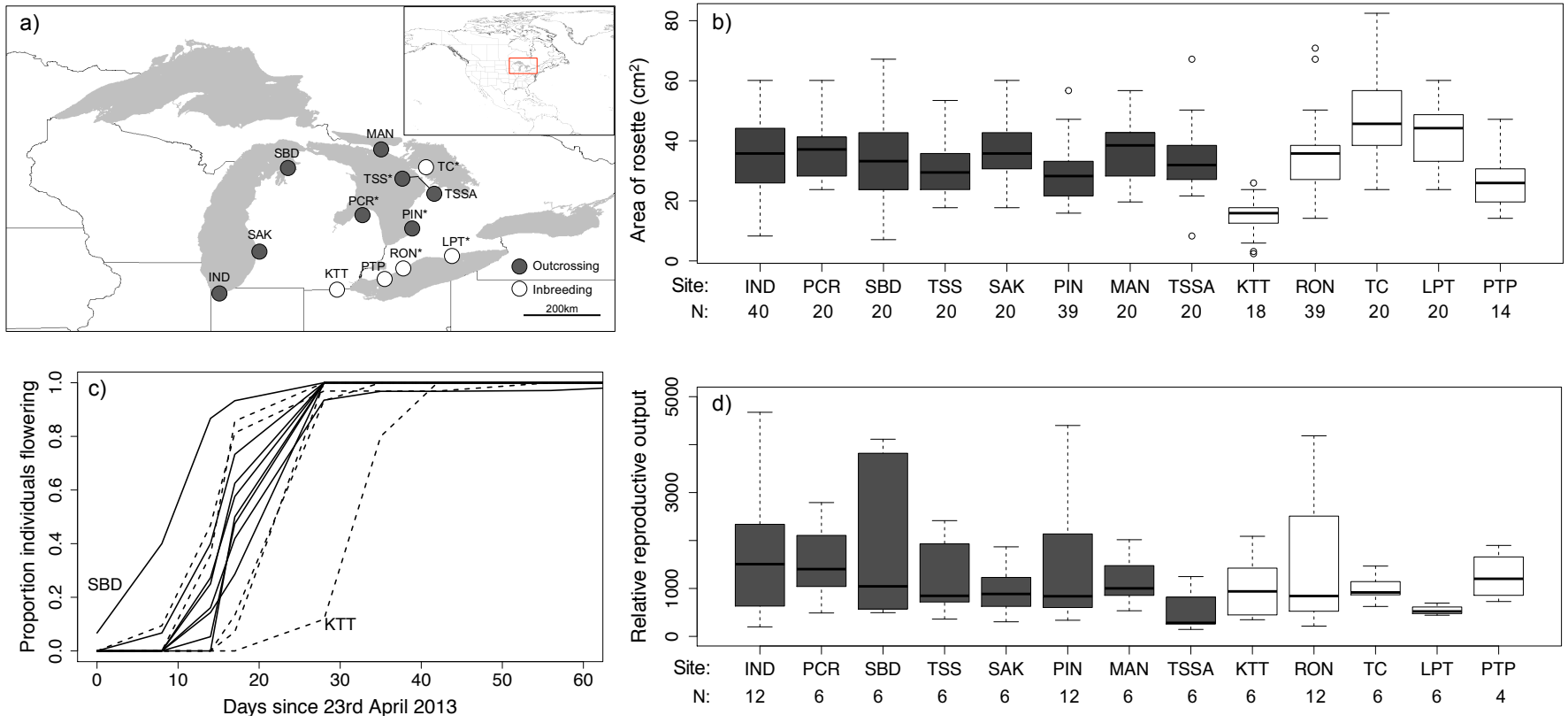
Our results reveal no consistent effects of mating system on fitness or short-term physiological responses to environmental changes, despite clear metabolomic divergence under the two growing environments at the later time point. The remarkable similarity in metabolic responses of inbred and outcrossed populations suggests that standing genetic variation is not important for adaptive physiological plasticity under potentially stressful novel environments.

MATERIALS AND METHODS

Seed sampling and plant origins

Seeds were collected from 25-40 individual *A. lyrata* ssp. *lyrata* plants per population from thirteen sites across the North American Great Lakes region (Fig 1a; Table S1), with eight outcrossing and five inbreeding populations selected based on a combination of outcrossing rates (t_m) estimated using progeny arrays, proportion of self-compatible individuals (reflecting potential for inbreeding), and observed heterozygosity (H_o; reflecting actual history of inbreeding) in a previous study (Foxe et al., 2010)(see Table S2

Figure 1: *Arabidopsis lyrata* sampling location and variation in phenology and fitness among populations and with respect to population mating system. (a) map of sampling sites with eight outcrossing and five inbreeding populations and an * indicating those populations used for metabolomics analysis; (b) boxplot of rosette area (mm^2) before transplantation to common garden; (c) proportion of plants flowering per population at 8 time points over an 8-week period (dashed lines = inbreeding populations; solid lines = outcrossing populations; (d) boxplot of reproductive effort (average length of fruits multiplied by number of fruits produced) on 1st July 2013. For (a), (b) and (d), dark grey indicates outcrossing populations, and white indicates inbreeding populations. Site codes and sample sizes are given under plots (b) and (d), in which populations are ordered by decreasing outcrossing rates (from left to right).



for values of t_m and H_o). Seeds were collected in 2011 (Table S2), except for two inbreeding populations (KTT in 2007 and PTP was supplemented in 2012). Outcrossing populations have significantly higher observed heterozygosity and nucleotide diversity relative to inbreeding populations based on both neutral microsatellite markers (Table S2; Foxe et al. 2010) and RAD-seq markers (Buckley *et al.* 2016). A mixed mating population TSSA (t_m = 0.41) was grouped with the outcrossing populations (t_m = 0.83-0.99) rather than the inbreeding populations (t_m = 0.09-0.31), as it showed similar levels of genome-wide diversity and heterozygosity to the outcrossing populations (Buckley et al., 2016). Most populations were found in freshwater coastal sand dune habitats, though plants at some sites (TC and TSSA) occupied rocky, alvar habitats and KTT was found in an oak woodlands sand flat. The inbreeding populations are predicted to represent three putatively independent origins of selfing (Foxe et al., 2010), with geographically proximate outcrossing populations predicted to be in the same genetic cluster included, where possible for metabolomics analysis: 1) inbreeding: RON, PTP, LPT; outcrossing: PCR, PIN; 2) inbreeding: TC; outcrossing TSS, TSSA; 3) inbreeding KTT is not located on a lakefront but it is on a similar latitude as IND, which is in the same genetic cluster as SAK, SBD, and MAN (Table S1).

The effects of population inbreeding on growth, survival and reproduction

Experimental Design

To compare relative fitness of outbred and inbred *A. l. lyrata*. we established a common garden in a novel environment in Scotland (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 more mild, than the corresponding times of the year around the North American Great lakes, where hot and dry summers and cold winters are more common. The common garden site was situated in a clearance surrounded by deciduous woodland.

We therefore expected this common garden to represent a novel environment for *A. lyrata.* Seeds were germinated under controlled growth chamber conditions (16h: 8h, 20°C:16°C, light: dark cycle) in Levington F2+S (seed & modular + sand) compost in 40-cell seedling propagators (Parasene, Cradley Heath, UK) to maximise germination rates. Three seeds from each of 20 maternal families per population were germinated per tray cell and thinned to one seedling per maternal family per population. For three populations (outcrossing IND and PIN and inbreeding RON) we used one seedling from each of 40 maternal seed families to obtain more precise estimates of fitness. These were three of the largest populations of *A. lyrata* in the field, and therefore were sites from which seeds from a greater number of maternal families were available. For one inbreeding population (PTP) only 14 maternal seed families were available (Table S2).

In total, 310 individuals, 199 of which were from outcrossing populations and 111 from inbreeding populations, were transplanted to the common garden on 21st September 2012. They were placed in a raised bed filled with Levington F2+S compost and horticultural grit to enhance drainage, and surrounded by high wire fencing to exclude mammalian herbivores. Two iButton dataloggers were placed in the centre of the plot to record above-ground and below-ground temperatures. Four propagator trays with 5-10 individuals from each population formed a block and four blocks were placed in a linear common garden plot (Fig S1). Plants in the common garden were watered once after transplanting, but thereafter no additional water or fertiliser was added during the experimental period. Seedlings from different populations were systematically arranged across the trays to distribute populations differing in mating system evenly across the common garden plot (Fig S1). Rosette size on transplant (7 weeks after germination) was calculated as the area of a circle from two perpendicular measurements of rosette diameter.

Survival and reproductive phenology in the common garden

Plants were checked approximately once a week in the spring from 23rd April to 4th June (and then every 2 weeks till 9th July) to record the proportion of plants with at least one open flower. All plants, except two individuals had flowered by early June. At the beginning of July, we counted the total number of fruits produced by two haphazardly selected individuals per population from each of three experimental blocks (in total 6 individuals per population, or 12 for populations IND, PIN and RON), to provide a snapshot measure of reproductive effort. Mean fruit length, which is highly correlated with seed number (Hoebe, 2009), was measured, on average, from five haphazardly selected, fully-developed fruits from each individual. Relative reproductive output per individual for this subset was then calculated as the number of fruits multiplied by the mean fruit length. The proportion of plants surviving overwinter was recorded in late spring on 28th May 2013 and 21st May 2014. Survival in 2014 therefore represents cumulative fitness over the time course of the experiment.

Statistical analyses

The effect of population mating system on each response variable (plant rosette area before transplantation, proportion plants flowering, survivorship, number of fruits, mean length of fruits per plant and the combined measure of relative reproductive output) was tested using generalised linear mixed effects models (GLMMs) using the R package *lme4* (Bates *et al.* 2015). Population mating system was included as a fixed factor in the models, and population was modelled as a random effect, to account for unexplained variation due to geographic source. For traits where all plants were measured (rosette area, proportion flowering, survivorship) experimental block was also included as a separate random factor in this model. Where traits were only measured for a subset of individuals (fruit length, number fruits, reproductive output) there were insufficient individuals to estimate random effects of experimental block. To account for multiple testing, a Bonferroni-correct P-value threshold of 0.008 (0.05 divided by 6) was used to identify significant mating system

effects. Binomial error distributions were used for modeling binary traits (survival and flowering status) and a normal error distribution used for continuous traits (rosette area and reproductive output). When modeling survival and propensity to flower, rosette size at transplant was included as a covariate to account for differences in initial growth rates in the cabinets. Model residuals were inspected to ensure good model fit and where necessary the response variables were log-transformed. To directly test for differences among populations in each response variable, we used separate models for a fixed effect of population on the different response variables controlling for the random effect of experimental block, but not considering mating system. Again a reduced P-value threshold of 0.008 was used to account for multiple testing and identify significant relationships. As multiple fruits were measured per individual, we additionally used a linear mixed effects model to estimate the proportion of variance in fruit length associated with population, maternal plant (family) and within-plant (residual variance). Likelihood ratio tests were used to estimate the significance of fixed effects following their removal from a model.

We additionally tested for variation in fitness-related traits consistent with local adaptation along the latitudinal range (41.6206 -45.6703 decimal degrees) occupied by these populations, and whether any of the fitness-related traits varied with population mating system. We conducted regression analyses to test for a significant interaction between mating system (inbreeding and outcrossing) and latitude for each of the above-described fitness-related traits, as well as the separate effect of latitude. The statistical models used for each trait were the same as described above.

The effects of population inbreeding on the metabolome

Experimental Design

To determine whether population mating system affected the physiological response under contrasting environmental conditions, we compared metabolomic profiles over time for three outcrossing (PIN, PCR and TSS) and three inbreeding (RON, TC, LPT) populations

when transplanted outdoors to the common garden, or when kept in a controlled growth chamber. We selected the three outcrossing and three inbreeding populations to represent similar levels of genetic structure and geographic range within each mating system group. Specifically, previous research showed that the chosen populations represent two different genetic clusters, with PIN, PCR, RON and LPT in one and TSS and TC in another (Foxe *et al.* 2010; Buckley *et al.* 2016). We germinated seeds from five maternal plants from each population under controlled growth cabinet conditions for six weeks. In August 2013 one seedling from each mother was either transplanted to new trays (6cm deep) under the same growth chamber conditions, or transplanted to the common garden environment (used in the fitness experiment).

Sampling strategy and methods for collecting leaf tissue

Two similar-sized leaves were sampled from the rosette of each individual at three time points in the common garden environment to examine changes in the metabolome over time. Each individual was sampled: 1) before transplanting to the outdoor environment (4th August 2013) to establish baseline profiles; 2) ~24h after transplantation to test for effects of 'transplant shock' (7th August 2013); and 3) 1 month after transplantation (5th September 2013), to give time for the plants to physiologically adapt to the growing environment. Any dirt was quickly removed from sampled leaves, before leaves were placed in a cryo-tube and flash frozen in liquid nitrogen. They were kept frozen on dry ice for a maximum of 2h during transport and then transferred to a -70°C freezer. The samples from the growth chamber plants were collected on the same days and in the same manner as described above. The plants were checked for signs of disease or herbivore damage and photos were taken of trays just before sampling to monitor their general status (e.g. colour of leaves).

Metabolomic data generation

For the three inbreeding and three outcrossing populations, individuals were selected for metabolite screening if leaf samples were available at all three time points for the two experimental growing conditions. Individuals from the same maternal families were used in the common garden experiment and the growth chamber. To allow us to assess changes in the metabolome over three timepoints and two treatments, metabolomics data was generated for only three individuals per population (nine individuals per mating system group). We therefore had insufficient power to resolve population-level differences in metabolomic plasticity through time and under different growing environments. One leaf per individual was placed in a Fastprep RNA bead tube (Lysing matrix D) and disrupted thoroughly in a Fastprep machine (MP Biomedicals). Each tube was dipped in liquid Nitrogen, and then disrupted in 2 x 10s runs, with samples refrozen in between runs. Then 1mL of chilled (-15°C) extraction buffer (chloroform: methanol: water in a 1:3:1 ratio) was added and the tube vortexed for 5s. The mixture was shaken on ice for 10min and centrifuged for 30s to separate tissue and lysate. The clear lysate was transferred to a new tube and used for metabolomics analysis. 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 20 mM 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. The LC–MS data were annotated using a bespoke bioinformatics pipeline (mzMatch, IDEOM and PiMP) developed at Glasgow Polyomics (Scheltema et al., 2011; Creek et al., 2012; Gloaguen et al., 2017). IDEOM assigns confidence scores ranging from 0-10 to each

putative compound identification. We considered all compounds with an IDEOM score of at least 6 that were present at either the first or third experimental time point, which resulted in a final dataset of 936 metabolites. The raw peak heights for each putative compound in each individual sample was then corrected by subtracting the average of the twelve blank readings for that identified compound. These corrected peak heights were used for further analysis. The identity of 106 metabolites was confirmed through comparison of retention times and masses to a panel of 122 standards, of which 96 compounds were uniquely identified and 13 had two related identifications (from now referred to as 'confidently identified metabolites').

Metabolomics Analyses

Multivariate Analyses

Principal Components Analysis (PCA) was performed on the dataset of 936 metabolites measured over the three time points using the R function '*prcomp'*. We plotted the first two principal components against each other to explore broad patterns of change in the metabolite data with respect to experimental growing condition, time point and population mating system. As a measure of plasticity, we compared the magnitude and direction of metabolome shifts in response to the two different growth conditions (growth chamber and outdoor common garden). We also plotted the difference in values of the first five principal components (PCs) for related individuals (same maternal family) growing in the different environments at time point 3 as a measure of relative plasticity. If reduced genetic variation from multiple generations of selfing compromises plasticity in key traits, we predicted that individuals from inbreeding populations would show a reduced magnitude of change in each PC relative to those from outcrossing populations. To test this, we used a two-way ANOVA with mating system as a fixed effect and the magnitude of this difference for each of the first five PCs separately as a response variable.

361 ***Metabolite diversity***

362 Metabolite diversity was also estimated using a set of diversity measures that have been
363 developed to assess the relative importance of differences in the abundance of species, as
364 well as the presence or absence of species in a community (Leinster and Cobbold, 2012).
365 Specifically, the emphasis placed on relative abundance is changed by adjusting a
366 parameter q. In the context of metabolite data, q = 0 is equivalent to the total number of
367 metabolites observed (with all metabolites weighted equally), whereas q = 1 or higher
368 results in lower abundance metabolites having less influence on the diversity measures (i.e.
369 the most abundant compounds shape diversity estimates). Here, we should note that using
370 an untargeted metabolomics approach means that variation in raw peak heights among
371 identified metabolites may reflect both inherent differences in metabolite detectability, as
372 well as variation in their actual abundance. Estimating diversity using q = 1 therefore
373 assumes that variation in metabolite detectability does not vary over orders of magnitude,
374 which may not always be the case. Nevertheless, our ability to estimate the 'true' diversity
375 of metabolites should not impact our statistical comparison of changes in diversity profiles
376 over time in each growing environment for inbred and outcrossed individuals. We
377 therefore statistically tested whether variation at q = 0 (metabolite "richness") and q = 1
378 (when relative abundance is considered) was explained by time, environment, mating
379 system or their interactions using GLMMs, with likelihood ratio tests used to determine the
380 significance of individual terms and population included as a random effect. Non-
381 significant interactions and individual terms were sequentially removed until a reduced
382 final model consisting of only significant interactions and terms remained.
383
384 ***Changes in individual metabolites and metabolite pathways***
385 To comprehensively test for the importance of mating system in explaining
386 variation in metabolite concentrations, we used GLMMs to model variation in corrected
387 peak heights for each of the 936 metabolites. We accounted for the random effect of

388 population and tested the fixed effects of mating system, time, treatment, and all two-way
389 and three-way interaction. Given the difficulty of interpreting three-way interactions, we
390 also tested the effects of time, mating system and their interaction for each experimental
391 condition separately. Given that time points 1 and 2 showed similar multivariate
392 metabolomic patterns, we focused on data from time points 1 and 3 in the analysis for each
393 environment separately. We corrected for multiple testing using the Benjamini-Hochberg
394 procedure for restricting the false discovery rate to 5%. For the subset of confidently-
395 identified metabolites, we identified those metabolites that were on average > 1-fold higher
396 or lower in the common garden samples relative to the growth chamber samples at time
397 point 3, but which showed no difference (< 1-fold changes) at time point 1 (when all
398 plants were in the growth chamber). For these confidently identified compounds we also
399 tested for a significant interaction between mating system, growing environment and time
400 (focused on time points 1 and 3) using a linear mixed model as described previously. After
401 correcting for multiple testing, we identified metabolites showing both a significant 3-way
402 interaction involving mating system, and a strong response to the common garden
403 environment (through fold-change comparisons).
404
405 **RESULTS**
406 ***Natural population inbreeding does not reduce fitness in a novel common garden***
407 ***environment***
408 Of the 310 transplanted individuals, 251 (79.0%) survived the first winter, with no
409 significant effect of either mating system (Table 1: Likelihood Ratio statistic (LR-stat =
410 0.50, df = 1, P = 0.479) or initial rosette size (LR-stat = 0.91, df = 1, P = 0.340) on
411 survival. Rosette size itself did not significantly vary with mating system (Fig 1b; LR-stat
412 = 0.025, df = 1, P = 0.874), despite showing significant variation among populations (Fig
413 1b; LR-stat = 124.8, df = 12, P < 0.0001). Survival was markedly lower over the second
414 winter, with only 34 individuals (11.0%) surviving to spring 2014, and again no effect of

Table 1: The proportion of surviving plants per population in the common garden on 28th May 2013 and a year later on 21st May 2014.

Site[a]	Decimal degrees[b]		N[c]	Proportion surviving	
	Latitude	Longitude		2013	2014
SAK	42.7044	86.2086	20	0.700	0.200
LPT	42.5797	80.3875	20	0.700	0.000
SBD	44.9389	85.8703	20	0.750	0.300
MAN	45.6703	82.2753	20	0.750	0.250
TC	45.2417	81.5175	20	0.750	0.000
IND	41.6214	87.2122	40	0.775	0.100
TSS	45.1925	81.5839	20	0.800	0.050
TSSA	45.1908	81.5906	20	0.800	0.050
RON	42.2614	81.8464	39	0.821	0.051
PIN	43.2689	81.8314	39	0.846	0.077
KTT	41.6206	83.7875	18	0.944	0.000
PCR	44.0042	83.0739	20	0.950	0.100
PTP	41.9278	82.5142	14	1.000	0.429
Outcrossing			199	0.799	0.131
Selfing			111	0.806	0.114

[a] sites are ordered by increasing proportion of individuals surviving in 2013; shaded populations represent outcrossing populations and unshaded inbreeding populations.
[b] coordinates in decimal degrees describing the latitude and longitude of each study location
[c] number of individuals at the start of the experiment in November 2012.

mating system (Table 1; LR-stat = 1.76, df = 1, P = 0.185), although outcrossing populations showed evidence for being more resilient to the challenges of overwintering. Specifically, over the second winter less than 5% of individuals survived in four of the five inbreeding populations, whereas only two of the eight outcrossing populations showed such low mortality (Table 1). The overall low survival over the second winter may in part be due to the milder winter temperatures experienced over the winter of 2013 (average 2.44°C) compared to 2012 (average 4.04°C; Fig S2). Despite no significant effect of inbreeding, the fixed effect of population explained 17.2% of variance in survival over the second winter (LR-stat = 36.8, df = 12, P = 0.0002), but was not significant for survival over the first winter. Interestingly, the inbreeding populations showed both the highest

435 (PTP) and lowest (LPT, TC, KTT) rates of survival over the second winter, which
436 emphasises population is more important than mating system for explaining patterns of
437 survival.

438 Population mating system also did not affect flowering phenology, with 88% of
439 plants flowering within a 20-day time period in May. However, there were clear population
440 effects, with individuals from SBD (outcrossing) flowering earliest and those from KTT
441 (inbreeding) flowering latest (Fig 1c). On 10th May 2013, when just over 50% of plants
442 were flowering, there was no effect of population mating system on the propensity of
443 individual plants to flower (LR-stat = 1.87, df = 1, P = 0.171), but there were significant
444 population-level effects (LR-stat = 89.91, df = 12, P < 0.0001), and also a small,
445 significant positive effect of rosette size when transplanted on the likelihood of flowering
446 (LR-stat = 4.20, df = 1, P = 0.041).

447 In mid-July, no measure of reproductive investment varied with respect to mating
448 system (number of fruits: LR-stat = 3.00, df = 1, P = 0.083; average fruit length: LR-stat =
449 0.04, df = 1, P = 0.842), although significant differences between populations were
450 apparent (number of fruits: LR-stat = 31.46, df = 12, P = 0.002, Length fruits: LR-stat =
451 52.49, df = 12, P < 0.0001; Fig S3a,b). The percentage of within-individual variance in
452 fruit length was 36.6%, which is similar to that explained by maternal plant (34.5%) and
453 population (28.9%). By contrast, in this analysis mating system explained ~0% variance in
454 fruit length. The combined measure of relative reproductive output revealed no significant
455 effects of mating system on relative reproductive effort at this single time point when
456 controlling for the random effect of population (Fig 1d; LR-stat = 3.08, df = 1, P = 0.079).
457 There was also no significant difference among populations in relative reproductive effort,
458 when population was considered as a fixed effect for the analyses (LR-stat = 7.50, df = 12,
459 P = 0.377).

460 While on average selfing populations showed lower reproductive output than
461 outcrossing populations in terms of numbers of fruits per individual (Table 2), this was

462 **Table 2**: Fitness-related trait means, sample sizes and 95% confidence intervals for the study populations, as well as when separated into inbreeding and
463 outcrossing groups. Rosette area (estimated area of a circle in cm^2 is given) was measured for all plants, whereas fruit length (based on an average of 5
464 fruits per plant), the total number of fruits at a mid-season timepoint and total reproductive output (fruit length*number of fruits) was measured for a
465 subset of plants (as described in the main text).
466

	Rosette area (cm^2)			Average fruit length (mm)			Number of fruits		Total reproductive effort	
Population	**N**	**Mean**	**95% CI**	**N**	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
IND	40	35.23	[31.55; 38.90]	12	25.85	[22.60; 29.11]	63.75	[31.68; 95.82]	1665.74	[866.23; 2465.25]
PCR	20	36.39	[31.69; 41.09]	6	25.09	[22.04; 28.13]	60.83	[29.78; 91.89]	1538.96	[685.55; 2392.36]
SBD	20	34.49	[27.99; 40.99]	6	26.9	[23.68; 30.13]	66.17	[6.78; 125.56]	1847.51	[83.11; 3611.90]
TSS	20	30.5	[26.39; 34.60]	6	18.75	[13.29; 24.20]	62.42	[24.60; 100.23]	1186.1	[347.14; 2025.05]
SAK	20	35.97	[31.07; 40.87]	6	30.13	[24.64; 35.62]	32.92	[12.95; 52.88]	966.36	[400.70; 1532.02]
PIN	39	28.83	[25.91; 31.76]	12	23.69	[21.08; 26.29]	56.08	[29.06; 83.11]	1419.48	[620.00; 2218.96]
MAN	20	36.58	[31.73; 41.42]	6	22.59	[19.07; 26.12]	49.42	[31.76; 67.07]	1147.84	[598.50; 1697.19]
TSSA	20	33.43	[27.92; 38.94]	6	17.94	[8.75; 27.12]	25.83	[9.68; 41.99]	550.8	[-31.42; 1133.02]
Outcrossing	**199**	**33.57**	**[32.04; 35.10]**	**54**	**24.73**	**[23.34; 26.11]**	**56.82**	**[46.01; 67.64]**	**1428.58**	**[1132.89; 1724.27]**
KTT	18	14.51	[11.34; 17.67]	6	27.27	[25.26; 29.27]	36.67	[12.62; 60.71]	1030.89	[300.22; 1761.57]
RON	39	34.53	[30.64; 38.43]	12	22.65	[20.27; 25.04]	64.71	[31.00; 98.41]	1559.56	[693.16; 2425.96]
TC	20	48.42	[40.78; 56.06]	6	25.68	[22.01; 29.35]	35.5	[15.34; 55.66]	1003.76	[609.44; 1398.09]
LPT	20	41.94	[37.49; 46.39]	6	33.45	[25.76; 41.14]	14.08	[9.68; 18.49]	544.68	[373.39; 715.96]
PTP	14	26.84	[22.08; 31.60]	4	24.62	[22.08; 27.16]	51.5	[14.83; 88.17]	1257.18	[440.70; 2073.66]
Selfing	**111**	**34.15**	**[31.22; 37.09]**	**40**	**24.61**	**[22.68; 26.53]**	**41.38**	**[29.75; 53.00]**	**1107.79**	**[792.28; 1423.29]**

467

468

469

driven by very low values for one of the selfing populations (LPT), in contrast to another selfing population on Lake Erie (RON), which showed a reproductive output on the higher end of values observed in the outcrossing populations.

Rosette area before transplantation showed a significant interaction between mating system and latitude (Fig S4a), with a significantly increase in size with latitude for inbreeding, but not outcrossing populations. Flowering time also showed a significant interaction between mating system and latitude (Fig S4c), with an increase in flowering at the early time point for outcrossing populations with latitude but a very low flowering rate in the selfing population found at the highest latitude (TC). There was also a weakly negative effect of latitude on mean fruit length, but the pattern was the same in outcrossing and selfing populations (Fig S4e). After controlling for multiple testing, survival rates in both 2013 and 2014, the number of fruits produced and relative reproductive output at a mid-season timepoint showed neither a significant effect of latitude, nor an interaction of latitude with mating system (Fig S4b, d, f).

Physiological responses to novel environments are driven by time and experimental treatments, with limited impact of inbreeding.

Plants growing outside for the metabolomics study were exposed to herbivores and disease during the time course of sampling, and we avoided sampling any heavily damaged or infected plants for metabolomics analysis. At time point 2 (7th August), the earliest time of sampling leaves in the field, there was no evidence for herbivore damage or disease on any plants used in the metabolomics analysis. At time point 3 (on 5th Sept), eight of the 18 plants analysed showed no herbivore damage, and five of the 18 plants showed damage to just 1 leaf. The remaining five plants showed 2 or 3 damaged leaves). Molluscs were most likely causing this minor damage, and damage was mostly restricted to older leaves, which were not sampled in our study (JB personal observation). Additionally, three individuals showed early signs of *Albugo candida* infection (a common oomycete parasite of the

497 Brassicaceae). These three individuals included one inbreeding (RON) and two outcrossing
498 individuals (PIN and TSS). Therefore, the observed rates of infection and low levels of
499 herbivore pressure should not impact the observed absence of inbreeding effects on the
500 metabolome.

501 At time point 3 (one month after transplantation) there was clear divergence
502 between plants in the growth chamber and common garden in growth and appearance (Fig
503 S5), which made this time point the most informative for examining inbreeding effects on
504 physiological plasticity. The first five principal components (PCs) extracted from all
505 compounds explained 50.1% variation in the metabolome (Table 3).

506

507 **Table 3**: The significance of population mating system (inbreeding or outcrossing), time,
508 experimental environment and their interactions for explaining variance in the first five
509 principal components generated using data from 936 metabolites.
510

Principal component	% variance in metabolites	Final model (explanatory factors)[a]	Significance of final model[b]
PC1	19.86	environment * time	LR-stat = 77.75, df=2, p<0.0001
PC2	13.07	time	LR-stat=42.80, df=2, p<0.0001
PC3	7.00	environment * time * mating system	LR-stat=7.33, df=2, p=0.026
PC4	5.29	environment * time	LR-stat=19.99, df=2, p<0.0001
PC5	3.08	environment * time * mating system	LR-stat=7.06, df=2, p=0.029

511 [a] minimal adequate linear mixed model to explain variation in the principal component,
512 generated by removing factors in order of complexity from a starting model of a 3-way
513 interaction between all three fixed factors.
514 [b] likelihood ratio test statistic, associated degrees of freedom and p-value on comparison of
515 the final model to the next most simple (nested) model.
516
517
518 Plotting PC1 (19.9% variance) against PC2 (13.1%) showed clear evidence for
519 divergence in metabolite profiles at time point 3 compared to the earlier time points,

520 particularly in the outdoor growth conditions, but with no strong effects of mating system
521 (Fig 2a). Variation in these five PCs were mostly influenced by the interacting effects of
522 growing environment and time, rather than mating system (Table 3). At time points 1,
523 there was some clustering by population in both the growth cabinet and common garden
524 environment, whereas at time point 3 this was only clear in the common garden, but in
525 neither case was the pattern strongly associated with mating system or latitude (Fig S6).
526 Time was a significant fixed factor for all five PCs and growing environment-by-time
527 interactions were significant for four of the PCs. Despite a strong metabolomic shift over
528 time in the experiment, there was no evidence for altered physiological plasticity at time
529 point 3 in inbreeding populations. The direction and magnitude of change in PC1 and PC2
530 were mostly consistent across families and independent of population mating system (Fig
531 2b -2e). PC3 to PC5 (Fig S7a-f) also showed no effect of mating system on metabolomic
532 plasticity, except that inbreeding populations showed greater variance in the magnitude of
533 the metabolomic shift for PC5 (Fig S7f).
534 The diversity of metabolites changed significantly over time in the outdoor
535 common garden environment, but again independent of mating system (Fig 3).
536 Specifically, the metabolite richness (number of metabolites, when $q = 0$) showed a
537 significant time*treatment interaction ($P < 0.0001$; Fig 3a) with fewer metabolites at time
538 point 3 in the outdoor common garden, but not in the growth chamber. After accounting
539 for relative abundance of metabolites (using $q = 1$ to reduce the contribution of rare
540 compounds to estimated diversity), there was a significant time*treatment and significant
541 mating system*treatment interaction (combined model significance: $P < 0.0001$). This was
542 driven by a greater number of abundant compounds at time point 3 in the outdoor common
543 garden, but also a tendency for inbred individuals to show an elevated number of abundant
544 compounds relative to outcrossed populations at all time points in the growth chamber, but
545 not in the common garden (Fig 3b).
546

Figure 2: Multivariate variation and plasticity in the *Arabidopsis lyrata* metabolome with respect to time, mating system and experimental treatment: (a) visualised by plotting principal components 1 and 2, with colours indicating three different time points of sample collection (see key, Time 1= pre-transplant, Time 2 = 1 day after transplant, Time 3 = 1 month after transplant), symbols indicating environments (circles = growth chamber, triangles = common garden) and fill denoting mating system (open= inbreeding; filled = outcrossing); (b,d) plots of PC1 and PC2 values, respectively, for each individual at time point 3 for the two environments, with lines joining related individuals from the same family. Dashed lines (and open shapes) connect individuals from inbreeding populations and solid lines (and filled shapes) indicate those from outcrossing populations; (c,e) boxplots representing the change in PC1 and PC2 respectively between growing environments for related individuals grouped by population inbreeding status. The significance of population inbreeding on the magnitude of plasticity is indicated.

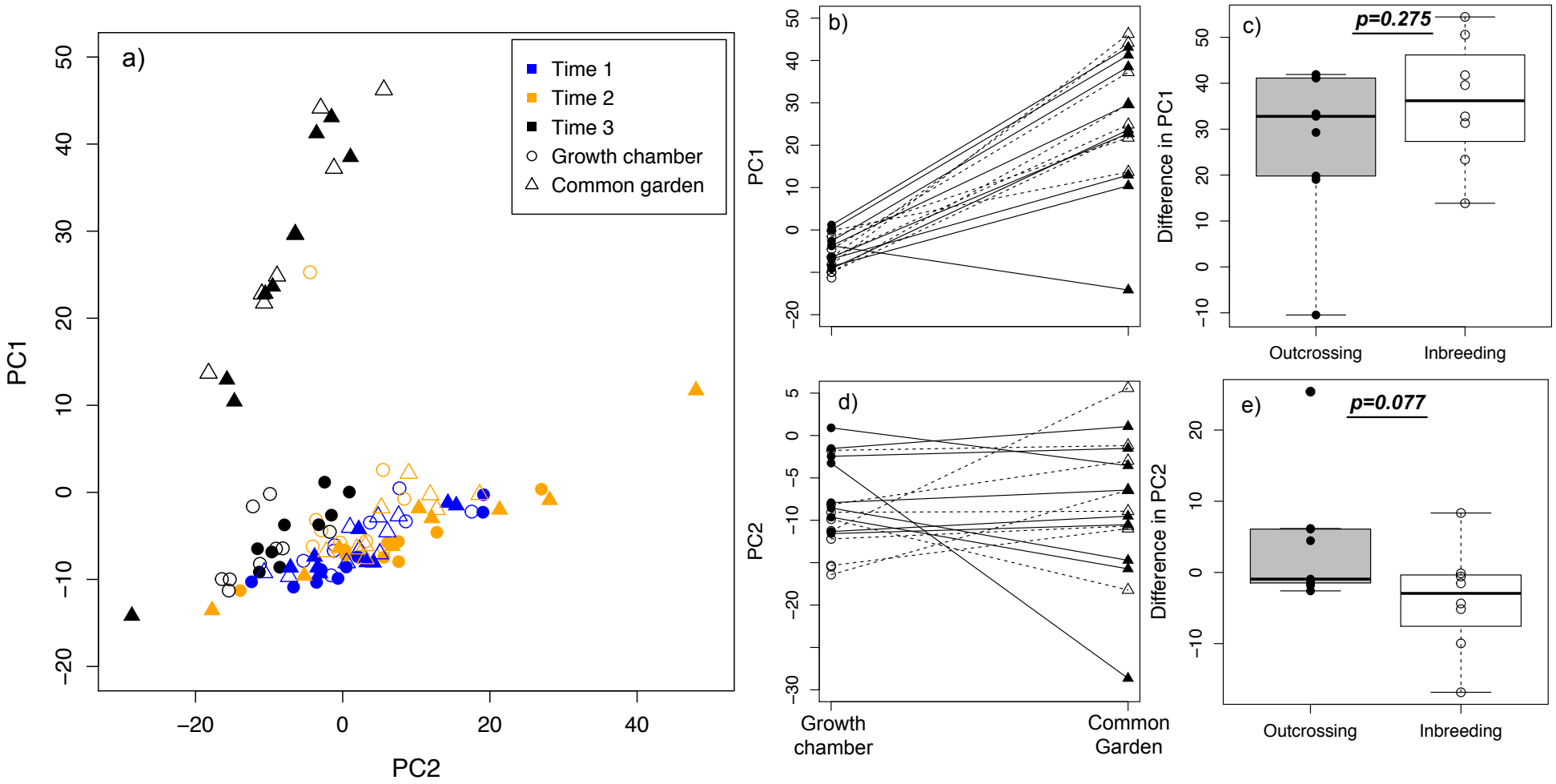
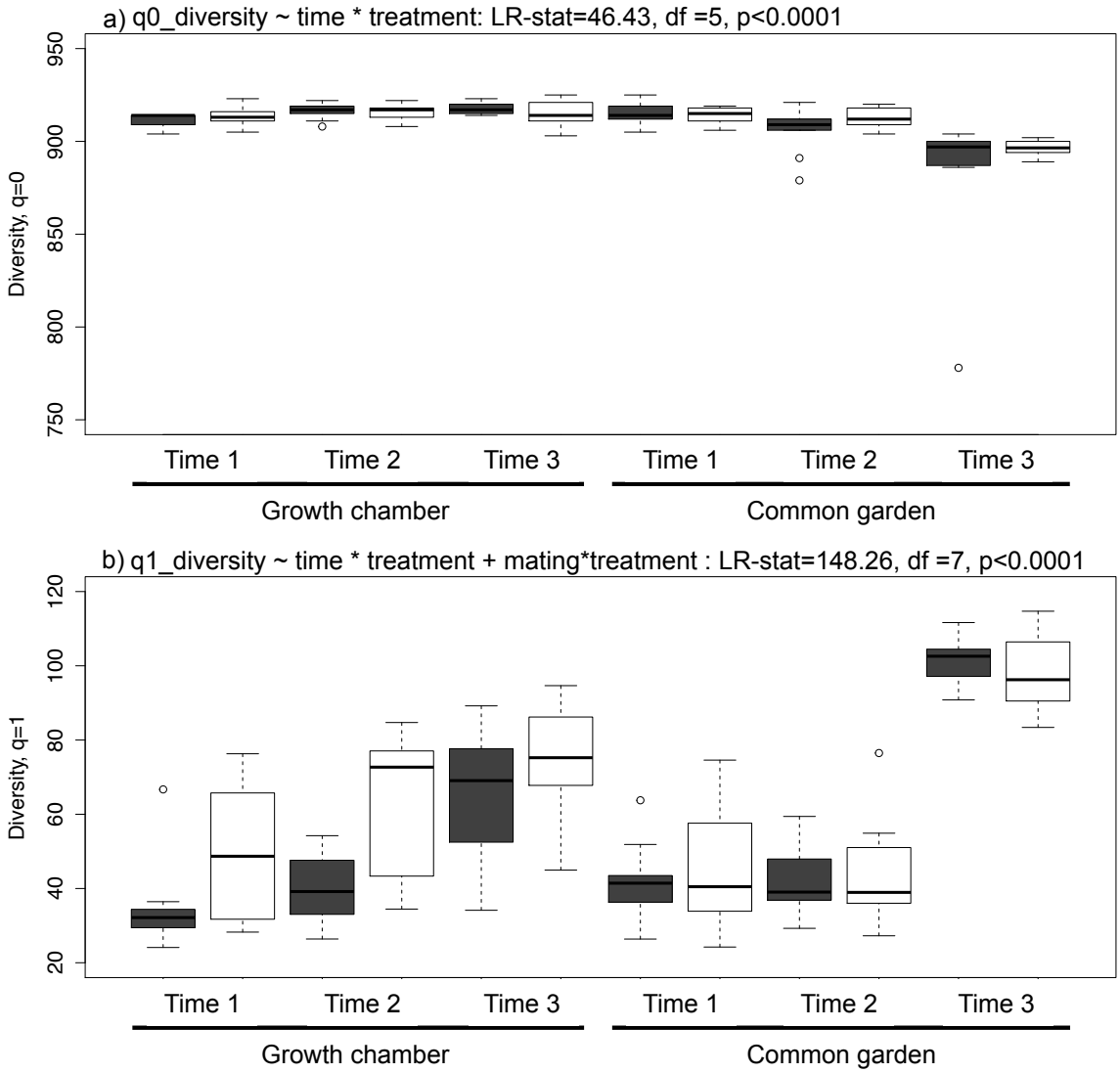


Figure 3: Metabolite diversity across the two growing environments for three different time points and individuals from outbred and inbred populations of *Arabidopsis lyrata*. Two measures of diversity are given based on (a) q=0 (analogous to number of metabolites observed) and (b) q=1 (reduced emphasis on metabolites at lower concentrations). Shaded boxes represent samples from outcrossing populations and open boxes those from inbreeding populations. The best model for explaining variation in diversity is given above each plot. The statistics represent the significant change in model likelihood when the full model was compared to the next simplest model.



Individual metabolite data reveals limited effects of inbreeding, but clear changes in metabolites between growing environments over time

We found that 59.3% of the 936 metabolites showed significant (with a FDR of 5%) time*treatment interactions (Table 4a). Interactions involving mating system were only significant with 5% FDR for less than 0.5% metabolites (Table 4a). When the two

Table 4: Summary of results testing the effect of interactions between time, growing environment and mating system for 936 individual metabolites.

a)

Interaction tested [a]	N p<0.05 [b]	N p<0.01 [b]	N (FDR 5%)[b]
3-way	125	48	7
mating system*time	76	22	4
mating system *environment	79	15	0
time*environment	601	496	555

[a] interactions between mating system (inbreeding or outcrossing), environment and time (time points 1 and 3 only)

[b] number of individual metabolites significant at different p-value thresholds, or with a false-discovery rate (Benjamini-Hochberg method) of 5%

b)

Growing environment	Interaction tested[a]	N p<0.05 [b]	N p<0.01 [b]	N (FDR 5%)[b]
Growth chamber	mating*time	85	27	0
Growth chamber	mating	77	20	0
Growth chamber	time	405	305	337
Common garden	mating*time	42	5	0
Common garden	mating	63	15	0
Common garden	time	586	496	554

[a] mating system by time interactions separately for each growth treatment, compared between time points 1 and 3

[b] number of individual metabolites significant at different p-value thresholds, or with a false-discovery rate (Benjamini-Hochberg method) of 5%

growing environments (growth chamber and outdoor common garden) were analysed separately, time-by-mating system interactions were not significant for any metabolites following multiple testing correction (Table 4b). Similarly, no metabolites showed significant effects of just mating system in either growing environment (Table 4b). By contrast, the effect of time alone was significant for 36.0% (growth chamber) and 59.2% (common garden) of the metabolites (Table 4b). Notably, 1.6x more metabolites showed

significant changes over time in the outdoor common garden compared to the growth chamber, which suggests that the outdoor environment was more physiologically challenging for the plant.

Of the 106 confidently identified compounds, 27 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 S3). Compounds that showed the strongest fold-changes included one annotated as the vitamin ascorbate (also annotated as D-Glucuronolactone), several members of the TCA cycle (S-malate, citrate and phosphoenolpyruvate) and several sugar phosphates associated with glycolysis and the pentose phosphate pathway (D-ribose 5-phosphate and D-glucose/D-Fructose 6-phosphate). The sugar sucrose was also 1.2-fold higher in the outdoor common garden samples. By contrast, 12 of 18 compounds that showed the greatest decrease in the common garden samples were amino acids or amino acid derivatives. Only three of the compounds showing > 1-fold change in the common garden samples also showed significant mating system effects at time point 3. For two of these compounds (dUMP and D-glucose/D-fructose-6-phospate), the effects of inbreeding were only apparent in the common garden sample set (Fig S8a,b). A significant negative effect of inbreeding in the common garden was only observed for the amino acid L-Asparagine (Fig S8c).

DISCUSSION

In this study, we found that highly inbred and genetically depauperate populations of *A. lyrata* sampled from multiple genetic lineages (Foxe et al., 2010; Buckley et al., 2016) show similar fitness and short-term physiological responses to a change in environment as highly outcrossing and genetically diverse populations. Specifically, inbreeding populations showed similar survival rates and reproductive output to outbred populations over a two-year period in a common garden environment outside their native range, although relationships between several fitness traits and latitude of origin did vary

between outcrossing and inbreeding populations. Instead, population-level effects, including latitude of origin, consistently explained more variation in fitness-related traits. Furthermore, we found that mating system had very little impact on the magnitude or plasticity of physiological responses to the novel environment, and only minor effects on metabolite diversity. Together, these results show that natural variation in inbreeding, and associated changes in genetic variation, do not negatively impact on short-term responses to changing environmental conditions.

Natural population inbreeding does not reduce fitness in a novel common garden environment

Survival over the first winter was high in the common garden, but was much lower over the second winter, likely due to milder winter conditions resulting in root degradation over the winter of 2013/2014. Such mild winter conditions are rarely encountered in their native range around the North American Great Lakes region, where sub-freezing temperatures and snow cover are expected overwinter in all of the populations sampled. Variation in survival was not significantly explained by population mating system in either year, although populations did significantly differ in survival over the second winter, suggesting that factors other than mating system may be driving over-winter tolerance. One hypothesis is that local adaptation to conditions in their native range might influence their rates of survival in this new environment. However, we found no evidence for an association between population latitude and survival in either year of this study. Three populations were sampled on the Bruce Peninsula on Georgian Bay (TSSA, TSS, TC) where the growing season is shorter than in the other populations sampled; previous surveys have found that plants typically flowered and seeds were produced approximately 1 month later in these northern populations than those growing on the shores of Lake Erie (RON, LPT, PTP) (personal observation). Although the Bruce Peninsula populations showed similar survival to one another, this was not true of the Lake Erie populations,

suggesting that source latitude (and geographic proximity) is not a major driver of overwinter survival. In a different common garden experiment within the native range of *A. l. lyrata,* outcrossing populations showed higher survival rates after the first year, but over two years there was no effect of mating system on survival (Oakley, Spoelhof, and Schemske, 2015). Together with our results, these data suggest that reduced genetic variation due to inbreeding is not a consistent driver of variation in survival in this species.

A shift to selfing (and associated inbreeding) might also be associated with altered selection on growth rates, flowering traits and reproductive output (e.g. Sicard and Lenhard, 2011; Tedder et al., 2015). However, based on data from one mid-season time point, we observed no effect of mating system on the time to first flowering or measures of relative reproductive investment. Despite significant population effects on flowering time, the number of fruits produced and mean fruit length, the combined measure of relative reproductive output (a proxy for seed production) did not significantly vary among populations. By contrast, effects of mating system have been observed on flower and seed production traits in *A. l. lyrata* in two recent common garden experiments, conducted both in the native (Oakley *et al.* 2015) and non-native range (Willi *et al.* 2013), although effects were often only observed in one year and often varied between study years, supporting our finding of an absence of consistent effects of inbreeding on fitness. Furthermore, population-level, but not mating system effects, have also been observed for other flowering traits (including flower size and corolla length) in *A. l. lyrata* sampled from the same geographic region (Carleial, van Kleunen, and Stift, 2017b). These population effects may represent local adaptation to their native population environments, or simply phenotypic variation stochastically fixed in different regions following postglacial colonisation of the Great Lakes region.

Supporting the hypothesis of local adaptation shaping among-population trait variation, rosette area at time of transplant to the common garden tended to increase with latitude, but only in the selfing populations, which may reflect faster growth rates of

117 individuals as an adaptation to shorter growing seasons. Furthermore, mean fruit length
118 (correlated with the number of seeds in a fruit) declined with increasing latitude, which
119 again may reflect an adaptation for more rapid reproduction under shorter growing
120 seasons. However, the number of fruits did not vary with population latitude of origin, and
121 this resulted in no significant variation in relative reproductive effort in this common
122 environment for populations from different latitudes. Interestingly, flowering time was
123 faster for outcrossing populations from higher latitudes, consistent with a faster transition
124 to flowering with the more contracted growing season. Yet, the high latitude selfing
125 population, TC, showed both a slower transition to flowering, and larger rosette size at
126 transplantation, which could either be a consequence of its ability to self-fertilise, reducing
127 its dependency on pollinators in a short growing season, or that it must reach a larger
128 rosette size before it transitions to flowering. Nevertheless, we included just one selfing
129 population at a higher latitude in our study, which was associated with a distinct rocky
130 alvar habitat rather than sand dune habitat, so the effect of inbreeding on local adaptation is
131 difficult to determine. Further sampling of high latitude inbreeding populations is therefore
132 necessary to test whether populations with differing mating systems may diverge in their
133 adaptive response to the broad environmental gradients represented by latitude.

134 The populations included in our study represented multiple genetic lineages,
135 predicted to have colonised the Great Lakes region through different postglacial dispersal
136 corridors (Hoebe, 2009; Foxe et al., 2010). However, our fitness data suggests that
137 genetically (and often geographically) clustered populations are not more similar to each
138 other in survival and flowering traits, than populations from distinct genetic clusters. For
139 example, RON, LPT, PTP form a genetic cluster with PIN and PCR, but do not show more
140 similarity in fitness-related traits than populations sampled from other genetic clusters (e.g.
141 TC, TSS, TSSA).

142 The absence of strongly negative inbreeding effects on fitness in our common
143 garden, even when plants were exposed to novel and stressful winter conditions is

consistent with other studies, which have found that fitness differences between planting sites, experimental treatments (e.g. exposure to herbivory) or genetic families are frequently larger than the effects of experimental inbreeding (Ivey, Carr, and Eubanks, 2004; O'Halloran and Carr, 2010; Murren and Dudash, 2012). Our study also supports the hypothesis that North American populations of *A. l. lyrata* show only weak negative effects of natural inbreeding in most traits studied (Stift et al., 2013; Oakley, Spoelhof, and Schemske, 2015; Carleial, van Kleunen, and Stift, 2017a). The inbreeding populations used in this study have persisted following postglacial expansion into the Great lakes region (Hoebe, 2009; Foxe et al., 2010), so selection could have removed those individuals with the greatest inbreeding load. Alternatively, growing plants in a novel environment to which all populations are maladapted could have consistently reduced inbreeding depression effects in our study (as predicted by a theoretical study: Ronce et al., 2009). By contrast, experimentally-induced selfing has negatively affected germination rates and vegetative biomass (Carleial, van Kleunen, and Stift, 2017a), as well as reduced survival and reproductive effort (Willi, 2013; Oakley, Spoelhof, and Schemske, 2015) relative to experimentally-outbred progeny in both outcrossing and selfing populations of *A. l. lyrata.* Together, these data suggest that natural variation in inbreeding, in the absence of strong inbreeding depression, does not necessarily result in reduced fitness in a novel environment.

Physiological responses are driven by time and growing environment, not history of inbreeding

Contrary to results from experimental-inbreeding studies (e.g. Kristensen et al., 2008; Campbell, Thaler, and Kessler, 2012), we found limited evidence that inbreeding altered physiological responses, as measured by shifts in the metabolome, to a novel common garden environment. We found no mating system effect on physiological plasticity through variation in the first two Principal Component (PC) axes, which together

171 explained more than 30% of variation in the metabolome, and only minor effects of
172 inbreeding on variation in PC3 and PC5, which together explained just 10.1% of variation
173 in the metabolome. Instead, the first two PCs (explaining 30% of variation in the
174 metabolome) were strongly influenced by interactions between growing environment and
175 time since transplantation. These results suggest that naturally inbred populations retain a
176 similar physiological plasticity under different environmental conditions as outbred
177 populations. Such a result contrasts with the effects of experimental inbreeding on
178 important biosynthetic pathways related to specific stressors, such as anti-herbivore
179 defense induction (Campbell, Thaler, and Kessler, 2012; Kariyat et al., 2012). One
180 explanation for the absence of mating system effects in our study is that the common
181 garden treatment (one month growing in late summer) was not stressful enough to detect
182 inbreeding effects on stress-related processes (Murren and Dudash, 2012; Schou,
183 Kristensen, and Loeschcke, 2015). This suggests that testing physiological responses to
184 known stressors in *A. lyrata*, for example herbivores or pathogens, might reveal
185 physiological costs to inbreeding. However, experimental evidence using *A. lyrata* from
186 these same populations suggested no consistent negative effect of inbreeding on resistance
187 to the pathogen *Albugo candida*, although the underlying physiological defense responses
188 were not measured (Hoebe et al., 2011). Furthermore, the induction of defenses by
189 generalist herbivores in *A. lyrata* was unaffected by population inbreeding status
190 (Joschinski, van Kleunen, and Stift, 2015). We found no significant effects of mating
191 system on variation in amounts of individual metabolites in either environment, with
192 significant mating system by time interactions only observed for a small number of
193 compounds. Rather, changes over time in the common garden, relative to the growth
194 chamber, dominated the response of individual metabolites. Overall, the similarity of
195 responses of individuals from inbreeding and outcrossing populations in our study suggests
196 that the reduced heterozygosity resulting from multiple generations of selfing has not

197 compromised their ability to physiologically respond to contrasting environmental
198 conditions.

199 The absence of effects of natural variation in mating system on plant physiology
200 are perhaps expected, as the plant metabolome shows high plasticity under different
201 growing environments (Brunetti et al., 2013). In our experiment, the plants grown in the
202 outdoor common garden were exposed to a range of potential abiotic and biotic stressors,
203 which are known to significantly alter the leaf metabolome (Sutter and Muller, 2011;
204 Escobar-Bravo, Klinkhamer, and Leiss, 2017), often in ways specific to different stressors
205 (Obata and Fernie, 2012). The observed changes in confidently-identified metabolites in
206 our experiment are consistent with plants in the common garden responding to increased
207 light intensity and levels of radiation, whereas plants in the growth chamber showed
208 metabolic signatures of enhanced growth rates. Specifically, the common garden samples
209 showed elevated levels of the vitamin ascorbate, a compound associated with UV-B
210 tolerance (Wulff-Zottele et al., 2010; Kusano et al., 2011), as well as elevated levels of
211 compounds linked to glycolysis (e.g. Glucose/Fructose -6-phosphate and Ribose-5-
212 phosphate), and the TCA cycle (e.g. citrate, (S)-malate and succinate), suggesting elevated
213 rates of photosynthesis (Wulff-Zottele et al., 2010). Conversely, the reduced levels of
214 many important metabolites in growth chamber samples could reflect higher growth rates
215 (e.g. Meyer et al., 2007), which is consistent with their higher rates of leaf production
216 under controlled growth chamber conditions. Elevated growth rates in the growth chamber
217 are also reflected in the elevated levels of amino acids in leaves in this growing
218 environment, which is consistent with the higher levels of protein synthesis needed during
219 growth (Hildebrandt et al., 2015). However, reduced amounts of amino acids in the
220 common garden could also reflect resource constraints such as nitrogen availability (Obata
221 and Fernie, 2012), although we used the same soil mix for both the common garden plot
222 and growth chamber trays without additional fertiliser, so this seems unlikely to play a role
223 in our experiment. Interestingly, statistical evidence for inbreeding effects on these highly

224 responsive compounds in the common garden was limited to the amino acid, L-
225 Asparagine. To understand the adaptive nature of these divergent responses to different
226 growing environments, additional controlled experiments would be necessary to identify
227 the key stressors driving observed physiological changes.
228
229 ***Minor inbreeding effects on metabolite diversity under benign but not stressful***
230 ***conditions***
231 Most metabolomic studies have used traditional multivariate approaches to
232 interpret changes in metabolite composition (e.g. Davey et al., 2008; Kunin et al., 2009;
233 Field and Lake, 2011). However, here we also estimated metabolite diversity using an
234 approach based on changing the emphasis on the relative abundance across "species" using
235 the parameter 'q' (Leinster and Cobbold, 2012). We found that inbreeding did not alter the
236 total number of metabolites observed (equivalent to species richness, q = 0) across growing
237 environments and time. However, when less emphasis was placed on low abundance
238 metabolites (q = 1), inbred populations showed elevated metabolic diversity in the benign
239 growth chamber environment relative to the common garden. This is consistent with
240 inbreeding, specifically increased genome-wide homozygosity or exposure of deleterious
241 alleles, having direct metabolic consequences (Pedersen, 1968; Cheptou and Donohue,
242 2011; Reed et al., 2012), and supports the importance of the environmental context of
243 inbreeding depression (Kristensen et al., 2008; Bijlsma and Loeschcke, 2012).
244 Interestingly, experimentally inbred progeny from two self-incompatible *A. l. petraea*
245 populations grown in a controlled environment also showed elevated expression of stress
246 and photosynthesis related genes relative to outbred progeny (Menzel et al., 2015).
247 Furthermore, inbred *Drosophila* lineages show changes to fundamental metabolic
248 processes under both benign conditions and temperature stress (Pederson *et al.* 2008). It is
249 therefore notable that mating system effects on metabolite diversity in our study were only
250 observed in the constant growth chamber environment, and not in the outdoor common

garden. However, the similarity of outcrossing and inbreeding populations in metabolite diversity in the common garden suggests that while inbreeding populations could suffer from the effects of reduced genome-wide heterozygosity, consistent with the observation of increased heterosis in these populations in other studies (Willi, 2013; Oakley, Spoelhof, and Schemske, 2015), this has limited effects on their short-term physiological capacity to respond to new environments. Instead, the metabolomic response to the environmental conditions experienced in our common garden overwhelms the relatively minor effects of genome-wide reduced heterozygosity in *A. lyrata.*

CONCLUSIONS

Our results show that populations with a long history of inbreeding and associated reductions in genetic variation are not compromised in their ability to survive, reproduce and physiologically respond to a novel environment, at least relative to closely-related outcrossing populations. Such a finding could help to explain the general success of selfing lineages to colonise and adapt to a broad range of environments. The use of metabolomics to understand plastic physiological responses to novel environments and stressors offers promise for asking more specific questions linked to the understanding of the different type of pathways activated under a range of stressful conditions, as well as the impacts of genome-wide patterns of diversity on levels of metabolite diversity and plasticity. Together, these results offer new insights into the importance of intraspecific patterns of genetic variation for capacity to tolerate changing environmental conditions.

ACKNOWLEDGEMENTS

We thank Glasgow Polyomics for generating and help analysing the metabolomics data, as well as the Scottish Centre for Ecology and the Natural Environment for providing space for the common garden and David Fettes for preparing the experimental site. We thank

Natalie Hutchison who measured the length of fruits collected from the common garden. The comments of several anonymous reviewers have helped to improve the manuscript.

AUTHOR CONTRIBUTIONS

JB and BKM designed the experiment. JB conducted the experiment, collected the data and performed the metabolite extractions. JB, CC, RD, KB and BKM analysed the metabolite data, and JB analysed the field data. JB and BKM wrote and revised the manuscript. Funding from a Natural Environment Research Council grant to BKM (NE/H021183/1)

DATA ACCESSIBILITY STATEMENT

We will make all data available on an online repository, such as Data Dryad, should the manuscript be accepted for publication.

SUPPORTING INFORMATION

Table S1: Location of populations used in this study, around the North American Great Lakes region, ordered by outcrossing rate (see Table S2).

Table S2: Details on the year of population sampling, population mating system variation, genetic diversity and heterozygosity and sample sizes for the experiments.

Table S3: List of 109 confidently identified compounds and their average log-fold changes between samples from the outdoor common garden and growth chamber at the initial pre-transplant time point and one month after transplant.

Figure S1: Layout of experimental common garden, illustrating how samples and populations were allocated to the plot.

Figure S2: Variation in temperature in the common garden field plot over the winter period (1st Dec – 1st March 2012 and 2013).

Figure S3: Summary plots of variation in reproductive investment among populations of *Arabidopsis lyrata* with respect to their mating system classification. Populations are ordered by outcrossing rates.

Figure S4: Regression plots illustrating the interaction between different fitness-related traits and latitude for the individuals from outcrossing and inbreeding populations.

Figure S5: Visual differences in *Arabidopsis lyrata* growing in the growth chamber and common garden one month after transplanting.

Figure S6: Principal component analysis of metabolite variation among sample populations at time points 1 and 3 for the growth chamber and outdoor common garden set of plants.

Figure S7: Plots representing plasticity in different principal components of metabolite variation in *Arabidopsis lyrata*.

Figure S8: Three compounds that show clear differences between the growth chamber and common garden at time point 3, as well as significant interactions between mating system and growing environment.

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