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

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Running title: Inbreeding and physiological plasticity

1 **ABSTRACT**

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

20

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

## 23 **BACKGROUND**

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

37 One explanation for this pattern is that neutral genetic variation may not always  
38 predict adaptive genetic variation and therefore the evolutionary potential of a population  
39 [9]. Supporting this, even highly endangered species sometimes show adaptation despite  
40 extremely low levels of genome-wide variation [reviewed in 1]. Purging of genetic load in  
41 highly inbred lineages can also reduce the impacts of inbreeding depression at the  
42 population level [4, 10]. However, few studies have directly tested the effects of the  
43 resulting low additive genetic variation on short-term plastic responses to new  
44 environments.

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

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

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

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

81 Strong inbreeding depression in growth and germination-related traits, as well as  
82 altered patterns of gene expression under stable environmental conditions, has been  
83 observed for experimentally-inbred European populations [28-30]. By contrast, both  
84 outcrossing and inbreeding populations from North America show more subtle fitness  
85 reductions in response to experimental inbreeding when grown in a stable environment  
86 [30] or outdoor common garden environments [31, 32], suggesting some purging of the  
87 genetic load. Even when challenged by herbivores, inbreeding depression in defence traits  
88 was low for populations of either mating system [33, 34]. Previous physiological studies in  
89 *A. l. petraea* have revealed variation in metabolite profiles and cold tolerance responses  
90 among and within populations from different geographic regions [35-37], although  
91 metabolomic divergence was mostly independent of population genetic structure [36]. Yet,  
92 these analyses were restricted to European outcrossing populations, so the effects of  
93 natural variation in inbreeding on physiology remain untested. North American *A. l. lyrata*  
94 is therefore a good model to assess the impacts of inbreeding-associated loss of genetic  
95 diversity on short-term plastic responses to environmental change, without the potentially  
96 overwhelming effects of strong differences in inbreeding depression.

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

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

109

## 110 **MATERIALS AND METHODS**

### 111 **Seed sampling and plant origins**

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

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

129

### 130 **Measuring growth, survival and reproduction in the common garden**

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

151

## 152 **Measuring metabolomic responses to growing environment, time and population** 153 **inbreeding history**

154 To determine whether population inbreeding history affected short-term  
155 physiological responses under contrasting environmental conditions, we compared  
156 metabolomic profiles over time for three outcrossing (PCR-O, PIN-O, and TSS-O) and  
157 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 17<sup>th</sup> 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

185 using LC-MS. Briefly, 10 $\mu$ L of each sample was introduced to a liquid chromatography  
186 system (UltiMate 3000 RSLC, Thermo, UK) and separated on a 4.6 mm x 150 mm ZIC-  
187 pHILIC analytical column with a 2 mm x 20 mm guard column. The eluents were A: water  
188 with 20mM ammonium carbonate and B: acetonitrile. The gradient ran from 20% A, 80%  
189 B to 80% A, 20% B in 15min with a wash at 95% A for 3min followed by equilibration at  
190 20% A for 8min. Metabolites were detected using an Orbitrap Exactive (ThermoFisher,  
191 UK) instrument in positive/negative switching mode at resolution 50,000 with a m/z scan  
192 range of 70-1400. In total, 108 samples, plus a sample of pooled individual extractions for  
193 quality control, were run in a randomised order interspersed with twelve blank extraction  
194 buffer samples. No extraction internal standards were used, because our analysis focused  
195 on relative quantitation among treatments rather than absolute quantitation. We follow  
196 additional published guidelines to avoid detector sensitivity differences and drift over time  
197 among sample batches [38]. Data were annotated using a bespoke bioinformatics pipeline  
198 (mzMatch, IDEOM and PiMP) developed at Glasgow Polyomics [39-41], which resulted  
199 in a final dataset of 936 metabolites, of which 106 metabolites were confidently identified  
200 through comparison to a panel of standards (supporting methods). Raw peak heights for  
201 each putative compound in each sample were corrected by subtracting the average of the  
202 twelve blank readings for that compound; these corrected peak heights were used for  
203 subsequent analyses.

204

## 205 **Statistical analyses**

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

212 broad environmental gradients varied with mating system, we also tested for an interaction  
213 between inbreeding history and latitude. History of inbreeding was modelled using either  
214 mating system class (“inbreeding” or “outcrossing”, a categorical predictor) or observed  
215 heterozygosity ( $H_o$ , a continuous predictor).  $H_o$  consistently separates mating system  
216 groups across study years, whereas estimates of outcrossing rates ( $t_m$ ) and the proportion of  
217 self-compatible plants can vary across years (Mable, unpublished data). As these predictors  
218 are correlated, we first fitted two full models using either mating system class or  $H_o$ : (i)  
219 response ~ mating system + genetic cluster + latitude + latitude:mating system +  
220 (1|population) + (1|block); (ii) response ~  $H_o$  + genetic cluster + latitude + latitude: $H_o$  +  
221 (1|population) + (1|block)

222 We selected the model with the lowest AIC and then performed backwards model  
223 selection, removing non-significant factors and comparing nested models using likelihood  
224 ratio tests. We assumed a Gaussian error distribution for rosette area and binomial error for  
225 the three binary response variables of flowering status and survivorship (in 2013 and  
226 2014). Gaussian models were fitted using maximum likelihood, and we assessed Gaussian  
227 model fit by examining plots of residuals against fitted values and quantile-quantile plots.

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

234 For the metabolomics data, we first conducted Principal Components Analysis  
235 using the R function *prcomp* (with variables scaled to have unit variance) to visualise  
236 changes in the metabolome with respect to experimental growing condition, time point and  
237 population inbreeding history. As peak height data for some metabolites was non-normally  
238 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:  $\text{diversity} \sim \text{time} * \text{environment} * \text{mating system class} + (1 | \text{population})$ .  
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 log<sub>2</sub>-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

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

268

## 269 **RESULTS**

### 270 *Natural population inbreeding does not reduce survival or alter phenology in a novel* 271 *common garden environment*

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

282 Of the 310 transplanted individuals, 251 (79.0%) survived the first winter, with no  
283 significant effect of either inbreeding history, latitude or rosette size at the time of  
284 transplant on survival (Fig 2b; Table 1). Genetic structure significantly affected first year  
285 survival, with individuals from genetic cluster C (PCR, PIN, RON and PTP) and E (KTT)  
286 showing higher rates of survival than other clusters (Table 1; Table S3). Only 34  
287 individuals (11.0%) survived to spring 2014, and again no effect of inbreeding history was  
288 observed (Table 1). Over the second winter inbreeding populations showed both the  
289 highest (PTP-I) and lowest (LPT-I, TC-I, KTT-I) rates of survival, driving a significant  
290 interaction of latitude with inbreeding history (Fig 2c) and a significant effect of genetic  
291 cluster (Table 1; Table S3). However, this effect of genetic cluster disappeared when only  
292 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 10<sup>th</sup> 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

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

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

329 The diversity of metabolites changed significantly over time in the outdoor  
330 common garden environment, but mostly independent of population inbreeding history.  
331 Specifically, metabolite richness showed a significant time\*treatment interaction ( $P$   
332  $<0.0001$ ; Fig S6a), with fewer metabolites detected at time point 3 in the outdoor common  
333 garden than the growth chamber. By contrast, abundance-corrected diversity showed a  
334 significant time\*treatment and mating system\*treatment interaction (combined model  
335 significance:  $P < 0.0001$ ). This was driven by a greater number of abundant compounds at  
336 time point 3 in the outdoor common garden, and a tendency for inbred individuals to show  
337 an elevated number of abundant compounds relative to outcrossed populations at all time  
338 points in the growth chamber, but not in the common garden (Fig S6b).

339 In both growing environments, time-by-inbreeding history interactions, or effects  
340 of inbreeding history alone, were not significant for any metabolites following multiple  
341 testing correction (Table 2). By contrast, time since transplant had significant effects on  
342 36.0% of metabolites in the growth chamber and notably 1.6x more (59.2%) metabolites  
343 changed over time in the outdoor common garden.

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

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

353

## 354 **DISCUSSION**

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

365 Rates of survival in the common garden were not significantly explained by  
366 population inbreeding history in either study year, even when survival was much lower  
367 over the second winter compared to the first. Such low survival may reflect root  
368 degradation under the relatively mild winter conditions observed in 2013 (Fig S7);  
369 conditions that are rarely encountered in the native range of *A. l. lyrata* around the North  
370 American Great Lakes. We found only a weak effect of genetic structure on first year  
371 survival, but second year survival was explained by genetic structure and an interaction  
372 between inbreeding history and latitude (a proxy for climatic conditions). Specifically,  
373 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

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

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

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

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

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

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

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

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

467

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478

#### 479 **AUTHOR CONTRIBUTIONS**

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

483

#### 484 **DATA ACCESSIBILITY STATEMENT**

485 Data available from the Dryad Digital Repository:

486 <https://doi.org/10.5061/dryad.w0vt4b8m8>. Raw metabolomics datafiles have been  
487 deposited to the EMBL-EBI MetaboLights database (DOI: 10.1093/nar/gks1004.

488 PubMed PMID: 23109552) with the identifier MTBLS883, and can be accessed at:

489 <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 <sup>a</sup> | Rosette area <sup>c</sup>   | Survival (2013) <sup>c</sup> | Proportion plants flowering <sup>c</sup> | Survival (2014) <sup>c</sup> |
|----------------------------------|-----------------------------|------------------------------|--|------------------------------|
| Inbreeding*Latitude <sup>b</sup> | <b>LR= 12.5, P= 0.0004</b>  | LR= 0.474, P= 0.491          | <b>LR= 17.9, P&lt;0.0001</b>             | <b>LR= 14.9, P= 0.0001</b>   |
| Genetic structure                | <b>LR = 20.0, P= 0.0005</b> | <b>LR= 10.0, P= 0.040</b>    | <b>LR= 27.7, P&lt;0.0001</b>             | <b>LR= 18.9, P= 0.0008</b>   |
| Inbreeding <sup>b</sup>          | 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                     | <i>Not included</i>         | LR= 0.033, P= 0.857          | LR= 3.16, P= 0.075                       | <i>Not included</i>          |

<sup>a</sup> Explanatory factors included in the full model: Genetic structure + Latitude + Inbreeding + Inbreeding:Latitude + Rosette area

<sup>b</sup> 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.

<sup>c</sup> 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.

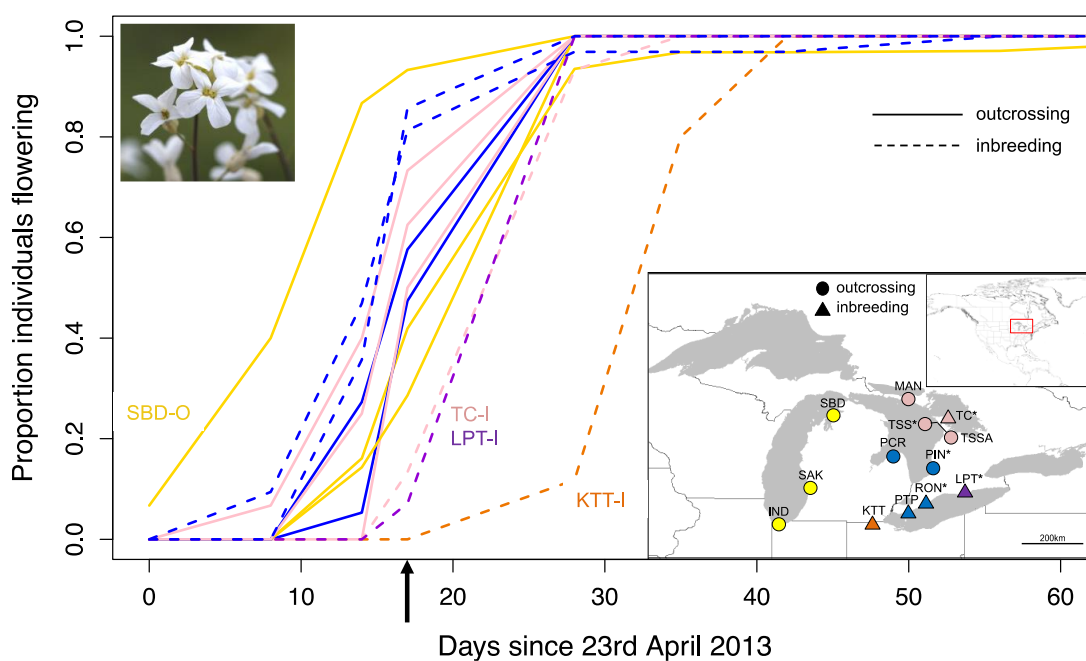
**Table 2:** The number of metabolites showing significant interactions between time (points 1 vs 3) and inbreeding history in each growing environment.

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

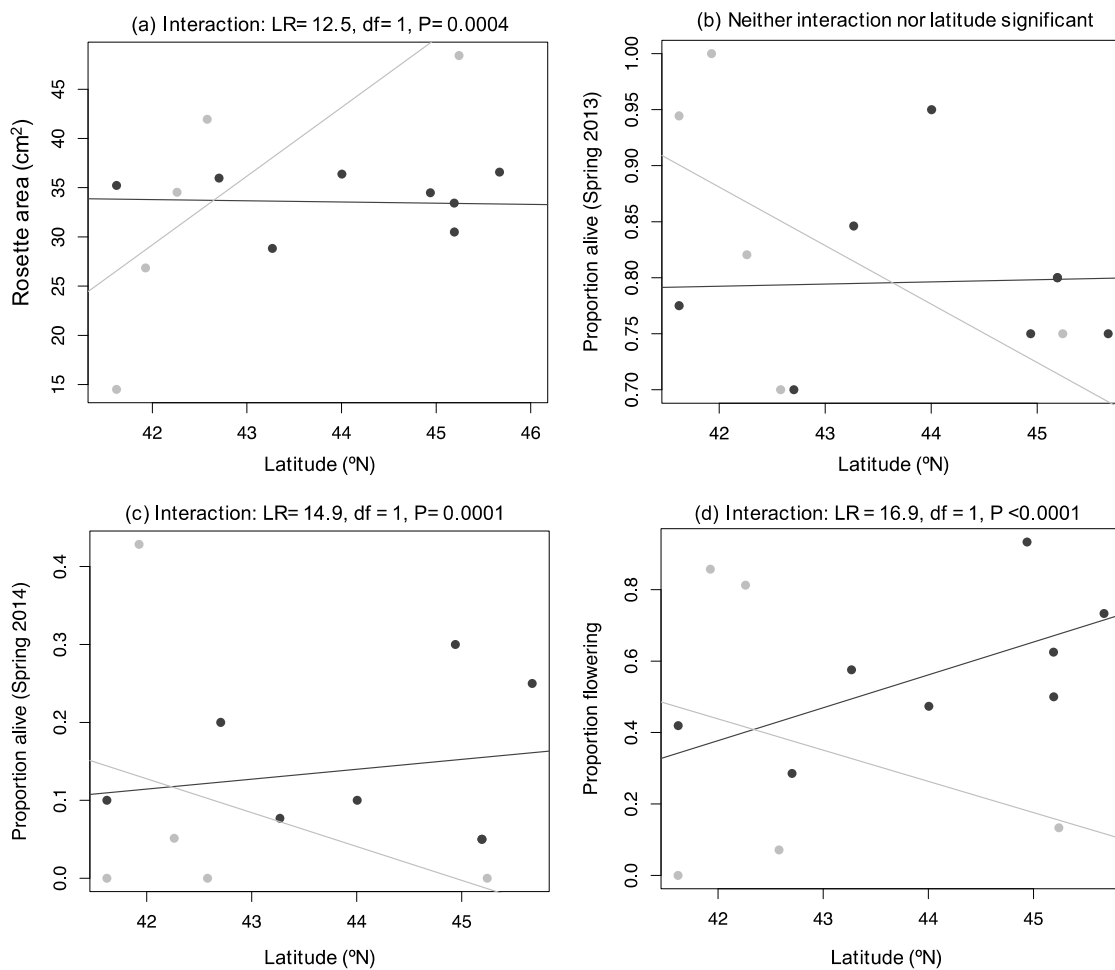
<sup>a</sup> number of 936 metabolites significant at P < 0.05, or with a false-discovery rate of 5%

## 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<sup>th</sup> 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.

