

1 Life-history trade-offs and the genetic
2 basis of fitness in *Arabidopsis thaliana*

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1 Abstract

Local adaptation may entail trade-offs in allocation to survival and reproduction, but also an increase in the ability to acquire resources in the local environment. We examined the relative contribution of trade-offs and increased resource status to adaptive evolution in a recombinant-inbred-line population of *Arabidopsis thaliana* planted at the native sites of the parental ecotypes in Italy and Sweden in two years. Local ecotypes produced more seeds per fruit than did non-local ecotypes, reflected in stronger adaptive differentiation than was previously shown based on survival and fruit number only. Genetic correlations between survival and overall fecundity, and between number of fruits and number of seeds per fruit, were positive, and there was little evidence of a trade-off between seed size and number. Quantitative trait loci for these traits tended to map to the same regions of the genome, and showed positive pleiotropic effects. The results indicate that adaptive differentiation between the two focal populations largely reflects the evolution of increased ability to acquire resources (“condition”) in the local environment, rather than shifts in the relative allocation to different life-history traits.

2 Introduction

Adaptation to the local environment is reflected in an increase in mean population fitness in response to local selection pressures (Williams 1966a; Kawecki & Ebert 2004). This may be associated with a shift in relative allocation to optimise the balance between components of fitness, such as between reproduction and future survival, or offspring size and number (Williams 1966a; b; Smith & Fretwell 1974; Schluter *et al.* 1991). However, it may also be associated with an increased ability to acquire resources in the new environment through changes in physiology, phenology, and/or morphology. This causes variation in overall resource status (“condition”) that may overwhelm the effects of variation in relative allocation to different functions (Van Noordwijk & Jong 1986; Schluter *et al.* 1991). The two processes have distinct implications for local adaptation. Trade-offs place a constraint on adaptive evolution, and will be reflected as negative genetic correlations between components of fitness. By contrast, an increased ability to acquire resources in the new environment should allow for increased allocation to multiple components of fitness, and as long as there is variation in condition, positive genetic correlations between fitness components. A full understanding of local adaptation and life-history evolution therefore requires insight into the genetic basis of overall fitness, but also of different components of fitness and their correlations.

If fitness components are correlated, this raises two questions about the ge-

55 netic architecture of fitness and its components. First, are fitness components
56 associated with distinct sets of loci, or with alleles at the same loci that
57 show pleiotropic effects on multiple components? Second, if loci do show
58 pleiotropy, is the sign of the genetic correlation reflected in the direction of
59 allelic effects on each trait at individual loci? If there are genetic trade-
60 offs among fitness components we expect to observe antagonistic pleiotropic
61 effects of individual quantitative trait loci (QTL), whereby an allele is asso-
62 ciated with an increase in one component of fitness, but a decrease in one
63 or more other components (Hazel 1943; Falconer & Mackay 1996). On the
64 other hand, if variation in resource acquisition is large we expect positive
65 pleiotropy, whereby alleles at QTL affecting resource status are associated
66 with changes in two or more fitness components in the same direction, caus-
67 ing phenotypes to be positively correlated (Houle 1991). In reality, both
68 processes are likely to be acting, and it is the relative strength of trade-offs
69 and variation in condition that will determine whether negative or positive
70 genetic correlations are observed.

71 In plants, three trade-off relationships are likely to be especially relevant for
72 overall fitness. First, plant survival is typically positively correlated with
73 plant size, and growth depends on meristems, which can develop into either
74 vegetative or reproductive tissue. Since these outcomes are mutually ex-
75 clusive, a trade-off between reproduction and growth (and hence survival) is
76 expected (Geber 1990). Furthermore, resources allocated to reproduction are
77 not available for investment in defence against parasites and predators, nor

78 abiotic stressors such as cold (Bazzaz *et al.* 1987), which may further reduce
79 future survival. Thus, we expect a negative correlation between fecundity
80 and survival.

81 Second, total seed production is a function of both the number of fruits
82 produced and the number of seeds per fruit, and there may be a trade-off
83 between these two components of fecundity. For practical reasons, studies
84 of local adaptation in plants typically focus on either fruit production or
85 estimates of total seed production as a measure of fecundity (e.g. Latta 2009;
86 Hall *et al.* 2010; Fournier-Level *et al.* 2011; Hancock *et al.* 2011; Ågren *et al.*
87 2013). To quantify components of fecundity, it is necessary to estimate both
88 number of fruits and number of seeds per fruit, and substantial additional
89 effort is required to collect and process data on two components compared
90 to just one (e.g. Maddox & Antonovics 1983; Verhoeven *et al.* 2004; Hall &
91 Willis 2006; Leinonen *et al.* 2011; Ågren & Schemske 2012). If investment in
92 fruit production is negatively correlated with investment in seed production
93 per fruit, relying only on estimates of one of these components of fecundity
94 will overestimate variation in total fecundity. If investment in seed and fruit
95 production are positively correlated, the opposite would be true.

96 Third, theory predicts a trade-off between investment in individual offspring
97 and the total number of offspring (Lack 1954; Smith & Fretwell 1974). In
98 plants this would be expressed as a negative correlation between seed size and
99 number (Harper *et al.* 1970; Leishman *et al.* 2000). Selection for larger seeds

100 may thus constrain the evolution of increased fecundity. Negative genetic cor-
101 relations between seed size and number have been documented across species
102 (Šerá & Šerý 2004) and within crop species (Sadras 2007). Meanwhile studies
103 within natural plant populations have found positive, negative, and negligible
104 genetic correlations between seed size and number (Silvertown 1989; Venable
105 1992). The extent to which variation in seed size places a constraint on
106 fecundity may thus vary among species.

107 In this study, we investigate the contribution of individual components of
108 fitness to estimates of local adaptation, and the genetic basis of correlations
109 among components of fitness. We use a population of recombinant inbred
110 lines (RILs) derived from a cross between two locally-adapted populations of
111 *Arabidopsis thaliana* from close to the southern (Italy) and northern (Swe-
112 den) margins of the native range in Europe. Reciprocal transplants have
113 shown that the two source populations display strong adaptive differenti-
114 ation expressed through higher survival and fruit production of the local
115 ecotype (Ågren & Schemske 2012; Ågren *et al.* 2013), and there is some
116 evidence that the local ecotype also produces more seeds per fruit compared
117 to the non-local ecotype (Ågren & Schemske 2012). QTL mapping in the
118 RIL population identified a total of 15 QTL affecting an estimate of overall
119 fitness (number of fruits per seedling planted) at the sites of the two source
120 populations (Ågren *et al.* 2013). However, this estimate of overall fitness did
121 not include possible variation in seed production per fruit, and it is therefore
122 not clear how inclusion of this fitness component would affect estimates of

123 selection against the non-local ecotype, correlations between fecundity and
124 survival, or the genetic basis of fecundity and overall fitness.

125 Here, we quantify seed output per fruit and mean seed size of the parental
126 ecotypes and of >300 RILs planted at the sites of the source populations
127 in two years. We combine these data with previously published data on
128 survival and fruit production to ask: (1) Does the local ecotype produce
129 more seeds per fruit than does the non-local ecotype, which would result in
130 an even larger estimate of selection against the non-local ecotype than an
131 estimate previously reported based on survival and fruit production only?
132 (2) Are there genetic correlations between fecundity and survival, between
133 components of fecundity (number of fruits and number of seeds per fruit),
134 and between offspring number and size, and are these negative or positive?
135 (3) Are there pleiotropic effects of QTL for number of seeds per fruit and
136 seed mass on other components of fitness, and are these effects positive or
137 negative?

138 **3 Materials and methods**

139 **3.1 Data collection**

140 We estimated seed traits for recombinant inbred lines (RIL) and parental
141 accessions in reciprocal transplant experiments conducted at the native sites
142 of the source populations in two years (2010-2011 and 2011-2012). These

143 experiments have previously been described by Ågren & Schemske Ågren
144 & Schemske (2012) and Ågren *et al.* Ågren *et al.* (2013), who quantified
145 survival to reproduction, number of fruits per reproductive plant, and number
146 of fruits per seedling planted in the parents and 398 RIL. We expanded these
147 data by quantifying number of seeds per fruit and mean seed mass. In each
148 site \times year combination we sampled a single mature fruit from between 1917
149 and 2359 RIL plants and between 79 and 189 parental plants. For each
150 fruit, we counted the number of seeds and determined total seed mass to the
151 nearest 0.01 mg on an AT261 balance (Mettler Toledo, Columbus, United
152 States). We calculated mean seed mass as the mass of all seeds in a fruit,
153 divided by the total number of seeds. We estimated genotypic values for
154 each line in each site \times year combination as the mean across all individuals
155 of the same RIL or parental ecotype. After excluding lines with seed data for
156 fewer than three replicate individuals per line, we had estimates of genotypic
157 values for number of seeds per fruit in 389, 375, 380 and 340 RILs for Italy
158 in 2010, Italy in 2011, Sweden in 2010 and Sweden in 2011 respectively. We
159 likewise had corresponding data on seed mass for 387, 368, 378 and 338 RILs
160 in the same experiments respectively.

161 We combined data on number of seeds per fruit with previously published
162 data to obtain estimates of overall fecundity and overall fitness that include
163 information on seed number. In previous analyses of data from these experi-
164 ments, fecundity was defined as number of fruits per reproductive plant, and
165 overall fitness as number of fruits per seedling planted (Ågren & Schemske

2012; Ågren *et al.* 2013, 2017). Here, we estimated fecundity of reproductive plants by multiplying number of fruits by line-mean number of seeds per fruit. We chose to estimate fecundity this way because we did not have data on number of seeds per fruit for all individuals for which data on number of fruits were available. Moreover, it was impractical to sample more than one fruit per plant, precluding any estimate of within-plant variation in number of seeds per fruit. We quantified total fitness as number of seeds per seedling planted (zero for plants that did not survive to reproduce).

We estimated broad-sense heritability (H^2) as the proportion of total phenotypic variation among individuals that is explained by RIL genotype in each site-year combination. We used a mixed-effect ANOVA estimated using the package *lme4* (Bates *et al.* 2015), with block as a fixed effect and RIL genotype as a random effect. To assess the uncertainty around these estimates we performed parametric bootstrapping on model parameters using the function *bootMer*, and estimated 95% confidence intervals as the 25 and 975 quantiles of 1000 bootstrap draws. We carried out data handling and statistical analyses in RStudio 1.1.442 using R 3.6.1 (RStudio Team 2016; R Core Team 2018).

3.2 Fitness differences between parental lines

To assess the influence of different fitness components on estimates of adaptive differentiation, we quantified selection against the non-local ecotype by

187 calculating selection coefficients based on individual components of fitness,
 188 and on estimates of overall fitness. We calculated selection coefficients as
 189 $s = 1 - w_{min}/w_{max}$, where w_{min} is the fitness of the less fit ecotype and
 190 w_{max} that of the fitter ecotype. For cases where the non-local ecotype had
 191 higher fitness than the local ecotype, we multiplied the selection coefficient
 192 by -1. We calculated selection coefficients based on two estimates of over-
 193 all fitness: number of fruits per seedling planted and number of seeds per
 194 seedling planted, reflecting fitness estimates excluding and including infor-
 195 mation on number of seeds per fruit. We also calculated selection coefficients
 196 based on survival, and on two components of fecundity (number of fruits per
 197 reproductive plant and number of seeds per fruit).

198 We estimated confidence intervals for selection coefficients by non-parametric
 199 bootstrapping. We drew 1000 bootstrap re-samples by sampling data with
 200 replacement from within experimental blocks. We calculated selection coeffi-
 201 cients for each bootstrap sample and estimated 95% confidence intervals for
 202 each coefficient as the 25th and 975th quantiles of these values. We tested
 203 the null hypothesis that there is no adaptive differentiation using two-tailed
 204 p-values, calculated as twice the proportion of bootstrap values overlapping
 205 zero. It is more difficult to determine whether selection coefficients for the
 206 two measures of overall fitness, number of fruits per seedling planted and
 207 number of seeds per seedling planted, differ from one another because both
 208 estimates include common data on fruit number, and as such are not in-
 209 dependent. Rather than perform a formal test, we simply asked whether

210 the selection coefficient based on number of seeds per seedling planted was
211 beyond the 95% confidence interval of that based on number of fruits per
212 seedling planted. We compared differences between parental lines in mean
213 seed mass in each site \times year combination using Wilcoxon-signed-rank tests.

214 **3.3 Correlations between traits**

215 For each site \times year combination we assessed the strength of trade-offs among
216 fitness components by calculating Pearson's correlation coefficient, r_P , be-
217 tween RIL means. We estimated 95% confidence intervals around r_P by
218 drawing 1000 non-parametric bootstrap samples from vectors of RIL means,
219 recalculating correlation coefficients, and taking the 2.5% and 97.5% quan-
220 tiles of the distribution of correlation coefficients these across these resam-
221 ples. We examined relationships between three pairs of traits: (1) survival
222 and overall fecundity (number of seeds per reproductive plant), (2) the two
223 components of fecundity (number of fruits per reproductive plant and num-
224 ber of seeds per fruit), and (3) overall fecundity and offspring size (mean seed
225 mass).

226 **3.4 QTL mapping**

227 We mapped QTL for fitness and its components using the *R/qtl* package in
228 R (Broman *et al.* 2003; Broman & Šen 2009) using additional visualisation
229 tools from the package *arghqt* (Ellis 2018). Mapping results for survival,

230 number of fruits per reproductive plant and number of fruits per seedling
 231 planted were previously reported by Ågren *et al.* (2013) based on 398 RILs.
 232 However, the number and positions of QTL detected can be affected by on
 233 the number of RIL lines included because different subsets of lines contain
 234 different recombination events. To allow comparisons of QTL positions and
 235 examination of evidence of pleiotropic QTL effects, we therefore performed
 236 QTL mapping for seed mass, number of seeds per fruit, number of seeds
 237 per reproductive plant, survival and number of fruits per reproductive plant
 238 including only RILs with information regarding all five traits in each site
 239 \times year combination (Italy 2010, $n=387$; Italy 2011, $n=368$; Sweden 2010,
 240 $n=378$; Sweden 2011, $n=338$). To investigate the effect of including infor-
 241 mation on seed number in resolving the genetic basis of overall fitness, we
 242 compared QTL models for fruit number per seedling planted to models for
 243 seed number per seedling planted using only those RILs for which both fitness
 244 measures could be quantified in each site \times year combination (Italy 2010, $n=$
 245 389 ; Italy 2011, $n=375$; Sweden 2010, $n=380$; Sweden 2011, $n=340$).
 246 We performed mapping based on RIL mean data for each site \times year combi-
 247 nation separately. We used Haley–Knott regression using genotype probabili-
 248 ties of the genetic markers and pseudomarkers in gaps >2 cM (Haley & Knott
 249 1992). We performed a two-QTL scan of the genome with 10,000 permuta-
 250 tions of the phenotypic data to determine 5% LOD-significance thresholds
 251 for inclusion of QTL and epistatic interactions (Doerge & Churchill 1996;
 252 Broman & Šen 2009). Based on these thresholds, we used R/qtl’s automated

253 stepwise model selection procedure to identify significant additive QTL and
254 epistatic interactions. We applied a quantile normal transformation to phe-
255 notypes before model selection. Finally, we fitted a multiple-QTL model to
256 untransformed data to calculate, for each locus, the proportion of the total
257 phenotypic variance among RILs explained (PVE), and the effect size (in
258 units of the trait) of a substitution of the Swedish homozygous genotype.

259 To investigate whether QTL showed pleiotropic effects on multiple traits we
260 examined whether QTL for different traits, map (colocalise) to the same
261 region. There are currently no clear guidelines on how to delineate QTL
262 in linkage-mapping studies, so we rely on a set of heuristic rules used in
263 previous studies (Ågren *et al.* 2013, 2017; Dittmar *et al.* 2014; Oakley *et*
264 *al.* 2014; Postma & Ågren 2018). We note, however, that this does not
265 represent a formal test that QTL are identical, and that caution is needed
266 in their interpretation. We considered any pair of QTL to colocalise and
267 represent the same QTL if the maximum-likelihood estimate of their positions
268 were within 2 cM of each other, or if the 95% Bayesian credible intervals for
269 these estimates overlapped. Based on these criteria, we identified ‘pleiotropic’
270 regions associated with multiple traits if they contained colocalising QTL for
271 two or more traits, among those traits that were directly observed (number of
272 fruits per plant, seed number per fruit, survival and seed mass). We excluded
273 “poorly-defined” QTL whose credible intervals for QTL position were greater
274 than one quarter of the length of the shortest chromosome (15.2cM) from
275 assessments of colocalisation, because such QTL provide little information

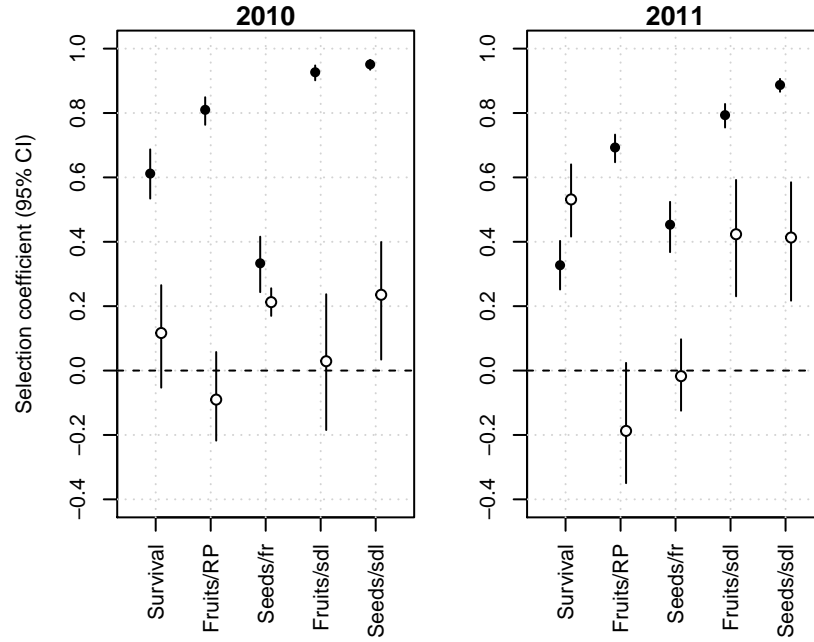


Figure 1: Selection coefficients based on survival to reproduction, number of fruits per reproductive plant (Fruits/RP), number of seeds per fruit (Seeds/fr), number of fruits per seedling planted (Fruits/sdl) and number of seeds per seedling planted (Seeds/sdl) at the Italian (closed symbols) and Swedish (open symbols) sites. Error bars show 95% bootstrap confidence intervals. Positive values indicate selection favouring the local ecotype, negative values selection favouring the non-local ecotype.

276 about position.

277 4 Results

278 4.1 Number of seeds per fruit influences estimates of 279 selection

280 The local ecotype had significantly higher overall fitness (expressed as number
281 of seeds per seedling planted) compared to the non-local ecotype at both sites
282 and in both years ($p \leq 0.001$ in all cases; figure 1). This advantage was more
283 than twice as strong in Italy than in Sweden (selection coefficient against the
284 foreign ecotype, s , of 0.95 vs. 0.24 in 2010; $s = 0.89$ vs. 0.41 in 2011 in Italy
285 and Sweden respectively).

286 At the Italian site, the local ecotype consistently outperformed the non-local
287 ecotype for all components of fitness (figure 1). The Italian ecotype had
288 significantly higher survival, corresponding to selection coefficients of 0.61 in
289 2010 and 0.33 in 2011 ($p < 0.001$), produced more fruits per reproductive
290 plant ($s = 0.81$ in 2010 and 0.69 in 2011; $p < 0.001$), and produced more
291 seeds per fruit ($s = 0.33$ in 2010 and 0.45 in 2011; $p < 0.001$) than did the
292 non-local Swedish ecotype. When information on number of seeds per fruit
293 was included in estimates of overall fitness, estimates of selection favouring
294 the local ecotype in Italy increased by $\Delta s = 0.024$ (2.6%) in 2010 and by
295 $\Delta s = 0.094$ (11.8%) in 2011 compared to when fecundity was based on fruit
296 production only (figure 1). In both cases, the selection coefficient based on
297 number of seeds per seedling planted was beyond the 95% confidence interval

298 of that based on number of fruits per seedling planted (figure 1)
 299 At the Swedish site, the contributions of survival and the two components of
 300 fecundity to the overall advantage of the local ecotype varied among years.
 301 In 2010, the local ecotype produced more seeds per fruit ($s = 0.21$; $p <$
 302 0.001), but no significant differences between the two ecotypes were detected
 303 in survival ($s = 0.12$; $p = 0.152$), or number of fruits per reproductive plant
 304 ($s = -0.09$; $p = 0.202$). In 2011 the local ecotype had higher survival ($s =$
 305 0.53 ; $p < 0.001$), whereas neither number of fruits per reproductive plant
 306 ($s = -0.19$; $p = 0.086$) nor number of seeds per fruit ($s = -0.02$; $p = 0.78$)
 307 differed between the two ecotypes. Including information on number of seeds
 308 per fruit substantially increased the estimated selection against the non-local
 309 ecotype in Sweden in 2010 ($\Delta s = 0.206$; 711%), but had a negligible influence
 310 in 2011 ($\Delta s = -0.01$; -2.4%; figure 1).

311 **4.2 Limited differentiation in seed mass**

312 Differences in seed mass between the two ecotypes depended on both site and
 313 year (supporting figure 1). In 2010, The Italian parent produced larger seeds
 314 than did the Swedish parent at both the Italian site (means \pm SE, Italian
 315 ecotype, 23.26 ± 0.48 μg ; Swedish ecotype, 19.77 ± 1.11 μg ; $W = 1449.5$, $p =$
 316 0.003) and Swedish site (Italian ecotype, 25.98 ± 0.30 μg ; Swedish ecotype,
 317 25.06 ± 0.21 μg ; $W = 5130$, $p = 0.023$). No significant difference in seed
 318 mass between the two parental ecotypes was recorded in the second year at

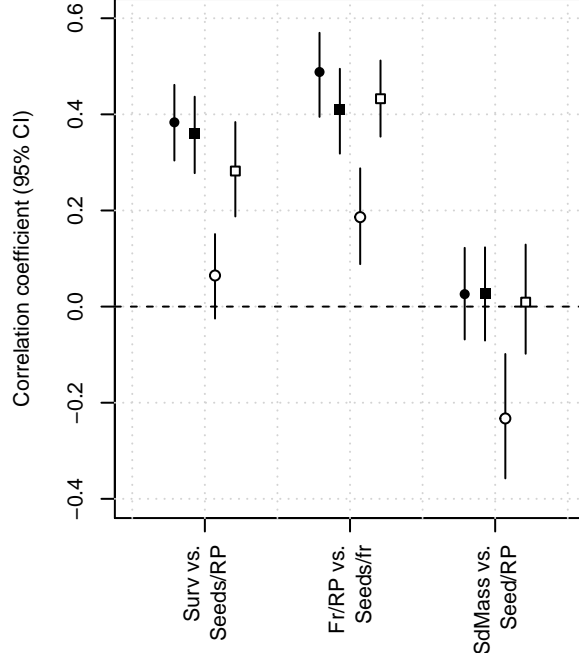


Figure 2: Genetic correlations between survival and number of seeds per reproductive plant (Surv vs. Seeds/RP), between number of fruits per reproductive plant and number of seeds per fruit (Fr/RP vs. Seeds/fr.) and between seed mass and number of seeds per reproductive plant (Mass vs. Seed.RP). Indicated are Pearson correlation coefficients in Italy (filled) and Sweden (open) in 2010 (circles) and 2011 (squares) with 95% confidence intervals derived from 1000 bootstrap resamples.

319 either the Italian site ($W = 753.5$, $p = 0.306$; Swedish site: $W = 720.5$, $p =$
320 0.519 ; supporting figure 1). Both ecotypes produced larger seeds at the site
321 in Sweden compared to that in Italy.

4.3 Positive correlations dominate among fitness components

We found positive correlations between number of fruits per reproductive plant and number of seeds per fruit, as well as between survival and number of seeds per reproductive plant in both years in Italy, and in Sweden in 2011 ($r_P \geq 0.28$; $p < 0.0001$; figure 2, supporting figure 2). In Sweden in 2010, the positive correlation between number of fruits per reproductive plant and number of seeds per fruit was weaker but still significant ($r_P = 0.19$, $p < 0.0001$), while survival and number of seeds per reproductive plant were not significantly correlated ($r_P = 0.06$, $p = 0.065$).

In Sweden in 2010, seed mass was negatively correlated with fecundity ($r_P = -0.23$, $p \leq 0.0001$), whereas no significant correlation was detected between seed mass and fecundity in Sweden in 2011, nor in Italy in either year ($r_P \leq 0.03$, $p \geq 0.597$; figure 2, supporting figure 2). A small number of lines showed unusually high seed mass and low fecundity (supporting figure 2). To examine whether these lines unduly inflate estimates of correlations between seed mass and fecundity, we repeated the analyses excluding lines with mean seed mass greater than 35 μ g. Neither test statistics nor p-values changed substantially when these lines were excluded (Sweden 2010: $r_P = -0.18$, $p = 0.001$; Sweden 2011: $r_P = 0.08$, $p = 0.129$; Italy 2010: $r_P = 0.08$, $p = 0.127$; Italy 2011: $r_P = 0.03$, $p = 0.554$).

343 4.4 QTL for seed number per fruit contribute to dif- 344 ferences in fecundity

345 Swedish alleles at QTL for number of seeds per fruit were associated with
346 reduced seed output per fruit in Italy, whereas the direction of effects varied in
347 Sweden. In Italy, we identified a total of six distinct QTL for number of seeds
348 per fruit, of which three were detected in both years (figure 3, supporting
349 table 1). For all QTL, the non-local Swedish allele was associated with fewer
350 seeds per fruit. In Sweden, we identified a total of six distinct QTL for
351 number of seeds per fruit, of which three were detected in both years. At
352 four of these loci, the local Swedish allele was associated with an increase in
353 number of seeds per fruit, whereas at the other two it was associated with
354 fewer seeds per fruit.

355 QTL for overall fecundity (number of seeds per reproductive plant) could
356 be explained by QTL for individual components of fecundity. In Italy we
357 identified a total of 12 distinct QTL for overall fecundity, of which four were
358 detected in both years (figure 3; supporting table 2). All of these loci colo-
359 calised with QTL for either number of fruits per reproductive plant or number
360 of seeds per fruit, and seven loci colocalised with QTL for both components
361 of fecundity (figure 3; supporting table 2-3). In Sweden, we detected a total
362 of seven distinct QTL for number of seeds per reproductive plant, one of
363 which was detected in both years. All of these loci colocalised with QTL for
364 either number of fruits per reproductive plant or number of seeds per fruit,

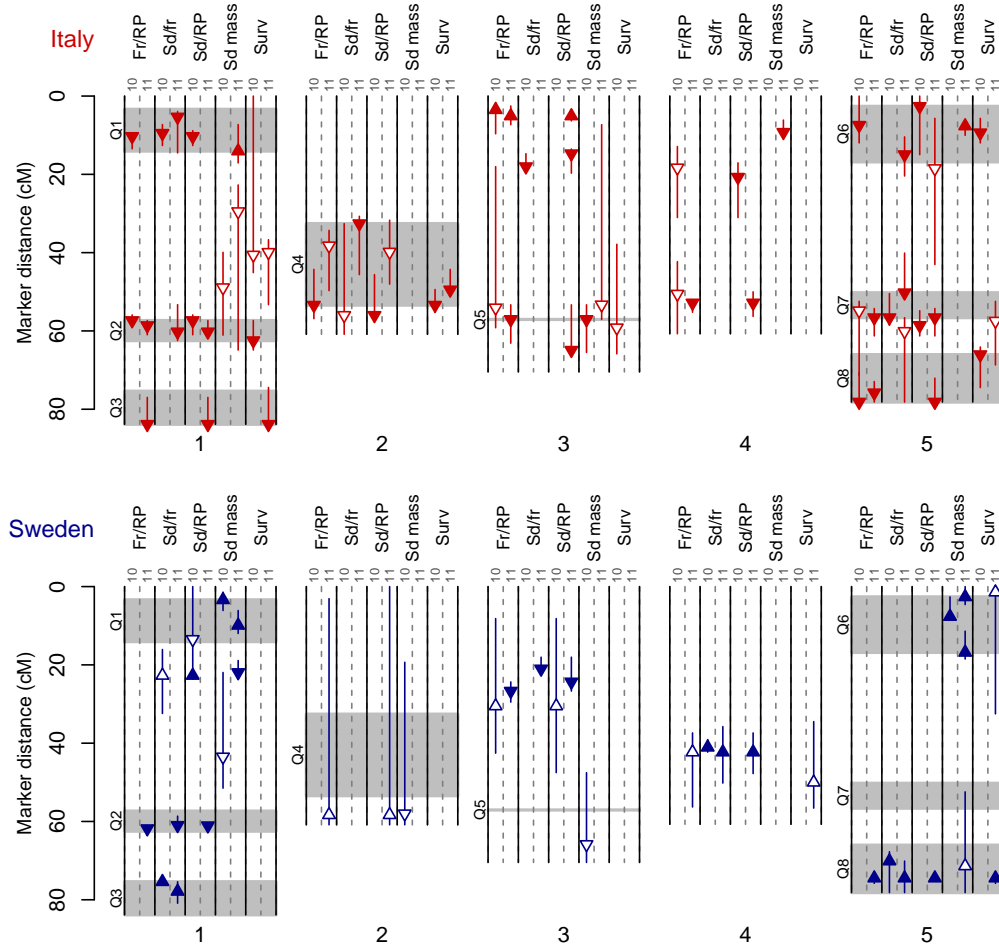


Figure 3: QTL for fecundity, seed mass, and survival. Lanes show QTL for number of fruits per reproductive plant (Fr/RP), number of seeds per fruit (Sd/fr), number of seeds per reproductive plant (Sd/RP), seed mass (Sd mass) and survival (Surv). Arrows indicate most-likely QTL position and the effect of the Swedish genotype (upward: increased phenotype; downward: decreased phenotype) in Italy (red, upper panels) and Sweden (blue, lower panels) in the 2010 (10) and 2011 (11) experiments respectively. Vertical bars show the 95% Bayesian credible intervals for QTL position. Open arrows show QTL with credible intervals wider than 15.2cM. Grey boxes indicate regions harbouring QTL with pleiotropic effects on two or more of those traits that were directly observed (number of fruits per plant, seed number per fruit, survival and seed mass) (Q1-Q8).

365 and four colocalised with QTL for both components of fecundity (figure 3;
366 supporting table 2-3).

367 Most QTL for number of seeds per seedling planted corresponded to a QTL
368 detected for fruit number per seedling planted (figure 4; supporting tables
369 4-5), but including information about seed output per fruit did result in
370 some changes in the map positions of QTL for overall fitness. In Sweden in
371 2010, when fitness differences between the parental genotypes were mainly
372 expressed through differences in number of seeds per fruit, the three QTL
373 detected for number of seeds per seedling planted were not observed when
374 fitness was quantified as fruit number per seedling planted (figure 4). More-
375 over, two QTL for overall fitness were detected in Italy in 2010 (chr. 3 18.0
376 cM; chr 5. 12.9) and one in Italy in 2011 (chr. 1 13.5 cM), which did not
377 overlap with QTL for number of fruits per seedling planted, but colocalised
378 with QTL for number of seeds per reproductive plant in the same year. The
379 position of one of the former QTL for overall fitness (chr 5. 12.0) was shifted
380 by 4.5 cM compared to the closely located QTL for number of fruits per
381 seedling planted (chr. 5, 7.5 cM; figure 4). Other changes included the ab-
382 sence of QTL for number of seeds per seedling planted at the positions of
383 four QTL detected for fruit number per seedling planted (Italy 2010, chr 3.
384 54.1 cM, chr 4. 50.0 cM; Italy 2011, chr 3. 2.2 cM; Sweden 2010, chr 3. 9.6
385 cM; figure 4). Including information about seed output per fruit in estimates
386 of overall fitness thus allowed some additional fitness QTL to be detected,
387 but also affected estimates of a few of the QTL observed when fitness was

388 quantified based on survival and fruit production only.

389 **4.5 QTL for seed size vary in the direction of effects**

390 In Italy, we identified two QTL for seed mass in 2010 and five in 2011 (figure
391 3, supporting table 6). At two of the QTL detected in 2011, the Swedish
392 allele was associated with an increase in seed mass, and at three with a
393 decrease. In Sweden, we detected five QTL for seed mass in each year, one
394 of which was detected in both years. The local Swedish alleles at three of
395 the five QTL detected in 2010, and at one of the five QTL detected in 2011,
396 were associated with a decrease in seed mass.

397 **4.6 QTL show positive pleiotropy**

398 QTL for components of fitness that could be resolved to within 15.2 cM
399 tended to map to one of eight distinct regions of the linkage map (Q1-Q8,
400 indicated in grey in figure 3; supporting tables 1-3, 6-7). Four regions in
401 Italy (Q2, Q3, Q4, Q6, Q8) and one region in Sweden (Q8) included QTL for
402 both survival and overall fecundity (number of seeds per reproductive plant).
403 Meanwhile, five regions in Italy (Q1, Q2, Q4, Q6, Q7) and two regions in
404 Sweden (Q2, Q8) included QTL for both number of fruits per reproductive
405 plant and seed number per fruit. This indicates that loci in these regions
406 have pleiotropic effects on multiple components of fitness, and/or that these

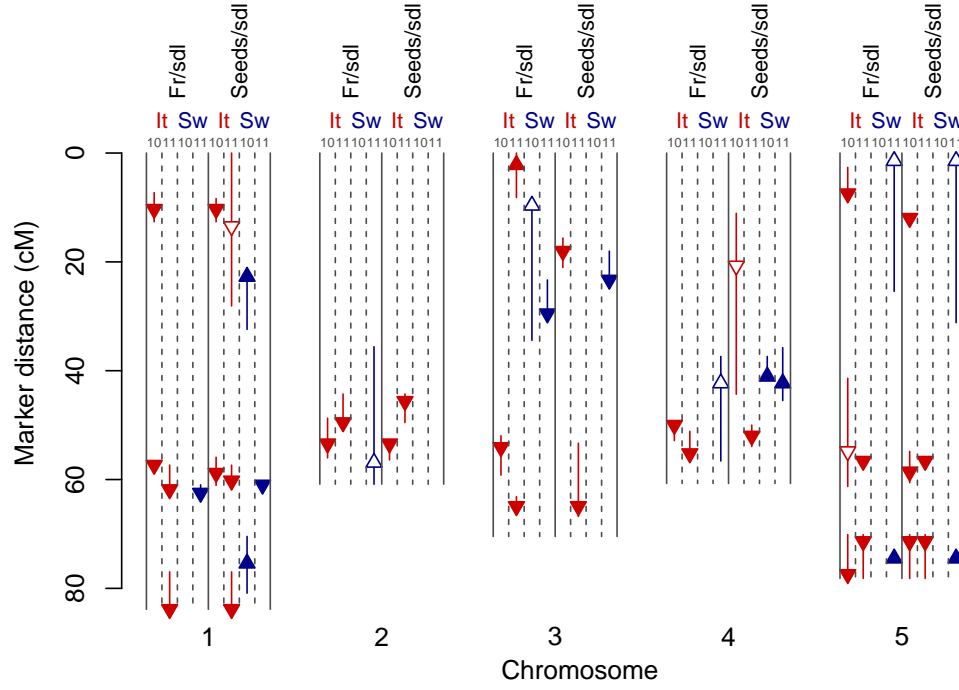


Figure 4: QTL for number of fruits per seedlings planted (Fr/sdl) and number of seeds per seedling planted (Seeds/sdl) in each of the four site \times year combination. Arrows indicate most-likely QTL position and the effect of the Swedish genotype (upward: increased phenotype; downward: decreased phenotype) in Italy (red) and Sweden (blue). Vertical bars show the 95% Bayesian credible intervals for QTL position. Open arrows show QTL with credible intervals wider than 15.2cM.

407 regions harbour multiple loci in tight linkage that affect individual fitness
408 components.

409 We next examined whether alleles at QTL within these regions were associ-
410 ated with positive or negative pleiotropy between survival and fecundity, and
411 between components of fecundity. In Italy, the non-local Swedish alleles at
412 QTL in regions showing pleiotropic effects on survival and number of seeds
413 per reproductive plant or on number of fruits per plant and number of seeds
414 per fruit were associated with a decrease in both fitness components (figure
415 3). In Sweden, the local allele at the well-defined QTL for survival (Q8) was
416 associated with increases in both survival and seed number per reproductive
417 plant. Local alleles at QTL detected for number of fruits per reproductive
418 plant and number of seeds per fruit were associated with an increase in both
419 fitness components (Q8), or with a decrease in both components (Q2). Thus,
420 when QTL for both survival and fecundity or both components of fecundity
421 map to the same places, the Swedish allele was associated with effects in the
422 same direction, even if the direction varied. This indicates that these QTL
423 have positive pleiotropic effects on components of fitness.

424 QTL for seed mass tended to map to regions harbouring QTL for fitness
425 QTL. Well-defined QTL for seed mass mapped to regions Q1, Q5 and Q6
426 in Italy, and to Q1, and Q6 in Sweden (figure 3). The only well-defined
427 seed-mass QTL that did not colocalise with QTL for at least one other trait
428 is the locus detected on chr. 4, 14.1 cM in Italy in 2011. At Q1 and Q6 in

429 Sweden we detected two distinct QTL for seed mass within the intervals of
430 the regions identified as pleiotropic.

431 Pleiotropic interactions between fecundity and seed mass were more variable
432 than among components of fitness. In Italy, the Swedish allele at one QTL
433 (Q5) was associated with a decrease in both seed mass and seed number per
434 reproductive plant, whereas at Q6 the Swedish allele was associated with an
435 increase in seed mass but a decrease in seed number per reproductive plant.
436 In Sweden, only the QTL on chr 1. at 21.9 cM colocalised with a QTL for
437 number of seeds per reproductive plant, and at this locus the local allele
438 was associated with a decrease in seed mass but an increase in seed number
439 per reproductive plant. QTL for seed mass thus showed both positive and
440 negative pleiotropic associations with fecundity (figure 3).

441 4.7 Epistasis

442 We observed five instances of pairs of loci that showed significant epistatic
443 interactions (supporting table 8, supporting figures 3-5). Firstly, in Sweden
444 in 2011 we found an interaction between QTL detected in region Q2 and
445 within 2 cM of the marker at 23 cM on chr. 3 for number of seeds per fruit,
446 number of seeds per reproductive plant and number of seeds per seedling
447 planted that explained 8.0%, 9.3% and 7.3% of the overall phenotypic vari-
448 ances respectively. This corresponds to the pair of epistatic loci detected for
449 number of fruits per seedling planted in the previous analysis by Ågren *et*

450 *al.* (2013). In addition, we detected an epistatic interaction in Italy in 2011
451 between QTL for number of seeds per fruit in regions Q1 and Q6 explaining
452 2.7% of the variance, and another interaction in Italy in 2010 between QTL
453 for number of seeds per seedling planted in region Q6 and the marker on chr.
454 3 at 18.0 cM explaining 0.58% of the phenotypic variance.

455 5 Discussion

456 The present study demonstrates that genetic differences influencing number
457 of seeds per fruit can make an important contribution to adaptive differen-
458 tiation and the genetic basis of fitness variation among natural populations
459 of *Arabidopsis thaliana*. In a reciprocal transplant between an Italian pop-
460 ulation located close to the southern margin of the European native range
461 and a Swedish population located close to the northern range margin, the
462 local ecotype produced more seeds per fruit than did the non-local ecotype
463 in three of four site \times year combinations (figure 1). Including information
464 about number of seeds per fruit thus increased the estimated magnitude of
465 the fitness advantage of the local ecotype compared to estimates based on
466 differences in fruit production and survival alone. Genetic correlations be-
467 tween fecundity and survival, and between components of fecundity (number
468 of fruits per reproductive plant and number of seeds per fruit) were generally
469 positive, whereas the correlation between fecundity and seed size was sig-
470 nificant (and negative) in only one of four site \times year combinations (figure

2). The genetic correlations were reflected in widespread pleiotropic effects of QTL for fecundity and survival, with allelic effects typically in the same directions (figure 3). Below we discuss the results in relation to processes affecting adaptive differentiation and pleiotropic interactions among traits.

5.1 Adaptive differentiation for seed number per fruit

The examination of variation in seed number per fruit provided several new insights into variation in fecundity and how this contributes to adaptive differentiation between these populations. In both years in Italy, the local ecotype produced more seeds per fruit than did the non-local ecotype, and had a greater overall fitness advantage when fitness was estimated including information about number of seeds per fruit (figure 1). However, because selection through number of seeds per fruit was not as strong as selection through number of fruits per reproductive plant and survival (figure 1), and number of seeds per fruit was strongly positively correlated with number of fruits per reproductive plant (figure 2), much of the adaptive differentiation expressed in Italy was captured by differences in number of fruits per seedling planted. In Sweden on the other hand, number of seeds per fruit was the only fitness component for which the parental ecotypes differed in 2010 (figure 1). This was reflected in a significant overall advantage to the local ecotype that year that was only detected when this component was included in the estimate of overall fitness. Despite differences between sites and between

492 years, the results show that fecundity variation in *A. thaliana* is due to
493 differences in both fruit production and number of seeds per fruit.

494 Including information on seed production into estimates of overall fitness
495 allowed us to detect additional QTL for fitness. In particular, we detected
496 three additional fitness QTL in Sweden in 2010, where only one was detected
497 when fitness was based on survival and fruit production only. All three QTL
498 colocalised with QTL for seed number per fruit (figures 3 and 4), and we
499 found significant selection against the non-local Italian genotype through
500 seed number per fruit but not through number of fruits per reproductive
501 plant in Sweden in 2010 (figure 1). This indicates that the effects of QTL
502 on number of seeds per fruit were responsible for the differences in overall
503 fitness associated with these loci.

504 Surprisingly, some QTL for fitness estimated as the number of fruits per
505 seedling planted were not detected when fitness was estimated as number of
506 seeds per seedling planted (figure 4). One explanation for this could be that
507 QTL have weakly negative pleiotropic effects on one or more combinations
508 of number of seeds per fruit, number of fruits per reproductive plant and
509 survival. However, in almost all cases where pleiotropy was observed the
510 effects were positive (figure 3), so this seems unlikely. Alternatively, our
511 estimates of number of seeds per fruit might be less precise than for number
512 of fruits per reproductive plant, which would inflate the residual variance
513 of our estimates of number of seeds per seedling planted. Although sample

514 sizes for number of seeds per fruit and seed mass were much smaller than for
515 survival and fruit production, this was not reflected in reduced heritability
516 of seed traits (supporting figure 6), so this explanation also appears unlikely.
517 A third explanation is that there are many loci affecting fitness with effects
518 close to the threshold of statistical significance, which would be consistent
519 with classical population-genetic theory (Fisher 1930). Such subtle effects
520 would be sensitive to the precise way in which fitness is defined, as well as to
521 fluctuations in environmental noise. Because linkage mapping is designed to
522 detect relatively few loci of large effect, this would cause some stochasticity in
523 the loci detected and their map positions indicated by the stepwise-regression
524 approach used in QTL mapping (Beavis 1998; Harrell 2001; Broman & Šen
525 2009). The apparent disappearance of fitness QTL when information on
526 seed number is included could thus reflect a highly polygenic nature of QTL
527 affecting fitness.

528 **5.2 Positive pleiotropic effects on multiple fitness com-** 529 **ponents**

530 Both genetic correlations in the RIL population and the QTL mapping
531 showed evidence of positive pleiotropic effects on different components of fit-
532 ness. Correlations between components of fecundity, and between fecundity
533 and survival were positive, except for Sweden in 2010 when no significant cor-
534 relation was observed between fecundity and survival (figure 2). Moreover,

535 QTL for these components of fitness tended to map to the same regions of the
536 genome, and allelic effects were in the same direction in all cases (figure 3).
537 Taken together, the overall positive genetic correlations among phenotypes
538 are reflected in positive pleiotropic effects of the underlying genetic loci.

539 The preponderance of positive genetic correlations and positive pleiotropy
540 indicates that variation in overall vigour or “condition”, where the fittest
541 genotypes have more resources overall to invest across fitness components,
542 predominates over variation in relative allocation to different components of
543 fitness in the RIL population (Van Noordwijk & Jong 1986; Houle 1991).
544 This suggests that adaptive differentiation between the two focal popula-
545 tions largely reflects the fixation of variants that allowed for increased ability
546 to acquire resources and grow under local environmental conditions, that
547 in turn positively affect several components of fitness. In a meta-analysis
548 of local adaptation across 74 studies of plants, animals, fungi and protists,
549 Hereford Hereford (2009) was not able to test explicitly for correlations be-
550 tween selection through components of fitness, but did demonstrate that the
551 advantage of local populations was greater when estimated based on overall
552 fitness than when estimated based on survival or fecundity only. Although
553 not a formal test, this pattern would be expected if selection acts to increase
554 overall condition, causing components of fitness to be positively correlated
555 within populations. These observations indicate that adaptation frequently
556 entails the evolution of increased condition in the local environment, and that
557 while local adaptation is reflected as trade-offs in performance across envi-

558 ronments, it may often also be associated with positive genetic correlations
559 among fitness components within a given environment in situations where
560 there is genetic variation for overall fitness..

561 Several traits may contribute to variation in resource status in *A. thaliana*.
562 The Swedish ecotype has higher freezing tolerance (Oakley *et al.* 2014) and a
563 greater ability to optimize photosynthesis at cold, but non-freezing tempera-
564 tures (Cohu *et al.* 2013; Adams *et al.* 2014; Oakley *et al.* 2017). This should
565 provide a fitness advantage at the Swedish site where plants are exposed to
566 cold conditions for an extended period, but may be associated with a fitness
567 cost in a milder climate (Oakley *et al.* 2014). QTL affecting these traits can
568 thus be expected to have pleiotropic effects on overall fitness and its compo-
569 nents. QTL for phenological variation may also play a key role in variation
570 in resource status. Previous work on the same populations has demonstrated
571 strong selection to tune the timing of germination at both sites and flowering
572 time in Italy to match the local climate (Akiyama & Ågren 2014; Postma &
573 Ågren 2016; Ågren *et al.* 2017). Differences in timing of life-history transi-
574 tions should contribute to differences in resource status by allowing locally
575 adapted ecotypes to grow and reproduce at the times best suited to local
576 conditions. For example, Akiyama & Ågren Akiyama & Ågren (2014) ex-
577 perimentally demonstrated that early germination allowed for faster autumn
578 growth and increased winter survival at the Swedish site. QTL affecting
579 both physiological and phenological traits can thus be expected to influence
580 resource status and thereby have pleiotropic effects on several components of

581 fitness.

582 **5.3 Pleiotropy and linkage**

583 Two caveats need to be borne in mind regarding our results on the pleiotropic
584 effects of QTL. Firstly, resolution in a mapping population derived from a
585 cross is limited by the number of recombination breakpoints, resulting in
586 linkage disequilibrium between nearby markers. As such, it is very difficult
587 to distinguish a single pleiotropic locus from two or more tightly-linked loci
588 affecting individual traits separately (Jiang & Zeng 1995). However, two
589 loci with little recombination will tend to be co-inherited, and hence will
590 segregate much like a single locus (Paaby & Rockman 2013). Therefore,
591 from an evolutionary perspective, it makes little difference whether a QTL
592 reflects a single pleiotropic locus or multiple strongly linked loci, because
593 both traits will co-evolve in a similar manner in both cases.

594 A second caveat is that even if a QTL genuinely is due to a single causative
595 variant, linkage will mean that there is uncertainty about its exact posi-
596 tion. As such, we lack a definitive rational basis to define when two QTL
597 should be defined as mapping to the same place in the genome. We have
598 therefore followed previous studies (Ågren *et al.* 2013, 2017; Dittmar *et al.*
599 2014; Oakley *et al.* 2014; Postma & Ågren 2018) in adopting a heuristic ap-
600 proach by defining QTL detected for different traits to colocalise, and hence
601 be pleiotropic, if the confidence intervals of QTL positions overlapped. This

602 approach is practical, but such a simplification necessarily means that infor-
 603 mation about the subtleties of genetic architectures are lost. For example,
 604 we were not able to assess the contribution of ‘poorly-defined’ QTL whose
 605 positions could not be resolved to within 15.2cM. Such QTL might reflect
 606 either a single causative locus of weak effect, or multiple loci spread across
 607 the chromosome (figure 3). At the same time, we detected two QTL for seed
 608 mass in Sweden whose credible intervals were distinct, but which both fell
 609 into the single pleiotropic region Q10 (figure 3). These examples highlight
 610 that the pleiotropic regions should be interpreted with caution, and not seen
 611 as rigid boundaries separating pleiotropic and non-pleiotropic loci.

612 **5.4 No evidence for genetic constraint through seed** 613 **mass**

614 The Italian ecotype tended to produce somewhat larger seeds than did the
 615 Swedish ecotype, and this difference was particularly marked at the Italian
 616 site (supporting figure 1). Moreover, both ecotypes produced larger seeds
 617 at the field site in Sweden compared to that in Italy. Previous work in
 618 the same populations has demonstrated strong selection favouring the local
 619 ecotype during seedling establishment (Postma & Ågren 2016). In general,
 620 both probability of successful seedling establishment (Krannitz *et al.* 1991)
 621 and early growth rate (El-Lithy *et al.* 2004) should be positively related to
 622 seed size. At the Italian site, the larger seeds of the Italian ecotype can thus

623 be expected to contribute to a higher fitness of its offspring relative to that
624 of the Swedish ecotype. However, the higher seedling establishment success
625 of the local ecotype documented at the Swedish site (Postma & Ågren 2016)
626 cannot be explained by differences in seed size, but is more likely related to
627 differences in seed dormancy and the timing of germination (cf. Postma &
628 Ågren 2018). Further work is required to determine the relative importance
629 of seed size and other seed traits for differential seedling establishment in the
630 two environments.

631 Although overlap between seed mass QTL and fecundity QTL indicated
632 pleiotropic effects of several genomic regions (figure 3), genetic correlations
633 between seed size and fecundity in the RIL population tended to be weak
634 (figure 2. Seed mass and fecundity were negatively genetically correlated in
635 Sweden in 2010, but no significant genetic correlation was detected in the
636 other three site \times year combinations. The overall weak genetic correlation
637 between seed size and number can be explained by the fact that the direction
638 of pleiotropic effects varied, and was positive roughly as frequently as nega-
639 tive. In contrast to theoretical predictions that there should be a trade-off
640 between seed size and seed number (Smith & Fretwell 1974), these observa-
641 tions indicate that genetic correlations with seed mass place little constraint
642 on the evolution of increased fecundity in these populations. One possible
643 reason for the lack of a strong correlation between seed size and fecundity
644 is that variation in seed size was too limited to affect fecundity and that
645 trade-offs might be detected if genotypes with larger differences in seed size

646 were crossed.

647 **5.5 Conclusions**

648 In conclusion, this study has examined how variation in number of seeds per
649 fruit and in resource allocation to different components of fitness contribute
650 to overall adaptive differentiation in *A. thaliana*. Our results show that there
651 is adaptive variation in seed production independent of variation in fruit
652 number, and that the advantage to local genotypes can be underestimated
653 if this is ignored. Moreover, we demonstrate consistent positive pleiotropy
654 among components of fitness reflected in both genetic correlations among
655 phenotypes and effects of underlying QTL, and very little evidence of a trade-
656 off between offspring size and number. These findings indicate that the
657 process of population divergence has been due in large part to the fixation
658 of alleles that increase overall vigour or “condition” in different ways at each
659 site.

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671 **8 Data availability**

672 Data, R scripts, and the R markdown document used to create this
673 manuscript will be uploaded to a suitable public server on publication.
674 In the meantime, they are available at [https://github.com/ellisztamas/](https://github.com/ellisztamas/fecundity_components)
675 `fecundity_components`.

676 **9 Competing interests**

677 The authors declare no conflict of interest.

678 10 Authors' contributions

679 TJE performed analyses and wrote the manuscript. JÅ conceived the study,
680 and JÅ, FMP and CGO co-ordinated data collection and critically revised
681 the manuscript.

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