Local adaptation and ecological differentiation under selection, migration and drift in *Arabidopsis lyrata*

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Author contributions

T.H and O.S devised the study and wrote the manuscript. T.H conducted the field experiments and analysed the sequence data with T.M.M.

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Abstract

How the balance between selection, migration and drift influences the evolution of local adaptation has been under intense theoretical scrutiny. Yet, empirical studies that relate estimates of local adaptation to quantification of gene flow and effective population sizes have been rare. Here, we conducted a reciprocal transplant trial, a common garden trial, and a whole-genome based demography analysis to examine these effects among *Arabidopsis lyrata* populations from two altitudinal gradients in Norway. Demography simulations indicated that populations within the two gradients are connected by gene flow ($0.1 < 4N_em < 11$) and have small effective population sizes ($N_e < 6{,}000$), suggesting that both migration and drift can counteract local selection. However, the three-year field experiments showed evidence of local adaptation at the level of hierarchical multi-year fitness, attesting to the strength of differential selection. In the lowland habitat, local superiority was associated with greater fecundity, while viability accounted for fitness differences in the alpine habitat. We also demonstrate that flowering time differentiation has contributed to adaptive divergence between these locally adapted populations. Our results show that despite the estimated potential of gene flow and drift to hinder differentiation, selection among these *A. lyrata* populations has resulted in local adaptation.

Introduction

Differential selection among environments can lead to adaptive divergence when opposing evolutionary forces have weaker effects. This happens when populations inhabit environments

separated by migration barriers, or when spatially varying selection is strong enough to overcome the homogenizing effects of the gene flow (Kawecki and Ebert 2004). The former scenario is a well-studied one, but less is known about local adaptation that occurs despite ongoing gene flow (Savolainen et al. 2013).

The early theory by Haldane (1930) showed that locally beneficial alleles may exist in a continent-island model only when the strength of selection on the island exceeds the rate of incoming migration from the continent. Since then, the emergence and maintenance of adaptive divergence has been examined under a multitude of demographic scenarios (Bulmer 1972; Slatkin 1973; Felsenstein 1976; Lenormand 2002; Yeaman 2015). In general, theoretical models point towards a critical migration threshold above which selection will be overwhelmed by migration and no local adaptation is possible [i.e. allelic "swamping" happens (Lenormand 2002)]. Alternatively, below this threshold, selection may overcome the effects of migration and the beneficial polymorphisms are maintained in a set of populations, resulting in local adaptation (Antonovics and Bradshaw 1970; Hendry et al. 2002; Sambatti and Rice 2006; Gonzalo-Turpin and Hazard 2009; Comeault et al. 2015; Monnahan et al. 2015). Another confounding factor in the evolution of local adaptation is genetic drift (Lande 1976). Populations with small effective sizes experience elevated levels of drift (Wright 1931), which can lead to loss of adaptive alleles or fixation of maladaptive alleles. In consequence, large populations tend to have higher adaptive potential than small ones (Robertson 1960), leading more often to local adaptation (Leimu and Fischer 2008). Indeed, the theory of quantitative trait evolution suggests that especially strong selection is needed for local adaptation to evolve among small populations connected by gene flow (Blanquart et al. 2012). Gene flow can, however, also replenish genetic diversity lost through drift, so migration from a large "source" population into a small "sink" population may actually facilitate adaptation (Holt and Gomulkiewicz 1997). Despite the well-developed theoretical background, few empirical studies have attempted to examine the conditions of local adaptation by combining estimates of differential

selection with quantification of migration rates and effective population sizes. For example, a meta-analysis by Leimu and Fischer (2008) reveals that geographical distance between plant populations is a poor predictor of their performance at reciprocal transplant trials, whereas even rough approximations of the population sizes proved more reliable indicators of fitness. On the other hand, in a similar meta-analysis, Hereford (2009) emphasized the role of greater environmental distance (i.e. more differential selection pressures) in promoting adaptive differentiation, while excluding the effects of geographical distance and population sizes from the study. Geographical distance might not, in fact, inform about gene flow in such analyses, as it can be confounded by many other factors when different species are compared. Therefore, to better understand the roles of selection, migration and drift in local adaptation, more accurate estimates of the relevant evolutionary forces in studies on individual species are clearly needed.

Climate imposed selection frequently leads to large-scale adaptation over latitudes (Morgenstern 1996; Hall and Willis 2006; Leinonen et al. 2011; Ågren and Schemske 2012; Alberto et al. 2013; Colautti and Barrett 2013; Toräng et al. 2015). However, the shallowness of these environmental gradients often precludes gene flow between populations in highly distinct environments (Savolainen et al. 2007). An alternative source for differential selection is the environmental variation along an elevation gradient, where abiotic (e.g. temperature and solar radiation) and biotic (e.g. pathogens and herbivores) factors can change rapidly at short spatial scales (Körner 2007). Reciprocal transplant experiments between alpine and lowland populations can therefore be useful when examining differential selection in the presence of migration, but they also have the potential to predict how populations might respond to climate change (Byars et al. 2007; Gonzalo-Turpin and Hazard 2009; Kim and Donohue 2013; Frei et al. 2014). The alpine and montane ecosystems are thought to be especially vulnerable to global warming (Beniston 2003), so to evaluate how high-altitude populations perform at low-altitude sites can provide valuable insights into their adaptive potential upon climate change. Moreover, the level and direction of gene flow

can have different consequences under rapid climate change than in more constant conditions (Hoffmann and Sgrò 2011; Alberto et al. 2013). The spread of alleles towards high altitude populations might prove adaptive, whereas gene flow to the opposite direction is likely to be even more detrimental for future local adaptation.

In the present study, we examined local adaptation among alpine and lowland populations of *Arabidopsis lyrata* (L.) O'Kane and Al-Shehbaz (Brassicaceae). Unlike its close relative *A. thaliana*, *A. lyrata* is predominantly outcrossing (Clauss and Koch 2006), so selection can be counteracted much more effectively by gene flow, especially via pollen movement. Furthermore, as *A. lyrata* is perennial, selection acting over multiple years can be evaluated. Its wide circumpolar distribution consists of several isolated populations (Jalas and Suominen 1994), which have demonstrated large-scale local adaptation at common garden sites across Europe and North America (Leinonen et al. 2009, 2011; Vergeer and Kunin 2013). Many of these populations are genetically diverged (Muller et al. 2008; Pyhäjärvi et al. 2012; Mattila et al. 2017) and phenotypically differentiated in life history and morphological traits (Kärkkäinen et al. 2004; Quilot-Turion et al. 2013; Remington et al. 2015; Hämälä et al. 2017). Studies conducted in natural settings have proven the importance of flowering phenology in *A. lyrata* adaptation (Riihimäki and Savolainen 2004; Leinonen et al. 2011, 2013; Puentes et al. 2016). Northern populations generally exhibit faster vegetative to reproductive development than southern populations, and the earlier flowering start has shown to be under directional selection in a Norwegian alpine habitat (Sandring et al. 2007).

To examine the patterns of local adaptation and levels of gene flow, drift and selection in *A. lyrata*, we performed a study consisting of a demography analysis, a reciprocal transplant trial and a common garden trial. We sequenced plants from Norwegian alpine and lowland populations to estimate migration rates, effective population sizes and divergence times. Individuals from these populations were then reciprocally transplanted to contrasting high- and low-altitude sites, where their flowering time, fruit production and survival were followed for three

years. Plants were also grown in a novel lowland habitat in Finland to more precisely measure variation in flowering traits and to provide an additional fitness comparison. We used these data to address the following questions: What are the patters of migration and population size variation among the study populations? Has differential selection been strong enough to overcome the effects of gene flow and drift; i.e. do we see evidence of local adaptation? And, what phenotypic traits are under sufficiently strong selection to contribute to local adaptation?

Materials and methods

Study species and populations

Arabidopsis lyrata favours low-competition habits, such as river and lake shorelines, sand dunes, rock outcrops and serpentine soils. In southwestern Norway, it can be found in riverbeds and cliffs at altitudes ranging up to 1,500 m.a.s.l, whereas in Central Europe, individuals mostly grow at lower elevations on dolomitic and gypsum outcrops (Clauss and Koch 2006). *A. lyrata* forms compact leafy rosettes with long flowering shoots and small white flowers. Transfer of non-self pollen by insects is necessary for the sexual reproduction of *A. lyrata*, but seed dispersal can still permit gene flow beyond the range of insect pollinators.

Ten *A. lyrata* populations were used in this study: eight from Norway, one from Germany and one from North Carolina. The Norwegian populations were collected from two alpine areas (Jotunheimen and Trollheimen), each consisting of four populations (Fig. 1). The growing sites of the Jotunheimen populations represented an altitudinal gradient from 300 m.a.s.l to 1200 m.a.s.l ('J1', 'J2', 'J3', and 'J4', from here on), whereas in Trollheimen, altitudes ranged from 10 m.a.s.l to 1360 m.a.s.l ('T1', 'T2', 'T3' and 'T4', from here on) (Table 1). Populations of the two alpine areas form two distinct genetic clusters, as measured by microsatellite variation (Gaudeul et al. 2007). The map distance between the highest and lowest sampling site is approximately 30 km in Jotunheimen and 35 km in Trollheimen. As control groups, we used populations from Germany (abbreviated as

'GER') and North Carolina, USA (abbreviated as 'NC'). Earlier studies have already demonstrated local adaptation between these populations and the Norwegian population from Spiterstulen (J3 in the present study) (Leinonen et al. 2009, 2011), so we can validate our experimental design by observing fitness differences between the local and control populations.

Whole-genome sequencing

To establish the presence of gene flow and to have an estimate of effective population sizes and divergence times, we acquired whole-genome sequence data from part of the study populations. We sampled nine individuals from J1, seven from J3, five from T1 and five from T3. Total genomic DNA was extracted from fresh leaves using NucleoSpin Plant II kit (Macherey-Nagel). The DNA was fragmented with Bioruptor sonication system (Diagenode), after which the libraries for Illumina whole-genome sequencing were prepared with NEBNext master mix kit (New England Biolabs). Samples from J1, J3 and T1 populations were sequenced with Illumina HiSeq2500 in Institute of Molecular Medicine Finland, University of Helsinki, using 100-bp paired-end protocol, whereas T3 samples were sequenced with Illumina NextSeq550 in Biocenter Oulu, University of Oulu, using 150-bp paired-end protocol. To supplement our data, we used six whole-genome sequences from GER and NC, as well as five from J3 (bringing the number of individuals from that population to 12), published previously by Mattila et al. (2017). Therefore, our total data set consisted of 44 resequenced individuals from six populations.

We first removed low quality reads and Illumina adapters with Trimmomatic (Bolger et al. 2014). The reads were aligned to *A. lyrata* v1.0 reference genome (Hu et al. 2011) with bwa-mem (Li and Durbin 2009). Duplicated reads were removed with Picard tools (http://broadinstitute.github.io/picard/) and indels realigned with GATK (DePristo et al. 2011). Neutral F_{ST} estimates and site frequency spectra of derived variants (unfolded SFS) and were then inferred from genotype likelihoods with ANGSD (Korneliussen et al. 2014). Compared to methods based on variant calling, this direct estimation approach is less biased by uncertain genotypes

resulting from low coverage sequencing data (Nielsen et al. 2011; Han et al. 2014). To minimize the effects of mapping errors, repeats and areas with excessive coverage were removed and sites with only heterozygote genotypes within populations were masked from the analysis. For more information about the sequence processing and analysis, see Text S1.

Demography simulations

Demography parameters were inferred with coalescent simulations, using a composite-likelihood based method implemented in fastsimcoal2 (Excoffier et al. 2013). We estimated the effective number of diploid individuals in each population (N_e), population migration rates ($4N_em$: number of migrant lineages per generation), and population divergence times in number of generations. Demography models were fitted to three-dimensional SFS data, estimated for fourfold degenerate sites. We used four different population models, with GER as an outgroup in each model: J1-J3-GER, T1-T3-GER, J1-T1-GER and J3-T3-GER. For each population comparison, we first tested four models with different migration parameters between the Norwegian populations: no migration, unidirectional migration (from 1 to 2 and from 2 to 1) and bidirectional migration. These models were then compared against ones with independent bottlenecks in each lineage to explore alternative explanations for the migration parameters. This method estimates gene flow after the neighboring low- and high-altitude populations diverged, but as it is based on allele frequency differences that accumulate over generations, the most recent events are not fully reflected in the estimates. Simulations were repeated 50 times to acquire global maximum likelihood estimates for the parameters. The relative fit of each migration model was assessed with the Akaike information criterion (AIC). To produce confidence intervals for the parameter estimates, the best migration models were fitted to 100 nonparametric bootstrap SFS replicates. For more information about the demography analysis, see Text S1.

Reciprocal transplant and common garden trials

Plants were grown and measured in three field sites to test for the presence of local adaptation and to evaluate which traits contribute to the adaptive divergence. Individuals from all ten populations were initially grown in controlled conditions (+20°C, 20 h light / 4 h dark -cycle) and crossed to produce independent full-sib families for the reciprocal transplant and common garden experiments. In June 2014, the experimental seeds were sown into agar plates and stratified in +4°C for four days. Seeds germinated in a growth cabinet (Sanyo MLR 350H), with +20°C temperature, 8 h light / 16 h dark -cycle, and 80% relative humidity. Germinated seedlings were transferred to pots filled with 1:1 mix of peat and gravel and pre-grown in a greenhouse, under natural light conditions (day length approximately 20 h), for about one month. The pre-growing took place at the University of Oulu.

In August 2014, we established two experimental fields in Jotunheimen, Norway and one in Oulu, Finland (Fig. 1). The Norwegian fields represented low (Lom; 300 m.a.s.l) and high (Spiterstulen; 1100 m.a.s.l) altitude growing sites. Seed families from four Norwegian populations (J1, J3, T1, T3), as well as GER and NC plant were planted into these fields (see Table 1 for sample sizes). The high-altitude field was situated in a riverbed among the natural habitat of the J3 population, whereas the low-altitude field was established approximately three kilometers away from the sampling area of the J1 population and consisted of soil transferred from the natural growing site. In order to minimize the effects of environmental variation within sites, individuals from each family were randomized into two blocks with 20 cm spacing between plants. To analyze the phenology of these populations more closely, and to provide an additional fitness comparison, a third experimental field was established in Oulu, Finland (65°06´N, 25°46´E; altitude 12 m.a.s.l). Based on temperature data (Table 1), Oulu is intermediate between the low- and high-altitude field sites [mean annual temperature 1.8ºC; annual precipitation 457 mm; growing season 5 months (Hijmans et al. 2005)], but due to higher latitude (Fig. 1), annual variation in day lengths differs from the Norwegian locations. Seedlings from all ten populations (eight Norwegian, GER and NC) were

randomly planted into five blocks made from a mix of peat and sand, situated in the Botanical gardens of Oulu University.

Trait measurements

In the Norwegian fields, flowering start date, reproductive output and survival of the plants were measured during three consecutive years: 2015, 2016 and 2017 (in the high-altitude field, the flowering start dates were not measured during 2017). The flowering start dates were determined by inspecting the plants twice a week in the low-altitude field and once a week in the high-altitude field. Sampling periods were determined by the advancement of spring each year and they lasted approximately from mid-May to late-June. We visited the fields during peak fruit production in August and counted the number of fruits produced by each surviving individual. In Oulu, plants were monitored three times a week throughout the three field seasons (from May to September). We measured flowering start date, number of inflorescences and length of the longest flowering shoot at the time of first flower, fruit maturation date, fruit production, flowering cessation date (when last flower had wilted) and survival.

Statistical analysis

To test for the presence of local adaptation, we performed fitness analyses with aster models (Geyer et al. 2007) in the R environment (R Core Team 2017). These regression models can take into account the hierarchical structure of the fitness components, and allow to combine data from life history traits that follow different sampling distributions (Shaw et al. 2008). Our models included binary representations of survival and flowering success, modelled as Bernoulli distribution, and the count of produced fruits, given successful flowering, modelled as zero-truncated Poisson distribution. The hierarchical structure for the first-year data was: 1 → survival → flowering → fruit production. The hierarchy for the second and third years was the same, except survival was conditional on surviving the previous year. Population differences were analyzed with likelihood-

ratio tests, by comparing the fit of a full model to a reduced model with the pair of populations combined as one category.

Variation in individual traits was analysed with general and generalized linear mixed models (GLMM) in the R package lme4 (Bates et al. 2015). For survival data, we fitted a binomial distribution with logit link function, while a Gaussian distribution with identity link function was used with other phenotypes. Some traits were log or square root transformed to improve homoscedasticity. Population was included in the models as fixed effect and family and planting block as random effects. The differences between populations were assessed using likelihood-ratio tests by comparing the full and reduced models, as in the case of total fitness estimates.

To quantify flowering trait differentiation between the Norwegian populations in more detail, we estimated levels of trait divergence using Q_{ST} – the quantitative genetic equivalent of F_{ST} (Wright 1951; Spitze 1993). The estimates were calculated as $\sigma^2_{GB}/\ (\sigma^2_{GB} + 2\sigma^2_{GW})$, where σ^2_{GB} and σ^2_{GW} are the between and within population additive variance components, respectively. The latter was estimated as twice the average between full-sib family (nested within population) variance component, while assuming no dominance variance (Lynch and Walsh 1998). Note, however, that this assumption may cause downward bias of the Q_{ST} estimates, as a nonzero dominance variance component leads to overestimation of σ^2_{GW}. The variance components were inferred with Markov chain Monte Carlo based regression models in the R package MCMCglmm (Hadfield 2010). This approach was chosen over the more traditional way of using restricted maximum likelihood models, because a Bayesian framework provides an effective way to determine credible intervals by drawing Q_{ST} estimates directly from the full posterior distribution of the model variance (O'Hara and Merilä 2005). Q_{ST} estimates for flowering start dates were obtained for all three field sites, whereas estimates for flowering shoot lengths, inflorescence numbers, fruit maturation dates and flowering cessation dates could only be inferred from the Oulu data set. We also calculated F_{ST} estimates using the four Norwegian populations (J1, J3, T1, T3) for which we had

whole-genome data and compared a global average against the Q_{ST} estimates to determine whether the trait divergence is more likely due to selection ($Q_{ST} > F_{ST}$) or drift ($Q_{ST} = F_{ST}$) (Merilä and Crnokrak 2001).

We also tested whether phenotypic selection on flowering traits can contribute to local adaptation. Selection was quantified using aster models (Geyer et al. 2007), with the hierarchical absolute fitness as dependent variable and the trait of interest, standardized to mean of zero and standard deviation of one, as independent variable (population and planting block were included as additional predictors). This approach is akin to a selection analysis by Lande and Arnold (1983), but unlike the classical method, aster models are not dependent on normally distributed response variables (Mitchell-Olds and Shaw 1987; Shaw and Geyer 2010). The highest-ranking models were used to estimate linear selection gradient β, which measures the strength and direction of selection on the trait, and by including a quadratic term γ, these models can also approximate nonlinear relationships between the phenotype and fitness. The curvature of this quadratic relationship can indicate stabilizing selection if positive or disruptive selection if negative (Lande and Arnold 1983; Shaw and Geyer 2010). Confidence intervals for the inferred β and γ terms were estimated using parametric bootstrapping with 1,000 replicates. The analysis included all individuals planted at the experimental fields (focal populations and controls) to reach sufficient phenotypic variation to quantify the environment specific selection gradients in detail. For the Oulu data set, the analysis was conducted with simple- and multiple-regression models to evaluate the potential for selection acting on correlated traits (Lande and Arnold 1983).

Results

Demography analysis indicates gene flow and limited population sizes

Pairwise F_{ST} estimates indicated low differentiation between the neighboring low- and high-altitude populations (Table 2), reflecting recent and possibly ongoing gene flow. This assumption was

confirmed by the demography analysis, as models including the closely adjacent populations J1-J3 and T1-T3 had unambiguously highest likelihoods when parameters included bidirectional gene flow between the populations (Table S1). The population migration rates ($4N_em$) were, however, more than 36× higher from J3 to J1 than from J1 to J3 (Table 3). The same pattern was also evident in the T1-T3 comparison, but the asymmetry was less severe (~10× higher from T3 to T1). The inclusion of independent bottlenecks reduced the fit of both population models, suggesting that the estimated migration patterns are likely not an artifact of cryptic population size variation (Table S1). The comparison that included the high-altitude populations J3-T3 had the best fit from models without gene flow, while the low-altitude comparison (J1-T1) showed modest, but significant, gene flow only from J1 to T1 (Table 3 and Table S1). The maximum likelihood estimates for divergence times revealed a more recent split between T1 and T3 populations (254 generations ago) than between J1 and J3 populations (866 generations ago). Lineages containing the two alpine areas (Jotunheimen and Trollheimen) were estimated to have diverged around 1,400 generations ago, while the Norwegian and German split happened approximately 28,000 generations ago. The inferred effective population sizes (N_e) showed a large difference between the German ($N_e \approx 50{,}000$) and Norwegian populations (maximum $N_e < 6{,}000$). The Trollheimen populations had lower N_e estimates than the Jotunheimen populations, with T3 exhibiting the smallest effective population size and J3 the largest (Table 3). These models make several simplifying assumptions, but comparisons between simulated and observed one-dimensional SFS confirmed that our best-fitting models provide reasonable approximations of the population histories, although some absolute lack of fit still remains (Fig. S1).

Fitness estimates show local adaptation

The local populations had significantly higher fitness estimates at their home sites (J1 in low-altitude and J3 in high-altitude) than any non-local ones (Fig. 2), proving the existence of local adaptation. The other Norwegian populations (T1 and T3) did not, however, mirror these results. Fitness

differences were not significant in the low-altitude field, whereas in the high-altitude field, the lowland population T1 had significantly higher fitness than the alpine population T3. Overall, the absolute fitness estimates were clearly higher in the low-altitude field (Fig. 2). In the Oulu field, the Jotunheimen populations had significantly higher fitness estimates than Trollheimen populations ($p < 1 \times 10^{-16}$, two-way ANOVA), but there were only slight differences between populations within the Jotunheimen and Trollheimen groups (Fig. S2 and Table S2). The control populations GER and NC did worse than the Norwegians in all field sites (aside from T3 in the high-altitude field), coinciding with patterns observed in previous studies (Leinonen et al. 2009, 2011)

Examination of the fitness components revealed differences between the Norwegian field sites. In the low-altitude field, survival proportions were close to equal between the four Norwegian populations (Fig. S3), whereas in the high-altitude field, the local population J3 had significantly higher survival rates than the other Norwegian populations (Fig. S5). In contrast, fruit production in the low-altitude field was significantly highest in the local J1 population (Fig. S4), but this fitness component showed only subtle differences between populations in the high-altitude field (Fig. S6). The Jotunheimen populations produced more fruits in the Oulu field than Trollheimen populations, but there were no marked differences in survival proportions (Figures S7 and S8). The GER and NC control populations had lower survival proportions (all NC plants died during the second winter in all three field sites) and fruit set than the Norwegian populations (Figures S3 – S8). Overall, the fitness differences increased during the second and third growing seasons, highlighting the importance of multi-year experiments in perennial species. For results from the pairwise GLMM models, see Tables S3 – S5.

Population differentiation in flowering traits is promoted by selection

The Q_{ST} analysis indicated high differentiation in some flowering traits among the Norwegian populations (Fig. 3). For flowering start dates and shoot lengths, point estimates exceeded the average neutral F_{ST} (0.180), but the 95% credible and confidence intervals (CI) between Q_{ST} and F_{ST}

overlapped in both cases. Flowering start dates had higher Q_{ST} estimates in the Norwegian fields than in the Oulu field. Inflorescence numbers, fruit maturation dates and flowering cessation dates had low point estimates that fell within the F_{ST} CI (Fig 3).

To further examine the role of differential selection in local adaptation, we focused on the two traits showing indications of higher than neutral divergence ($Q_{ST} > F_{ST}$); flowering start dates and shoot lengths. In general, all plants started flowering earlier in the low-altitude field than in the high-altitude field (Fig. 4). Plants that flowered earlier produced shorter flowering shoots in Oulu, with a positive correlation of $r = 0.17$ ($p < 1 \times 10^{-10}$) between the two traits. The alpine population J3 flowered earlier than the lowland population J1 during the three field seasons in all three field sites (Fig. 4). In the Oulu field, the four Jotunheimen populations showed clinal patterns in flowering start dates (Fig. 4) and shoot lengths (Fig. 5), with high-altitude populations flowering earlier and producing shorter flowering shoots than low-altitude populations. However, patterns among the Trollheimen populations were almost the opposite. Although the alpine T4 population flowered earliest and produced the shortest flowering shoots in Oulu, the lowland population T1 flowered earlier than the alpine population T3 in Norway, and corresponding geographical patterns were seen in flowering start dates (Fig. 4) and shoot length (Fig. 5) between the T1, T2 and T3 populations in the Oulu field. The GER and NC control populations generally flowered later and produced longer flowering shoots than the Norwegian populations (Figures 4 and 5). Based on the pairwise GLMM-models, most population comparisons in flowering start dates and shoot lengths were statistically significant (Tables S3 – S5). Concordant with the Q_{ST} estimates, other flowering traits showed less evidence of population differentiation (Figures S9 – S11 and Table S5).

Strength of selection on flowering start differs between the field sites

Phenotypic selection analysis indicated that all flowering traits had a significant correlation with fitness, but estimates for the linear (β) and quadratic (γ) selection gradients varied between years and field sites (Table 4). For flowering start dates, all β estimates were negative, while most γ

estimates showed positive values. This combination of linear and quadratic terms translates into a negative curvature for the function between fitness and the trait, indicating possible disruptive selection. However, the relationships between average flowering start dates and total three-year fitness estimates showed only shallow curvatures without local fitness minima, indicating that early flowering leads to higher fitness at all three field sites (Fig. 6; for distributions of the trait values, see Fig. S12). The strength of this correlation gives insights into local selection pressures, as the high-altitude environment is predicted to impose stronger selection for early flowering than the low-altitude environment (Fig. 6). In the Oulu data set, both simple- and multiple-regression models indicated directional selection for longer flowering shoots, higher number of inflorescences, earlier fruit maturation dates and later flowering cessation dates (with possibility of stabilizing selection in all traits except fruit maturation; Table 4). The inclusion of multiple predictors had only a slight influence on the β and γ terms, with no change in estimated direction of selection, suggesting that among the phenotypes measured here, selection is mostly acting on individual traits. Results from the multiple-regression models are shown in Table 4, while simple-regression models are depicted in Table S6.

Discussion

Local adaptation in the face of gene flow and genetic drift

Decades of theoretical work has provided insights into evolutionary processes underlying the spread and maintenance of adaptive variation (Haldane 1930; Wright 1931; Bulmer 1972; Slatkin 1973; Felsenstein 1976; Lenormand 2002; Yeaman 2015). Importantly, how the interplay between selection, migration and drift effects the emergence of local adaptation has been under increasing discussion (Yeaman and Otto 2011; Blanquart et al. 2012). Yet many empirical studies have focused on populations that have evolved under independent selection pressures, without recent or ongoing gene flow (e.g. Hall and Willis 2006; Leinonen et al. 2011; Ågren and Schemske 2012;

Anderson et al. 2013; Toräng et al. 2015). In contrast, when local adaptation forms over short geographical distances, selection is continuously challenged by migration and drift, and the outcome depends on the balance between these forces. Here, we have shown that differential selection between lowland and alpine populations of *A. lyrata* has led to local adaptation despite gene flow and low effective population sizes. We acknowledge, however, that current evolutionary dynamics are not fully depicted in our study, as allele frequency based demography estimates tend to reflect events on longer time scales than phenotypic traits. In all likelihood, the recent (and very likely ongoing) gene flow has still opposed local selection, as our results suggest that these fitness differences have evolved during the last 1,000 generations. Based on classical theory by Haldane (1930), any locus with selection coefficient higher than the migration rate (i.e. $s > 0.00072$ in our case) could contribute to the local adaptation. However, this model assumes an infinite population size, and in natural systems the probability of reaching the selection threshold imposed by migration is further influenced by genetic drift (Yeaman and Otto 2011; Blanquart et al. 2012). Importantly, as shown by Blanquart et al. (2012), under all but very high levels of gene flow (i.e. if $2 + 4N_em \approx 4N_em$), drift hinders local adaptation. We therefore expect higher selection coefficients than predicted by the simple model of $s > m$ to underlie the observed local adaptation, even though we lack the necessary data to test this hypothesis.

Compared to studies of large-scale local adaptation in *A. lyrata* [J3 vs. GER (Leinonen et al. 2009) and J3 vs. NC (Leinonen et al. 2011)], the levels of local superiority observed here were modest, coinciding with the expected effects of gene flow and shorter environmental distances. Furthermore, a reciprocal transplant experiment between J2 and J3 populations did not reveal evidence of local adaptation (P.H. Leinonen, unpublished), potentially reflecting higher gene flow and/or less differential selection pressures. Local adaptation under verified gene flow has previously been reported in few plant species (Richardson et al. 2014). Notably, Sambatti and Rice (2006) found reciprocal fitness differences and indications of gene flow between serpentine and riparian

populations of a sunflower species, *Helianthus exilis*, while Gonzalo-Turpin and Hazard (2009) observed similar patterns among low- and high-altitude populations in alpine grass species, *Festuca eskia*. To the best of our knowledge, our study is still the first to show local adaptation under gene flow in *Arabidopsis* species. These results can be contrasted with the findings of adaptive differentiation in the self-fertilizing *A. thaliana*. Although documented examples of large-scale local adaptation (Ågren and Schemske 2012) and genomic divergence (Fournier-Level et al. 2011) among *A. thaliana* populations exist, no evidence of local adaptation under gene flow has been reported. The flowering time variation across latitudes (Stenøien et al. 2002; Stinchcombe et al. 2004) and altitudes (Lewandowska-Sabat et al. 2017) has also been limited. Here, we also emphasize the importance of multi-year experiments in perennial species, as the observed fitness effects were highly cumulative.

Local superiority was observed for fecundity in the low-altitude field, whereas viability differences were more pronounced in the high-altitude field. Furthermore, sequence variation in these populations was consistent with bidirectional gene flow, with migration from high to low altitudes being more frequent than from low to high altitudes. Based on theoretical expectations of gene swamping (Lenormand 2002), the lowland population J1 should be worse adapted to its natural growing environment than the alpine population J3, as it receives more maladaptive alleles from high-altitudes. However, the realized fitness estimates did not support this expectation, as J1 showed clearer signs of local adaptation than J3. Although this fact may reflect real differences in local selection, an alternative explanation is also possible: at the high-altitude site, local adaptation was mostly attributed to differences in survival, which has less emphasis on total fitness estimation (in aster analysis) than reproductive output (Geyer et al. 2007). Additionally, higher survival of the J3 population in the high-altitude site suggests that subsequent years would likely increase the fitness difference between local and non-local populations (Leinonen et al. 2011).

Despite clear evidence of local adaptation among the Jotunheimen populations, fitness estimates in the Trollheimen populations did not support altitude specific adaptation. The underlying reason could be a technical one, as our Jotunheimen fields may have failed to replicate important environmental factors present at the Trollheimen growing sites [related to e.g. abiotic and biotic composition of the soil (Stanton-Geddes et al. 2012)]. Alternatively, the adaptive potential of these populations may be lowered by a combination of short divergence time (~100 – 600 generations ago), small effective sizes (~500 – 3,000 individuals per population) and gene flow, leading to low fitness in both lowland and alpine environments. This hypothesis is further supported by measurements made in the Oulu field; the Trollheimen populations had lower total fitness estimates than populations from Jotunheimen, which diverged longer ago (~600 – 1,000 generations ago) and have larger effective population sizes (~3,000 – 6,000 individuals per population). Furthermore, the fairly equal population sizes among the two Trollheimen populations, as well as higher gene flow towards the slightly larger T1 population, likely do not lead to an adaptive scenario predicted by the "source-sink" model of local adaptation (Holt and Gomulkiewicz 1997).

Flowering traits contribute to adaptive divergence

Flowering phenology and floral display have a major influence on plant fitness (Linhart and Grant 1996; Hall and Willis 2006; Sandring et al. 2007; Anderson et al. 2011; Munguía-Rosas et al. 2011; Ågren et al. 2017), so we explored what roles these traits play in adaptive divergence between our lowland and alpine populations. As all areas of the genome share roughly the same levels of gene flow and drift, variation in quantitative traits can give insights into selective forces shaping the underlying loci. First, the Q_{ST}-F_{ST} comparisons suggested that differentiation in flowering start dates and shoot lengths is promoted by selection. Second, phenotypic examination revealed clinal variation among the Jotunheimen populations, with alpine populations flowering earlier and producing shorter flowering shoots than lowland populations. And third, the selection analysis showed stronger directional selection towards earlier flowering in the high-altitude field site. Taken

together, these results strongly support the idea that local selection has overcome the effects of gene flow and drift, leading to different flowering time responses in the alpine and lowland populations. In contrast, despite indications of selection, other flowering traits showed little evidence of population differentiation, suggesting more homogeneous selection pressures among the growing sites. As the observed altitudinal cline in flowering time (and in correlated shoot length) is similar to latitudinal clines found in several plant species (Endler 1977; Stinchcombe et al. 2004; Munguía-Rosas et al. 2011), we conclude that growing season length is a potential explanation for the trait divergence. Furthermore, the relationships between flowering start dates and fitness estimates were close to linear in all field sites, suggesting that the strength of directional selection is primarily driving differentiation between the populations (as opposed to stabilizing selection towards local optima). However, the strength of selection varied between years. This fact likely reflects year-to-year changes in local microclimates, but mortality caused differences in measured individuals also play a role (especially in the high-altitude field, where non-locals had high mortality). The yearly differences should therefore be interpreted cautiously, with first year data potentially providing the most accurate estimates. As in the case of fitness traits, the flowering start date and shoot length patterns in the Trollheimen populations differed from those in the Jotunheimen populations. The two lowland populations, T1 and T2, flowered earlier and produced shorter flowering shoots than the alpine population T3 (but not T4, which might have different responses because of its isolation from the other populations [Fig. 1]). This result shows that the correlation between growing season length and flowering time is not ubiquitous at short spatial scales, and other environmental factors have more influence on flowering time in the Trollheimen area.

Previous studies conducted on *A. lyrata* (Leinonen et al. 2011, 2013), as well as on other plant species (Hall and Willis 2006; Anderson et al. 2011; Ågren et al. 2017), have documented adaptive variation in flowering traits among distant populations. For example, by reciprocally transplanting *A. lyrata* populations from Norway (J3) and United States (NC), Leinonen et al. (2011)

inferred that selection on flowering start dates has contributed to local adaptation between these isolated populations. Here, we have advanced our knowledge about flowering time variation by showing that strong local selection can lead to adaptive differentiation even among recently diverged populations that are connected by gene flow.

Implications to climate change

The anthropogenic climate change threatens alpine and montane ecosystems in Northern Europe by rapidly raising the annual mean temperatures (EEA 2017). In consequence, these *A. lyrata* populations must react to ever warmer conditions either by migrating or adapting *in situ*. Under the latter scenario, we predict overall detrimental effects for the asymmetric gene flow, because it mainly introduces alpine-specific alleles into lowland populations. However, the gene flow may also promote earlier flowering in the lowland populations, which is likely to be adaptive under longer growing seasons (Anderson et al. 2012). Although the estimated gene flow from low to high altitudes is relatively weak, it can be beneficial for the alpine populations by assisting adaptation to more lowland-like conditions. The lowland populations, on the other hand, may be better at dispersing, as their adaptive potential was mainly attributed to higher reproductive output. Furthermore, comparisons between the three field sites indicated some phenotypic plasticity in fitness and flowering traits, which can facilitate important first responses to changing environmental conditions (Nicotra et al. 2010).

Conclusions

We have shown that *A. lyrata* populations from Jotunheimen, Norway are adapted to their local environments despite gene flow and low effective population sizes. At the low-altitude site, local superiority was facilitated by greater reproductive output, whereas local individuals had higher survival proportions at the high-altitude site. In contrast, the Trollheimen populations did not show significant signs of altitude adaptation, which may be constrained by the recent divergence, small

effective population sizes and gene flow. Observed clinal variation in flowering start dates and shoot lengths, as well as selection inferences with Q_{ST}-F_{ST} comparisons and aster models, strongly indicated that differential selection on flowering time has overcome the effects of gene flow and drift, thus contributing to the adaptive divergence. Furthermore, our results suggest that phenotypic plasticity, potential dispersal and partially beneficial migration may support future adaptation under climate change, but gene flow from high to low altitudes is likely to become even more detrimental for the lowland populations.

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Table 1. Information about the study populations and the number of seed families and individuals planted at each field site.

Population	Location	Latitude (N)	Longitude (E)	Altitude (m.a.s.l.)	Temperature (ºC)	Precipitation (mm)	Growing season	Seed families	N_{Low}	N_{High}	N_{Oulu}
J1	Lom[†]	61°84´	8°57´	300	2.9	559	5	9	74	73	88
J2	Visdalen	61°76´	8°41´	500	1.8	570	5	13	–	–	111
J3	Spiterstulen[‡]	61°62´	8°40´	1,100	–1.3	850	3	6	102	100	89
J4	Spiterstulen High	61°59´	8°39´	1,200	−1.7	880	3	8	–	–	108

T1	Sunndalsøra	62°66′	8°62′	10	5.5	1,060	6	11	55	57	62
T2	Gjøra	62°54′	9°09′	210	3.5	921	5	9	–	–	101
T3	Grøvudalen	62°44′	8°90′	900	–1.4	986	3	14	71	73	88
T4	Nedre Kamtjern	62°74′	9°30′	1,360	–2.2	982	3	12	–	–	90
GER	Plech, Germany	49°39′	11°29′	400	8.3	691	7	14	75	76	81
NC	Mayodan, NC, USA	36°25′	–79°58′	225	14.4	1129	9	11	34	36	122

Temperature and precipitation are annual means. Growing season is the number of months with mean temperature >5 ºC. Data were extracted from WorldClim (Hijmans et al. 2005), using the finest 30 second (~1 km^2) scale. *N* is the number of individuals planted at each field site.

†Location of the low-altitude field site.

‡Location of the high-altitude field site.

Table 2. Neutral pairwise F_{ST} estimates for populations planted at the Norwegian field sites.

Population	J1	J3	T1	T3	GER
J3	0.086				
T1	0.267	0.276			
T3	0.297	0.307	0.128		
GER	0.412	0.425	0.369	0.384	
NC	0.661	0.651	0.667	0.682	0.597

Values are weighted genome-wide averages, estimated for 4-fold degenerate sites.

Table 3. Maximum likelihood estimates (MLE) and their 95% confidence intervals (CI) for the demography parameters.

Parameter	MLE	95% CI
N_{J1}	3,370	2,691 – 4,988
N_{J3}	4,295	3,776 – 5,788
N_{T1}	1,413	716 – 3,166
N_{T3}	1,155	481 – 2,158
N_{GER}	51,351	43,854 – 61,369
$M_{J1\text{-}J3}$	0.266	0.077 – 1.503
$M_{J3\text{-}J1}$	9.748	6.033 – 10.918
$M_{T1\text{-}T3}$	0.027	0.004 – 0.648
$M_{T3\text{-}T1}$	0.276	0.088 – 3.027
$M_{J1\text{-}T1}$	0.004	0.001 – 0.322
$T_{J1\text{-}J3}$	866	637 – 1,097
$T_{T1\text{-}T3}$	254	112 – 598
$T_{JOT\text{-}TRO}$	1,393	1,022 – 1,725
$T_{GER\text{-}NOR}$	27,813	23,450 – 33,279

N is the effective population size (N_e) of diploid individuals, *M* is the population migration rate $4N_em$, and *T* is the divergence time in number of generations. JOT-TRO indicates the divergence between Jotunheimen and Trollheimen and GER-NOR the divergence between Germany and Norway.

Table 4. The linear (β) and quadratic (γ) selection gradients for different flowering traits. Multiple-regression models were used for the Oulu data set. Shown are point estimates and 95% confidence intervals for statistically significant terms.

Field	Trait	Year	β	γ
Oulu	Flowering start	1st	–0.169 (–0.201, –0.135)	0.031 (0.021, 0.040)
		2nd	–0.287 (–0.344, –0.232)	–0.109 (–0.149, –0.075)
		3rd	–0.244 (–0.304, –0.189)	0.060 (0.033, 0.076)
Low	Flowering start	1st	–0.126 (–0.150, –	0.076 (0.060,

			0.104)	0.092)
		2nd	–0.309 (–0.332, –0.287)	0.057 (0.040, 0.074)
		3rd	–0.307 (–0.358, –0.258)	0.022 (0.007, 0.033)
High	Flowering start	1st	–0.656 (–0.800, –0.444)	0.078 (0.008, 0.117)
		2nd	–0.153 (–0.324, –0.027)	–
Oulu	Shoot length	1st	0.074 (0.049, 0.099)	–
		2nd	0.092 (0.047, 0.136)	–0.030 (–0.049, –0.012)
		3rd	0.140 (0.096, 0.189)	–0.098 (–0.127, –0.072)
Oulu	Inflorescence number	1st	0.244 (0.215, 0.274)	–0.035 (–0.047, –0.023)
		2nd	0.761 (0.719, 0.801)	–0.070 (–0.080, –0.060)
		3rd	0.884 (0.841, 0.929)	–0.149 (–0.161, –0.138)
Oulu	Fruit maturation	1st	–0.182 (–0.207, –0.156)	–0.067 (–0.082, –0.053)
		2nd	–0.163 (–0.196, –0.132)	–0.042 (–0.065, –0.019)
		3rd	–0.315 (–0.363, –0.270)	–0.045 (–0.081, –0.010)
Oulu	Flowering cessation	1st	0.118 (0.096, 0.139)	–0.023 (–0.031, –0.016)
		2nd	0.165 (0.118, 0.219)	–0.046 (–0.076, –0.019)
		3rd	0.192 (0.149, 0.232)	–0.058 (–0.082, –0.032)

Figure 1. Locations of the study populations (circles) and experimental fields (diamonds). Altitudes in meters above sea level are also shown.

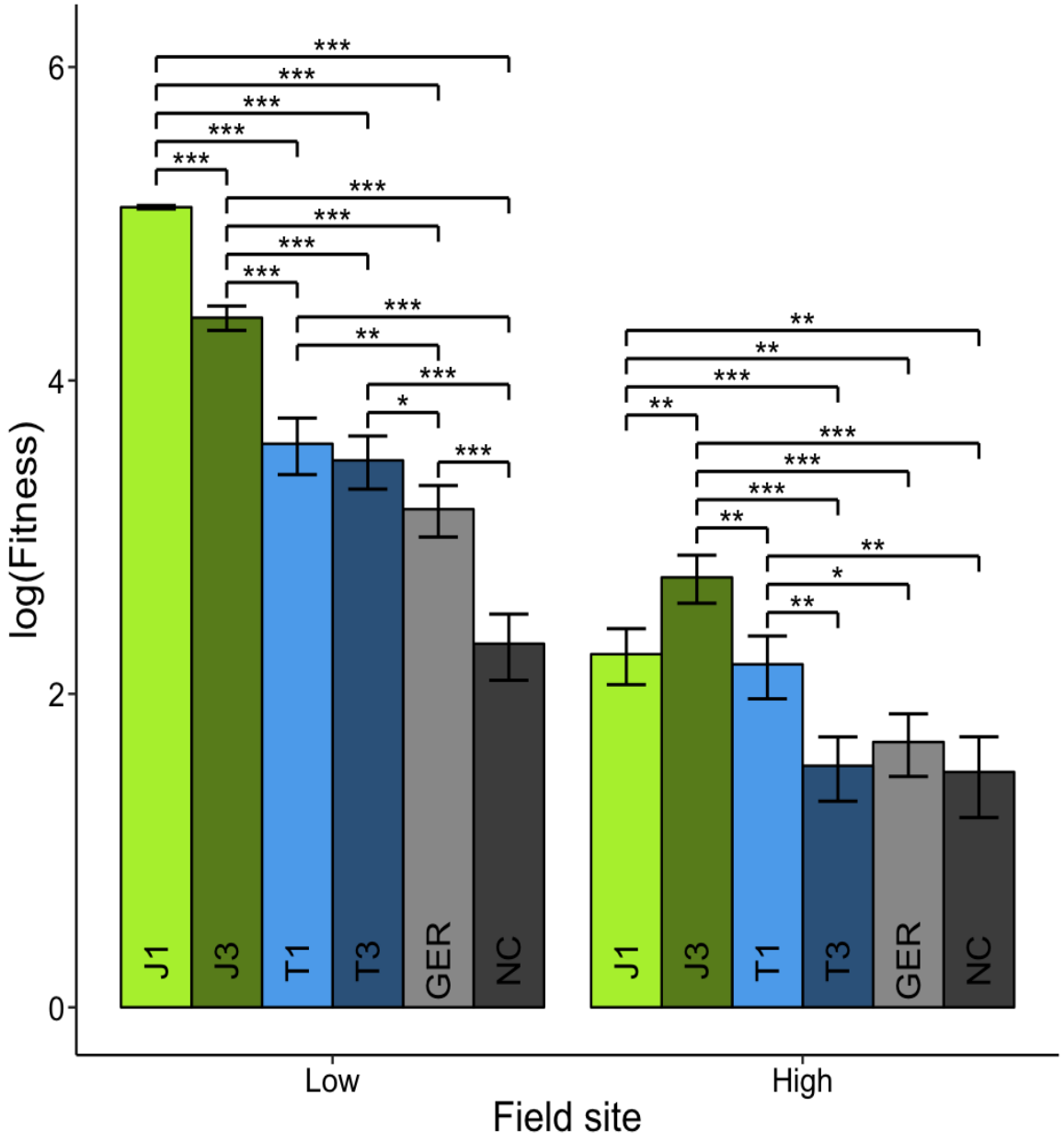

Figure 2. Total fitness estimates (log-transformed) and ± 1 standard errors in the two Norwegian field sites. The hierarchical aster models included three-year survival, flowering propensity and fruit production. Stars above the brackets indicate significant pairwise differences: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

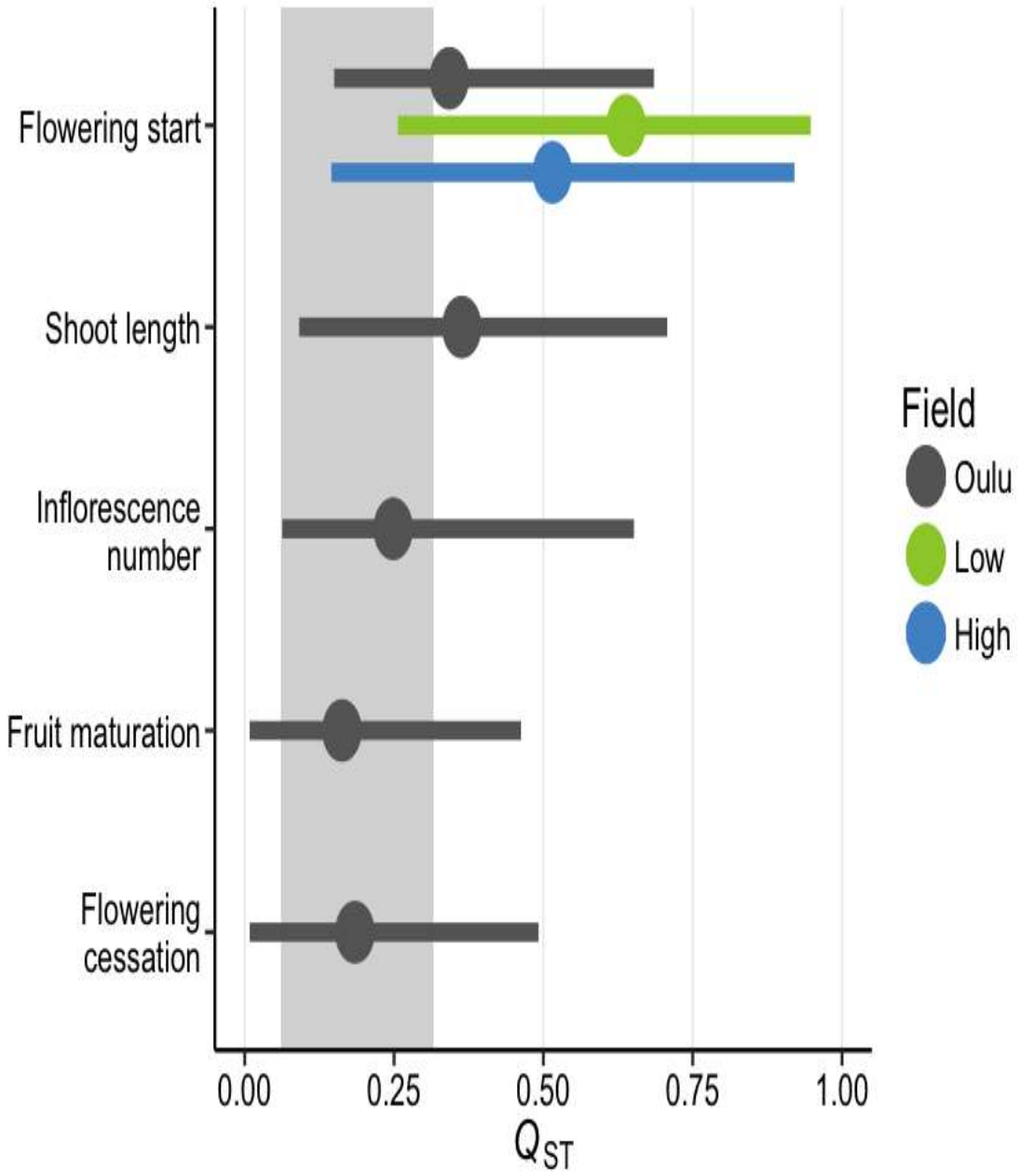

Figure 3. Q_{ST} estimates for different flowering traits among the Norwegian populations. Estimates are averaged over the three years. Circle marks the point estimate and line indicates 95% Bayesian credible intervals. Shaded are shows 95% confidence interval for global neutral F_{ST} among J1, J3, T1 and T3 populations (estimated in 10 Kb non-overlapping windows for 4-fold degenerate sites).

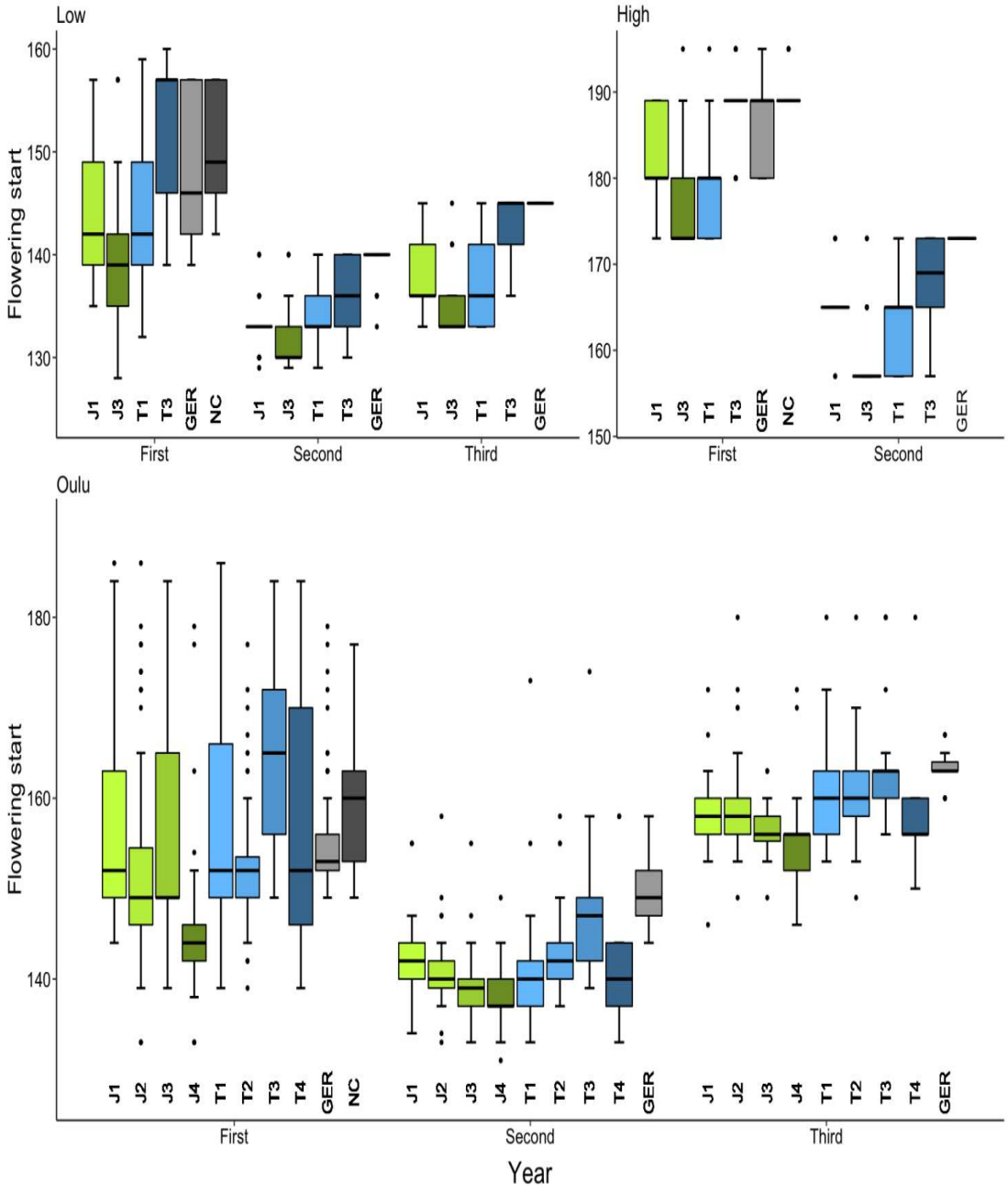

Figure 4. Flowering start dates at the three field sites. Julian date is shown in the *y*-axis. Thick line: median, box: upper and lower quartiles, whiskers: ±1.5 interquartile range, dots: outliers.

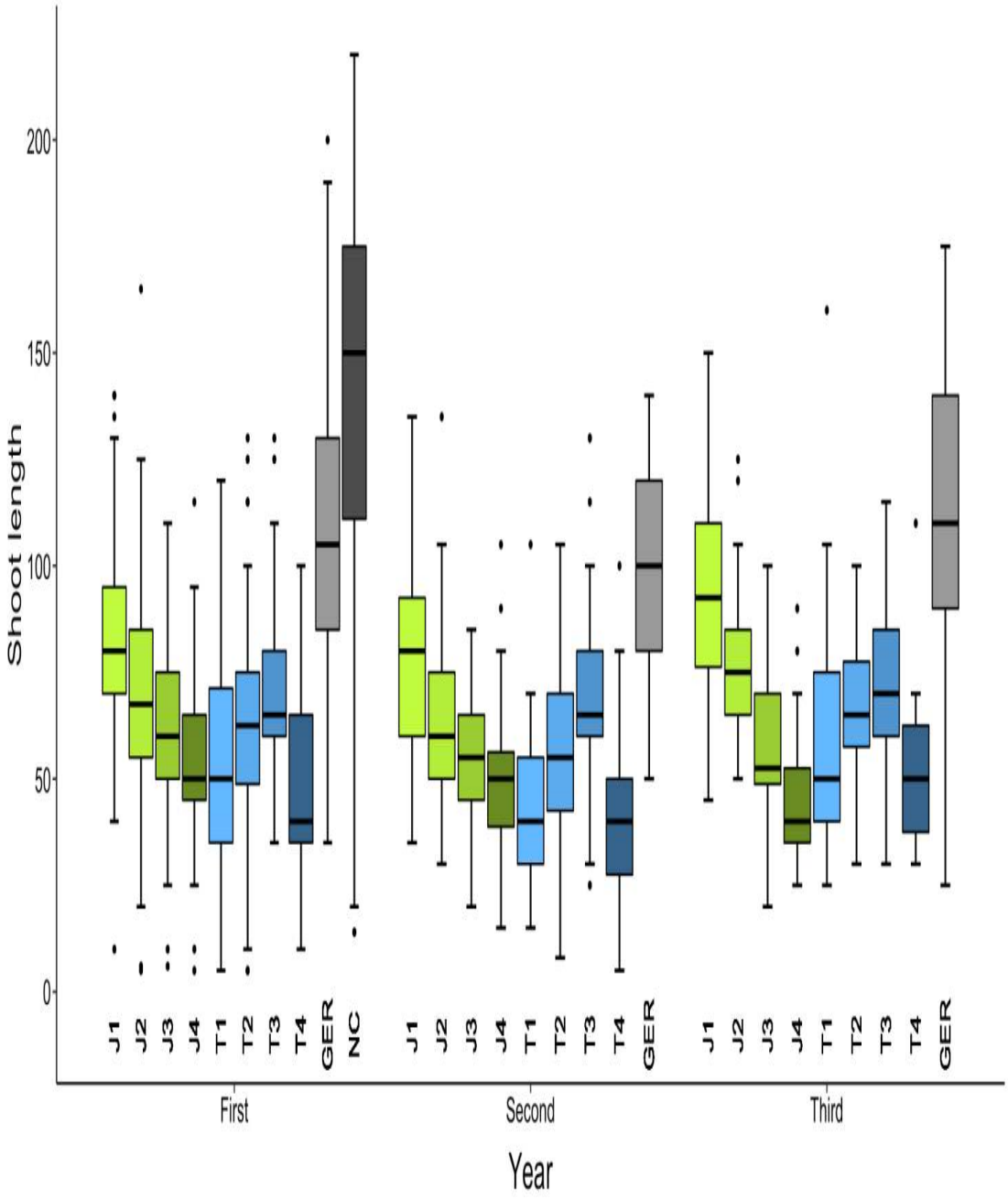

Figure 5. Flowering shoot length at the time of first open flower in the Oulu field. Length in mm is shown in the *y*-axis. Thick line: median, box: upper and lower quartiles, whiskers: ±1.5 interquartile range, dots: outliers.

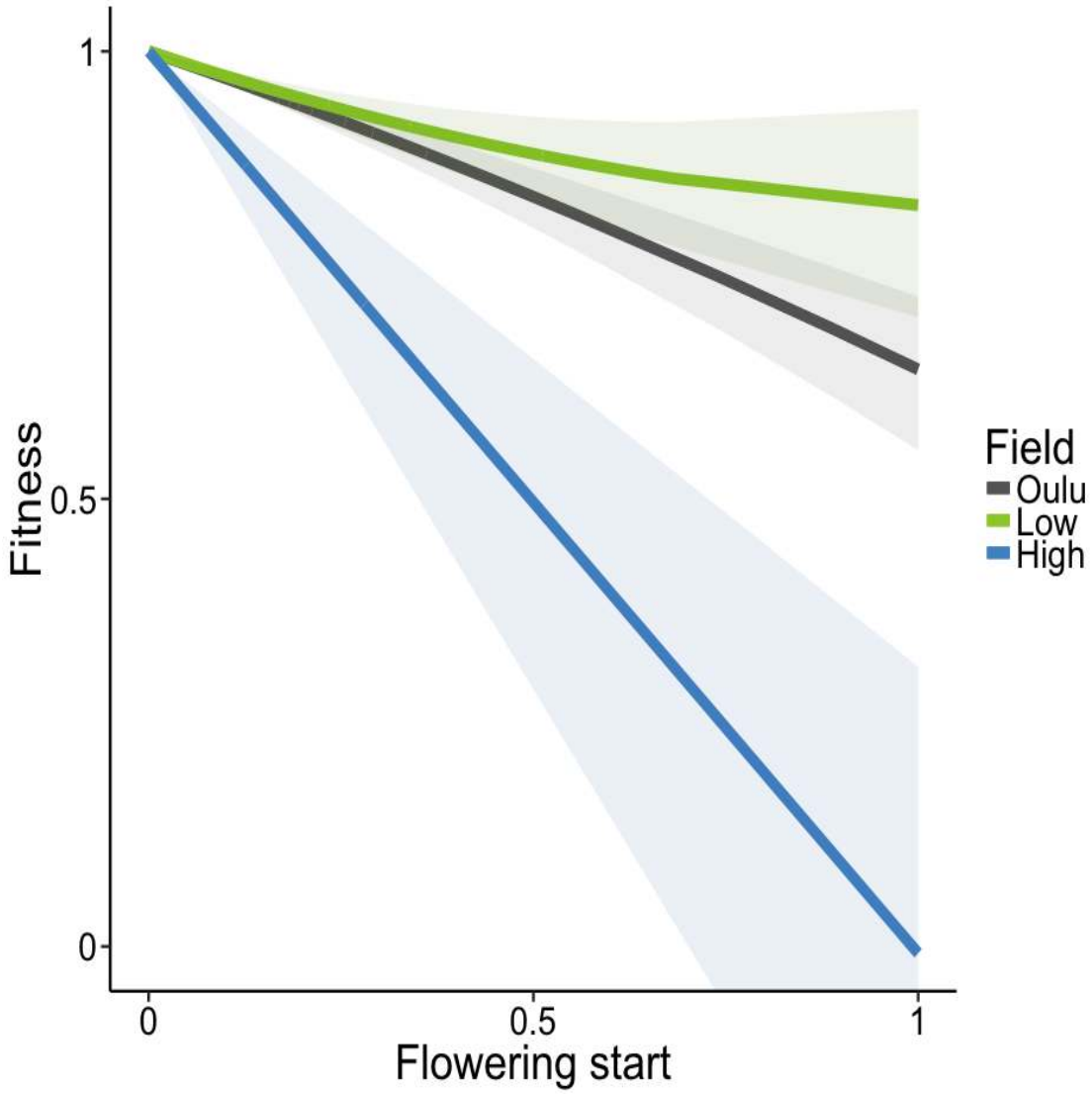

Figure 6. Relationships between average flowering start dates and total three-year fitness at the three field sites. Selection gradients were inferred from flowering propensity and fruit production with aster models. Shaded areas mark the 95% confidence intervals. For distributions of the trait values, see Fig. S12.