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A polygenic architecture with habitat-dependent effects underlies ecological differentiation in \textit{Silene}

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Summary

- Ecological differentiation can drive speciation but it is unclear how the genetic architecture of habitat-dependent fitness contributes to lineage divergence. We investigated the genetic architecture of cumulative flowering, a fitness component, in second-generation hybrids between \textit{Silene dioica} and \textit{Silene latifolia} transplanted into the natural habitat of each species.
- We used reduced-representation sequencing and Bayesian sparse linear mixed models (BSLMMs) to analyze the genetic control of cumulative flowering in each habitat.
- Our results point to a polygenic architecture of cumulative flowering. Allelic effects were mostly beneficial or deleterious in one habitat and neutral in the other. Positive-effect alleles often were derived from the native species, whereas negative-effect alleles, at other loci, tended to originate from the non-native species.
- We conclude that ecological differentiation is governed and maintained by many loci with small, habitat-dependent effects consistent with conditional neutrality. This pattern may result from differences in selection targets in the two habitats and from environmentally dependent deleterious load. Our results further suggest that selection for native alleles and against non-native alleles acts as a barrier to gene flow between species.

Introduction

Adaptation to different habitats can promote divergence and speciation and can mediate population persistence under changing conditions (Nosil, 2012; Savolainen \textit{et al}., 2013). Evolutionary trajectories toward adaptive differentiation depend on the number and effect sizes of loci controlling fitness, on allelic effects in alternative habitats and on gene flow (Savolainen \textit{et al}., 2013; Tigano & Friesen, 2016; Kokko \textit{et al}., 2017). However, despite recent progress in the theoretical understanding of adaptation (Yeaman, 2015; Mee & Yeaman, 2019; Booker \textit{et al}., 2021), empirical studies on the genetic control of fitness in natural habitats remain scarce (Wadgymar \textit{et al}., 2017). Here we provide such a study using two ecologically differentiated, but naturally hybridizing, campions (\textit{Silene}).

Adaptive differentiation often is depicted as a classic cartoon where each population outperforms the other in their habitat but not in the foreign habitat (Kawecki & Ebert, 2004). A similar pattern has been proposed to operate at the genetic level: alleles with positive effects on fitness in one habitat have negative effects in the alternative habitat, termed antagonistic pleiotropy (reviewed in Anderson \textit{et al}., 2011; Savolainen \textit{et al}., 2013). Empirical data, however, suggest that such antagonistic pleiotropy is rare in natural populations, although this could be caused partially by limited statistical power (Anderson \textit{et al}., 2011; Anderson \textit{et al}., 2014; Wadgymar \textit{et al}., 2017). An alternative pattern, conditional neutrality, where alleles have neutral effects in one environment and are under positive or negative selection in the other, may be much more prevalent (Anderson \textit{et al}., 2011; Savolainen \textit{et al}., 2013; Wadgymar \textit{et al}., 2017). In fact, even at the phenotypic level, reciprocal transplant studies often detect a home site advantage (local population outperforming the foreign population) at one of the population’s sites but not at the other’s (Leimu & Fischer, 2008). Importantly, polymorphism at loci with antagonistic pleiotropy for fitness in alternative habitats is expected to be maintained by natural selection and to contribute to population differentiation even with high gene flow, whereas conditionally neutral loci can only lead to transient allele frequency differences in high-gene-flow scenarios (Mitchell-Olds \textit{et al}., 2007; Savolainen \textit{et al}., 2013; Tiffin & Ross-Ibarra, 2014; Mee & Yeaman, 2019; Booker \textit{et al}., 2021). If adaptive allele frequency changes are predominantly transient, even in the presence of consistent selection, prospects will be poor for finding loci underlying adaptive differentiation with commonly used methods for quantifying and comparing genetic differentiation along the genome (i.e. genome scans of differentiation).

A further major determinant of adaptive differentiation concerns the number and effect sizes of the underlying loci. In general, the distribution of effect sizes for complex traits (including...
fitness) is expected to follow a negative exponential distribution (Orr, 1998, 2005) such that adaptation mainly is the result of fitness) is expected to follow a negative exponential distribution (Orr, 1998; Orr, 2005; Rockman, 2012; Savolainen et al., 2013; Boyle et al., 2017; Selby & Willis, 2018). A large number of segregating loci underlying adaptation further promotes transient genetic architectures of adaptation (Yeaman & Whitlock, 2011; Yeaman, 2015; Booker et al., 2021). Polygenic or even omnigenic genetic architectures render identification of all individual loci both practically impossible and undesirable (Rockman, 2012). As effect sizes decrease and the number of loci increases, it becomes progressively more difficult to detect individual genotype–phenotype associations and to distinguish them from spurious associations resulting from linkage. For this reason, promising methods, such as Bayesian sparse linear mixed models (BSLMMs, see the Materials and Methods section), identify genotype–phenotype associations of all loci simultaneously rather than individually, and assume polygenic genetic architectures and remove effects that can be attributed to linkage disequilibrium (Zhou et al., 2013; Gompert et al., 2017). This approach has been successfully applied in studies on adaptive divergence, for example in pine (Lind et al., 2017) and in Arabidopsis (Exposito-Alonso et al., 2019).

In this study, we investigate the genetic basis of differential adaptation in two dioecious sister species of Silene (Caryophyllaceae): S. dioica and S. latifolia. Demographic models indicate that the two species diverged with gene flow within the last 120 000 yr reaching a neutral sequence divergence (D\text{a}, autosomes) of 0.0027 and genetic differentiation, F\text{ST}, of 0.28 (Hu & Filatov, 2015; Liu & Karrenberg, 2018). Both species are widely distributed in Europe where the pink-flowered S. dioica occurs in moister and colder habitats such as meadows, pastures or forests and occupies a wide range of elevations up to > 2300 m above sea level in the Alps, whereas the white-flowered S. latifolia is found on drier, warmer and more disturbed sites such as dry meadows, arable fields or road sites at elevations up to 1000 m asl (Friedrich, 1979; Karrenberg & Favre, 2008). Ecological differentiation and assortative pollination constitute strong reproductive barriers (barriers to gene flow) in this species pair, but the two species are still fully cross-fertile (Goulson & Jerrim, 1997; Karrenberg et al., 2019). Studies in an experimental garden point to complex, genome-wide genetic architectures of traits associated with reproductive barriers in this system, including flower color, flowering phenology, first-year flowering and specific leaf area (Liu & Karrenberg, 2018).

Here we investigated the genetic architecture of adaptation using recombinant second-generation hybrids (F\text{2}) between S. dioica and S. latifolia from a multi-site field transplant experiment conducted for 4 yr (Favre et al., 2017). We included naturally occurring variation in our experiment: within- and between-species crosses were derived from 36 individuals of three populations from each species (Favre et al., 2017). The two species exhibit strong evidence of habitat adaptation; each species outperforms the other in its own habitat in terms of survival and flowering, here combined as cumulative flowering over the experimental period (Fig. 1; for detailed analyses see Favre et al., 2017). We focused on the following questions: (1) What is the genetic architecture underlying differential habitat adaptation? (2) Is antagonistic pleiotropy or conditional neutrality the predominant pattern when comparing allelic effects across habitats? and (3) Are fitness effects associated with allele frequency differences between the two species; for example, are beneficial alleles more likely to be derived from the native species?

**Materials and Methods**

Transplant experiment, measurements and sampling

Second-generation (F\text{2}) hybrids (19 families) between Silene dioica (L.) Clairv. and Silene latifolia Poir., derived from 36 parental individuals (F\text{0}) from three populations of each species (three males and three females from each population), were

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**Fig. 1** Habitat adaptation in campions (Silene): cumulative flowering (number of times flowered over 4 yr, plus one if the plant survived to the end of the experiment), a fitness component, in crosses within each of two campion species, Silene dioica (SD) and Silene latifolia (SL), as well as in their first-generation hybrids (F\text{1D} with SD mother, F\text{1L} with SL mother) and second-generation hybrids (F\text{2}) in the habitat of each species, (a) SD habitat and (b) SL habitat; least square means and 95% confidence intervals are given and different letters indicate significant differences between cross types within each habitat. These data are published in Favre et al. (2017) and re-analyzed here for illustration (see the Introduction and Materials and Methods sections).
transplanted as juveniles into four natural sites in Valais, Switzerland, two in each species’ habitat (Supporting Information Tables S1–S3), as part of a larger experiment with six sites (Favre et al., 2017). Transplant sites in the *S. latifolia* habitat were situated at 646 and 962 m above sea level and those in *S. dioica* habitat at 1248 and 1402 m asl. F2 hybrids were generated by first crossing the two species with each other and intercrossing the resulting F1 hybrids thereafter (Favre et al., 2017; Tables S1–S3). Sites of *S. dioica* were situated at higher altitudes with a colder climate and shorter growing season compared to the *S. latifolia* sites (Table S1). Leaf samples were collected before transplantation and silica-dried; we selected four of the six sites for this study based on availability and quality of leaf samples. Flowering and survival were assessed over 4 yr and cumulative flowering was calculated as the number of years in which an individual flowered plus one if it survived to the end of the experiment, as in Favre et al. (2017) where detailed analyses of survival and flowering in this experiment are provided. We added one to the years flowered if the plant survived to account for potential further flowering events, given that the oldest plants found in natural populations of the two species were between 6 and 9 yr old (unpublished data, using root anatomy). Flower numbers were generally low. In F2 hybrids, flower number ranged from 1.2 (95% confidence interval: 1.1–1.3) in Bodmen to 3.5 (CI 3.1–4.0) in Leuk and the two parental species had similarly low flower numbers (Favre et al., 2017; Supporting Information). More detailed fitness measurements such as seed number and siring success would have been prohibitively laborious and difficult to interpret in our experimental set-up with mostly hybrids. The strength of the fitness component used here (cumulative flowering) is that it integrates mortality and reproduction over 4 yr (Favre et al., 2017). Of the individuals included in this study, only 23% (69 plants) survived to the end of the experiment; of these surviving plants, five had never flowered. We sampled four to six F2 individuals from each of the 19 F2 families at each of the four selected sites (298 F2 individuals in total), striving to include both high- and low-fitness individuals from each family and site. To assess allele frequency in the two species we further included samples of 32 parental F0 individuals and used existing data for the remaining four F0 individuals (Liu & Karrenberg, 2018; Table S3).

For illustration purposes (Fig. 1), we re-analyzed cumulative flowering in the two species and their first- and second-generation hybrids for the four sites used here, (18–36 families per cross type, 5–20 individuals per family, five blocks per site; Favre et al., 2017; Table S2). We used linear mixed models of cumulative flowering in each habitat with cross type as a fixed factor and family and block nested in site as random factors using the lme4 package (Bates et al., 2015) for R v.4.0.2 (R Core Team, 2020). We extracted least square means of cumulative flowering and performed multiple comparisons between cross types within sites with Holm correction of *P*-values in REMMEANS (Lenth, 2020). Cumulative flowering was log(1+y)-transformed to yield normally distributed residuals and improve model fit. Means and standard errors are reported back-transformed to the original scale.

DNA extraction and sequencing

We extracted genomic DNA from silica-dried leaf tissue with a DNase Plant Mini Kit (Qiagen) and quantified DNA using a Qubit dsDNA HS fluorometer (Life Technologies). Double-digest RAD sequencing (ddRAD-seq) libraries were prepared with EcoRI and TaqI restriction enzymes as described in Liu & Karrenberg (2018). After enzymatic digestion, DNA fragments were ligated with barcoded adaptors and size-selected to c. 550 bp (Peterson et al., 2012). In total, eight 48-plex libraries were sequenced on an Illumina HiSeq 2500 system at the SNP&SEQ technology platform of SciLifeLab, Uppsala, Sweden, using 125-bp paired-end chemistry and two libraries per lane. F0 individuals were included in two libraries to achieve higher coverage.

Bioinformatic analysis – Processing of raw reads and variant filtering

The total sequencing output was 1382 838 294 reads for 298 F2 individuals (mean with one SE: 4656 021 ± 233 639 reads) and 461 127 142 reads for 32 F0 individuals (mean 14 410 223 ± 1093 409 reads); data on the remaining four F0 individuals (Table S3) were available from Liu & Karrenberg (2018). We processed the ddRAD-sequence reads following the dDocent pipeline (Puritz et al., 2014). After de-multiplexing of raw reads using STACKS 2.0b (Catchen et al., 2013) and trimming with FASTP (Chen et al., 2018), we used BWA MEM 0.7.17 with default parameters (Li, 2013) to map reads to ddRAD-seq-generated reference contigs, which were assembled previously from eight deeply sequenced individuals of both species and hybrids and corresponded to 95 040 562 bp in total, corresponding to c. 3.4% of the *S. latifolia* genome (Liu & Karrenberg, 2018; Liu et al., 2020). Only a partial genome sequence (one third of the 2.8-Gbp genome) with short scaffolds (*N*50 = 10 785 bp) is currently available for *S. latifolia* (Krasovec et al., 2018).

Variants were called with FreeBayes 1.1.0 (Garrison & Marth, 2012) without population priors using the following parameters: minimum mapping quality 30, minimum base quality 20, maximum complex gap 3, minimum repeat entropy 1, binomial-disequilibrium < 0.05 & PAIRDR/PAIRIR > 0.25, and a map-disequilibrium contigs, which were assembled previously from eight deeply sequenced individuals of both species and hybrids and corresponded to 95 040 562 bp in total, corresponding to c. 3.4% of the *S. latifolia* genome (Liu & Karrenberg, 2018; Liu et al., 2020). Only a partial genome sequence (one third of the 2.8-Gbp genome) with short scaffolds (*N*50 = 10 785 bp) is currently available for *S. latifolia* (Krasovec et al., 2018).

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Variants were called with FreeBayes 1.1.0 (Garrison & Marth, 2012) without population priors using the following parameters: minimum mapping quality 30, minimum base quality 20, maximum complex gap 3, minimum repeat entropy 1, binominal-obs-priors 1, and use-best-n-alleles 10. Variants were filtered following O’Leary et al. (2018): First, VCFTOOLS 0.1.15 (Danecek et al., 2011) was used to retain single nucleotide polymorphism (SNP) sites with a minimum depth of 3, quality of 30, mean depth of 10 and allele count of 3. Second, we used VCFFILTER implemented in vcffib/2017-04-04 (https://github.com/vcffib/vcffib) to retain sites with an allele balance either between 0.25 and 0.75 or < 0.01, a quality/depth ratio of > 0.25, and a mapping quality ratio between 0.9 and 1.05. We further used VCFFILTER to remove loci with differences in read pairing between the alleles (PAIRRED > 0.05 & PAIREDR > 0.05 & PAIRED/ PAIRED < 1.75 & PAIREDR/PAIRED > 0.25)PAIRRED < 0.05 & PAIREDR < 0.05) or with or excessive read depths > 4-fold larger than the median read depth (O’Leary et al., 2018). We reduced the dataset to bi-allelic sites and removed eight F2 individuals with > 99% missing data. SNPs in perfect linkage disequilibrium (r² = 1) within the F2 individuals were reduced
to one SNP using PLINK 1.9 (Purcell et al., 2007, http://pngu.mgh.harvard.edu/purcell/plink/). The dataset, filtered as described above, contained 290 F2 individuals (S. dioica habitat: 134, S. latifolia habitat: 156) and 89 524 loci. Of these, 42 090 loci with both alleles in both habitats and data for > 95 F2 individuals in each habitat were used for further analyses, yielding on average 107 and 127 individuals per locus for the S. dioica and S. latifolia habitat, respectively. These loci had an average read depth of 15.02 ± 0.03 (median 13.83) for the F2 individuals, 36.85 ± 0.08 (median: 33.09) for the 32 F0 individuals sequenced in this study and 41.39 ± 0.17 (median: 36.25) for the four F0 individuals from Liu & Karrenberg (2018). We used genotype probabilities ranging from 0 to 2, calculated from genotype likelihoods (https://github.com/visoca/popgenomeworkshop-gwas_gemma/tree/master/scripts/bcf2bbgeno.pl, accessed 28 January 2019), rather than genotypes (Nielsen et al., 2011). Genotype probabilities of 0 and 2 denote homozygotes for the reference and alternative allele, respectively, whereas 1 indicates heterozygosity. The reference allele in our ddRAD seq reference can be derived from either species (see DNA extraction and sequencing section above).

Genetic structure

We expect that genetic variation within recombinant F2 hybrids families derived from three different populations per species much exceeds variation between families, allowing us to detect species-wide pattern of genetic architecture. To check this assumption (lack of strong family structure) we analyzed the genetic structure in the F2 individuals using principal components analysis (PCA) with the ade4 package (Dray & Dufour, 2007), based on genotype probabilities. For this analysis we reduced the dataset to 220 SNP loci with data available in all 290 F2 individuals used in the genetic association analysis. Results did not differ from analyses on more or all loci with missing values replaced by average genotype probabilities.

Genetic association analysis

We used Bayesian sparse linear mixed models (BSLMM) in GEMMA 0.98.1 (Zhou et al., 2013) to investigate the genetic architecture of cumulative flowering. BSLMMs are a combination of linear mixed models, which assume that every variant has an effect, and Bayesian variable selection regression, which assumes that only a small proportion of the variants has an effect (Zhou et al., 2013). BSLMM analyses were performed separately for each habitat using cumulative flowering values standardized within sites and genotype probabilities. A centered relatedness matrix was used as a covariate to take account of the family structure and the wide cross, according to the standard BSLMM method implemented in GEMMA (Zhou et al., 2013).

We characterized genetic architectures using estimates of the following hyperparameters: the proportion of phenotypic variance explained by all SNPs in the model (PVE); the proportion of PVE explained by SNPs with measurable, nonzero effects on the phenotype, referred to sparse-effect loci (PGE); and the number of the sparse-effect loci (n-γ). For each SNP locus, we estimated the posterior inclusion probability (PIP, the proportion of iterations in which a SNP had a nonzero effect on phenotypic variation) and the effect size of the alternative allele on cumulative flowering. We report both raw effect estimates (β̂), the effect of a locus on the phenotype when it is included in the model, and model-averaged effect estimates (β̄) that take into account the posterior inclusion probability of a locus in the models (Zhou et al., 2013; Gompert et al., 2017). BSLMMs were run five times with 10 000 000 burn-in steps and 40 000 000 iterations with a thinning interval of 10. Convergence of the five runs was checked graphically. Hyperparameters were estimated after combining posterior distributions across runs; PIP, β̂ and β̄ were averaged per locus across runs. A threshold of PIP > 0.01 was used to identify SNPs with sparse effects (Gompert et al., 2013; Comeault et al., 2014).

Allelic effects and allele frequencies

We graphically evaluated whether allelic effects are universal or consistent with antagonistic pleiotropy or conditional neutrality by plotting per-locus effect sizes (β̂ and β̄) in the S. dioica against effect sizes in the S. latifolia habitat. Note that β-values reflect effect estimates of the alternative allele replacing the reference allele. The de novo ddRADseq reference sequences used here contain alleles derived from both species (Liu & Karrenberg, 2018; Liu et al., 2020) and do not inform on allelic origin.

In order to analyze whether allelic origin is associated with fitness effects, we calculated the frequency of the alternative allele (AFalt) in the 18 F0 individuals of each species (Tables S2, S3) for loci that had genotypes for > 12 F0 individuals per species at individual read depths of ≥ 6 (36 161 loci). For each SNP locus, we expressed AFalt as the average genotype probability per species divided by 2. We further estimated the allele frequency difference between species (AFDalt) as AFalt (S. dioica) – AFalt (S. latifolia). AFDalt values thus range from −1 (alternative allele fixed in S. latifolia F0 individuals, and reference allele fixed in S. dioica F0 individuals) to 1 (alternative allele fixed in S. dioica F0 individuals, and reference allele fixed in S. latifolia F0 individuals). We tested whether AFDalt differs between loci with positive and negative effects on cumulative flowering in each habitat using a general permutation test in R/COIN (Hothorn et al., 2008).

Method validation using simulations

We assessed the power of BSLMMs to detect genotype – phenotype associations in our data using a simulation approach similar to that in Gompert et al. (2017, with supplementary R code). Briefly, phenotypes were simulated in a two-step process: First, the specified number (n) of loci with detectable (‘sparse’) effects was randomly selected from all loci, each locus was assigned an effect drawn from the standard normal distribution and the resulting total additive effect of these loci calculated for all individuals based on their observed genotypes at these loci. In a second step, phenotypes for each individual were simulated from these total additive effects plus an additional effect drawn from a
normal distribution with a mean of 0. The standard deviation of this distribution was adjusted such that the loci with simulated effects were expected to account for the proportion of phenotypic variation among individuals specified as heritability, \( h^2 \).

Phenotypes were simulated on the basis of the observed genetic data in 290 individuals for four combinations of heritability (\( h^2 = 0.2 \) or \( h^2 = 0.05 \)) and number of loci with effects on the phenotype (\( n = 10 \) or \( n = 50 \)). We simulated a normally distributed trait for which nine individuals of each family were randomly drawn and reduced the simulated dataset to 150 individuals at random to match the sample size in our data.

For each combination of \( h^2 \) and \( n \), 30 sets of simulated phenotypes were used as input for BSLMMs with 10 000 000 burn-in steps and 40 000 000 iterations. For each simulation, PVE and \( n-\gamma \) were estimated as well as the correlation between the simulated effect size and effect size estimates (\( \hat{\beta} \) and \( \beta \)).

Results

Cumulative flowering and genetic structure

Each species outperformed the other in its own habitat and performed best in its habitat in terms of cumulative flowering, providing strong evidence for habitat adaptation (Fig. 1; Favre et al., 2017). F1 hybrids were intermediate for cumulative flowering and F2 hybrids flowered less often than F1 hybrids on average (Fig. 1; Favre et al., 2017).

The F2 families exhibited extensive genetic variation in the PCA, with clustering of individuals within families but without any other emerging patterns (Fig. S2). For most loci, the alternative allele occurred at low frequency in one or both species, but all combinations of allele frequencies between species were found in the data, including loci with high differentiation where the alternative allele was at high frequency in one species and at the same time absent from the other species (Fig. 2).

Association analysis

The proportion of phenotypic variation explained (PVE) by BSLMMs was 0.12 and 0.10 for the \( S. dioica \) and the \( S. latifolia \) habitat, respectively (medians of posterior distributions, Table 1; posterior distributions are shown in the Figs S3, S4). Less than half of the PVE could be attributed to sparse-effect loci (PGE; Table 1). The number of loci with nonzero effects (\( n-\gamma \)) was estimated to be 11 in the \( S. dioica \) habitat and 16 in the \( S. latifolia \) habitat (Table 1). PGE and \( n-\gamma \) had very wide credible intervals and thus were difficult to estimate, whereas credible intervals of PVE were narrower (Table 1). Median PIP for individual SNPs was 0.001 in both habitats and we detected three loci with PIP > 0.01, one in the \( S. dioica \) habitat and two in the \( S. latifolia \) habitat (Table S4). PIP for individual SNPs could not be summed over genomic windows because no continuous reference genome is available (Krasovec et al., 2018). We used a ddRADseq-generated \textit{de novo} reference in this study (Liu & Karrenberg, 2018; Liu et al., 2020, see the Materials and Methods section).

Allele-specific effects in alternative habitats and origin of alleles

Raw effect size estimates \( \hat{\beta} \) for cumulative flowering (in units of standard deviations, i.e. z-scores) ranged from −0.57 to 0.28 in the \( S. dioica \) habitat and from −0.52 to 0.34 in the \( S. latifolia \) habitat. Positive or negative allelic effects in one habitat (\( \hat{\beta} \gg 0 \) or \( \hat{\beta} \ll 0 \)) were associated with very low \( \hat{\beta} \) values (\( \hat{\beta} \approx 0 \)) in the other habitat, consistent with conditional neutrality (Fig. 3, cross
Table 1 Genetic architecture of cumulative flowering, a fitness component, in recombinant second-generation (F2) hybrids between the campion species *Silene dioica* and *Silene latifolia* in a field transplant experiment at sites of each species.

<table>
<thead>
<tr>
<th>Hyperparameter</th>
<th>PVE</th>
<th>PGE</th>
<th>n-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Silene dioica</em> habitat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.117</td>
<td>0.411</td>
<td>11</td>
</tr>
<tr>
<td>Mean</td>
<td>0.136</td>
<td>0.434</td>
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</tr>
<tr>
<td>90% CI</td>
<td>0.017–0.321</td>
<td>0–0.945</td>
<td>0–205</td>
</tr>
<tr>
<td><em>Silene latifolia</em> habitat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.096</td>
<td>0.411</td>
<td>16</td>
</tr>
<tr>
<td>Mean</td>
<td>0.124</td>
<td>0.431</td>
<td>51.4</td>
</tr>
<tr>
<td>90% CI</td>
<td>0.008–0.337</td>
<td>0–0.938</td>
<td>0–225</td>
</tr>
</tbody>
</table>

Given are medians, means and 90% equal tail credible intervals (90% CI) of hyperparameters estimated in Bayesian Sparse Linear Mixed Models (BSLMMs): PVE, proportion variation explained; PGE, proportion variation explained by sparse effects; n-γ, number of sparse-effect loci. Posterior distributions of hyperparameters are shown in Supporting Information Figs S3, S4.

A single locus showed opposing effects in the two habitats, $\hat{\beta} \ll 0$ in the *S. dioica* habitat and $\hat{\beta} \gg 0$ in the *S. latifolia* habitat, suggestive of antagonistic pleiotropy (Fig. 3). Analyses for model-averaged effect sizes, $\bar{\beta}$, exhibited a very similar pattern (Fig. S5), but with much smaller values due to multiplication with low posterior inclusion probabilities.

A striking pattern in our data is that alleles with effects on cumulative flowering were associated with higher allele frequencies in F0 individuals of the native species, whereas alleles with negative effects on cumulative flowering were more common in the non-native species (Figs 3, S5–S7). In more detail, loci with positive effects of the alternative allele on cumulative flowering in the *S. dioica* habitat had a positive median AFD$_{alt}$ (alternative allele more common in *S. dioica*, red on Fig. 3), that were significantly different from the negative median AFD$_{alt}$ (alternative allele more common in *S. latifolia*, blue on Fig. 3) at negative-effect alleles (Figs S6, S7). In the *S. latifolia* habitat, we observed the reverse pattern: loci with positive effects had negative median AFD$_{alt}$ values that differed significantly from the positive median AFD$_{alt}$ values for negative-effect loci (Figs S6, S7). These patterns were present for both $\hat{\beta}$ and $\bar{\beta}$, when using a subset of loci with absolute values of $\hat{\beta} > 0.1$ and absolute values of $\bar{\beta} > 0.003$; corresponding to loci with effect sizes above the 99.3 and 99.5 percentile for *S. dioica* and *S. latifolia*, respectively (Fig. S6). We obtained similar results when using all loci – negative-effect loci with $\hat{\beta}$ or $\bar{\beta} > 0$ and positive-effect loci with $\hat{\beta}$ or $\bar{\beta} < 0$, Fig. S7).

Alleles with positive effects on cumulative flowering in each habitat ($\hat{\beta} > 0.1$) were rare in the foreign species but occurred at appreciable frequencies in the native species; this effect was stronger in the *S. latifolia* habitat than in the *S. dioica* habitat (Fig. 4). Alleles with negative effects ($\hat{\beta} < -0.1$), by contrast, were rare in the native species but occurred at moderate or high frequencies in the foreign species (Fig. 4). These allele frequency patterns across species differ strongly from the joint allele frequency pattern for all loci (Fig. 2c).

![Fig. 3 Locus-specific effect sizes of the alternative allele on cumulative flowering, a fitness component, measured in recombinant hybrids between two campion species, *Silene dioica* (SD) and *Silene latifolia* (SL) that were transplanted into the habitat of each species (SD habitat, y-axis; SL habitat, x-axis). Given are raw effect estimates, $\hat{\beta}$, from Bayesian sparse linear mixed models (BSLMMs). Allele frequency differences for the alternative allele (AFD$_{alt}$) between the two species (SD–SL) are indicated as a color gradient from blue (allele fixed in SL and absent in SD) to white (equal frequency in both species) to red (allele fixed in SD and absent in SL). Points are plotted in the order of increasing absolute AFD$_{alt}$, such that highly differentiated loci are most visible. Loci with evidence for antagonistic pleiotropy would have $\hat{\beta}$-values of opposite signs in two habitats (top left, and bottom right), whereas loci consistent with conditional neutrality lie at zero on one axis, but deviate substantially from zero on the other.](image-url)
Fig. 4 Frequencies of the alternative allele (AFalt) in two campion species, Silene dioica (SD, y-axis) and Silene latifolia (SL, x-axis) for loci associated with cumulative flowering, a fitness component, in the habitat of each species. Shown are loci with absolute raw effect sizes, $\hat{\beta}$, over 0.1 in Bayesian sparse linear mixed models (BSLMMs) with the number of loci ($n$): $\hat{\beta}_{SD} < -0.1$, loci with negative fitness effects in the SD habitat (top left), $\hat{\beta}_{SD} > 0.1$, loci with positive fitness effects in the SD habitat (top right), $\hat{\beta}_{SL} < -0.1$ loci with negative fitness effects in the SL habitat (bottom left), and $\hat{\beta}_{SL} > 0.1$, loci with positive fitness effects in the SL habitat (bottom right). Allele frequency differences for the alternative allele (AFDalt) between the two species (SD–SL) are indicated as a color gradient from blue (allele fixed in SL and absent in SD) to white (equal frequency in both species) to red (allele fixed in SD and absent in SL). Positive fitness effects are associated with native alleles, whereas alleles with negative fitness effects are more common in the non-native species (see the Results section, and Supporting Information Figs S6, S7).

Method validation

BSLMM estimates of PVE in simulated datasets responded to changes in simulated heritabilities, as expected (Fig. 5). BSLMMs on simulated data with $h^2 = 0.2$ recovered PVE estimates in a similar range, whereas models on simulated data with $h^2 = 0.05$ yielded median PVE estimates near 0.1 (Fig. 5). The number of sparse-effect loci was correctly estimated for simulated datasets with few loci ($n = 10$), but strongly underestimated when the simulated number of loci was high (Fig. 5). Correlations of simulated and estimated $\hat{\beta}$ and $\overline{\beta}$-values were highest for datasets with high simulated heritabilities and declined for datasets with lower heritability and a higher number of sparse-effect loci (Figs 5, S8). In simulations with $h^2 = 0.2$, $\beta$ exhibited higher correlations with simulated effect sizes than $\overline{\beta}$ (Fig. S8).

These simulations thus show that our analyses can recover heritabilities and general patterns in effect size. However, estimates of the number of sparse-effect loci ($n\overline{\gamma}$) must be treated with great caution. We expect that the apparent underestimation of $n\overline{\gamma}$ also decreases posterior inclusion probabilities and thus $\overline{\beta}$.

Discussion

Our results suggest that ecological differentiation between the campions Silene dioica and S. latifolia has a polygenic architecture, based on a reciprocal transplant experiment with recombinant second-generation hybrids between the two species. Many alleles had small effects on cumulative flowering, a fitness component, but only in one of the two habitats; this is consistent with a conditional neutrality pattern. Despite small effect sizes, the direction of allelic effects was consistent with selection for native alleles and against non-native alleles. This suggests that habitat adaptation is maintained by species-specific variants and that habitat-dependent selection on hybrids reduces the inflow of non-native alleles. Below we discuss the implications of these findings for lineage divergence as well as the advantages and limitations of our approach.

Low heritability of a fitness component in the field, based on many small-effect loci

Using Bayesian sparse linear mixed models (BSLMM) we estimated the proportion of variation in cumulative flowering, a fitness component, explained by all single nucleotide polymorphisms (SNPs) (PVE) to be 0.12 and 0.10 for S. dioica and S. latifolia habitats, respectively. This finding suggests that cumulative flowering has low to moderate heritability. Our simulations indicate that such low heritability values can be recovered fairly well with BSLMM analyses, as also reported by Gompert et al. (2017). Similar low-to-moderate heritabilities previously have been found for complex traits, for example for height in Pinus albicaulis (Lind et al., 2017) and in Populus (Bresadola et al., 2019) as well as in Arabidopsis thaliana accessions, where heritability for fitness components varied strongly between experimental sites (Exposito-Alonso et al., 2019). Our analyses suggest a polygenic genetic architecture of cumulative flowering in Silene the number of sparse-effect loci ($n\overline{\gamma}$) estimated to 11 in the S. dioica habitat and to 16 in the S. latifolia habitat; however, wide credible intervals of $n\overline{\gamma}$, as well as our simulations indicate...
that the number of sparse-effect loci in our dataset is difficult to estimate with BSLMM analyses, similar to results from Gompert et al. (2017). In many other systems, oligo- or polygenic control of fitness components was detected in natural settings, especially when the number of contributing loci was explicitly modelled (Lind et al., 2017; Bresadola et al., 2019; Exposito-Alonso et al., 2019). A polygenic genetic architecture of fitness components in our study system does appear likely given that quantitative trait loci (QTL) studies detected many loci distributed throughout the genome that were associated with ecologically relevant traits (Liu & Karrenberg, 2018; Baena-Díaz et al., 2019).

Limitations of studies on the genetic architecture of fitness in natural habitats include the number of individuals and sites that can be studied as well as the genetic resolution in the experimental material. Where markers are used, as in our study, most associations will be the result of linkage to causal variants rather than due to causal variants themselves. This reduces estimates of effect sizes and may lead to a situation where multiple loci linked to the same causal variant are included in alternative BSLMM iterations reducing the posterior inclusion probability (PIP) of each linked locus (Bresadola et al., 2019). We used a highly variable multicross F2 population with a limited number of recombination events, where markers probably were associated with larger genomic regions derived from each species. Recombination and genetic variation included in our material differs markedly from other studies using largely homozygous selfing accessions (Arabidopsis; Exposito-Alonso et al., 2019), within-species crosses or recombinant inbred lines derived from a small number of individuals (Arabidopsis; Fournier-Level et al., 2013; Leinonen et al., 2013; Ågren et al., 2017) or naturally recombinant wild-collected hybrids (Populus; Bresadola et al., 2019). These differences can make it difficult to compare results and model performance across studies. Despite its limitations, our setup has the advantage that the multiple F2 crosses that were transplanted into the natural habitats, are similar to a situation with naturally occurring hybrids upon which habitat-dependent selection acts and this constitutes a reproductive barrier (Karrenberg & Favre, 2008; Favre et al., 2017; Karrenberg et al., 2019).

**Fig. 5** Performance analysis of Bayesian sparse linear mixed models (BSLMM) on simulated data based on a dataset from campions (*Silene*, 150 individuals, 42 090 loci). Simulated phenotypes had heritabilities ($h^2$) of 0.05 or 0.20 due to additive effects of 10 or 50 loci ($n$); 30 replicate simulations per scenario were used and boxplots or violin plots summarize variation in medians of posterior distributions of hyperparameters among simulation replicates as well as of correlations of simulated and estimated per-locus effect sizes. (a) Percentage variation explained by all SNPs (PVE), (b) number of sparse-effect loci ($n_\gamma$) and (c), correlation between raw estimated effect sizes for individual loci $\hat{\beta}_{est}$ and simulated effect sizes $\beta_{sim}$. Boxplots (a, b) and inner boxplots (c) depict median (black horizontal lines), interquartile range (hinges), the range of values within $1.5 \times$ the interquartile range from each hinge (whiskers) and outliers (points). Violins (c) show smoothed probability densities. Red, horizontal lines (a, b) indicate simulated values of $h^2$ and of the number of loci ($n$), respectively.
Allelic effects are consistent with conditional neutrality as the prevailing pattern

Allelic effects mostly converged to a conditional neutrality pattern: both positive and negative allelic effects on cumulative flowering in one habitat were associated with near-zero effects in the other habitat; only one locus deviated from this pattern. This finding is in line with the view that conditional neutrality is more common in near-natural settings than antagonistic pleiotropy, as has been shown in monkeyflowers (Hall et al., 2010) and Arabidopsis (Fournier-Level et al., 2013; Ågren et al., 2017; Exposito-Alonso et al., 2019), switchgrass (Lowry et al., 2019) and Lycaeides butterflies (Gompert et al., 2015). However, strong evidence for conditional neutrality is fundamentally difficult to provide, because this would require evidence for the absence of an effect in one of the habitats (Anderson et al., 2014; Mee & Yeaman, 2019). Interestingly, antagonistic pleiotropy appears to be detected more readily in controlled experimental evolution studies with microorganisms than in natural settings with higher plants or insects (Gompert & Messina, 2016; Bono et al., 2017; Wadgymar et al., 2017; Tusso et al., 2021). This is probably a consequence of the control of selective agents in experiments as opposed to natural sites where both selective agents and traits under selection may vary (Wadgymar et al., 2017). In our system, high-elevation sites of S. dioica exhibited high winter mortality in S. latifolia and in hybrids in comparison to S. dioica (Favre et al., 2017) suggesting that frost tolerance or the regulation of carbohydrate storage could be under selection. At lowland S. latifolia sites, by contrast, S. dioica and hybrids suffer higher summer mortality than the native S. latifolia, most likely as a result of drought exposure (Favre et al., 2017). We therefore find it plausible that phenotypic targets of selection differ between habitats. Such a situation is expected to lead to different suites of genes associated with fitness in each habitat and can thereby contribute to a conditional neutrality pattern of effect sizes for fitness components as observed here.

Fitness is increased by native alleles and decreased by non-native alleles, but not at the same loci

A striking result in our study is the association of allele frequencies in the two species with allelic effects on cumulative flowering, even for loci with very small effects. Alleles with positive effects were rare in the non-native species and common or of intermediate frequency in the native species, especially in the S. latifolia habitat. On the one hand, alleles with positive effects on cumulative flowering in one habitat were neutral in the alternative habitat and thus could easily spread through both species if not lost by drift (Savolainen et al., 2013; Mee & Yeaman, 2019). Alleles with negative effects on cumulative flowering, on the other hand, had intermediate to high frequencies in the non-native species but were rare in the native species. Negative-effect alleles also included numerous variants that were rare in both species, but these loci are most likely linked to causal loci with unknown allele frequencies rather than being causal loci themselves (Fig. 4, left panels). Negative-effect alleles are most readily interpreted as deleterious load that is only exposed to selection in the alternative habitat and rare, conditionally deleterious variants are expected to arise frequently (Orr, 2005). Conditionally deleterious loci may experience reduced gene flow due to negative selection in the alternative habitat and thereby increase between-lineage differentiation (Mee & Yeaman, 2019). However, rare variants with conditionally deleterious effects probably remain rare even in the lineage they arose in and will thus not exhibit high differentiation between lineages. Variants with conditionally neutral effects probably play an important role for adaptation, but they are most certainly under-reported, not only because many of these variants are rare, but also because studies in alternative and relevant environments are needed to detect them.

Implications for hybrid zones and speciation

Our results suggest that selection favors hybrids that are genetically similar to the native species. In early-generation hybrids with still large genomic regions of each species, as in our experiment, selection will favor genomic regions that contain positive-effect alleles that were more often derived from the native species. At the same time, selection will disfavor regions with negative-effect alleles that often were derived from the non-native species. Such variants with habitat-dependent effects thus act as barriers to gene flow in each habitat. In natural hybrid zones between S. dioica and S. latifolia, intermediate individuals are rare and later-generation hybrids are heavily biased toward individuals that are genetically similar to the native species (Minder et al., 2007; Karrenberg & Favre, 2008). Our study suggests that this may be due not only to back-crossing with the native species, but also to ecological selection in each habitat.

Regarding the evolution of adaptive differentiation, our results suggest adaptation through selection on many loci in each species. Alleles with small positive effects had intermediate-to-high frequencies in the native species and low frequencies in the non-native species. This may point to a redundant genetic control of adaptation as also suggested by a QTL study under controlled conditions in our study system (Liu & Karrenberg, 2018) and by studies in other systems, for example in Drosophila (Barghi et al., 2019). Polygenic architectures of adaptation with redundant effects are expected to be transient in time such that variants associated with adaptation in current populations may not have been present or under selection at earlier stages of divergence (Yeaman & Whitlock, 2011; Yeaman, 2015). In addition, different populations of each species may have different realization of polygenic adaptation even if selection pressures are similar. Thus, even when ecological differentiation is a probable driver of divergence, as in our study system (Goulson & Jerri, 1997; Karrenberg & Favre, 2008; Karrenberg et al., 2019), this may not necessarily be manifested in strong, range-wide genetic differentiation at the underlying loci.

Conclusion

Overall, our study adds to the understanding of how ecological differentiation can promote reproductive barriers and lineage
divergence. We detected a polygenic architecture for a fitness component, cumulative flowering, in the campions Silene dioica and S. latifolia using a reciprocal transplant experiment. Allelic effects were mostly beneficial or deleterious in one habitat and neutral or with very small effects in the other. Habitat-dependent beneficial alleles may result from differences in selection targets in the two habitat types, whereas habitat-dependent deleterious alleles probably correspond to deleterious load with effects only under challenging, non-native conditions. In each habitat, alleles with positive effects on cumulative flowering were preferentially derived from the native species, whereas negative-effect alleles were more often derived from the non-native species. This suggests that ecological selection acts as a barrier to gene flow through hybrids in the corresponding genomic regions.

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Author contributions

The study was designed by SK with input by SG, CAB, AF and XL. AF conducted the transplant experiment with help from SK; XL performed molecular laboratory work; XL and SG did bioinformatic analyses; SG performed the association analysis and simulations with help from CAB and SK; SK analyzed phenotypes and genotype probabilities and genotype probabilities for the parental individuals are available on Dryad (doi:10.5061/dryad.4tmpg4fcn).

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Data availability statement

Double-digest RAD (ddRAD) sequencing data is available on NCBI’s Short Read Archive, SRA (accession no. SRP287913, BioProject, https://www.ncbi.nlm.nih.gov/sra/PRJNA669447). The variant call format (VCF) files as well as GEMMA input files

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Principal components analysis including F0 and F2 generations.

Fig. S2 Principal components analysis of F2 families.

Fig. S3 Posterior distributions of hyperparameters, Silene dioica habitat.

Fig. S4 Posterior distributions of hyperparameters, Silene latifolia habitat.

Fig. S5 Model-averaged effect sizes (β) in the Silene dioica habitat, plotted against β in the Silene latifolia habitat.

Fig. S6 Permutation test comparing between-species allele frequency differences among positive- and negative-effect alleles, based on raw effect size estimates (β̂).

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**Fig. S7** Permutation test comparing between-species allele frequency differences among positive- and negative-effect alleles, based on model-averaged effect size estimates ($\bar{\beta}$).

**Fig. S8** Correlations among simulated effects and raw and model-averaged estimates of effect sizes ($\beta$ and $\bar{\beta}$).

**Tables S1** Description of source populations and transplant sites.

**Table S2** Crossing scheme.

**Table S3** Individuals used for crosses.

**Table S4** Sparse-effect loci with posterior inclusion probabilities $P > 0.01$.

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