Complex Genetic Effects on Early Vegetative Development Shape Resource Allocation Differences Between *Arabidopsis lyrata* Populations

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ABSTRACT Costs of reproduction due to resource allocation trade-offs have long been recognized as key forces in life history evolution, but little is known about their functional or genetic basis. *Arabidopsis lyrata*, a perennial relative of the annual model plant *A. thaliana* with a wide climatic distribution, has populations that are strongly diverged in resource allocation. In this study, we evaluated the genetic and functional basis for variation in resource allocation in a reciprocal transplant experiment, using four *A. lyrata* populations and F₂ progeny from a cross between North Carolina (NC) and Norway parents, which had the most divergent resource allocation patterns. Local alleles at quantitative trait loci (QTL) at a North Carolina field site increased reproductive output while reducing vegetative growth. These QTL had little overlap with flowering date QTL. Structural equation models incorporating QTL genotypes and traits indicated that resource allocation differences result primarily from QTL effects on early vegetative growth patterns, with cascading effects on later vegetative and reproductive development. At a Norway field site, North Carolina alleles at some of the same QTL regions reduced survival and reproductive output components, but these effects were not associated with resource allocation trade-offs in the Norway environment. Our results indicate that resource allocation in perennial plants may involve important adaptive mechanisms largely independent of flowering time. Moreover, the contributions of resource allocation QTL to local adaptation appear to result from their effects on developmental timing and its interaction with environmental constraints, and not from simple models of reproductive costs.

DIFFERENTIAL allocation of resources to current reproduction vs. continued growth and maintenance is a central feature of life history differences among organisms (Stearns 1992; Roff and Fairbairn 2007). Reproduction is widely recognized as costly, leading to trade-offs between investment in reproduction vs. growth and maintenance and ultimately survival (Bell 1980; Reznick 1985; Lovett Doust 1989). Optimal resource allocation strategies are likely to vary by environment due to differential effects of allocation on growth rates, survival, and fecundity, thus leading to selection for different allocation strategies in different envi-

ronments (Williams 1966; Charnov and Schaffer 1973; Bell 1980; Reznick 1985; Biere 1995; Johnson 2007).

Among iteroparous organisms, which must allocate some resources to somatic maintenance in order to survive to reproduce multiple times, a wide range of proportional investments in growth and maintenance vs. reproduction is possible (van Noordwijk and De Jong 1986). Genetic variation in traits subject to trade-offs results in what has been termed “structured pleiotropy,” in which genetic covariances between traits result from functional constraints imposed by the limiting resources (De Jong 1990; Stearns *et al.* 1991; see Figure 1B). However, differences in resource acquisition and hierarchical allocation of resources over the course of development can mask trade-offs by generating positive correlations between growth and reproduction (van Noordwijk and De Jong 1986; Houle 1991; Worley *et al.* 2003; Björklund 2004; see Figure 1A). Moreover, little is known about the genotype-to-phenotype basis for resource allocation trade-offs (Harshman and Zera 2007). For example, are the genetic mechanisms of resource allocation simple switches that

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measured, but the model in C would result in detection of QTL affecting net winter diameter growth, because increases in net winter diameter growth (i.e., less rosette branching) result in more reproductive shoots and reduced net reproductive season diameter growth (compound arrows).

shuttle resources down tracks of reproductive vs. somatic development, or are more complex developmental processes involved (Diggle 1999; Obeso 2002; Harshman and Zera 2007)?

The most straightforward scenario for divergent evolution of resource allocation strategies is simply for survival costs of reproduction and/or number of offspring per unit of reproductive investment to vary across environments (Williams 1966; Bell 1980; Obeso 2002). In this case, the contrasting effects of quantitative trait loci (QTL) on vegetative vs. reproductive growth may have similar direction and relative magnitude in each environment, but the consequences for survival or absolute fecundity would be different. However, genotypes may also differ in plasticity of their resource-allocation effects across environments (Sultan 2000; Sultan and Spencer 2002), resulting in QTL with environment-specific effects. Trade-off patterns might also differ between environments that have different resource limitations, altering the structured pleiotropic pattern of QTL effects in contrasting environments. Finally, complex interactions between genes, development, and environment might be responsible for fitness in different environments, and costs of reproduction may be largely indirect consequences of these interactions (Obeso 2002; Harshman and Zera 2007).

Two related questions surround the nature of resource allocation in plants. First, are the limiting resources subject to trade-offs physiological or meristematic? Trade-offs are typically understood to involve allocation of limited physiological resources (energetic and/or nutrient) to biomass

production among tissues (Rathcke and Lacey 1985; Biere 1995; Arntz *et al.* 1998; Koelewijn 2004; Jongejans *et al.* 2006; Figure 1B). However, trade-offs may also occur due to differential allocation of meristems to vegetative growth, reproduction, or dormancy (Lovett Doust 1989; Geber 1990; Bonser and Aarssen 1996, 2006; Huber and During 2000; Kim and Donohue 2012). Vegetative or dormant meristems can later become reproductive, but commitment to reproductive growth is generally irreversible. Thus, iteroparous plants, which undergo multiple cycles of reproduction, need to have some meristems that remain vegetative beyond the first growing season (Thomas *et al.* 2000; Munné-Bosch 2008). Bonser and Aarssen (2006) found that herbaceous iteroparous species had greater apical dominance (i.e., repression of lateral shoot growth by an active main shoot) than semelparous relatives, which die after a single reproductive episode. In other cases, however, reduced apical dominance prior to flowering has been found to favor iteroparity by increasing the number of lateral shoots committed to vegetative rather than reproductive fates (Wang *et al.* 2009; Kim and Donohue 2011, 2012; see Figure 1C). Semelparous and iteroparous plants can also differ quantitatively in physiological processes regulating rates of leaf senescence (Thomas *et al.* 2000; Munné-Bosch 2008). However, the extent to which quantitative variation in meristem fate allocation vs. physiological processes explains variation in resource allocation within iteroparous species remains largely unknown.

Second, are flowering time genes responsible for differences in resource allocation? Genes that regulate flowering

time variation, including *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*), have pleiotropic effects on traits involved in reproductive age–size trade-offs in the semelparous *Arabidopsis thaliana* (McKay *et al.* 2003; Baker *et al.* 2005; Callahan *et al.* 2005; Scarcelli *et al.* 2007; Wilczek *et al.* 2009). Few studies have addressed the role of flowering time genes vs. other processes in resource allocation in iteroparous perennial plants (Karrenberg and Widmer 2008), but some recent studies indicate that flowering time genes may have important roles in regulating perennality and fall growth cessation (Böhlenius *et al.* 2006; Wang *et al.* 2009; Lowry and Willis 2010; Hsu *et al.* 2011).

Arabidopsis lyrata (L.) O’Kane and Al-Shehbaz, a perennial iteroparous relative of *A. thaliana*, provides an ideal system to study the genome-to-phenotype processes that give rise to variation in resource allocation strategies. Based on our unpublished observations, the perennial habit in *A. lyrata* is associated with the presence of indeterminate axillary vegetative shoots within the rosette, which persist into subsequent growing seasons, and that resource allocation to vegetative vs. reproductive growth differs widely among populations. Vegetative rosette branching is associated with a transition from broad rosettes dominated by large primary-shoot leaves to more compact branched rosettes with smaller leaves. *A. lyrata* has a wide but highly fragmented distribution across Europe, Asia, and North America, and occurs in environments that range from subarctic and alpine to warm temperate in climate. *A. lyrata* also exhibits strong patterns of phenotypic variation between populations in flowering time (Riihimäki and Savolainen 2004; Riihimäki *et al.* 2005) and fitness components (Kuittinen *et al.* 2008; Leinonen *et al.* 2009, 2011). The complete genome sequence of *A. lyrata* has now been published (Hu *et al.* 2011) and genetic and chromosomal synteny maps have been developed (Kuittinen *et al.* 2004; Schranz *et al.* 2006), greatly facilitating application of the extensive genomic resources and functional information available in *A. thaliana* to *A. lyrata*.

A set of recent studies has used multiple European and North American *A. lyrata* populations and interpopulation crosses in reciprocal transplant experiments to investigate the genetics of adaptation. We found evidence for local fitness advantage in European populations (Leinonen *et al.* 2009) and in populations from contrasting Norway and North Carolina (NC) environments (Leinonen *et al.* 2011). In both studies, differences between environments in the contribution of different fitness components (flowering propensity, inflorescence and fruit production, and survival) to overall fitness variation were responsible for local adaptation. We also characterized the differential QTL effects underlying differences in fitness components and flowering time of the Norway vs. North Carolina populations, using F_2 crosses between these populations planted in both environments (Leinonen *et al.* 2013). In this article, we dissect the genetic and functional basis for differences in resource allocation between these populations, expanding on the pre-

vious analyses by including vegetative growth data from the same F_2 progeny in the two parental environments. Our basic approach was to use QTL mapping for multiple traits in conjunction with structural equation modeling to evaluate genetic variation in resource allocation in a developmental context.

The objectives of this study were to address the following questions: (a) Do resource allocation trade-offs between vegetative growth and reproductive output observed at the population level in North Carolina reflect trade-offs in effects of individual QTL? (b) Do flowering time QTL affect resource allocation? (c) Do QTL coordinately affect resource allocation through effects on “upstream” traits in developmental networks, which are then transmitted through the network? (d) To what extent do developmental models based on variation in prereproductive resource acquisition, allocation of limited physiological resources, or allocation of meristems to vegetative branching vs. reproduction explain patterns of trait variation? (See Figure 1 for a detailed description of these models.) (e) Do differential costs of survival associated with resource allocation QTL explain the contrasting effects on fitness in Norway vs. North Carolina? The results provide insights on the genetic and developmental nature of variation in plant resource allocation and its relationship with fitness in contrasting environments.

Materials and Methods

Study system

A. lyrata is a perennial rosette plant in the mustard family (Brassicaceae), consisting of two recognized subspecies; ssp. *lyrata* occurs in North America from the Great Lakes region south into the southern Appalachian mountains and adjacent foothills, and ssp. *petraea* occurs across northern Eurasia and Alaska (Schmickl *et al.* 2010). Interpopulation crosses we have tested between populations of ssp. *lyrata* and ssp. *petraea*, including those used in this study, show no evidence of hybrid breakdown in fitness in F_1 or outcross F_2 offspring (Leinonen *et al.* 2011). However, F_1 and F_2 plants show reduction in pollen fertility and some F_2 progeny show cytoplasmic male sterility (Leppälä and Savolainen 2011). *A. lyrata* seeds typically germinate in late summer or fall, and plants overwinter as vegetative rosettes before flowering the following spring. Like *A. thaliana*, the apical meristem of the primary shoot in *A. lyrata* usually undergoes the initial transition to a reproductive shoot, followed by a variable number of axillary meristems. However, some axillary meristems in *A. lyrata* also develop as vegetative shoots, either prior to or after the start of flowering, and at least a subset of these vegetative shoots remain indeterminate until the following growing season. Reproductive shoots vary from unbranched to highly branched, producing variable number of flowers and, after fertilization, fruits (siliques) containing ≥ 10 –40 seeds.

Plant material

Seeds were collected from *A. lyrata* populations at Spiterstulen, Norway (61° 38'N, 8° 24'E, 1106 meters above sea level, m.a.s.l.), Plech, Germany (49° 39'N, 11° 29'E, 400 m.a.s.l.), Ithaca, NY (42°22'N, 76°21'W, 420 m.a.s.l.), Chena River, Alaska (AK) (64°55'N, 146°17'W), and Mayodan, NC (36°25'N, 79°58'W, 225 m.a.s.l.; collected by Charles Langley). Plants from field-collected seed were grown and crossed within populations to generate full-sib families. In addition, two crosses were made between different Spiterstulen and Mayodan plants to generate two unrelated F_1 families. To obtain F_1 plants with different cytonuclear genomes for reciprocal crosses, the Spiterstulen plant was used as the female parent for one of these crosses (Sp1 $\times$ Ma1) and the Mayodan plant was used as the female parent for the other (Ma2 $\times$ Sp2). Single F_1 plants from each family were crossed reciprocally to generate two out-cross F_2 families that differ only in their cytoplasmic origin. These crosses are the same as those described previously (Leinonen *et al.* 2011, 2013; Gove *et al.* 2012). Data from the two F_2 reciprocals are combined for all analyses reported in this article.

Field study design

Establishment of the North Carolina and Norway field study sites has been described in detail elsewhere (Leinonen *et al.* 2011, 2013). However, in this article we include data for vegetative and seed-mass traits not addressed in the previous studies, except for comparison with simulated data in a theoretical study of developmental networks (Gove *et al.* 2012). We also include data for additional populations planted at the North Carolina site. Briefly, seeds from the within-population families, F_1 and F_2 crosses for the North Carolina site, were sown in peat-vermiculite growing mix in September 2005, corresponding to the litter and decomposed duff in which *A. lyrata* typically grow amid the rock outcrops in their natural environment in North Carolina. Seedlings were grown in growth chambers at the University of North Carolina at Greensboro for ~2 months, during which day lengths and temperatures were gradually lowered to acclimatize the plants for late fall field conditions. In November 2005, plants with their surrounding growing media were transplanted to a field site at the North Carolina A&T State University Farm in Greensboro (44 km SE of the site of the Mayodan population), where they were planted in eight replicated blocks at a 30 $\times$ 30 cm spacing within each block. This study could not be established at the site from which the Mayodan population originated because that site is on steep slopes with large rock outcrops. The two sites have similar climates, but the soil at the planting site was finer textured and much less rocky than the Mayodan site. A total of 131 Spiterstulen plants from 6 full-sib families, 87 Plech plants from 4 families, 156 Ithaca plants from 10 families, 86 Mayodan plants from 5 families, 9 F_1 plants, and 397 F_2 plants were planted. Plants from each family and

cross were distributed as evenly as possible across the eight blocks and were located randomly within blocks.

Establishment of the Norway field site at Spiterstulen was similar to that of the North Carolina site. However, seeds were sown in a mixture of commercial planting soil and sand from the local area, corresponding to soil texture in the Norway environment. Seedlings were initially grown in a greenhouse in Lom, Norway, and field planting was done in June 2005 at a closer 10 $\times$ 10 cm spacing due to the much slower growth of plants in the Norway environment. A total of 479 F_2 plants were planted in Norway from the same crosses used for the North Carolina planting, as described previously (Leinonen *et al.* 2011, 2013). As in North Carolina, these plants were evenly divided across eight blocks along with plants from full-sib families from Spiterstulen, Mayodan, and two other populations, and were located randomly within blocks.

Trait measurements

To characterize patterns of resource allocation, traits describing allocation of resources to sexual reproduction and vegetative growth and maintenance were measured. In North Carolina, survival was recorded at the time of outplanting in November 2005, near the time of first flowering in early March 2006 and in late June 2006 when reproduction was nearly complete. At these three times, vegetative growth was also evaluated by measuring rosette diameters as the largest distance between live leaf tips (fall diameter, spring diameter, and postreproductive diameter, respectively). The respective differences between consecutive measurements were used to calculate the differences between spring and fall diameter (net winter diameter growth) and between postreproductive and spring diameter (net reproductive season diameter growth). Plants were inspected for flowering two to three times per week, and the date at which flowering was first observed was recorded for each plant. In late June, reproductive output traits were evaluated when reproductive shoot development was largely complete. The number of reproductive shoots was counted for each flowering plant. The number of fruits (siliques) produced per shoot was estimated using the mean of a sample of three representative shoots for plants with more than five reproductive shoots, and using the mean of all reproductive shoots otherwise. Six to 12 ripe siliques were collected from each plant to estimate the average number of seeds per silique and the mean mass per 100 seeds. Total seed production was calculated as the product of reproductive shoots, siliques per shoot, and seeds per silique. Finally, survival was also recorded in November 2006. Due to heavy mortality later in the summer and fall, most likely a result of nematode infection and seasonal drought stress, only a single season of data was collected from the North Carolina site.

Mean values for spring diameter, reproductive shoots, and siliques per shoot were calculated separately for each block at the North Carolina site. The block mean spring

diameter was used as a measure of block productivity in subsequent analyses.

Field measurements in Norway were generally similar to those conducted in North Carolina. Due to the much lower reproductive output in Norway, a complete count of siliques was made to calculate the average number of siliques per shoot. Mean seed mass was not estimated in Norway. Data have been collected for five field seasons (2006–2010) at the Norway site, but we use data only for the first field season and survival to the following year.

Analysis of resource allocation patterns

All statistical analyses were conducted using the R computing environment (R Development Core Team 2008). We used mixed models implemented in the lmer function in R to estimate and test the significance of population differences in vegetative and reproductive traits used to evaluate resource allocation (see [Supporting Information, File S1](#)). Principal components (PC) analyses on six traits (spring rosette diameter, net reproductive season diameter growth, total reproductive shoots, siliques per shoot, seeds per silique, and mass per 100 seeds) were conducted on the samples from the four populations combined and separately on the F₂ family in the North Carolina data, using the prcomp function in R. Principal components analyses were also conducted on the first-year F₂ data from the Norway data, except that mass per 100 seeds (which was not scored in Norway) was not included.

QTL analysis

QTL analysis on the F₂ progeny was conducted using the qtl package in R (Broman *et al.* 2003), augmented by customized linear models developed using the lm and glm functions in R. Genetic markers for 60 SNPs, cleaved amplified polymorphic sequence markers, and microsatellite loci from a previously developed linkage map (Leppälä and Savolainen 2011) spanning the length of all eight *A. lyrata* chromosomes (linkage groups 1–8, LG1–LG8) were scored in 361 F₂ progeny from the North Carolina field site and 446 F₂ progeny from the Norway site, as described previously (Leinonen *et al.* 2013). We used the scanone function in the qtl package for interval mapping to identify QTL regions with genome-wide significance for individual resource allocation traits, the first principal component (PC1) estimated from the F₂ data, and date of initial flowering. QTL effects were estimated at 2-cM intervals using Haley–Knott regression (Haley and Knott 1992). Genome-wide empirical significance thresholds ($P = 0.05$) were estimated by using scanone to do permutation tests, with 1000 permutations run for each trait (Churchill and Doerge 1994). We adjusted flowering date by subtracting the date of earliest flowering and taking the square root of the difference to achieve a more normal residual distribution and obtain genome-wide QTL significance thresholds that were similar to those of the other traits. Untransformed values were used for QTL mapping on all other traits. Block mean productivity (mean spring rosette diameter) was used

Table 1 Summary of principal components analyses

	NC populations	NC F ₂	Norway F ₂
PC1 standard deviation ^a	1.610	1.524	1.494
Coefficients ^b			
SpD	0.574	0.584	0.625
RS	0.453	0.431	0.520
SilS	0.429	0.437	0.184
SdSil	0.057	0.178	−0.028
SdMass	−0.051	0.016	NA ^c
dDr	−0.525	−0.501	−0.552

^a Square root of PC1 eigenvalue, from the prcomp package in R.

^b Abbreviations: SpD, spring diameter; RS, reproductive shoots; SilS, siliques per shoot; SdSil, seeds per silique; SdMass, seed mass; and dDr, net reproductive season diameter growth.

^c Seed mass was not measured in Norway. The principal components analysis for Norway data included only the remaining five traits.

as an additive covariate in all analyses. We analyzed QTL effects as outcross F₂ data, estimating additive effects, dominance, and heterogeneity between the heterozygote classes (*a*, *d*, and *i*, respectively) for each QTL peak (see [File S1](#)). At each QTL peak location that was significant or nearly significant at the genome-wide level for one of the measured traits or PC1, we evaluated resource allocation patterns by also estimating effects for each of the other scored vegetative and reproductive traits. These latter comparisons test specific individual regions for effects expected under models of coordinated QTL effects on resource allocation, so their significance was evaluated at single-comparison rather than genome-wide levels.

Structural equation modeling

Structural equation modeling (SEM) was implemented for the F₂ progeny using the sem package in R (Fox 2006), to evaluate causal models that could potentially explain multiple-trait QTL effects. SEM tests the extent to which a model of cause–effect relationships explains covariances between a set of variables; in this case QTL genotypes and traits involved in resource allocation. This approach is conceptually similar to systems genetics techniques used to evaluate networks of gene expression QTL (eQTL) at a single point in time (reviewed in Rockman 2008; Mackay *et al.* 2009; Li *et al.* 2010) but is also applicable to analysis of QTL for developmentally related traits expressed over time (Li *et al.* 2006; Remington 2009; Gove *et al.* 2012). Variance–covariance matrices were estimated for fall diameter, net winter diameter growth, net reproductive season diameter growth, number of reproductive shoots, siliques per shoot, the *a*, *d*, and *i* terms of each QTL peak location, and block mean productivity using the R cov function. An iterative process was used to add and delete QTL-to-trait paths and trait-to-trait paths leading from traits expressed earlier in development to later traits, to obtain best-fitting models for each environment ([File S1](#)).

To evaluate whether the data are consistent with the hypothesis that net winter diameter growth acts as a surrogate for vegetative rosette branching (model shown in Figure 1C),

we also tested a model with a latent variable (“branching,” placed in quotes to indicate the trait is unmeasured) upstream of all measured traits except fall diameter. We tested models with paths from all QTL to “branching” and to all of the measured traits except net reproductive season diameter growth; from “branching” to the measured traits (in place of paths from net winter diameter growth); and additional trait-to-trait paths corresponding closely to traits from the best-fitting models without the latent variable.

All marker data, trait data, and scripts used for data analysis have been deposited in the Dryad Repository: <http://dx.doi.org/10.5061/dryad.1k4gq>.

Results

A. lyrata populations show contrasting resource allocation patterns

The four *A. lyrata* populations planted in the fall of 2005 at the North Carolina field site showed distinct patterns of trait values during the spring–summer 2006 reproductive season that suggested genetically based differences in resource allocation. The local Mayodan population had the largest spring diameters and highest numbers of reproductive shoots and siliques per shoot on average, followed in order by Ithaca, Plech, and Spiterstulen for all three traits (Figure 2, A–D). However, the rank order was reversed for net reproductive season diameter growth, with Mayodan plants showing the greatest diameter reduction while Spiterstulen plants showed net growth. Plants from the four populations could be readily distinguished visually on the basis of these resource allocation patterns, especially when comparing Mayodan and Spiterstulen plants (Figure 3). Most Mayodan plants showed large crowns of flowering shoots but nearly complete senescence of the vegetative rosette during the reproductive season, with very little growth of new leaves. By contrast, Spiterstulen plants from Norway had few or in many cases no reproductive shoots, while the rosettes remained vigorous. Ithaca and Plech plants tended to have intermediate phenotypic patterns. F_2 plants from the Spiterstulen–Mayodan crosses showed a broad range of phenotypes, but mean values were intermediate to those of the parental populations (Figure 3; Leinonen *et al.* 2011). The contrasting resource allocation patterns had little effect on survival. All populations showed high survival through the flowering season but experienced heavy mortality in the later summer and fall, ranging from ~60% mortality for Plech plants to >90% for Spiterstulen plants.

Principal components analyses produced similar results in the set of four populations and the set of F_2 plants. In both analyses, PC1 represented very similar trade-offs between spring diameter, reproductive shoots, and siliques per shoot vs. net reproductive season diameter growth. The two seed traits had minimal contributions to PC1 (Table 1). The magnitude of the trade-offs was also very similar in the population and F_2 samples, with PC1 explaining 43.2 and 38.7% of

Figure 2 Resource allocation patterns for *A. lyrata* plants from four populations planted in North Carolina. Mean values of vegetative and reproductive traits (± 2 SE): (A) spring diameter (in millimeters); (B) number of reproductive shoots; (C) number of siliques per shoot; and (D) net reproductive season diameter growth (in millimeters).

the total variance, respectively. Moreover, both PC1 patterns were highly similar to the pattern of differences between the parental populations (Figure 2, A–D). These results are consistent with the pattern we expected to find if the differences between populations are primarily due to genetic trade-offs, and not separate sets of genes regulating variation in vegetative and reproductive traits. The small PC1 coefficients of the two seed traits indicate that their variation is largely independent of these trade-off processes.

Variation in spring diameter showed evidence of both resource acquisition and shoot architecture components (models in Figure 1, A and C, respectively). Spring diameter

Figure 3 Contrasts in resource allocation patterns. Photos show typical Spiterstulen (top left) and Mayodan *A. lyrata* plants (bottom right; vegetation near base of plant is a separate plant of a different species), and three F_2 plants (bottom) growing at the North Carolina field site in June 2006.

varied significantly by block at the North Carolina field site, indicating productivity-related variation in resource acquisition. However, populations showed heterogeneous patterns of preflowering rosette diameter changes, with Mayodan plants showing substantial overwinter growth while Spiterstulen plants showed net diameter losses during this period. This suggested that developmental factors contributed to overwinter diameter changes (Figure 4; see also Figure S1 and File S2).

Individual QTL coordinately regulate resource allocation variation

We previously reported that Mayodan alleles at several QTL regions increased reproductive output components in the North Carolina environment, contributing to local adaptation (Leinonen *et al.* 2013), but did not examine the effects of QTL on vegetative growth in that analysis. Given the evidence for genetic variation in resource allocation described above, we predicted that QTL analysis of the Spiterstulen–Mayodan F_2 progeny would reveal chromosomal regions that coordinately affected vegetative and reproductive traits in patterns similar to the overall trade-offs represented by PC1. We also predicted that the direction of QTL effects would be consistent with the Mayodan vs. Spiterstulen differences in resource allocation patterns if divergent selection has shaped trait evolution.

QTL analysis of F_2 plants at the North Carolina site identified QTL regions with genome-wide significance for one or more of the traits subject to resource allocation trade-offs on *A. lyrata* LG 1, 2, and 4, and additional putative QTL regions approaching genome-wide significance on LG6 and LG8 (Figure 5A). On LG4, QTL effects are apparent along the entire length of the chromosome, and thus probably represent the effects of two or more QTL. The QTL region on LG2 had effects with genome-wide significance on all four traits, all in the same direction as the between-population trait

differences. Due to the QTL effects on both the number of flowering shoots and siliques per shoot, Mayodan homozygotes in the LG2 region had more than double the total seed production of Spiterstulen homozygotes (Figure S2, Figure S3). The other four QTL regions also had additive effects on multiple traits in the expected directions that were significant at least at single-comparison levels (Figure 6). All five QTL regions also showed effects with at least single-comparison significance on PC1, providing further evidence of coordinate effects of QTL on resource allocation. In each case, Mayodan alleles shifted resource allocation toward more reproductive growth, consistent with the hypothesis that increased investment in reproduction was under directional selection in the North Carolina environment (Figure 6).

QTL effects were largely additive for the LG1, LG2, LG6, and LG8 QTL regions (data not shown). By contrast, most QTL effects along the length of LG4 appeared to be segregating from just one of the two F_1 parents, with significant trait value differences between the two heterozygous genotypes, suggesting that genetic variation within either the Spiterstulen or Mayodan population is responsible.

We found that different QTL regions affected preflowering development by different mechanisms. The large-effect LG2 QTL affected only net winter diameter growth (Figure 5B), during the period when populations showed heterogeneous patterns of shoot architecture changes (Figure 4 and File S2). Mayodan homozygotes increased their rosette diameters an average of 20 mm over the winter, while Spiterstulen homozygotes had an average net diameter loss of 5 mm, consistent with the parental population differences. In contrast, the LG1 QTL region exclusively affected fall diameter, which largely reflected overall differences in growth rate, and thus was most likely related to initial resource acquisition. Mayodan homozygotes in the LG1 QTL region were on average 16 mm larger than Spiterstulen homozygotes, the opposite of the parental population differences during this

period (Figure 4). The bottom of LG4 showed a third pattern, with the Mayodan alleles reducing fall diameter and also increasing winter diameter growth, both consistent with the parental population differences. The fall diameter effects on LG4 segregated from both F_1 parents but the effects on net winter diameter growth segregated from only one parent (data not shown), providing further evidence that the QTL effects on LG4 involve multiple genes.

We found little overlap between QTL for flowering time and resource allocation. Mayodan plants had flowered significantly earlier than Spiterstulen plants in North Carolina, and three QTL regions with genome-wide significance for flowering date were identified, as described previously (Leinonen *et al.* 2013). We had expected flowering time QTL to have pervasive pleiotropic effects on resource allocation (see Introduction), but only one (on LG6) overlapped with resource allocation QTL regions (Figure 5C). The LG6 QTL region contains two important flowering time regulatory genes, *FLOWERING LOCUS C (FLC)* and *CONSTANS (CO)*. A region on LG8 had nearly genome-wide significance for flowering time and overlapped with the suggestive resource allocation QTL region on the same linkage group, but showed a different pattern of genetic effects. The LG8 effects on flowering date were largely due to early flowering of one of the two heterozygous genotypes, in contrast to the largely additive pattern of resource allocation effects, suggesting that different genes are probably involved. The resource allocation QTL on LG 1, 2, and 4 showed no evidence of effects on flowering time.

Resource allocation QTL also did not affect survival in North Carolina. In spite of the heavy mortality in the summer and fall after the reproductive period, no QTL were detected for survival either in the spring or the fall following the first reproductive season in North Carolina.

QTL modulate resource allocation via effects on early growth patterns

We next used SEM to investigate the functional basis for resource allocation trade-offs and QTL effects on trade-off patterns at the North Carolina site.

A SEM that included causal paths from all QTL to all traits, but no trait-to-trait paths, resulted in a poor fit to the trait covariance structure ($\chi^2 = 372.32$, 10 d.f., $P < 0.0001$; see File S3). Thus genetic trade-offs could not be explained strictly by QTL that act independently on each of the affected traits.

The SEM resulting in the best fit to the data [Akaike Information Criterion (AIC) = 230.34, 46 d.f., $P = 0.061$; Figure 7A] indicated that trade-offs occurred primarily due to large and contrasting direct effects of preflowering diameter growth on subsequent reproductive growth (especially the number of reproductive shoots) vs. vegetative growth. Greater fall diameter and greater net winter diameter growth both led to substantial increases in reproductive shoots, while greatly reducing net reproductive season diameter growth. The effects of both fall diameter and net

Figure 4 Patterns of heterogeneity among populations in net rosette growth before and during the reproductive season. Mean rosette diameters (± 2 SE) for each population at the North Carolina site in the fall at the time of planting (November 2005), spring (near the start of flowering, March 2006), and at the end of the reproductive season (June 2006). One or more populations showed net diameter reductions in the overwinter period and reproductive season, suggesting that developmental changes contributed to both spring and postreproductive rosette diameters.

winter diameter growth on net reproductive season diameter growth were the opposite of those predicted under the resource acquisition model (Figure 1A). However, the strong contrasting effects of net winter diameter growth on reproductive shoots and net reproductive season diameter growth were consistent with the effects predicted by the rosette branching model (Figure 1C). Greater numbers of reproductive shoots increased both siliques per shoot and net reproductive season diameter growth, which were the opposite of what was predicted under the physiological allocation model (Figure 1B). Greater net winter diameter growth did increase the number of siliques per shoot, which was predicted only under the resource acquisition model, though these effects were weak. More siliques per shoot led to reduced net reproductive-season diameter growth, also consistent with the physiological allocation model, but these effects were also weak. Greater fall diameter decreased net winter diameter growth, which was not specifically predicted under any of the models.

Block productivity had significant direct effects on net winter diameter growth, controlling in part for potentially confounding environmental effects on resource acquisition. Block productivity also had significant effects on siliques per shoot.

Four of the five QTL regions had substantial effects on preflowering development (fall diameter and/or net winter diameter growth), partially explaining the coordinated QTL effects on resource allocation. Each QTL region had significant direct effects on a different combination of traits, providing

Figure 5 QTL mapping results for the North Carolina field site. (A) LOD profiles for vegetative and reproductive traits: spring diameter (blue), reproductive shoots (red), siliques per shoot (green), and net reproductive season diameter growth (orange). (B) LOD profiles for early rosette development. Spring diameter is partitioned into fall diameter (blue) and net winter diameter growth (red). (C) LOD profiles for square root-transformed flowering date (per Leinonen *et al.* 2013). Horizontal lines represent genome-wide $P = 0.05$ significance thresholds.

further evidence that different QTL affect resource allocation via different mechanisms. Interestingly, the only QTL with direct effects on reproductive shoots was the LG6 QTL region, which also affected flowering time. The LG2 region in particular had strong effects on early development, with its largest path coefficient directed toward net winter diameter growth. The largest effects of the LG2 QTL region on reproductive shoots and net reproductive season diameter growth occurred through indirect paths, as a result of the QTL effects on net winter diameter growth. The LG1, LG4, and LG8 QTL regions had their largest direct effects on fall diameter, siliques per shoot, and net reproductive season diameter growth, respectively.

We next added a latent variable (“branching”) to examine more specifically whether coordinated QTL effects might be due to effects on rosette vegetative branching (Figure 1C). In the best-fitting latent-variable SEM ($AIC = -45.55$, 39 d.f., $P = 0.76$), all five of the QTL regions had their largest standardized effects on “branching” or on fall diameter, with Mayodan alleles reducing “branching” in each case (Figure 7B). None of the QTL regions had direct effects on net winter diameter growth in the latent variable model, and all five QTL regions had larger effects on “branching” than they had on net winter diameter growth in the SEM with only the observed traits. Nearly all effects of the LG2 QTL region in particular were explained by its effects on “branching.” However, three of the other four QTL regions still had direct effects on at least one observed trait that were larger than their indirect effects transmitted through “branching.” Effects of “branching” on the observed traits were mostly as predicted under the lateral rosette branching model (Figure 1C). However, increases in “branching” also led to fewer siliques per shoot in the latent-variable SEM, which was not predicted.

Resource allocation QTL have contrasting effects in Norway

A. lyrata from the same F_2 family, along with its parental populations and two other populations, showed different phenotypic patterns from those in North Carolina when grown at a field site in Norway (Figure S4). Productivity was much lower in the cool, short growing season in Norway. Mean first-season spring rosette diameters of F_2 plants at the Norway site were 22 mm, barely one-quarter of the mean of 82 mm in North Carolina, and mean values for reproductive traits were also three- to sixfold higher in North Carolina than in Norway. The Mayodan population showed >90% mortality after the first flowering season in Norway, but survival of the other populations was $\geq 60\%$ (Leinonen *et al.* 2011). The two parental populations showed less consistent differences in reproductive traits in Norway, with Mayodan plants having fewer siliques per shoot than Spiterstulen plants (Leinonen *et al.* 2011). In contrast with the North Carolina field site, mean trait values for F_2 plants were equal to or greater than those of the higher-value parental population for several traits (Leinonen *et al.* 2011). Thus, patterns of phenotypic variation representing resource allocation were not readily apparent at the population level (Figure S4).

Nevertheless, principal components analysis of the F_2 plants in Norway showed trade-off patterns similar to those found in North Carolina, except that the coefficient for siliques per shoot was somewhat smaller (Table 1). In contrast with the North Carolina data, however, there was little evidence that traits involved in resource allocation were coordinately regulated by QTL (Figure 8A). The QTL region with the largest effect was located on LG2, corresponding closely to the LG2 resource allocation QTL region found in North Carolina, but effects were limited to siliques per shoot and net reproductive season diameter growth. Moreover,

Figure 6 Coordinated patterns of QTL effects on resource allocation. Additive coefficient estimates (± 2 SE) of Mayodan resource allocation QTL alleles for PC1, spring diameter (SpD), number of reproductive shoots (RS), siliques per shoot (SiLS), and net reproductive-season diameter growth (dDr) in North Carolina, in standard error units.

Mayodan LG2 alleles reduced both the number of siliques per shoot and net reproductive-season diameter growth rather than showing trade-off effects as in North Carolina. A region on LG8 corresponding to the suggestive resource allocation QTL region in North Carolina also had nearly significant effects on siliques per shoot at a genome-wide level, with Spiterstulen alleles again increasing rather than decreasing the trait value. QTL regions on LG1 (in a different location than the LG1 QTL detected in North Carolina) and LG7 also affected one or more resource allocation traits, though effects of the LG1 QTL were largely due to overdominance rather than additive effects of Mayodan vs. Spiterstulen alleles. When we analyzed QTL effects on fall diameter and net winter diameter growth separately (Figure 8B), the LG7 and LG8 regions had significant effects on fall diameter, with Spiterstulen alleles increasing the rosette size in each case. No significant QTL effects were detected for net winter diameter growth.

QTL for flowering time in Norway were detected on LG1 and LG3, with the former coinciding with the LG1 resource allocation QTL detected in North Carolina (Figure 8C; Leinonen *et al.* 2013). In both cases, Norway alleles contributed additively to earlier flowering, consistent with the population differences detected in Norway (Leinonen *et al.* 2013).

Two QTL regions, on LG1 and LG8, explained much of the cumulative survival variation in each year following the first reproductive season (Figure 8D; Leinonen *et al.* 2013). These regions corresponded to the LG1 and LG8 resource allocation QTL regions in North Carolina. The LG8 region also coincided with the QTL region affecting siliques per

shoot in Norway. Thus, rather than representing a trade-off between reproduction and survival, the QTL effects of the LG8 region involved reduction in both aspects of fitness in the Mayodan homozygotes in Norway.

In spite of the lack of coordinate QTL effects on resource allocation, the best-fitting SEM for Norway (AIC = -28.67 , 31 d.f., $P = 0.35$) showed trait-to-trait paths similar in many respects to the North Carolina model (Figure 9A). However, the QTL effects in the Norway model were directed largely toward reproductive season traits, explaining the lack of coordinated QTL effects on resource allocation in Norway. None of the four QTL in the Norway model had direct effects on net winter diameter growth, in sharp contrast to North Carolina, even though the same QTL regions on LG2 and LG8 were included. The direction of QTL effects also contrasted with the North Carolina data, as Mayodan alleles consistently reduced fall diameter and siliques per shoot in Norway. In the latent-variable SEM (AIC = -32.62 , 29 d.f., $P = 0.66$), “branching” was highly redundant with net winter diameter change, and no QTL had significant effects on “branching” (Figure 9B). The Norway SEM also showed more evidence of physiological limitations. More reproductive shoots led to fewer siliques per shoot, as predicted by the physiological allocation model (Figure 1B). Increases in fall diameter led to significantly more siliques per shoot in Norway, as predicted by the resource acquisition model (Figure 1A), although this path was not significant in the latent-variable SEM. The unexpected positive effects of reproductive shoots on net reproductive season diameter growth found in North Carolina were not detected in Norway.

Figure 8 QTL mapping results for the Norway field site. LOD profiles are shown for (A) vegetative and reproductive traits: spring diameter (blue), number of reproductive shoots (red), siliques per shoot (green), and net reproductive season diameter growth (orange); (B) fall diameter (blue) and net winter diameter growth (red); (C) adjusted flowering date; and (D) survival to year 2. Shaded boxes show location of resource allocation QTL detected in North Carolina.

in the network (Li *et al.* 2006; Remington 2009). The extent to which QTL effects on “branching” in the latent-variable SEM accounted for effects on downstream traits in North Carolina is consistent with the latter explanation. However, future fine-mapping studies will be required to distinguish pleiotropic vs. linked QTL and verify these interpretations about the developmental effects of individual QTL.

In both environments, SEMs indicated that resource allocation trade-offs were largely due to large and contrasting effects of preflowering development on vegetative vs. reproductive growth during the reproductive period. These effects cannot easily be explained by variation in resource acquisition, which presumably would have had positive effects both on subsequent vegetative and reproductive growth (Figure 1A; van Noordwijk and De Jong 1986; Houle 1991). Neither were trade-offs the result of a simple “switching” mechanism, involving variation in the investment of physiological resources in reproductive growth (Figure 1B). Had that been the case, SEMs would have revealed strong negative paths from

reproductive traits to net reproductive-season diameter growth, which we did not find.

Instead, the data are consistent with a model in which greater vegetative development of axillary meristems prior to flowering (*i.e.*, weaker apical dominance) partially precludes subsequent reproductive development (Figure 1C). This model is similar in many respects to that described for the perennial *Arabis alpina* (Wang *et al.* 2009). A negative relationship between prereproductive branching and the proportion of shoots developing reproductively has also been reported in *Erysimum capitatum* (Kim and Donohue 2011, 2012) and *Mimulus guttatus* (Baker *et al.* 2012). However, meristem allocation alone cannot explain why preflowering development affected siliques per shoot in addition to the number of reproductive shoots, nor why fall rosette growth prior to changes in rosette architecture affected subsequent development. One possible explanation for the latter observation is that the negative paths from fall diameter to the two later stages of rosette diameter growth might reflect inherent developmental limitations on the size of rosette leaves under a given set of environmental conditions. Thus, plants that approach maximum diameters early might tend to grow less than smaller plants during the subsequent stages.

Resource allocation largely independent of flowering time regulation

Surprisingly, flowering time genes showed only minor effects on resource allocation patterns. The largest-effect resource allocation QTL in North Carolina on LG2, which resulted in twofold differences in reproductive output, had no detectable effect on flowering time in either environment. QTL regions on LG4 and LG8 also lacked additive effects on flowering time. The one clear overlap between flowering time and resource allocation QTL at the North Carolina site was on LG6, in a region containing the flowering time regulatory genes *CO* and *FLC*. *FLC* in particular has been found to have broadly pleiotropic effects on other aspects of development in *A. thaliana* (McKay *et al.* 2003; Scarcelli *et al.* 2007; Wilczek *et al.* 2009; Willmann and Poethig 2011). SEM analysis indicated that the LG6 region had direct effects on the number of reproductive shoots. Thus, the early-flowering effects of Mayodan alleles in this region (Leinonen *et al.* 2013), may have committed more axillary meristems to reproductive fates. A second North Carolina resource allocation QTL region (on LG1) affected flowering time only in Norway.

Shifts in developmental timing may explain local adaptation

Even though some of the same QTL regions affecting resource allocation in North Carolina affected survival in Norway, these effects cannot be explained as costs of reproduction. Mayodan alleles on LG1 and LG8 increased reproductive output and reduced vegetative growth in North Carolina without affecting survival. Mayodan alleles in the same regions did reduce survival in Norway, but did not increase reproductive investment in that environment. Thus, the

Figure 9 Best-fitting structural equation models for Norway QTL data. (A) SEM using observed traits only. (B) SEM including latent “branching” variable. Standardized trait-to-trait path coefficients are shown, with solid blue arrows representing positive path coefficients and broken red arrows representing negative path coefficients. Standardized coefficients of QTL-to-trait paths represent the sign of the additive effects of Mayodan alleles. Width of arrows is proportional to standardized path coefficients. Block mean productivity was included in the latent-variable model to control for common-environment effects.

contribution of these QTL regions to local adaptation must involve gene–environment interactions more complex than those predicted by trade-off theory alone (e.g., Bell 1980; Reznick 1985; Lovett Doust 1989; Biere 1995).

One possible explanation for these QTL is that Mayodan alleles delay growth cessation, which would be likely to delay fall dormancy and reduce survival in the short Norway growing season (P. H. Leinonen, H. Kuittinen, U. Kemi, B. Quilot-Turion, A. Okuloff, A. M. Baker, T. Mitchell-Olds, D. L. Remington, and O. Savolainen, unpublished results). However, extending the growth period might be advantageous in the much longer North Carolina growing season, because it would allow more time for reproductive development to occur.

Mayodan alleles at another QTL region, on LG2, greatly increased allocation to reproductive output at the expense of vegetative growth in North Carolina, but reduced the number of siliques per shoot in Norway. SEMs indicated that this QTL region in particular had large effects on early vegetative development in North Carolina, possibly by affecting the timing of lateral shoot development, but its effects were delayed in Norway (compare Figure 7B and Figure 9B). Plants at the Norway site would have been completely dormant due to snow cover and subfreezing temperatures during the winter period when genetic variation for lateral shoot development was expressed in North Carolina. Thus, the timing of QTL effects on lateral shoot development might have been shifted to later in the season, after flowering had started, in the cold Norway environment. If this were the case, Mayodan alleles that delay lateral shoot development until the start of flowering in North Carolina would increase reproductive output by favoring reproductive rather than vegetative fates for lateral

meristems. However, the same Mayodan alleles would reduce fitness in Norway by delaying reproductive development, thus reducing the time available for flowering and silique production in the short Norway growing season. Variation in the relative timing of vegetative vs. reproductive development has been shown to have pervasive effects on plant life history and morphological evolution (Diggle 1999). However, more detailed developmental studies of QTL effects will be needed to validate the scenarios we propose here.

Our results provide insights into the developmental genetic basis for local adaptation in *A. lyrata*. We previously found that fitness advantages of local populations in these environments were due primarily to much higher reproductive output of the Mayodan population in North Carolina and much higher survival and silique production for the Spiterstulen population in Norway (Leinonen *et al.* 2011). We found no survival advantage in North Carolina for plants with more reproductively conservative Spiterstulen alleles at resource allocation QTL, which may explain why the Mayodan population has evolved toward greater reproductive investment. Any fitness advantages of increased photosynthetic tissue in more conservative genotypes could be negated by the costs of additional transpirational demand under hot summer conditions in the southeastern United States. Reduced lateral vegetative shoot production has been shown to increase juvenile survival under droughty conditions in the rosette plant *E. capitatum* (Kim and Donohue 2012).

Even if the QTL detected in North Carolina and Norway involve different genes, the resource allocation QTL found in North Carolina are still likely to be locally adaptive. The two populations have migrated separately from their presumed

origins in central Europe and Russia to Scandinavia and North America (Koch and Matschinger 2007; Ross-Ibarra *et al.* 2008; Schmickl *et al.* 2010). Allelic variants that arose in either subset of populations would not have been tested by natural selection in the other environment. It has been suggested that evolution of the annual life history in *A. thaliana* may have allowed it to expand its range into hotter and more severe climates (Mitchell-Olds and Schmitt 2006). Our results indicate that populations along the southern extreme of the North American range of *A. lyrata* could be evolving toward semelparity in a parallel process, as indicated by the high investment in reproductive growth at the expense of vegetative development in the Mayodan population.

Our data from North Carolina are limited to a single year, during which temperature and precipitation patterns were fairly typical. The relatively fine-textured soils at the North Carolina planting site differed from the rocky sites on which *A. lyrata* typically grows, which could have influenced growth and survival patterns. However, heavy summer mortality is also observed some years in natural populations (D. Remington, unpublished data). Patterns from subsequent reproductive seasons in Norway were largely consistent with the first-season data reported here, suggesting that trade-off patterns in the first year are indicative of trends over the *A. lyrata* lifespan.

Functional basis of resource allocation QTL

As discussed above, we found evidence that QTL affected resource allocation at least in part via effects on rosette architecture. Basipetal auxin transport from shoot apical meristems is an important mechanism by which plants maintain apical dominance, thus regulating lateral shoot development. The LG2 QTL region, which had the largest QTL effects in both environments and appeared largely to affect apical dominance, is syntenic to the region from At1g68060–74600 in *A. thaliana*. This region contains the *A. lyrata* orthologs of genes encoding two auxin efflux carriers, PIN1 (At1g73590) and PIN3 (At1g70940), and a recently identified regulator of intracellular auxin homeostasis, PILS2 (At1g71090) (Barbez *et al.* 2012). The PIN1 protein in particular is known to function in shoots to regulate auxin gradients and lateral organ development (Gälweiler *et al.* 1998; Vieten *et al.* 2005; Prusinkiewicz *et al.* 2009). Mutations affecting auxin transport can have pervasive effects on shoot development patterns and not just shoot initiation (Gälweiler *et al.* 1998; Friml *et al.* 2002; Brooker *et al.* 2003; Prusinkiewicz *et al.* 2009; Barbez *et al.* 2012), possibly explaining both the effects of QTL and the “branching” variable on the number of siliques per shoot identified in SEMs.

The large effects of LG1 and LG8 QTL regions on Norway survival suggest that the underlying genes may regulate fall growth cessation and dormancy. Both QTL regions contain genes encoding important circadian clock regulators, and models for their fitness effects in different climates and photoperiods have been evaluated in a separate study (P. H. Leinonen, H. Kuittinen, U. Kemi, B. Quilot-Turion, A. Okuloff,

A. M. Baker, T. Mitchell-Olds, D. L. Remington, and O. Savolainen, unpublished results).

Conclusions

Three key insights about the genetics of plant resource allocation emerge from our results. First, genes with effects unrelated to flowering time can have major effects on resource allocation patterns, representing largely uncharacterized genetic mechanisms that warrant more detailed investigation. Second, these genes may not directly control resource allocation “switchpoints” but instead regulate early meristem development processes, which have cascading effects on resource allocation through developmental networks. Third, complex interactions of these developmental networks with local environmental constraints, and not direct costs of reproduction, may be largely responsible for local adaptation. Because development in *A. lyrata* is typical of the iteroparous syndrome described for flowering plants (Thomas *et al.* 2000; Munné-Bosch 2008), resource allocation mechanisms identified in *A. lyrata* are likely to provide insights into adaptive evolutionary processes in plants more broadly.

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Complex Genetic Effects on Early Vegetative Development Shape Resource Allocation Differences Between *Arabidopsis lyrata* Populations

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Figure S1 Phenotypic plasticity of populations to variation in block productivity. Plasticity of vegetative and reproductive traits to variation in block productivity, measured as mean block spring diameter, in North Carolina for Spiterstulen, Mayodan, and F₂ populations. (A) PC1 ($P < 0.001$ for population effects on plasticity); (B) spring diameter ($P < 0.001$); (C) reproductive shoots (with square root transformation, $P = 0.0077$); (D) siliques per shoot (with square root transformation, $P = 0.0020$); and (e) net reproductive season diameter growth ($P < 0.001$). Main effects of block productivity were highly significant for all traits ($P < 0.001$). Estimates are from a mixed model, with population and block productivity treated as fixed effects, and family (or F₂ reciprocal) as random effects.

Figure S2 Phenotypic plasticity of LG2 QTL region to variation in block productivity. Plasticity of vegetative and reproductive traits to variation in block productivity, measured as mean block spring diameter, in North Carolina for genotypic classes in the LG2 QTL region: **(A)** PC1 ($P < 0.017$ for QTL effects on plasticity); **(B)** siliques per shoot ($P = 0.035$); and **(C)** net reproductive season diameter growth ($P = 0.038$). QTL genotype probabilities for each F_2 individual were estimated in R/qtl, and coefficients for Spiterstulen homozygotes (red), Mayodan homozygotes (green), and the two heterozygous genotype classes (blue and orange) were estimated from QTL models with block productivity included as an interactive covariate with QTL genotype, using fitqtl with Haley-Knott regression. QTL genotype effects on the slope of the response were significant for each trait ($P < 0.05$).

Figure S3 Reproductive output effects of LG2 QTL region in North Carolina. Mean total reproductive output for Spiterstulen homozygotes, heterozygotes, and Mayodan homozygotes at the marker closest to the LG2 QTL peak at the North Carolina field site. Reproductive output was calculated as reproductive shoots x siliques per shoot x seeds per silique.

Figure S4 Distributions for vegetative and reproductive traits in Norway. Boxplots show the phenotypic distributions for spring diameter (mm), number of reproductive shoots, siliques per shoot, and net reproductive season diameter growth (mm) for the Norway field site. Groups are F₁ plants (F₁S and F₁M) and F₂ plants (F₂S and F₂M) with Spiterstulen and Mayodan cytoplasm, respectively; and population samples from Spiterstulen (S), Mayodan (M), Ithaca (I), and Chena River, Alaska USA (C).

Supplemental Methods

Phenotypic plasticity

Differences in phenotypic plasticity between the Mayodan, Spiterstulen and F_2 populations were tested using mixed-effects models in the lmer function from the lme4 package in R. We used block mean spring rosette diameter as measure of block productivity, and tested models in which the productivity covariate was either nested within population (separate slopes and intercepts model) or estimated as a non-nested effect separate from population (single slope, separate intercepts model). A significant likelihood-ratio test for these two models indicated significant differences in the slope of the productivity regression and hence significant differences among populations in plasticity. A likelihood-ratio test comparing the single slope, separate intercepts model with a model lacking the productivity covariate was used to test for overall phenotypic plasticity across populations. For these analyses, full-sib family (or reciprocal in the case of the F_2 population) was included as a random effect. Values of reproductive shoots and siliques per shoot were square-root transformed to improve the residual normality and homoscedasticity of the data.

Outcross F_2 analysis of QTL data

Due to sequence variation both within and between populations in our outcross design, markers segregated in multiple configurations, including fully-informative, F_2 configuration at the population level, and backcross configuration (i.e. informative for only one F_1 parent). R/qtl treats marker-trait data from outcross designs as 4-way data, with four possible genotypic classes at each locus (M_1M_2 , S_1M_2 , M_1S_2 , and S_1S_2), representing Mayodan (M) and Spiterstulen (S) alleles from the two different F_1 parents (M_1S_1 and M_2S_2). Deviations from the mean of the local homozygous class were used to estimate the genotypic effects of the other three classes. To estimate the effects of detected QTL on individual traits, we used the calc.genoprob function to generate pseudomarkers at 2-cM intervals throughout the genome. In the 4-way cross setting, pseudomarker scores consist of the genotype probabilities for the M_1M_2 , S_1M_2 , M_1S_2 , and S_1S_2 classes, on which the phenotypic effects are regressed.

To analyze these data as an outcross F_2 design involving parental crosses between two populations, we reparameterized the R/qtl output to estimate a , d , and i coefficients, which estimate additive effects of Mayodan vs. Spiterstulen homozygotes, dominance effects of Mayodan/Spiterstulen heterozygotes, and deviations of the two heterozygous classes from their mean, respectively. In order to analyze phenotypic effects of QTL in terms of the Mayodan vs. Spiterstulen

allelic origins, we reparameterized pseudomarkers at QTL peak locations by partitioning them orthogonally into additive ($a = [p(M_1M_2) - p(S_1S_2)]$), dominance ($d = \frac{1}{2}[p(S_1M_2) + p(M_1S_2) - p(M_1M_2) - p(S_1S_2)]$), and heterogeneity ($i = [p(S_1M_2) - p(M_1S_2)]$) terms. We then used the `lm` function in R (or the `glm` function for binary traits) to estimate the a , d , and i coefficients. We also tested for block effects and phenotypic plasticity of QTL effects in an analysis similar to the one used with the population data as described above. We compared models in which block mean productivity was included as an interactive covariate with the QTL genotype (separate slopes and intercepts for each genotype), was included as an additive covariate only (single slope with separate intercepts), or was excluded from the model. We developed customized R functions to conduct these analyses.

Since the correlation structure among the vegetative and reproductive traits was suggestive of resource allocation trade-offs, and thus structured pleiotropy for genes associated with these traits, we considered pleiotropy to be highly probable at the QTL level. Thus, if a QTL location had genome-wide significance for any one trait or for PC1, we estimated its effects on each of the other traits. We considered these effects to be significant if an interval of twice the standard error around the estimate of a did not include zero and the deviation was in the expected direction, since these are tests of single loci and no longer genome-wide tests.

Structural equation model optimization

To search for optimal structural equation models in each of the two field environments, we started with a null model containing only QTL genotype-to-trait paths. We then added causal paths from traits expressed earlier in development to later traits, one path at a time. We retained the path that improved the model fit the most (based on Akaike's Information Criterion, AIC) at each step until no further improvement was obtained or until there was only one residual degree of freedom in the model. We then removed all QTL-to-trait paths for which the P -values of the a , d , and i terms were all greater than 0.05, and retested the model to verify that the AIC value was lower and/or the p -value for model fit was higher. To control for possible confounding effects of environmental covariation, we also tested the significance of paths from block mean productivity to traits, and retained productivity-to-trait paths that were significant. A model P -value greater than 0.05 was interpreted as evidence that the model adequately explained the covariance structure of the marker-trait data. We also examined the model goodness-of-fit and adjusted goodness-of-fit indices (GFI and AGFI), which are analogous to R^2 and adjusted R^2 values, respectively, in linear models. The signs and sizes of standardized path coefficients were used to compare estimated path effects with those predicted under the three cause-effect models described in Figure 1.

SEM accommodates the use of latent variables, estimated as factors of the correlation structure of observed variables, to represent unobserved variables that may underlie relationships among observed traits. To evaluate whether the

data are consistent with the hypothesis that net winter diameter growth acts as a surrogate for vegetative rosette branching (model shown in Figure 1c), we also tested a model with a latent variable (“branching”, placed in quotes to indicate the trait is unmeasured) upstream of all measured traits except fall diameter. We tested models with paths from all QTL to “branching” and to all of the measured traits except net reproductive season diameter growth; from “branching” to the measured traits (in place of paths from net winter diameter growth); and additional trait-to-trait paths corresponding closely to traits from the best-fitting models without the latent variable. Degrees-of-freedom and structural limitations constrained the number and locations of paths that could be estimated simultaneously. Paths were added and deleted one at a time to obtain the best-fitting estimable model. Finally, non-significant QTL effects were removed, and additional paths from QTL to net reproductive season diameter growth were tested for significance.

We treated all exogenous variables (those strictly upstream of other traits), including block mean productivity, in the SEM as fixed effects without an error variance. Treating exogenous variables as random effects instead would add residual degrees of freedom to the model. With random-effects QTL genotypes, however, covariances between QTL genotypes (including the a , d , and i coefficients for individual QTL) would contribute to model fit, but these covariances are not of interest in the models and have expected values of zero for unlinked QTL lacking segregation distortion. This in turn would reduce the contribution of the relevant parameters (QTL-trait and trait-trait path coefficients) to the overall model fit and inflate the estimated fit of tested models. In practice, we have found that parameter estimates and relative fit of different models are very similar under both approaches. Consequently, we have preferred to use the more conservative estimates of model fit obtained with fixed exogenous variables.

Supplemental Results

Rosette growth rates and growth patterns both affect spring diameter

Our alternative models to explain coordinated variation in vegetative and reproductive traits (Figure 1a-c) include both resource acquisition and rosette branching as potential explanations for variation in pre-reproductive rosette diameter. Therefore, we investigated the extent to which variation in spring rosette diameter was a function of biomass accumulation (resource acquisition) vs. differences in rosette architecture. Spring diameter at the North Carolina site was positively correlated with reproductive shoots and siliques per shoot but negatively correlated with net reproductive season diameter growth, both in the population means and in the population and F_2 PC1 patterns. This result is inconsistent with the hypothesis that spring diameter is a measure of resource acquisition, in which case we predicted it would be positively correlated both with subsequent reproductive and vegetative growth (Figure 1a; Houle 1991; van Noordwijk and de Jong 1986). These results are instead consistent with the model in which pre-flowering rosette diameter growth is affected by rosette branching (Figure 1c).

We also found that the timing of pre-reproductive rosette growth was heterogeneous between populations, which also suggested that spring diameter has a developmental component. Spiterstulen plants showed a net reduction in rosette diameter over the winter while the other populations showed varying degrees of diameter increase (Figure 4). We observed that rosettes on Spiterstulen plants in particular had become extensively branched and compact over the winter (as seen for example in Figure 3, upper left). We have observed over the course of these studies that branched rosettes tend to produce shorter leaves, suggesting that increased overwinter vegetative branching might explain the overwinter diameter reductions.

However, the eight replicated blocks in the North Carolina field site differed substantially in mean spring diameter. The block mean values for reproductive shoots and siliques per shoot increased significantly with mean spring diameter ($P < 0.001$ for both traits). Thus, variation in pre-flowering rosette diameter also appears in part to reflect differences in resource acquisition associated with site productivity in each block.

Mayodan, Spiterstulen, and F_2 populations also differed significantly from each other in their responses to differences in block productivity ($P < 0.01$ for population x block productivity interactions for all traits). In Mayodan plants, vegetative and reproductive traits as well as PC1 were highly responsive to variation in block productivity, but Spiterstulen plants showed almost no response to block productivity, and F_2 plants were intermediate (Figure S1). This pattern of variation in response to block productivity was also reflected at the QTL level. Mayodan alleles in the LG2 QTL region were significantly more

responsive to variation in block mean productivity than Spiterstulen alleles for PC1 and two of the individual traits, consistent with the population differences (Figure S2).

File S3

**Summary of structural equation model runs for North Carolina field data (first worksheet)
and Norway field data (second worksheet)**

Available for download at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.151803/-/DC1>

All marker data, trait data, and scripts used for data analysis have been deposited in the Dryad
Repository: <http://dx.doi.org/10.5061/dryad.1k4gq>.