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QTL architecture of resistance and tolerance traits in *Arabidopsis thaliana* in natural environments

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Abstract

Quantitative-genetic approaches have offered significant insights into phenotypic evolution. However, quantitative-genetic analyses fail to provide information about the evolutionary relevance of specific loci. One complex and ecologically relevant trait for plants is their resistance to herbivory because natural enemies can impose significant damage. To illustrate the insights of combined molecular and ecological research, we present the results of a field study mapping quantitative trait loci (QTL) for resistance and tolerance to natural rabbit herbivory in the genetic model, *Arabidopsis thaliana*. Replicates of the L*er* × *Col* recombinant inbred lines were planted into field sites simulating natural autumn and spring seasonal germination cohorts. Shortly after flowering, herbivores removed the main flowering inflorescence (apical meristem). We found several main-effect QTL for resistance within each seasonal cohort and significant QTL–season interactions, demonstrating that the loci underlying resistance to a single herbivore differ across seasonal environments. The presence of QTL × environment also shows that variation at specific loci is only available to selection in some environments. Despite significant among-line variance components, no QTL for tolerance were detected. The combined results of the quantitative-genetic and QTL analyses demonstrate that many loci of small effect underlie tolerance to damage by rabbits, and counter the hypothesis of locus-specific tradeoffs between resistance and tolerance. The results also provide insights as to the locus-specific nature of evolutionary constraints, i.e. some loci influence flowering time and resistance in both seasonal cohorts. Our results show how linking molecular-genetic tools with field studies in ecologically relevant settings can clarify the role of specific loci in the evolution of quantitative traits.

Keywords: *Arabidopsis thaliana*, phenotypic evolution, QTL mapping, resistance, tolerance

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Introduction

Most ecologically important traits are quantitative, exhibiting a continuous range of phenotypic variation. For instance, plants in natural populations often show quantitative variation in their resistance to damage by natural enemies, as well as quantitative variation in the underlying phenological and architectural traits that confer resistance (e.g. Fritz & Simms 1992). To date, evolutionary ecologists have relied primarily on quantitative-genetic approaches to predict phenotypic evolution of complex, quantitative traits (e.g. Falconer & Mackay 1997; Lynch & Walsh 1997). In particular, investigators have used Lande's generalization of the traditional breeder's equation to describe the evolution of suites of correlated traits (Lande 1979). In this model, $\Delta\bar{z} = G\beta$, where a change in the mean value of several traits ($z_1, z_2, \ldots z_n$) is equal to the product of the genetic variance–covariance matrix (G) and a vector of selection gradients (β). By potentially affecting both the patterns of genetic variation and covariation of traits (elements of G) and the pattern of natural selection on those traits (elements of β), environmental heterogeneity can play a significant role in altering the evolutionary dynamics of traits.

Despite the important role environmental heterogeneity might play in the evolution of ecologically important traits, little is known about how the expression of variation at specific loci differs across environments, which specific loci are under selection in a given environment, and whether such locus-specific selection varies across environments.

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This absence of knowledge is the result of one aspect of traditional quantitative genetics that is both one of its greatest advantages and one of its limitations: it is possible to sample randomly genotypes from a natural population and estimate patterns of genetic variation and covariation in traits (i.e. elements of *G*) without any direct knowledge of the underlying loci or molecular genetic details. Similarly, it has been possible to estimate the pattern of natural selection on quantitative traits (i.e. elements of β) in the absence of knowledge about the number of loci that affect fitness, and the direction of their effects. However, by integrating genetic resources developed by molecular biologists into studies of evolutionary ecology, there is now a growing opportunity to understand the mechanisms underlying phenotypic evolution of complex, quantitative traits in heterogeneous environments at the level of specific loci.

The expression of complex traits is usually determined by many genes, known as quantitative trait loci (QTL). Recently developed genetic resources, such as recombinant inbred lines (RILs), provide the opportunity to identify such QTL, and thus to investigate the evolution of quantitative traits at a finer scale of genetic resolution than has previously been possible (Mauricio 2001; Mitchell-Olds 2001; Kliebenstein *et al*. 2001, 2002a, 2002b; Weinig *et al*. 2002). RILs are typically developed from a cross between two genetically distinct parents. From the hybrid F_1 generation, inbred lines are developed through successive generations of selfing. The resulting F_8 express a greater range of phenotypic variation than either parental population, thereby increasing the opportunity to detect natural selection. The attendant generations of recombination also provide the basis for identifying how variation in specific genomic regions affects a given trait. Finally, because replicates within an RIL are genetically uniform, it is possible to grow genetic replicates in multiple environments and determine whether similar or different QTL affect phenotypic expression or a component of fitness across environments. Thus, although RILs were initially developed as a tool for gene discovery (Lister & Dean 1993; Alonso-Blanco *et al*. 1998; Wilson *et al*. 2001), they also provide an important tool for studies of evolutionary ecology (e.g. Mitchell-Olds 1996; Mauricio 2001; Kliebenstein *et al*. 2001, 2002a, 2002b; Weinig *et al*. 2002).

Plant responses to damage by natural herbivores are ecologically important and complex traits. Similar to many plant species (Paige & Whitham 1987; Lennartson *et al.* 1997; Juenger & Bergelson, 2000), plants of *Arabidopsis thaliana* in natural populations experience herbivore damage to the apical meristem (Weinig *et al*. In review-a). Resistance and tolerance of damage are alternative, although not necessarily mutually exclusive, responses to herbivory (Mauricio, 2000; Mauricio *et al*. 1997; Tiffin & Rausher 1999; Pilson, 2000). Traditional quantitative genetic approaches, combined with selection analysis, have provided important insights into the traits and ecological mechanisms contributing to resistance and tolerance, as well as the possibility of tradeoffs between them (Fineblum & Rausher 1995; Stinchcombe & Rausher 1902; Mauricio *et al*. 1997; Stowe 1998; Pilson, 2000; Weinig *et al*. 2003a). For instance, traits that influence apparency (Feeny 1976) may be important determinants of resistance to apical meristem damage in *A. thaliana* (e.g. Weinig *et al*. 2003a). However, until recently, the genetic mechanisms underlying resistance traits have not been elucidated.

The genetic model species *A. thaliana* provides powerful tools for investigating the genetic architecture of resistance and tolerance to herbivory. For example, several recent QTL mapping studies have yielded important insight into the genetic mechanisms contributing to natural variation in chemical resistance to insect herbivores in this species (e.g. Kliebenstein *et al*. 2001, 2002a, 2002b). However, less is known about the genetic basis of resistance and tolerance to vertebrate herbivores or the architectural and life-history traits contributing to such resistance and tolerance. From previous investigations using traditional quantitative genetic techniques, we have determined that both timing of flowering and inflorescence height contribute to the risk of rabbit herbivory in *A. thaliana* (Weinig *et al*. 2003a). In addition, plastic responses of architectural traits, such as increases in branch number, increase tolerance to rabbit herbivory (where tolerance is defined as the difference in fruit production between the damaged and undamaged states). Plants experiencing damage also have delayed senescence, which contributes to increased tolerance (Weinig *et al*. 2003a). The genetic resources available for this species make it possible to identify specific QTL underlying these traits and to ask whether selection acts directly on these QTL in natural environments.

Here we investigate the QTL architecture of resistance and tolerance to natural rabbit herbivory in *A. thaliana*. In particular, we address the following questions. (i) What is the genetic architecture of QTL for resistance and tolerance, and do the effects of these QTL differ among different natural seasonal environments? (ii) Is there evidence for pleiotropic tradeoffs between resistance and tolerance at specific QTL? (iii) What are the mechanisms of resistance, i.e. are QTL for resistance also associated with variation in life-history or architectural traits? (iv) Are QTL for resistance associated with variation in lifetime fitness? That is, are these loci under direct selection in natural environments?

Materials and methods

Study species and mapping population

Arabidopsis thaliana (L.) Heynh. (Brassicaceae) is a predominantly self-fertilizing, colonizing annual species, native

to Eurasia but now widely naturalized in the USA and elsewhere. Populations of *A. thaliana* occur over a wide latitudinal gradient, and ecotypes differ substantially in life history and reproductive phenotypes (Napp-Zinn 1985; Nordborg & Bergelson 1999). Populations at northern latitudes often produce both autumn and spring germination cohorts, as would be expected under a bet-hedging strategy against unpredictable environments (Venable 1985; Silvertown 1988). Autumn germinants risk low fitness because of overwinter mortality (Weinig *et al*. 2003b), but seedlings that survive may attain a larger size at reproduction than spring germinants. In contrast, spring germinants avoid potential overwinter mortality but because of their smaller size at reproduction, may have reduced fecundity relative to the autumn germinants that survive the winter. Such within-population variation in life history may also have significant implications for responses to herbivory. Different traits and QTL may determine resistance to natural enemies in different seasonal cohorts, and the relationship between resistance and fitness may also differ between seasonal cohorts because of differences in growth-season duration, herbivore abundance, and abundance of alternative food sources for herbivores between the autumn vs. spring seasonal cohorts.

Arabidopsis thaliana initially grows as a rosette, then bolts to produce an indeterminate inflorescence from the apical meristem. Additional meristems in the axils of rosette leaves are quiescent until released from apical dominance through natural senescence, or damage to, the apical meristem. Inflorescences are initiated from axillary meristems following the release from apical dominance. Here, we refer to the inflorescence differentiated from the apical meristem as the 'apical inflorescence', and inflorescences differentiated from the leaf axils in the rosette as 'basal branches.' Notably, the architecture of *A. thaliana* resembles that of the monocarpic (albeit perennial) rosette plants *Gentianella* and *Ipomopsis* that have been the subject of prior studies on plant responses to herbivory and apical meristem damage (e.g. Paige & Whitham 1987; Lennartson *et al*. 1997; Juenger & Bergelson, 2000).

In the current experiment, we used a set of RILs to map responses to natural herbivory. These lines were developed from a cross between the Landsberg *erecta* and Columbia accessions advanced through single-seed descent to the F_8 (Lister & Dean 1993). Again, because siblings within a line are homozygous and genetically uniform, it is possible to examine environmental variation in QTL expression: that is, whether similar or different QTL determine variation in resistance between the autumn and spring seasonal cohorts.

Field experimental design

As part of a larger experiment mapping QTL for fitness in and phenotypic responses to different seasonal environments (Weinig *et al*. 2002, 2003b), we planted seedlings of these RILs into a ploughed field at Brown University's Haffenreffer Reserve, Bristol, Rhode Island. Full details of the experimental design and QTL analyses are reported elsewhere (Weinig *et al*. 2002). In brief, replicate seeds of 98 RILs were planted into each of 30 98-cell plug trays and cold stratified to simulate winter temperatures. Seeds were then germinated under natural day lengths in the Brown University greenhouse. Seedlings from each tray were transplanted into one of 30 randomized blocks at the field site. For the autumn seasonal cohort, seedlings were transplanted to the field between 6 November and 9 November 1999, while seedlings in the spring seasonal cohort were transplanted between 5 April and 7 April 2000. The timing of planting coincided with the developmental stage of plants growing in nearby natural populations.

We measured life-history and morphological traits, including the number of days from planting to bolting and to flowering, final height of the apical inflorescence at harvest, number of basal branches, height of the tallest basal branch and senescence date (defined as the date when the last flower senesced). The majority of plants experienced damage to the apical inflorescence shortly after flowering. We did not observe herbivores in the process of damaging plants. However, based on frequent sightings of rabbits at the site, the presence of rabbit faeces in the field, the observed rapid consumption of the entire inflorescence (which suggests insect damage was unlikely), and the absence of tracks that might indicate deer herbivory, we attribute this apical meristem damage (AMD) to rabbits. The damage occurred within a 2-week period within the season and was recorded following this interval. Fruit number at natural senescence was used to estimate fitness. Because *Arabidopsis* reproduces primarily via self-fertilization, fruit production includes male and female fitness components; prior studies have shown that this character is highly correlated with seed number (Westerman & Lawrence 1970; Mauricio *et al*. 1997). Tolerance was calculated as the difference in RIL mean fruit production between the damaged and undamaged states (e.g. Simms & Triplett 1994; Tiffin & Rausher 1999). Although it would have been ideal to calculate fruit production in the undamaged state from plants grown in an experimental treatment where rabbits had been successfully excluded, we were not able to do so. However, it is unlikely that our estimates of fruit production in the absence of damage are biased by microenvironmental factors because we utilize RIL means calculated from plants grown in a randomized design, and as such environmental effects are likely to be averaged across replicates within RILs (Tiffin & Inouye, 2000). Plants that died from transplant shock (within 7 days of transplanting) were scored as missing data; plants that survived transplanting and subsequently died before

fruit-set were assigned a fitness value of zero and included in analyses.

Analysis of variance in phenotypic traits and fitness

Analysis of variance (ANOVA) for most of the traits is presented elsewhere (Weinig *et al*. 2003a). We used logit modelling and mixed-model ANOVA to determine whether the experimental population exhibited genetic variation for resistance and tolerance to AMD, respectively. For the logit modelling, we considered the presence or absence of AMD as a binary response variable (0 = no AMD, 1 = AMD) and spatial block and RIL as independent categorical variables. To test for genetic variation for tolerance to AMD, we used mixed-model ANOVA. In this ANOVA, we evaluated the effects on relative fitness of spatial block, RIL, AMD and the AMD × RIL interaction, with RIL and AMD × RIL considered as random effects and all other effects fixed. Two-way ANOVA including spatial block and RIL as categorical variables was used to test for genetic variation in the remaining phenotypic traits (see Weinig *et al*. 2003a).

We also calculated the among-line variance, V_L, which estimates the total genetic variation between parental lines. Within each seasonal cohort, we used random-effects ANOVA to partition variance for phenotypic traits into sources originating from line (L), spatial block (B), and error according to the model, $y = \mu + B + L + \text{error}$, where μ is the overall mean. The among-line variance component, V_L, was calculated with the VARCOMP procedure of SAS, using the 'method = REML' option (SAS 1999). The logit and ANOVA models described above provide significance tests for V_L (i.e. significant heritability). Again, although a significant among-line variance component demonstrates significant heritability within a given environment, heritability estimates fail to provide information regarding environment-specific phenotypic expression at specific loci. Calculating the among-line variance also allows QTL effects to be standardized, facilitating comparisons of QTL effect size (see below).

QTL mapping

As described in the preceding section, we used the proportion of replicates within a seasonal cohort that experienced herbivory as our estimate of resistance, while tolerance was estimated as the difference in the RIL mean between the damaged and undamaged states (e.g. Simms & Triplett 1994). For traits expressed prior to herbivore damage, such as bolting date and flowering date, we used the phenotypic mean of all replicates within a seasonal cohort to map QTL. Within the autumn cohort, every replicate of 13 RILs experienced herbivory, making it impossible to map QTL for phenotypic traits and fitness of the undamaged plants. Therefore, for the autumn seasonal cohort we used the mean of the damaged replicates to map fruit number, basal branch number, basal branch height and senescence date.

For the spring seasonal cohort, we split the data by damage class and used the within-class means to map basal branch height, basal branch number and senescence date. For fitness, we mapped QTL using data from undamaged plants, damaged plants and pooled data containing both damaged and undamaged plants. Preliminary analyses detected no QTL for fruit number using data only from the damaged class, and QTL from analyses using the pooled data and analyses using only the undamaged replicates were similar. Accordingly, for the spring cohort we only report QTL for fitness from analyses that utilized data pooled across damage classes. This approach is consistent with the fitness estimate used in selection analyses on resistance and tolerance reported elsewhere (Weinig *et al*. 2003a), facilitating comparisons of traditional quantitative-genetic analyses and QTL analyses. QTL were mapped using the composite interval mapping (CIM) (Zeng 1994) procedure of QTL CARTOGRAPHER (Basten *et al*. 1994, 1999). QTL cofactors were initially selected using forward–backward stepwise regression. Within each experimental environment, the significance threshold of the likelihood ratio test statistic (LR) for a QTL was determined through permutation analyses (Doerge & Churchill 1996). QTL whose peaks were separated by LR values below the significance threshold are shown as separate QTL (Fig. 1). All QTL are shown with the 2-LOD support limits, where LOD = 0.217 × LR. When comparing across environments, overlap of the 2-LOD support limits (analogous to 95% confidence intervals) provides evidence that similar QTL determine the expression of a given trait. Additive effects were calculated as the difference of the two homozygous classes divided by two, and are standardized to the line variance within each environment to facilitate comparisons of effect size among QTL.

QTL by environment interactions were tested using ANOVA, in which season and all significant QTL detected in the genome-wide screen were included in the model (Fry *et al*. 1998). When all possible two–way interactions are tested, ANOVA effectively corrects for potential type I error resulting from multiple tests. However, tests for QTL × environment are valid only across environments with similar power to detect QTL. On average across RILs, resistance was higher in the spring than autumn seasonal cohorts (0.78 vs, 0.18; $\chi^2 = 1066.08$, d.f. = 95, $P < 0.0001$). As a result, the number of replicates in the damaged class is much higher in the autumn than spring seasonal cohort, and there are more undamaged than damaged replicates in a given RIL within the spring seasonal cohort. Because replicate number affects power (Soller & Beckman 1990; Lynch & Walsh 1997), we test only for QTL × season effects on

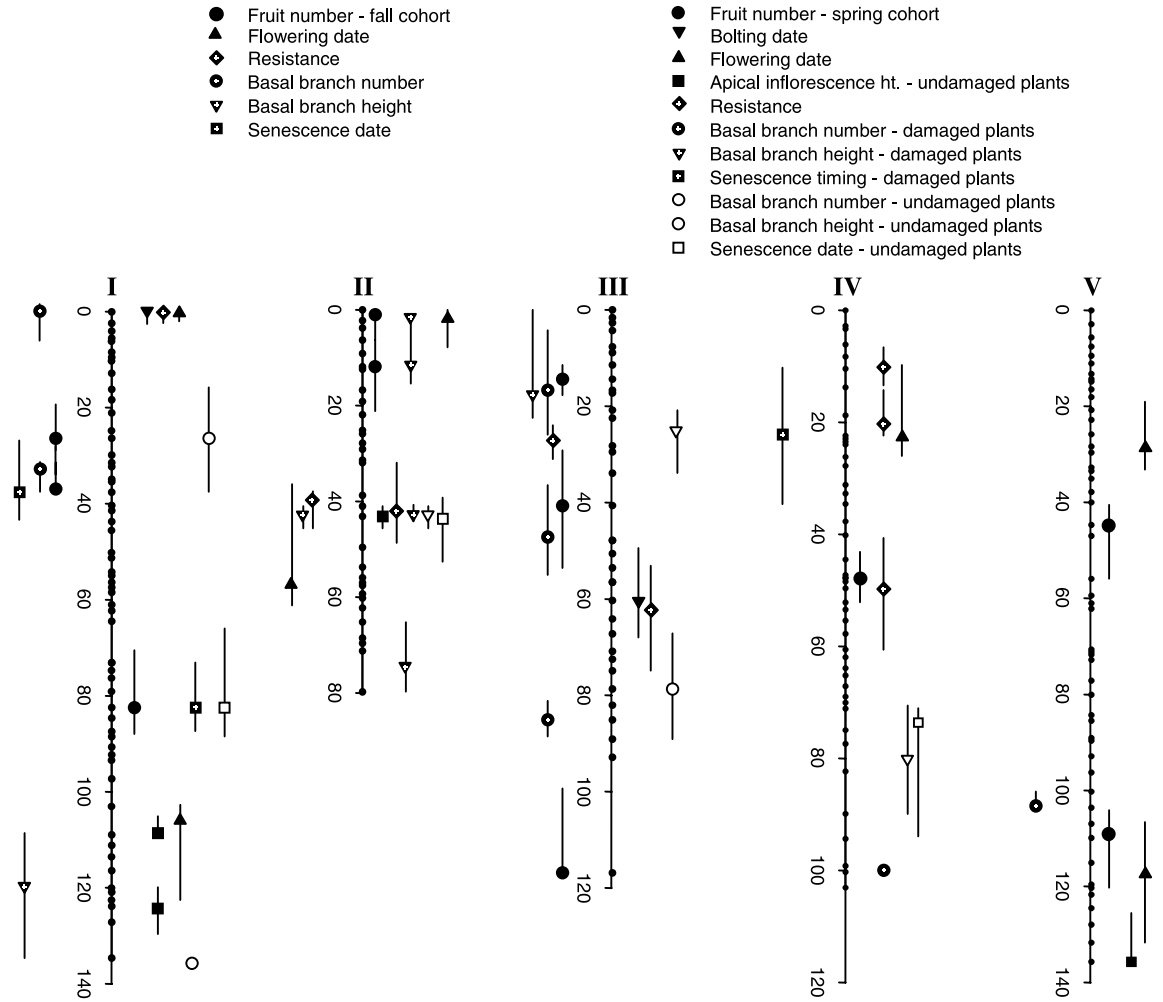

Fig. 1 QTL for fruit number (fitness), resistance and phenotypic traits hypothesized to affect resistance in plants experiencing and avoiding natural rabbit herbivory. Note that QTL mapped in the autumn seasonal cohort appear on the left side of the chromosome, while QTL mapped in the spring cohort are on the right. Icons reflect the position of QTL, while flanking lines denote the 2-LOD support limits (Table 1). Two QTL for bolting date in the spring cohort were originally presented elsewhere (Weinig *et al*. 2002) and are presented here for comparison with resistance QTL.

preherbivory traits and resistance (which are calculated using all replicates of an RIL).

Results

All traits show significant genetic variation

Both resistance and tolerance had significant among-line variance components in the spring seasonal cohort, as did most of the phenotypic traits hypothesized to underlie the expression of these two traits (Weinig *et al*. 2003a). In the autumn seasonal cohorts, the among-line variance components were significant for all phenotypic traits ($P < 0.05$) and marginally significant for resistance ($P = 0.11$). There was no detectable genetic variation for tolerance in the autumn cohort ($P = 0.99$).

Different QTL determine resistance in the autumn and spring seasonal cohorts

Within the autumn seasonal cohort, we detected two QTL at map positions of 39 cm on chromosome 2 and 28 cm on chromosome 3 that were significant determinants of resistance. Six QTL influenced resistance in the spring seasonal cohort (Table 1). A single QTL (near 40 cm on chromosome 2) significantly affected resistance in both seasonal cohorts, although the effects of additional QTL detected in the spring (at 10 and 21 cm on chromosome 4) and autumn seasonal cohorts (28 cm on chromosome 3) were statistically indistinguishable in the alternative cohort (Table 2). Significant QTL–season interactions were detected for three QTL (at 1 cm on chromosome 1, 63 cm on chromosome 3, and 49 cm on chromosome 4) that

Table 1 QTL for fitness, resistance, and phenotypic traits in spring and autumn seasonal cohorts

Trait*	Chrom.	QTL map position cM (nearest marker)	cM range of the 2-LOD support limit	Likelihood ratio	Additive effect/σ	[r^2]
Spring cohort						
Fruit number	1	82.48 (*PAP240*)	70.49–87.92	12.6	0.21	0.05
	2	1.01 (*ve012*)	0.01–21.14	12.2	0.15	0.06
	2	11.87 (*mi310*)	6.31–21.14	19.5	0.17	0.09
	4	48.91 (*g4564a*)	43.11–52.64	28.8	0.15	0.14
	5	44.71 (*nga139*)	41–55.46	14.0	−0.11	0.06
	5	108.96 (*h2a1*)	104.11–115.07	29.4	0.17	0.14
Bolting date†	1	0.01 (*ve001*)	0.01–3.01	32.9	−0.20	0.15
	3	61.07 (*g4564b*)	49.89–71.94	13.9	0.12	0.06
Flowering date	1	0.01 (*ve001*)	0.01–2.42	55.1	−0.52	0.28
	1	113.62 (*ve011*)	103.12–123.09	14.3	0.23	0.06
	2	31.91 (*0802F*)	29.11–37.41	21.4	0.35	0.09
	5	28.35 (*cor6.6*)	23.3–38.83	19.2	0.29	0.08
Apical inflorescence height	1	108.62 (*mi185*)	105.12–108.62	17.5	−0.05	0.05
	1	124.35 (*agp64*)	120.09–129.65	32.5	0.06	0.09
	2	43.13 (*er*)	41.51–45.63	106.7	−0.22	0.52
	5	135.71 (*CATHHANK*)	126.02–135.71	14.4	−0.04	0.04
Resistance	1	0.01 (*ve001*)	0.01–2.01	36.6	−0.63	0.20
	2	42.01 (*GPA1*)	31.91–48.63	23.0	0.51	0.12
	3	62.84 (*g4117*)	53.11–74.93	11.1‡	0.53	0.04
	4	10.43 (*mi390*)	7.08–13.77	28.8	0.52	0.15
	4	21.25 (*Gsl_ohp*)	13.77–22.39	28.0	−0.63	0.16
	4	49.63 (*m326*)	41.11–60.26	13.6	0.42	0.07
Basal branch number — U	1	26.39 (*g3829*)	15.81–37.43	13.7	1.23	0.10
	3	78.67 (*ve022*)	67.26–89.09	14.0	1.23	0.10
Basal branch height — U	2	43.13 (*er*)	40.22–71.35	80.7	0.32	0.037
	3	25.45 (*mi268*)	21.33–33.48	18.1	0.15	0.07
	4	80.38 (*O6455*)	71.1–89.8	12.1	−0.09	0.04
Senescence date — U	1	82.48 (*PAP240*)	65.99–88.49	12.3	0.25	0.06
	2	43.63 (*er*)	39.22–52.55	20.5	0.35	0.12
	4	74.10 (*mi232*)	71.1–93.4	19.0	−0.35	0.11
Basal branch number — D	4	100.25 (*um596A*)	99.65–103.06	14.5	0.15	0.13
Basal branch height — D	2	3.75 (*mi320*)	0.01–8.81	19.9	0.09	0.06
	2	11.87 (*mi310*)	0.01–16.75	15.1	0.09	0.05
	2	43.10 (*GPA1*)	40.72–44.13	70.5	0.20	0.30
Senescence date — D	1	82.48 (*PAP420*)	73.15–90.49	12.9	0.10	0.11
Autumn cohort						
Fruit number	1	26.36 (*mi203*)	19.84–29.39	28.2	0.40	0.16
	1	36.93 (*mi163*)	16.66–37.61	23.2	0.38	0.13
	3	14.38 (*nga162*)	11.95–17.26	26.8	0.48	0.15
	3	40.64 (*mi178*)	29.48–50.61	15.9	−0.38	0.09
	3	116.88 (*nga6*)	99.88–116.88	12.2	0.30	0.10
Flowering date	2	57.01 (*ve015*)	36.91–60.68	16.3	0.20	0.08
	4	44.11 (*CD.84*)	37.69–54.92	20.1	0.27	0.12
	5	128.94 (*SNP153*)	117.57–131.71	34.7	0.37	0.23
Resistance	2	39.22 (*g6842*)	35.91–45.63	46.7	1.14	0.29
	3	27.95 (*mi268*)	23.95–31.48	19.0	0.85	0.12
Basal branch number — D	1	0.01 (*ve001*)	0.01–3.42	17.5	0.98	0.08
	1	32.79 (*F19G10a*)	31.43–37.43	25.6	1.22	0.13
	3	15.88 (*nga162*)	4.31–29.45	12.9	0.73	0.06
	3	48.36 (*mi413*)	36.87–55.06	20.0	−0.97	0.09
	3	85.09 (*m339*)	81.67–88.59	28.7	1.22	0.13
	5	103.24 (*PAP3*)	100.74–105.11	17.3	−1.19	0.08

Table 1 *Continued*

Trait*	Chrom.	QTL map position cM (nearest marker)	cM range of the 2-LOD support limit	Likelihood ratio	Additive effect/σ	[r^2]
Basal branch height — D	1	120.09 (*mi425*)	108.97–134.65	13.9	−0.43	0.04
	2	43.13 (*er*)	40.22–45.13	100.5	1.72	0.50
	3	16.7 (*mi289*)	0.01–22.33	12.5	0.43	0.04
Senescence date — D	1	37.61 (*g17286*)	32.29–43.44	17.9	0.81	0.12
	4	22.92 (*mi306*)	13.77–27.15	14.0	0.73	0.10

The first three columns indicate the chromosomal (Chrom.) location of the QTL, the nearest marker locus, and the cM range defining the 2-LOD support limits around the QTL. The Likelihood Ratio is the test-statistic for composite interval mapping, the significance of which is determined through permutation analyses (Doerge & Churchill 1996); for all traits, the significance threshold for an experiment-wide error rate of $\alpha = 0.05$ was less than 14.0, and less than 12.0 for an error rate of $\alpha = 0.10$. The second to last column denotes the additive effects, which are standardized to the among-line variance component. Effects are positive if the *Col* allele confers an increase in the trait value, and negative if the L*er* allele increases the trait value. The final column shows the proportion of variance explained by a QTL.
*Some traits were measured prior to the occurrence of herbivory (bolting date, flowering date, apical inflorescence height), while others were measured following herbivory (basal branch number, basal branch height, and senescence date). QTL mapped in the damaged and undamaged classes are denoted with a D and U, respectively.
†As presented in Weinig *et al*. 2002.
‡Significant at $P < 0.12$.

Table 2 ANOVA for QTL–season interactions for resistance

Effect	III Mean square*	*F*-value	*P*-value
Season	10.19	1648.63	< 0.0001
ve001	0.11	17.60	< 0.0001
GPA1	0.18	28.90	< 0.0001
g4117	0.01	1.96	0.1639
mi390	0.02	3.24	0.0739
Gsl_ohp	0.00	0.60	0.4399
m326	0.06	9.31	0.0028
mi268	0.01	0.98	0.3235
Season × *ve001*	0.15	24.74	< 0.0001
Season × *GPA1*	0.01	1.77	0.1852
Season × *g4117*	0.02	3.66	0.0579
Season × *mi390*	0.01	1.81	0.1803
Season × *Gsl_ohp*	0.00	0.64	0.4237
Season × *m326*	0.06	9.69	0.0023
Season × *mi268*	0.00	0.04	0.8477

*Hypothesis and error degrees of freedom = 1, 147; error mean square = 0.006.

influenced resistance only in the spring. This result indicates that different genetic mechanisms underlie resistance in different seasonal environments.

There is no evidence of major QTL for tolerance, or for pleiotropic tradeoffs between resistance and tolerance

We failed to detect significant QTL for tolerance, despite the presence of significant among-line variance. Power analyses for most phenotypic traits (Soller & Beckman 1990; Lynch & Walsh 1997) indicate that QTL explaining less than 5% of the total phenotypic variation will be undetected in the mapping population used here. The absence of QTL for this trait therefore suggests that the observed genetic variation in tolerance is the result of many genes of small effect rather than of a few major genes. Our failure to detect QTL that affect both resistance and tolerance also counters the hypothesis that tradeoffs should exist between resistance and tolerance to AMD.

Three QTL determine both reproductive timing and resistance in the spring cohort

QTL at map positions of 1 cm on chromosome 1, at 64 cm on chromosome 3, and at 22 cm on chromosome 4 were significant determinants of bolting and/or flowering time and resistance (Table 1; Fig. 1; Weinig *et al*. 2002). At these QTL, alleles accelerating time to reproduction were associated with decreased resistance, suggesting that variation in these genomic regions influences the likelihood of herbivory by affecting flowering time. We note, however, that not all QTL for reproductive timing influenced resistance, a point we return to in the Discussion.

One QTL of large effect determines both apical inflorescence height and resistance

In the spring seasonal cohort, the Columbia allele at a QTL with a map position of 44 cm on chromosome 2 decreased apical inflorescence height and increased

resistance (Table 1). Although the high proportion of damaged replicates in the autumn seasonal cohort precluded mapping traits in the undamaged state, it is noteworthy that the Columbia allele at this QTL also increased resistance in the autumn seasonal cohort. These results are consistent with the negative RIL-mean correlation between inflorescence height and resistance (Weinig *et al*. 2003a), and the hypothesis that apparency affects the likelihood of damage.

One QTL affects both resistance and fitness

Previous selection analyses of the data from this experiment using traditional quantitative genetic techniques (Rausher 1992) failed to detect selection on resistance (Weinig *et al*. 2003a). However, the Columbia allele at one QTL at 50 cm on chromosome 4 increased both resistance and fruit production, indicating selection for resistance in this chromosomal region. The discrepancy between traditional analyses of natural selection and these QTL results suggests that there may be other undetected QTL of smaller effect (i.e. below the power of this mapping population to detect) elsewhere in the genome with positive additive effects on resistance but negative effects on fruit production (see below).

Discussion

A primary aim of evolutionary ecology has been to understand mechanisms of adaptation to heterogeneous environments (Levene 1953; Levins 1963; Lloyd 1984). Quantitative-genetic analyses have provided substantial insights into phenotypic evolution, but have done so without an understanding of genetic mechanisms underlying selected traits. By contrast, QTL mapping provides a means of identifying specific chromosomal regions associated with ecologically important traits. Such results can be coupled with further genetic dissection (e.g. fine-scale mapping, disequilibrium mapping, transgenic manipulation; Mackay 2001) and detailed knowledge of developmental pathways to characterize the specific loci targeted by selection in natural settings.

In our experimental population, we found little evidence of selection on resistance, using classic genotypic selection analysis (Weinig *et al*. 2003a), yet at least one QTL affected both resistance and fitness. Regardless of whether the QTL association between resistance and fitness is causal in this case (see below), this result illustrates that QTL analyses may be more sensitive than quantitative-genetic ones in detecting locus-specific patterns of selection. More specifically, selection at some loci may be undetectable using RIL or genotypic means because of antagonistic selection acting on variation segregating elsewhere in the genome.

It is also noteworthy that we failed to detect QTL for tolerance, despite the significant among-line variance component for this trait. This result illustrates the limit of resolution typical of QTL studies, which may only detect loci explaining greater than 3–5% of the variation, depending in part on the number of lines in the mapping population. When V_L is significant, the absence of significant QTL indicates that many loci of small effect must underlie the observed genetic variation in tolerance. Thus, independently and in combination, quantitative-genetic and QTL data from experiments in ecologically relevant settings make important contributions to our understanding of phenotypic evolution.

QTL mapping also provides a means to test for environment-specific phenotypic expression at specific loci. Such environment-specific expression underlies differences in heritability estimates that are commonly observed for the same trait measured in different environments (Falconer & Mackay 1997; Lynch & Walsh 1997). Here, we found that variation at specific loci determined resistance in either the autumn and spring seasonal cohorts, but not both. Such QTL–environment interactions are not uncommon (Brummer *et al*. 1997; Sari-Gorla *et al*. 1997; Gurganus *et al*. 1998; Vieira *et al*. 2000), even for major developmental loci (Weinig *et al*. 2002). However, such interactions demonstrate that variation at specific loci may be masked from selection in some environments. We found only slight evidence for selection on resistance at the QTL level. However, simulations of phenotypic selection demonstrate that selection for resistance depends on average tolerance within the population and the frequency of herbivory (Abrahamson & Weis 1997; Weinig *et al*. 2003a). In populations where tolerance is lower or the frequency of herbivory is higher than in our experimental setting, selection for resistance will be correspondingly greater. The QTL–environment interactions described here for resistance indicate that different QTL will be exposed to selection in different natural environments.

Determining whether similar or different QTL underlie ecologically important traits across multiple environments is also informative about the degree of genetic constraints on adaptation in complex, heterogeneous environments (e.g. Via & Lande 1985; Hawthorne & Via 2001; Mitchell-Olds 2001; Kliebenstein *et al*. 2001, 2002a, 2002b). For instance, plants in the spring seasonal cohort that flowered relatively early (at the end of April) were more likely to be damaged than those flowering later in the season. If early spring is the time of peak herbivore abundance or if *Arabidopsis* is one of the few species growing and available as forage, plants of *A. thaliana* that delay flowering in autumn seasonal cohorts (as late as mid-April) might have an increased likelihood of damage. Thus, selection to reduce damage would act to accelerate flowering in the autumn (i.e. flower before peak herbivore abundance) and delay flowering in the spring cohort (i.e. flower after peak herbivore abundance). However, the presence of large-effect QTL that affect flowering

time in both the autumn and spring cohorts in Rhode Island (Weinig *et al*. 2002) imposes an evolutionary constraint on flowering time and hence resistance.

Our results also illustrate that a genetic correlation between two traits, for instance, resistance and flowering time, need not imply that both traits share all the same QTL. For example, in our dataset not all of the QTL for reproductive timing affect resistance (Fig. 1). There are several explanations for the imperfect correspondence between QTL for reproductive timing and resistance. First, the genotypic correlation between reproductive timing and resistance is less than unity (Weinig *et al*. 2003a), indicating that some loci affecting flowering time do not affect resistance. There may also be a threshold additive effect on flowering time, below which allelic substitutions at a given QTL fail to affect resistance. It is also possible that linked genes with antagonistic phenotypic effects could also affect the disassociation between QTL for reproductive timing and resistance; that is, alleles at some large-effect QTL may result in early flowering, but may be associated with alleles at linked loci that increase resistance via another, unmeasured mechanism. Our data are somewhat consistent with this possibility, in that the QTL with the largest effect are those that determine resistance, with the exception of the QTL for bolting time in the middle of chromosome 3 (Weinig *et al*. 2002) and the QTL for flowering time in the bottom of chromosome 1.

As a population for mapping QTL, RILs can provide additional insights into the mechanisms underlying resistance. Here, we examined QTL for resistance to mammalian herbivory in two seasonal cohorts. The same lines have been used to determine QTL controlling the production of secondary compounds hypothesized to confer resistance, as well as damage caused by different insect herbivores. Again, comparisons of QTL detected in different experimental settings are possible, because individual RILs are homozygous and replicate seeds are genetically identical. The QTL influencing resistance to rabbit damage differ from those underlying glucosinolate production in leaves and seeds (Kliebenstein *et al*. 2002a) and those conferring resistance to the insect herbivore, *Trichoplusia ni* (Jander *et al*. 2001; Kliebenstein *et al*. 2002a). This result is consistent with the hypothesis that different mechanisms determine resistance to different herbivores, although differences between this study and previous ones in the organs that were damaged may also influence the observed QTL for resistance. In like manner, the presence of significant QTL × season effects on resistance demonstrates that different mechanisms underlie resistance in different seasonal cohorts.

Linking molecular-genetic resources with ecological studies provides a means to understand adaptive evolution at a finer scale of genetic resolution. A vast range of genetic resources have been developed in genetic model organisms, such as *Arabidopsis thaliana*, that will further our understanding of genetic mechanisms of adaptation. The RILs used here and the resulting QTL analyses illustrate the utility of such tools for evolutionary studies. One caveat to using RILs is that crosses between geographically separate parents may not replicate the genetic architecture of natural populations, i.e. the alleles that segregate in such wide crosses are likely to differ from the alleles that segregate in crosses made within populations. Thus, additional studies are needed to characterize how current selection in natural populations acts at a given locus, suggesting that a combination of quantitative-genetic and QTL approaches will be most informative. At the molecular-genetic level, the rapidly advancing elucidation of developmental pathways can help to suggest candidate loci that may be the target of selection in natural settings. Although *Arabidopsis* is a genetic model, it is also a wild plant species with an interesting natural history suggesting ecologically relevant settings in which to investigate evolutionary dynamics of complex traits at the genetic level. The opportunity now exists for examining the adaptive evolution of ecologically important traits from the molecular to the organism level in natural environments.

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