Comparative Analysis of Quantitative Trait Loci Controlling Glucosinolates, Myrosinase and Insect Resistance in *Arabidopsis thaliana*

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ABSTRACT

Evolutionary interactions among insect herbivores and plant chemical defenses have generated systems where plant compounds have opposing fitness consequences for host plants, depending on attack by various insect herbivores. This interplay complicates understanding of fitness costs and benefits of plant chemical defenses. We are studying the role of the glucosinolate-myrosinase chemical defense system in protecting *Arabidopsis thaliana* from specialist and generalist insect herbivory. We used two Arabidopsis recombinant inbred populations in which we had previously mapped QTL controlling variation in the glucosinolate-myrosinase system. In this study we mapped QTL controlling resistance to specialist (*Plutella xylostella*) and generalist (*Trichoplusia ni*) herbivores. We identified a number of QTL that are specific to one herbivore or the other, as well as a single QTL that controls resistance to both insects. Comparison of QTL for herbivory, glucosinolates, and myrosinase showed that *T. ni* herbivory is strongly deterred by higher glucosinolate levels, faster breakdown rates, and specific chemical structures. In contrast, *P. xylostella* herbivory is uncorrelated with variation in the glucosinolate-myrosinase system. This agrees with evolutionary theory stating that specialist insects may overcome host plant chemical defenses, whereas generalists will be sensitive to these same defenses.

PLANT chemical defense systems and their impact on specialist *vs.* generalist insect herbivores have intrigued scientists for decades. It is clear that some compounds elicit contrasting behavioral responses from various insect herbivores (Chew and Renwick 1994). Chemical coevolution theory suggests that specialist insects have adapted to withstand and even utilize some plant defensive chemicals, which nevertheless function as feeding deterrents to generalist herbivores (Ehrlich and Raven 1964; Berenbaum and Zangerl 1992). Several researchers have shown that secondary plant compounds that deter feeding of generalist herbivores also stimulate feeding and provide oviposition cues for specialist feeders (Da Costa and Jones 1971; Feeny 1976; Chew and Renwick 1994). However, understanding insect-plant interactions is complicated because different compounds within a chemical class can have heterogeneous effects on specialist herbivores (Bowers and Puttick 1988; Bartlet *et al.* 1994). Additionally, these compounds can interact synergistically to alter herbivory patterns (Berenbaum and Neal 1985).

The glucosinolate-myrosinase system is believed to protect plants from herbivore damage (Chew 1988; Giamoustaris and Mithen 1995). Glucosinolates are amino-acid-derived thioglycosides. Glucosinolates and their hydrolyzing agent, myrosinase, are spatially separated within plant cells (Bones and Rossiter 1996). When the cell is disrupted, myrosinase cleaves the sugar from the glucosinolate, and a series of toxic compounds are released. These toxins include nitriles, isothiocyanates, oxozaladines, and epithioalkanes. The fact that toxins are produced only when tissues are damaged suggests that they function in plant defense.

Effects of the glucosinolate-myrosinase system on specialist and generalist herbivores display heterogeneous results that do not strictly adhere to chemical defense theory. For example, increasing glucosinolate levels in *Brassica juncea* reduced feeding by a generalist lepidopteran herbivore, *Spodoptera eridania*, while the specialist *Plutella xylostella* was unaffected by glucosinolate concentration in *B. juncea*. Further, increased glucosinolate levels in *B. rapa* also led to decreased feeding by both the specialist, *Pieris rapae*, and the generalist, *Trichoplusia ni* (Stowe 1998). Elevated myrosinase levels had the opposite effect and decreased herbivory by the specialist, *P. xylostella* (Li *et al.* 2000). In contrast, elevated glucosinolate levels inhibited feeding of *P. xylostella* on *B. rapa* plants (Siemens and Mitchell-Olds 1996). Many studies have shown that specialist herbivores are attracted by glucosinolates and their breakdown products (Huang and Renwick 1994; Pivnick *et al.* 1994; Stadler *et al.* 1995; Bartlet *et al.* 1997; Rojas 1999; Griffiths *et al.* 2001; Moyes and Raybould 2001). In contrast, these compounds may play a defensive role by

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attracting a parasitoid of aphid herbivores (Bradburne and Mithen 2000). Simultaneous analysis of the joint effects of glucosinolates and myrosinase together might clarify the defensive role of this secondary metabolic system.

Effects of the glucosinolate-myrosinase system on generalist and specialist herbivores may be clarified by elucidating genetic control of defensive physiology and its effects on herbivory by generalists and specialists. *Arabidopsis thaliana* ecotypes differ with respect to glucosinolate content and composition, providing a suitable system for quantitative genetics (Kliebenstein *et al.* 2001a, b). Recombinant inbred (RI) lines in *A. thaliana* have been used to map loci controlling the glucosinolate-myrosinase system (Mithen *et al.* 1995; Mitchell-Olds and Pedersen 1998; Kliebenstein *et al.* 2001a). Quantitative trait locus (QTL) mapping of specialist and generalist herbivory responses on these same RI lines would uncover genes governing insect feeding responses. The relative effect of each locus could be quantified and interactions among the loci elucidated. In addition, the relative importance of the glucosinolate system can be compared for specialist and generalist insects.

To analyze genetic variation underlying defense against specialist and generalist insect herbivores, we measured feeding rates of *T. ni* (cabbage looper, *T. ni*, generalist) and *P. xylostella* (diamondback moth, *P. xylostella*, specialist) lepidopteran herbivores on Arabidopsis L*er* × Col and L*er* × Cvi RI lines. *T. ni* larvae have a wide host range that includes Brassica crops, while *P. xylostella* larvae feed only on Brassicaceae (Shorey *et al.* 1962). Quantitative levels of herbivore damage were used to map insect resistance QTL. The QTL for insect feeding were then compared to QTL regulating the glucosinolate-myrosinase system, to test the prediction that glucosinolates and myrosinase have larger effects on generalist insect herbivores than on crucifer specialists.

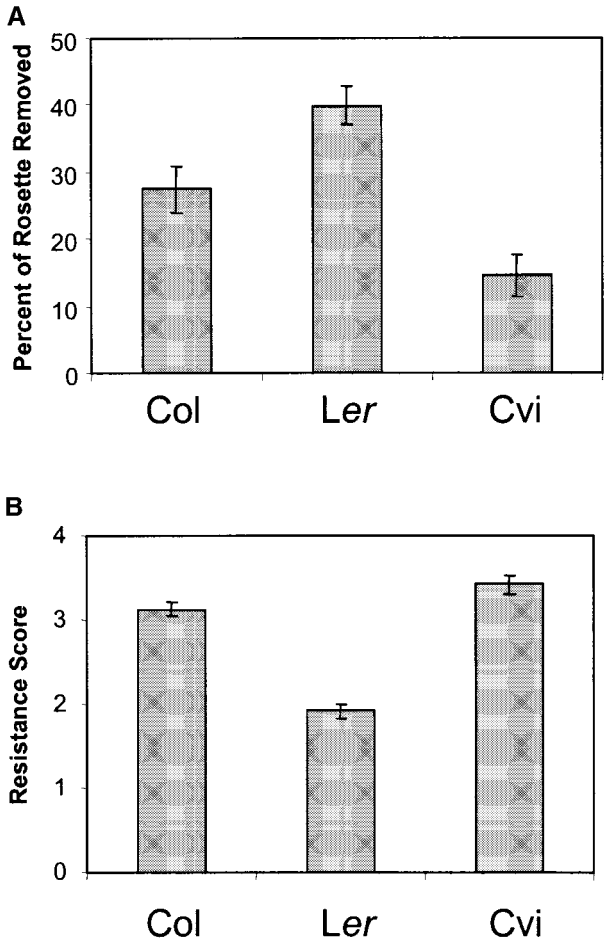

Figure 1.—Natural variation in insect resistance. (A) The mean resistance of the Col, L*er*, and Cvi ecotypes to *T. ni* herbivory measured after 50 hr. (B) The mean resistance of the Col, L*er*, and Cvi ecotypes to *P. xylostella* herbivory measured after 48 hr. The resistance score was determined as described (Stotz *et al.* 2000).

MATERIALS AND METHODS

Plant and insect growth conditions: All plant lines were obtained from the Arabidopsis Stock Center (Nottingham, UK). Plants were grown in potting soil mix with timed-release fertilizer (Osmocote). The plants were thinned to a density of one plant per cell in a 96-cell flat (507 plants m^{-2}) and grown under 14-hr day length with cool white and GrowLux fluorescent bulbs in a controlled environment growth room. After planting, flats were cold stratified at 4° for 5 days and then moved to the growth room. After 4 weeks (before the onset of flowering) the plants were analyzed for insect herbivory. *P. xylostella* eggs were obtained from Anthony Shelton (Department of Entomology, New York State Agricultural Experimental Station, Geneva, NY) and raised on an artificial diet according to published procedures (Perez and Shelton 1997). *T. ni* eggs were obtained from Entopath (Easton, PA) and reared on Southland artificial diet obtained from Entopath.

Measuring insect herbivory: Plant diameter was measured when the plants were 4 weeks old; then a single first instar *P. xylostella* or *T. ni* larva was placed on each rosette for 48 hr. The insects were taken directly from artificial growth medium and placed on the plants without a starvation period. The percentage of the rosette removed by the insect was estimated by eye with the aid of a transparent 1-cm^2 grid. Larvae were allowed to roam at will during the experiment. However, there was at least one insect on >95% of the plants at the end of each experiment. Additionally, all plants were investigated for the presence of insect larvae, and any plants lacking an insect were noted and removed from analysis. There was no significant variation among families for the proportions of insects remaining for the full 48 hr (our unpublished data).

Experimental design: Insect herbivory assays were carried out on the parental ecotypes (Col, L*er*, and Cvi) to determine whether they differed genetically. The percentage of the rosette eaten by a single insect larva over 48 hr was measured on at least 30 plants from each ecotype. In addition, 95 RI lines from the Col × L*er* cross and 160 lines from the Cvi × L*er* cross were scored for herbivory damage (Lister and Dean 1993; Alonso-Blanco *et al.* 1998b). From the complete set of 300 L*er* × Col RI lines, we chose 95 lines that were maximally informative for mapping previously identified myrosinase activity QTL (Mitchell-Olds and Pedersen 1998).

We used a randomized complete blocks design with 10–13 replicates for both populations (L*er* × Col, $N = 960$; L*er* × Cvi, $N = 1620$–2106). For the L*er* × Col RI lines, each 96-cell flat contained one plant from each of the 95 lines being tested and a Col plant. For the L*er* × Cvi RI population, pairs of 96-cell flats were analyzed as a single replicate to enable the use

TABLE 1

ANOVA for insect herbivory in two mapping populations

Cross	Insect	Lines	*N*	*F*-ratio	*P*	H^2
L*er* × Col	*P. xylostella*	95	901	1.26	0.0513	0.12
L*er* × Col	*T. ni*	95	739	3.04	0.0001	0.26
L*er* × Cvi	*P. xylostella*	162	1987	2.08	0.0001	0.18
L*er* × Cvi	*T. ni*	162	1333	3.36	0.0001	0.31

H^2 is the proportion of total variance attributable to genetic differences among lines.

of all 160 lines. The 160 lines were divided equally among the two flats and a L*er* and Cvi plant were planted in both flats. This was repeated independently to analyze both *T. ni* and *P. xylostella* herbivory. Mapping data for the L*er* × Col and L*er* × Cvi RI lines were obtained from the Nottingham Stock Center (http://nasc.nott.ac.uk/).

Aliphatic glucosinolate QTL mapping: A total of 300 L*er* × Col RI lines were grown for 4 weeks in a randomized design three independent times (Lister and Dean 1993). The aliphatic glucosinolates were then assayed from each plant using the previously described high-throughput methodologies (Kliebenstein *et al.* 2001a, b). QTL were mapped using the same marker data sets and techniques as for the insect herbivory QTL.

Statistical methods: Genetic variation among RI lines was analyzed as randomized complete blocks ANOVA using the model HERBIVORY = CONSTANT + FLAT + LINE + SIZE × SIZE. SIZE is a covariate included to control for developmental differences that may occur among individuals of the same line due to size-related environmental causes. Because lines were not replicated within replicates, it was necessary to assume that LINE × FLAT interaction was absent. QTL location and effects were estimated by utilizing the family mean for each RI line in conjunction with both interval mapping and composite interval mapping in QTL Cartographer (Basten *et al.* 1999). The genome-wide 5% significance threshold was estimated by randomly reshuffling the phenotypic data 500 times in QTL Cartographer (Basten *et al.* 1999). Epistatic interactions were tested with SYSTAT by ANOVA, utilizing the mean phenotypic value for each line. Only markers that were individually significant were tested for epistasis.

Genetic correlations, r_G, were estimated from the Pearson product-moment correlation coefficient among family means (typically using the least-squares family means, controlling for flat effects in ANOVA). When traits (such as resistance to several insect species) are measured in separate experiments, r_G provides an unbiased estimator of the genetic correlation (Falconer and Mackay 1996). In addition, we used ANCOVA to test whether the correlation between *P. xylostella* and *T. ni* damage in the L*er* × Cvi RI lines was controlled by a QTL near *erecta*:

P. XYLOSTELLA = CONSTANT + TNI (1)

P. XYLOSTELLA = CONSTANT + TNI + ERECTA (2),

where ERECTA is a categorical variable indicating genotype at the *erecta* locus. If a significant regression of *P. xylostella* resistance onto *T. ni* resistance is found in model 1, but is not significant in model 2, then the correlation between resistance to these herbivores is attributable to the QTL located near the *erecta*.

RESULTS

Variation among parental ecotypes for insect herbivory: Comparison of the mean levels of herbivore damage for *T. ni* and *P. xylostella* feeding showed significant differences between Landsberg *erecta* (L*er*), Cape Verdi Islands (Cvi), and Columbia (Col; Figure 1). All three ecotypes had significantly different *T. ni* herbivory levels ($N = 91$, $R^2 = 0.31$, $P < 0.0001$). Cvi was the most resistant while L*er* was the most susceptible. *P. xylostella* feeding also varied significantly, with L*er* showing more resistance than Col and Cvi, which had nearly identical feeding scores (Figure 1). These results indicate that resistance to insect herbivory varies among L*er*, Col, and Cvi.

Variation among RI lines for insect herbivory: Analysis of rosette damage by *P. xylostella* and *T. ni* larvae on 96 L*er* × Col RI lines and 160 L*er* × Cvi RI lines (Table 1) showed significant differences, indicating that these RI populations can be used to identify loci mediating insect defense (Lister and Dean 1993; Alonso-Blanco *et al.* 1998a).

QTL regulating resistance to *T. ni* herbivory: The mean levels of feeding damage per line were utilized for mapping QTL controlling resistance to *T. ni* herbivory. In both L*er* × Col and L*er* × Cvi, three QTL affecting the level of *T. ni* herbivory were mapped (Figure 2). None of the QTL overlapped between the two crosses, indicating that at least six loci controlling *T. ni* resistance segregate in these populations (Figure 2). Increased susceptibility to *T. ni* herbivory was mediated by the L*er* alleles at QTL in the L*er* × Col cross and at two of the three QTL in the L*er* × Cvi cross (Figure 2). The L*er* alleles at the QTL near *AOP* and *EC198L* caused increased susceptibility. All six QTL alter insect herbivory by 10–20% (Figure 2).

***T. ni* herbivory is negatively correlated with myrosinase activity:** In the L*er* × Col RI population there are two QTL controlling myrosinase activity (Mitchell-Olds and Pedersen 1998). The *NCC1* myrosinase QTL cosegregates with one of the *T. ni* QTL (Mitchell-Olds and Pedersen 1998). Comparison of myrosinase activity with *T. ni* herbivory revealed a modest but significant negative genetic correlation between the two (Figure 3, $r_G = -0.40$, $P < 0.001$, $N = 96$; Mitchell-Olds and Pedersen 1998), indicating that *T. ni* herbivory decreases with increasing levels of myrosinase activity. Myrosinase QTL have not been mapped in the L*er* × Cvi RI population.

***T. ni* herbivory is negatively correlated with glucosino-**

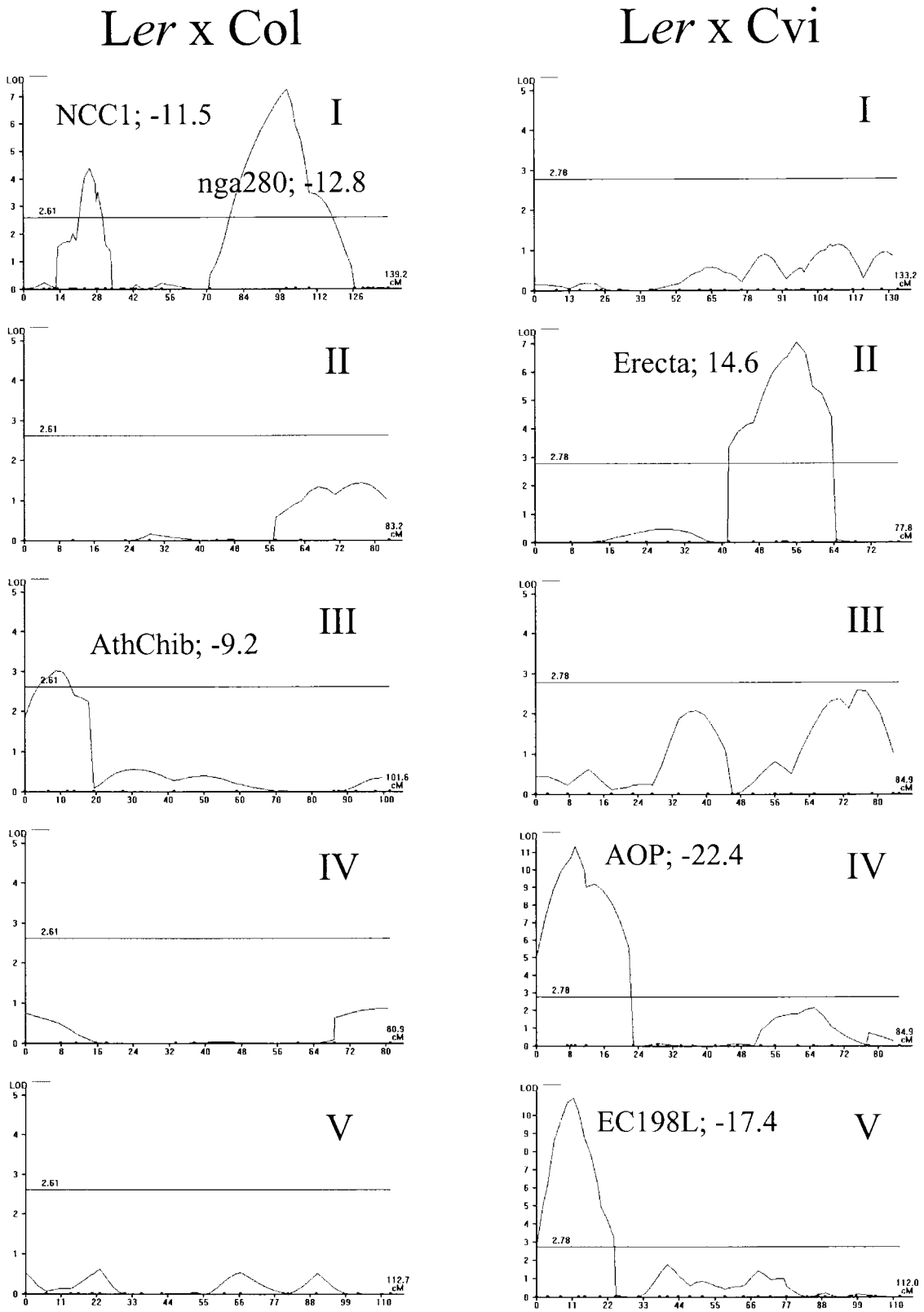

FIGURE 2.—QTL controlling resistance to *T. ni* herbivory. LOD plots of QTL controlling resistance of 4-week-old plants to *T. ni* herbivory obtained by composite interval mapping. The five chromosomes are marked by their corresponding Roman numeral and the axes are independently scaled to accommodate chromosome length and maximum LOD scores. The horizontal line represents the genome-wide $P = 0.05$ LOD score as determined by 500 random permutations of the data. Each QTL is labeled with the marker exhibiting the highest significance and the percentage effect of the L*er* allele on resistance. Positive values indicate that plants with the L*er* allele increase herbivory resistance in comparison to those carrying the *Cvi* allele and vice versa for a negative effect. (Left) Map for the L*er* × Col RI population. (Right) Map for the L*er* × Cvi RI population.

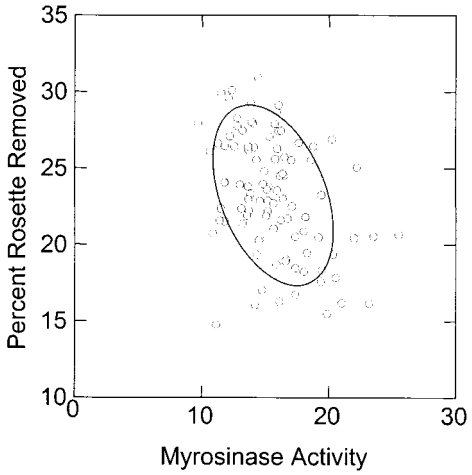

FIGURE 3.—Glucosinolate hydrolysis negatively impacts *T. ni* herbivory. A plot of mean *T. ni* herbivory *vs.* mean myrosinase activity for 96 L*er* × Col RI lines. The oval is the 95th percentile space obtained by least-squares regression.

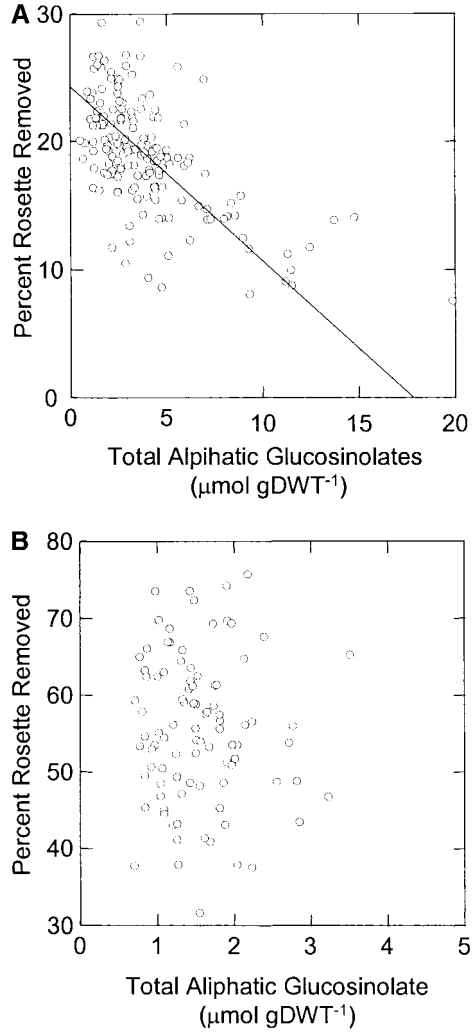

FIGURE 4.—Glucosinolates negatively impact *T. ni* herbivory. Comparison of the mean total leaf aliphatic glucosinolate concentration and insect herbivory. Notice the greater concentration of total aliphatic glucosinolates in L*er* × Cvi (scaling of the horizontal axis). (A) Plot of *T. ni* herbivory *vs.* leaf aliphatic glucosinolates in L*er* × Cvi. Diagonal line is the linear model obtained by least-squares regression. (B) Plot of *T. ni* herbivory *vs.* leaf aliphatic glucosinolates in L*er* × Col.

late concentration: In the L*er* × Cvi RI population, three QTL regulate leaf aliphatic glucosinolate concentration (KLIEBENSTEIN *et al.* 2001a). Two of these QTL, *AOP* and *EC198L*, are in the same region as *T. ni* herbivory QTL, suggesting that aliphatic glucosinolates deter *T. ni* herbivory (Figure 2 and KLIEBENSTEIN *et al.* 2001a). Comparison of leaf aliphatic glucosinolate concentrations *vs.* the rate of *T. ni* herbivory in the L*er* × Cvi RI lines showed a strong negative genetic correlation between concentrations of aliphatic glucosinolates and *T. ni* herbivory (Figure 4A, $r_G = -0.60$, $P < 0.001$, $N = 160$). ANOVA comparing the three QTL for glucosinolate concentration (*AOP*, *EC198L*, and *Elong*) in L*er* × Cvi to *T. ni* herbivory showed statistical significance, suggesting that the three glucosinolate loci significantly alter *T. ni* herbivory (Table 2). The relationship between these QTL appears to be the same for regulation of glucosinolates and herbivory responses: *AOP* interacts epistatically with *EC198L* and *Elong* to regulate leaf aliphatic glucosinolate concentration and *T. ni* herbivory (ANOVA, Table 2; KLIEBENSTEIN *et al.* 2001a). Therefore, glucosinolate concentration appears to be a major determinant of resistance to *T. ni* herbivory in the L*er* × Cvi RI population.

In the L*er* × Col RI population, the correlation between *T. ni* herbivory and glucosinolate concentration was not statistically significant (Figure 4B, $r_G = 0.08$, $P = 0.42$, $N = 93$), perhaps because the L*er* × Col cross has substantially less variation in glucosinolate concentration. Further, no herbivory or aliphatic glucosinolate QTL overlapped in this cross (Figure 5).

QTL regulating resistance to *P. xylostella* herbivory: The line means of feeding damage by *P. xylostella* on L*er* × Col and L*er* × Cvi RI lines were utilized to identify QTL regulating *P. xylostella* herbivory. In the L*er* × Cvi RI population two QTL were identified. They mapped near *erecta* on chromosome II and near the amplified fragment length polymorphism marker *DF184L* on chromosome V (Figure 6; LISTER and DEAN 1993; ALONSO-BLANCO *et al.* 1998b). For both QTL, the L*er* allele imparts increased resistance to *P. xylostella* herbivory (Figure 6). In the L*er* × Col RI lines, we found no significant variation among RI lines for *P. xylostella* resistance (Table 1) and no significant QTL affecting this trait (not shown).

Comparison of *P. xylostella* herbivory and the glucosinolate-myrosinase system: Comparison of *P. xylostella* herbivory to leaf aliphatic glucosinolate concentration in the L*er* × Cvi population found no significant correlation between these traits ($N = 93$, $r_G = 0.08$, $P = 0.416$). Further, no QTL controlling resistance to *P. xylostella*

TABLE 2

ANOVA: epistatic interactions of glucosinolate loci alter *T. ni* herbivory in L*er* × Cvi

Source	d.f.	Mean square	*F*-ratio	*P*
ERECTA	1	188.239	20.337	0.0000
GS-AOP	1	623.798	67.393	0.0000
EC198L	1	335.844	36.284	0.0000
GS-ELONG	1	54.697	5.909	0.0164
GS-AOP × EC198L	1	61.928	6.691	0.0108
GS-AOP × GS-ELONG	1	40.097	4.332	0.0393
GS-ELONG × EC198L	1	7.528	0.813	0.3688
ERECTA × GS-AOP	1	5.263	0.569	0.4521
ERECTA × EC198L	1	2.246	0.243	0.6231
ERECTA × GS-ELONG	1	21.788	2.354	0.1273
Error	135	9.2561		

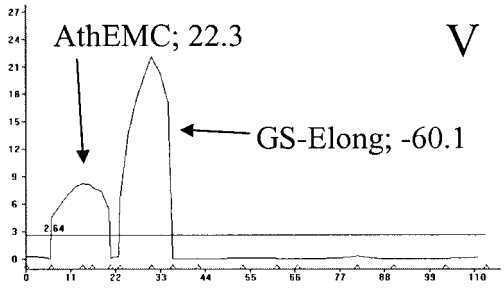

FIGURE 5.—QTL controlling total aliphatic glucosinolate levels in L*er* × Col leaves. LOD plot of QTL controlling aliphatic glucosinolate concentration in 4-week-old plants in the L*er* × Col RI population obtained by composite interval mapping. Only chromosome V contained significant QTL. The horizontal line represents the chromosome-wide $P = 0.05$ LOD score as determined by 500 random permutations of the data. Each QTL is labeled with the marker exhibiting the highest significance and the percentage effect of the L*er* allele on glucosinolate accumulation.

cosegregated with QTL regulating any known aspect of the glucosinolate-myrosinase system (Figure 6; MITCHELL-OLDS and PEDERSEN 1998; KLIEBENSTEIN *et al.* 2001a).

One QTL regulates resistance to both insects: Resistance to *T. ni* and *P. xylostella* herbivory in L*er* × Cvi is positively correlated (Figure 7, $r_G = 0.23$, $P = 0.003$, $N = 160$). Comparison of resistance QTL for the two insects indicates that the *erecta* region influences damage by both herbivores in the L*er* × Cvi lines (Figures 2 and 6). ANCOVA showed that correlated genetic patterns of resistance were completely attributable to the QTL located near *erecta*: ($r_G = 0.50$, model 2: ERECTA factor: $P < 0.001$, TNI covariate: $P = 0.495$, $N = 160$).

DISCUSSION

We found higher levels of genetic variation for resistance to the generalist herbivore, *T. ni*, than for the component mediating specialist, *P. xylostella* (Table 1). Furthermore, five QTL regulating *T. ni* herbivory overlap with QTL known to regulate the glucosinolate-myrosinase system, while *P. xylostella* feeding did not appear to be influenced by the glucosinolate-myrosinase system (Figures 2 and 6; KLIEBENSTEIN *et al.* 2001a). While generalist feeding was influenced by the glucosinolate-myrosinase system, there was variation for the relative impact of glucosinolate concentration and myrosinase activity between the two RI lines.

The *AOP* and *EC198L* QTL for glucosinolate levels overlapped with *T. ni* herbivory QTL in Cvi × L*er* (Figure 2). Both loci influence glucosinolate concentration, while *AOP* also influences glucosinolate type (KLIEBENSTEIN *et al.* 2001c). The allelic status at *AOP* determines the production of either alkenyl or hydroxy aliphatic glucosinolates. The fact that both QTL control *T. ni* herbivory and glucosinolate concentration suggests that glucosinolate concentration is more important than type in deterring *T. ni* herbivory (Figure 4). When a third (*Elong*) locus regulating glucosinolate amount was included in ANOVA, the *AOP* locus was epistatic to *Elong* and *EC198L* for both *T. ni* feeding and regulation of glucosinolate amount (Table 2; KLIEBENSTEIN *et al.* 2001a). Our findings suggest that glucosinolate loci play an important role in deterring feeding by *T. ni* and other generalist herbivores on Arabidopsis.

We did not find significant insect resistance QTL near *AOP* and *EC198L* in the L*er* × Col lines (Figure 2), and glucosinolate amount was not significantly correlated with herbivory in this cross (data not shown). The apparent discrepancy between glucosinolate concentration and feeding damage between L*er* × Cvi and L*er* × Col may be explained by the differences in maximal glucosinolate levels between the two populations. The L*er* × Cvi population has maximal aliphatic glucosinolate levels of ~20 μmol per gram dry weight ($gDWT^{-1}$) while the L*er* × Col population reaches only 5 μmol $gDWT^{-1}$ (KLIEBENSTEIN *et al.* 2001a). Contrasting results between the two crosses suggest that the rate of *T. ni* herbivory is little affected by glucosinolate levels up to at least 5 μmol $gDWT^{-1}$, but that higher concentrations inhibit *T. ni* herbivory.

While QTL regulating glucosinolate amount were not found to overlap with *T. ni* resistance QTL in L*er* × Col, one resistance QTL overlapped with the *NCC1* myrosinase activity QTL (MITCHELL-OLDS and PEDERSEN 1998). Further analysis showed that in L*er* × Col, increased myrosinase levels have a significant negative genetic correlation with *T. ni* feeding (Figure 3). This suggests that myrosinase may play a greater role in deterring herbivory when glucosinolate concentrations are relatively low. The results from these crosses indicate that both glucosinolate and myrosinase levels can inhibit *T. ni* herbivory and should be included in studies that assess the effects of this system on generalist herbivores.

The other two *T. ni* herbivory QTL in the L*er* × Col cross, *nga280* and *AthChib*, have been shown to regulate

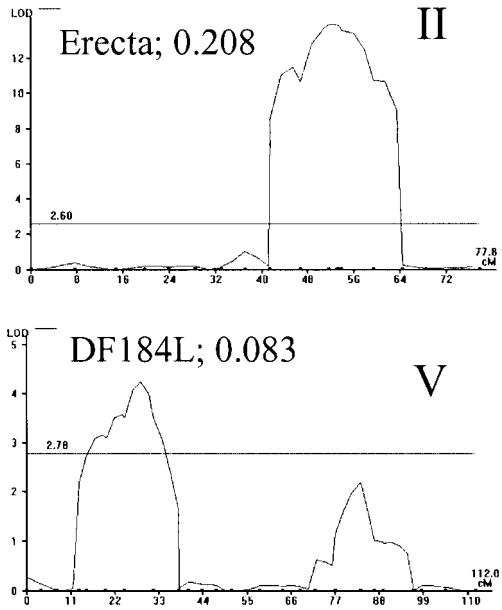

FIGURE 6.—QTL controlling resistance to *P. xylostella* herbivory. LOD plots of QTL controlling *P. xylostella* herbivory on 4-week-old plants in the L*er* × Cvi RI population obtained by composite interval mapping. Only the two chromosomes with significant QTL are shown. The axes are independently scaled to accommodate chromosome length and maximum LOD scores. The horizontal line represents the genome-wide $P = 0.05$ LOD score as determined by 500 random permutations of the data. Each QTL is labeled with the marker exhibiting the highest significance and the percentage effect of the L*er* allele on resistance. Positive values indicate that plants containing the L*er* allele are more resistant to herbivory than are those containing the other allele and vice versa for a negative effect. No significant QTL were identified in the L*er* × Col RI population.

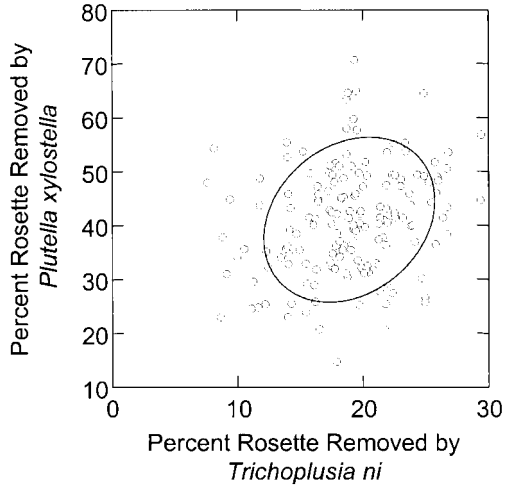

FIGURE 7.—Correlation of resistance to *P. xylostella* and *T. ni* herbivory. A plot of the mean resistance to *P. xylostella* and *T. ni* herbivory in 160 L*er* × Cvi RI lines. The oval is the 95th percentile space obtained by least-squares regression.

the type of aliphatic glucosinolate breakdown product produced after tissue damage (LAMBRIX *et al.* 2001). The QTL near *nga280* may be identical to the *TASTY* locus, which was previously identified as a *T. ni* herbivory QTL in L*er* × Col (JANDER *et al.* 2001). In combination, genes near *nga280* and *AthChib* determine the ratio of nitrile to isothiocyanate glucosinolate breakdown products. Comparison of *T. ni* herbivory and breakdown products showed that isothiocyanates are stronger feeding deterrents than nitriles (LAMBRIX *et al.* 2001). This indicates that glucosinolate production, rate of glucosinolate breakdown, and type of breakdown product all influence *T. ni* herbivory in Arabidopsis.

High myrosinase levels have previously been shown to be a feeding deterrent for *P. xylostella* (LI *et al.* 2000). In L*er* × Col, myrosinase and herbivory QTL did not overlap, and myrosinase levels did not influence *P. xylostella* herbivory. This disparity may reflect species-specific or concentration-specific variation in the mode of action of the glucosinolate-myrosinase system. Alternatively, statistical power to detect *P. xylostella* resistance QTL may differ between these two studies.

QTL mapping indicated one region that regulated resistance to both *T. ni* and *P. xylostella* herbivory. This QTL is tightly linked to the *erecta* locus in the L*er* × Cvi RI population but is not found in L*er* × Col, where *erecta* is also segregating. This disparity between RI populations suggests that resistance to these two insect herbivores is not caused by the *erecta* mutation. It is possible that this region contains a locus that imparts broad-specificity insect resistance or, alternatively, this region may contain two or more loci that independently control resistance to *T. ni* or *P. xylostella* herbivory. Fine-scale QTL mapping experiments are required to differentiate between these alternatives.

The experimental determination of the myrosinase levels, glucosinolate levels, and insect herbivory were conducted on independent plants in two different locations over a several year time span. The environments were maintained as similar as possible by utilizing the same soil type, lights, and growth chambers. However, fluctuations in the environment between the experiments could be affecting our results. However, three independent QTL mapping studies of glucosinolate concentration in the L*er* × Col RI populations conducted at both sites identified the same glucosinolate concentration QTL (our unpublished results). This suggests that the major effect of the differing environments may be to diminish the correlation between the glucosinolate/myrosinase system and insect herbivory. However, final confirmation of the herbivory QTL as glucosinolate/myrosinase loci remains to be functionally verified.

A. thaliana contains significant natural genetic variation for resistance to insect herbivory. Mapping QTL responsible for resistance in RI lines allows for comparison with published QTL maps and rapid testing of plant-insect interaction models. Combining the genomics tools available in Arabidopsis with the genetic tools de-

scribed in this article should allow the cloning of uncharacterized insect herbivory QTL. Finally, molecular characterization of these QTL will enhance our understanding of how plants defend themselves from insect herbivory.

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