

The Dominance of the Herbicide Resistance Cost in Several *Arabidopsis thaliana* Mutant Lines

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ABSTRACT

Resistance evolution depends upon the balance between advantage and disadvantage (cost) conferred in treated and untreated areas. By analyzing morphological characters and simple fitness components, the cost associated with each of eight herbicide resistance alleles (acetolactate synthase, cellulose synthase, and auxin-induced target genes) was studied in the model plant *Arabidopsis thaliana*. The use of allele-specific PCR to discriminate between heterozygous and homozygous plants was used to provide insights into the dominance of the resistance cost, a parameter rarely described. Morphological characters appear more sensitive than fitness (seed production) because 6 vs. 4 differences between resistant and sensitive homozygous plants were detected, respectively. Dominance levels for the fitness cost ranged from recessivity (*csr1-1*, *ixr1-2*, and *axr1-3*) to dominance (*axr2-1*) to underdominance (*aux1-7*). Furthermore, the dominance level of the herbicide resistance trait did not predict the dominance level of the cost of resistance. The relationship of our results to theoretical predictions of dominance and the consequences of fitness cost and its dominance in resistance management are discussed.

OVER the last 40 years, pest control programs have faced major problems in overcoming the evolution of resistance to xenobiotics. Resistance management has thus received much attention, and several theoretically based strategies to delay or prevent resistance spread have been proposed (PECK 2001). Such studies have identified the main parameters affecting resistance evolution; not surprisingly, those parameters are roughly the same as those described by early population geneticists as determining the maintenance of genetic polymorphism (LEVENE 1953; DEMPSTER 1955). The main forces affecting demography and ecological adaptation are gene flow between treated and untreated areas, population densities (carrying capacities), and selection pressure in both treated and untreated areas (LENORMAND and RAYMOND 1998). The fate of resistance alleles under selection depends on their selective advantage or disadvantage (cost) in the presence or the absence of pesticides in the environment, as well as the dominance (*i.e.*, the fitness of heterozygous RS individuals compared to resistant RR and sensitive SS homozygous individuals) of these effects (MAXWELL and MORTIMER 1994). The dominance of a resistance trait can depend on environmental parameters (BOURGUET *et al.* 1996), vary with genetic strain (BOURGUET *et al.* 1997), and evolve by fixation of costs modifiers (OTTO and BOURGUET 1999). Mutation rates, dominance toward

herbicide resistance, and fitness impairment have thus been the subject of much research.

Despite the importance of the dominance of the resistance cost in the evolution of resistance, no study has been specially designed to measure the dominance of resistance costs. Understanding the relative dominance of fitness costs is important because the resulting spread and establishment of resistance genes may initially depend on the fitness of heterozygous RS individuals: (i) In diploids, the first mutant will appear in a RS genetic context and (ii) in outcrossing plant species, migration of RR seeds or gene flow by pollen from a resistant population to a sensitive population will also produce RS individuals. The diffusion of a resistance allele will be much faster if the fitness cost associated with the resistance allele is recessive (RS = SS) instead of dominant (RS = RR; CARRIÈRE and TABASHNIK 2001). The importance of this parameter to the speed of resistance spread can be illustrated with the LEVENE (1953) or DEMPSTER (1955) models for the maintenance of polymorphism. Changing the dominance of the fitness cost of a resistance allele in a habitat mosaic (of both treated and untreated areas) can result in a range of outcomes from fixation of the allele to maintenance at a low equilibrium frequency.

In this study, we used eight different herbicide resistances developed in the model cruciferous plant species *Arabidopsis thaliana* to evaluate the range of dominance of the resistance cost: the well-characterized chlorsulfuron resistance (BERGELSON *et al.* 1996; PURRINGTON and BERGELSON 1997, 1999), imazapyr resistance (SATHASIVAN *et al.* 1991), three independent resistances to

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TABLE 1

Target site and point mutations conferring herbicide resistance in the mutant lines used in this study

HRAC group ^a	Herbicide group	Target site	Mutant line	Mutation
B (chlorsulfuron)	ALS inhibitor	<i>CSR</i>	<i>csr1-1</i>	C(589)T Pro(197)Ser ^b
B (imazapyr)	ALS inhibitor	<i>CSR</i>	<i>csr1-2</i>	G(1958)A Ser(653)Asn ^c
0 (2-4D)	Synthetic auxins	<i>AUX1</i>	<i>aux1-7</i>	G(3214)A Gly(459)Asp ^d
		<i>AXR1</i>	<i>axr1-3</i>	G(899)A Cys(154)Tyr ^e
		<i>AXR2</i>	<i>axr2-1</i>	C(816)T Pro(87)Ser ^f
L (isoxaben)	Inhibition of cellulose synthase	<i>CESA 3</i>	<i>ixr1-1</i>	G(4485)A Gly(998)Asp ^g
		<i>CESA 3</i>	<i>ixr1-2</i>	C(4317)T Thr(942)Ile ^g
		<i>CESA 6</i>	<i>ixr2-1</i>	C(4714)T Arg(1064)Trp ^h

^a HRAC, Herbicide Resistance Action Committee (<http://www.weedscience.org>).

^b HAUGHN *et al.* (1988).

^c SATHASIVAN *et al.* (1990).

^d BENNETT *et al.* (1996).

^e LEYSER *et al.* (1993).

^f NAGPAL *et al.* (2000).

^g SCHEIBLE *et al.* (2001).

^h DESPREZ *et al.* (2002).

the herbicide isoxaben, affecting two different target genes (HEIM *et al.* 1989, 1990b), and three resistances to the herbicide 2-4D conferred by three independent mutations, each on a different target gene (ESTELLE and SOMERVILLE 1987; PICKETT *et al.* 1990; WILSON *et al.* 1990). The chlorsulfuron herbicide (sulfonylurea group) and imazapyr herbicide (imidazolinone group) are chemical compounds inhibiting the branched-chain amino acid biosynthetic enzyme acetolactate synthase (ALS; LAROSSA and SCHLOSS 1984; LAROSSA and FALCO 1984). Isoxaben herbicide (benzamide group) is a specific inhibitor of cellulose biosynthesis (HEIM *et al.* 1990a), and 2-4D herbicide (phenoxy-carboxylic acids group) disrupts very diverse aspects of plant development (stem cell elongation, cell division during lateral root formation, and vascular strand differentiation; NAGPAL *et al.* 2000).

In this article, we present results from an analysis of morphological and productivity-related traits in a segregating R/S population at the F₂ generation in the absence of herbicide treatment. Our objectives were (i) to determine and compare the direct cost corresponding to the trade-off between fitness and resistance and (ii) to estimate the dominance of resistance costs for these eight herbicide resistances. The results and their implications for resistance management are discussed.

MATERIALS AND METHODS

Plant materials: The eight herbicide resistances in *A. thaliana* have all been isolated from ethyl methanesulfonate (EMS) mutagenized populations of the wild-type Columbia (Col) ecotype (Table 1). All mutant lines were provided to us via the Nottingham Stock Centre, with the exception of mutant line *ixr2-1*, kindly provided from the Institut National de la Recherche Agronomique-Versailles collection by S. Vernhettes. These lines are well characterized and the point mutations confer-

ring herbicide resistance are described in the literature (see Table 1).

For each resistance, a process of “genetic background randomization” was performed by generating a segregating F₂ population from a cross between each mutant line (Col genetic background) and the male sterile sensitive line NW77 (Ler genetic background). A control F₂ population was also generated by pollination of a Ler NW77 plant with a Col SS plant. Crossing schemes are illustrated in Figure 1. Each cross was replicated (named family effect) by using two different plants from the same mutant line. Crossing success was tested by genotyping each resistance allele in parental lines and F₁ plants. For the control crosses, we used the length difference of microsatellite *nga8* between Col and Ler (BELL and ECKER 1994) as a marker.

There are several reasons for choosing this crossing scheme involving the Ler and Col genetic backgrounds:

- The EMS origin of the mutant lines means that they may carry several mutations other than the one conferring resistance (JANDER *et al.* 2003). Only repeated backcrosses to the parental line could ensure complete removal of these undesired mutations (BERGELSON and PURRINGTON 1996; PURRINGTON 2000). By contrast, in an F₂ population, these mutations (except those linked to the mutation conferring resistance) are distributed with equal probability in each of the three genotype classes (SS, RS, and RR) and are therefore expected to bias each class in the same way. Moreover, in our study, the F₂ SS plants resulting from the self-fertilized RS F₁ plant share the same genetic background, with the exception of potential EMS mutations, as the F₂ SS plants resulting from the control cross. A comparison of these SS plants therefore reveals how much the remaining EMS mutations affect fitness.
- The genetic background has been shown to influence the expression of cost of resistance (BERGELSON 1994a). Crosses between Ler and Col genetic backgrounds will increase the genetic variance of the F₂ generation and thus provide a measure of the mean cost in a number of genetic backgrounds (between or even outside the parental range in cases of major nonadditive effects).
- Although a comparison of several *A. thaliana* ecotypes did not demonstrate extensive linkage disequilibrium (NORD-

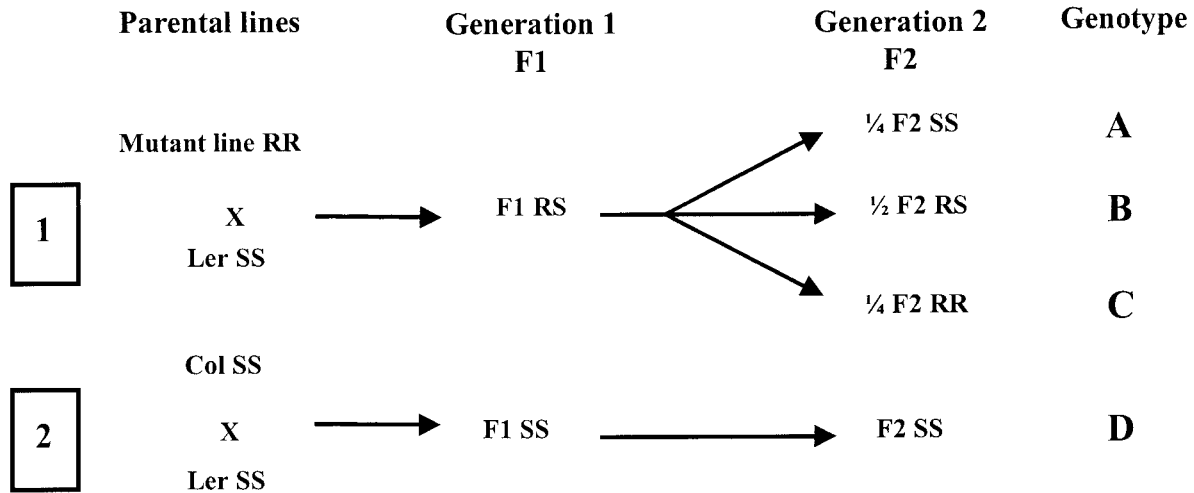


FIGURE 1.—Crossing schemes. In cross 1, buds of a Ler NW77 plant were cross-pollinated by hand with a mutant line plant (background Col). A control cross was performed by pollinating a Ler NW77 plant with a nonmutagenized Col plant (cross 2). There were two replicates for each cross. Resulting F₁ plants were selfed. Within each family, an A/C comparison will reveal the cost linked to a particular resistance allele and behavior of B in relation to A and C will measure dominance of that cost. An A/D comparison will indicate the mean fitness impairment due to other EMS mutations.

BORG *et al.* 2002), the high selfing rate may temporarily create and maintain large linkage groups through the entire genome. Other genes associated or not with a resistance trait or modifying the expression of its cost could be responsible for an artificial increase or decrease of the costs (reviewed in STRAUSS *et al.* 2002). The strategy of crossing inbred lines (Col and Ler) breaks such association and allows the estimation of costs due solely to a difference in resistance traits.

Growth, morphological traits, and fitness components: To measure costs of resistance and the degree of dominance for each of the resistance mutations, an experiment involving 3120 plants of different genetic origins was established. Forty-two seeds of Ler NW77 were included as a control. For each cross and family, 12 F₁ seeds and 150 F₂ seeds were included. These seeds were obtained by crossing a mutant Col RR line with a Ler NW77 SS line (see Figure 1). Eighteen seeds of each mutant Col RR line were added in the experiment. Seeds were sown in 30 trays (44 × 28.5 cm) and watered twice a week. All 3120 seeds were randomized among plots and grown in the absence of herbicides in the greenhouse from 10 April 2002 to 25 June 2002 under natural light supplemented by artificial light to provide a 16-hr photoperiod. The temperature was maintained between 20° and 25°. In each tray, the 104 seeds were regularly spaced 3 cm apart. The edges of trays were sown with (extra) Ler SS seeds to buffer against possible border effects and were discarded from the analysis. To avoid micro-environmental effects, the plots were regularly rotated during the growing period.

To decide the duration of the experiment, we assumed that SS plants would be the most fit. The *A. thaliana* ecological niche is most often a postwinter very narrow window, which necessitates rapid development and seed setting before strong competition among species develops (LINDE *et al.* 2001; REBOUD *et al.* 2004). We therefore decided that the optimal period for plant development would end when all the plants from the control cross had completed their flower production, *i.e.*, had reached their maximum seed production. While this choice greatly facilitates experimental design, it gives extra weight to traits linked to precocity.

A total of 12 phenological and morphological characters

were measured during the experiment: germination date (time from sowing to cotyledon emergence), rosette diameter at the 14th and 21st day after sowing, flowering time, number of rosette and cauline leaves, and height from the soil to first flower at flowering. These parameters provide indirect measures of plant precocity and compactness (X. REBOUD *et al.*, unpublished results). At the end of the experiment, the total number of siliques produced by each plant was counted. The number of fully formed seeds per silique was estimated by sampling the third, fifth, seventh, and ninth siliques on the main stem and measuring their length and productivity. Because silique length is highly correlated to the number of seeds in a silique ($r^2 = 0.988$, $n = 200$), plant productivity was calculated by multiplication of the mean silique size (measured on four siliques) by the number of siliques, giving a total silique length. Since measuring fitness as seed production is appropriate for a self-pollinating species (HEIL and BALDWIN 2002) like *A. thaliana* (SNAPE and LAWRENCE 1971), total individual fitness was assessed by seed production. Our measure of productivity as estimated by silique mean length and silique number is thus a linear approximation of seed counting. This time-saving procedure allowed the measurement of all the plants in the experiment.

Genotyping the resistance status: The identification of pesticide resistance genes when performed by biochemical or standard toxicological assays can distinguish only between susceptible and resistant phenotypes whenever resistance is either fully dominant or recessive. Therefore, an allele-specific PCR method was used to discriminate between the three genotypes (SS, RR, and RS) for each mutation. DNA was extracted from a section of the first cauline leaf that was cut during the last 3 days of the experiment. Each cauline leaf section was then placed in a microcentrifuge tube containing 150 μ l of the extraction buffer described by SAINI *et al.* (1999). The cauline leaf sections were crushed using a mixer mill. Tubes were placed in a water bath at 95° for 6 min, transferred onto ice for 5 min, and vortexed for 15 sec. DNA extracts were kept at -20° prior to PCR analysis. Allele-specific primers were designed with the knowledge that a 3' mismatch does not prime in a PCR at a specific annealing temperature (SOMMER *et al.* 1992). PCR amplification was performed in 20 μ l as

described by DÉLYE *et al.* (1997). One microliter of the supernatant of DNA extracts was used for each PCR reaction. Primers specific for each mutation and the three resulting distinct sizes of amplicons are described in Table 2. Primers F.OUT and R.OUT yielded the common amplicon, while primers F.IN and R.OUT or primers F.OUT and R.IN yielded the sensitive or resistant amplicons, respectively. The cycling program for all mutations consisted of one denaturation step of 1 min at 94°, followed by 37 cycles of 30 sec at 92°, 30 sec at the specific annealing temperature (see Table 2), and 1 min at 72°. Thus, taking the *csr1-1* mutant line as an example, DNA from plants in which both ALS gene copies contain C at position 589 (sensitive allele) would yield two fragments of 636 and 1036 bp, DNA from plants in which both ALS gene copies contain T at position 589 (resistance allele) would yield two fragments of 400 and 1036 bp, and amplification from heterozygous plants would yield three fragments of 400, 636, and 1036 bp.

Statistical analysis: The effect of each resistance mutation on fitness in the F₂ generation was assessed by a nested analysis of variance using the model: $y = \text{plot} + \text{family} + \text{genotype} (\text{family}) + \text{error}$. The presence of EMS mutations other than the one conferring resistance was analyzed using the nested analysis of variance mixed model: $y = \text{plot} + \text{cross} + \text{family} (\text{cross}) + \text{error}$. At the F₂ generation, fitness of SS plants of each cross involving a resistant line was compared to fitness of SS plants resulting from the control cross. These models treat plot and family as random effects while genotype (SS, RS, and RR) and cross were treated as fixed effects. Analyses were performed using Systat 10 software (SPSS). Fitness, flowering date, number of rosette and cauline leaves, height from soil to first flower, and total number of siliques were square-root transformed to homogenize variances, while length of the siliques was squared. The remaining variables (germination date and rosette diameter at the 14th and 21st day after sowing) were not transformed. All transformations succeeded in restoring homogeneity of variance as confirmed by nonsignificant Levene's test results.

To test the effect of resistance on "global" plant morphology, a principal component analysis (PCA) was performed using all the phenological and morphological traits. A multivariate analysis using the two first factors of the PCA as response variables was then performed using the model: $y = \text{plot} + \text{family} + \text{genotype} (\text{family}) + \text{error}$.

The fitness dominance index was taken as

$$h = |\text{SS mean fitness} - \text{RS mean fitness}| / |\text{SS mean fitness} - \text{RR mean fitness}|.$$

Following convention, the resistant allele is dominant toward cost when $h = 1$, semidominant when $h = 0.5$, and recessive when h approaches 0.

RESULTS

Genotyping and genotypic frequencies at the F₂ generation: For the reference cross, parents and F₁ plants were genotyped for the eight mutations conferring resistance. As these plants were all sensitive, we assumed sensitivity of the reference cross F₂ plants. All other plants from crosses involving a resistant mutant line were genotyped. Finally, among the 3120 seeds sown, a total of 2589 plants were analyzed by allele-specific PCR.

The number of surviving plants and the genotypic frequencies in the F₂ generation are given in Table 3. A significant distortion of Mendelian segregation was found for crosses involving *csr1-2* (only one family), *axr1-3*, and

axr2-1 mutant lines. For the cross using the *axr1-3* mutant line, the RR class had fewer plants than expected and the SS class had more plants than expected. As this cross had only a 6% reduction in the survival rate relative to the control cross, the distortion probably originates from lower viability or fertility of gametes containing the *axr1-3* allele. On the other hand, for the *axr2-1* cross, the SS class had the same number of plants compared to all the other crosses involving a mutant line, while both the RS and RR classes had fewer plants than expected compared to other crosses. As the number of surviving plants for this cross is reduced by 29% relative to the reference cross [99–105 plants compared to 130–150 plants for the other crosses (Table 3)], the reduced survival rate of RS and RR plants appears to be responsible for the observed segregation distortion.

Fitness: EMS mutations other than those conferring resistance induced a significant reduction in the fitness of SS plants from four crosses involving a resistant line (Table 4). The SS plants from the *ixr1-2*, *ixr2-1*, *aux1-7*, and *axr2-1* crosses had fitness reduced by 27.17% (total silique length 1425 mm ± 154 SE), 23.09% (1505 mm ± 128 SE), 31.12% (1348 mm ± 107 SE), and 33.89% (1294 mm ± 108 SE), respectively, relative to the SS plants from the control cross (1957 mm ± 91 SE).

A significant genotype effect on fitness was detected in the F₂ generation for five resistances (Table 5): the resistance to chlorsulfuron (*csr1-1* line), one resistance to isoxaben (*ixr1-2* line), and the three resistances to 2-4D (*aux1-7*, *axr1-3*, and *axr2-1* lines). As no plot and family effects were detected for these five resistances, classes were pooled and comparisons among SS, RS, and RR classes were performed using one-way ANOVAs. RR plants for the chlorsulfuron resistance, the isoxaben resistance, and one of the three resistances to 2-4D (*axr1-3* line) exhibited, on average, 36.87, 43.23, and 78.18% reduction in fitness, respectively, when compared to SS plants, whereas no reduction was observed between RS and SS plants (Table 6). Thus, the *csr1-1*, *ixr1-2*, and *axr1-3* resistance alleles demonstrated recessive fitness costs of resistance. Conversely, RR plants for the *axr2-1* mutation showed on average 89.01% reduction in fitness relative to SS plants, while no difference was observed between RS and RR plants (Table 6). The *axr2-1* resistance allele was therefore dominant for the fitness cost of resistance. Finally, no cost of resistance was detected between RR and SS plants in the cross involving the *aux1-7* resistance line, but RS plants exhibited a higher fitness relative to SS plants (Table 6). The *aux1-7* resistance allele is overdominant to the RS plants and this allele would thus be underdominant toward fitness cost.

As some distortion of segregation was found for the *axr1-3* and *axr2-1* resistances, a cost of resistance and the dominance of a resistance cost can be determined at the population level by taking into account both the relative frequencies of the SS, RS, and RR classes and

TABLE 2
Primers

Mutant line	Primers	Sequence (5' to 3')	Annealing temperature	Final concentration in PCR (nM)	Expected size of amplicons (bp)		
					Common	Sensitive	Resistant
<i>csr1-1</i>	csr1-1.F.OUT	ATCAAATCGAGCTCTCCCTCCATCTCC	63°	125	1036	636	400
	csr1-1.R.OUT	AGCCAGCTTAACATCACACACACAGACAC		125			
	csr1-1.F.IN	AGCAATCACAGGACAAGTCC		25			
	csr1-1.R.IN	TGTACCAATCATACGACGAGA		150			
<i>csr1-2</i>	csr1-2.F.OUT	GGAGTTTGGAGGAATGAGTTGAACGTACAG	67°	125	1044	427	617
	csr1-2.R.OUT	TTGGAAACACGGCCCATATGAGC		125			
	csr1-2.F.IN	TGTTGCCGATGATCCCGAG		35			
	csr1-2.R.IN	ACATCGTTGAAAAGTGCCACCAT		150			
<i>aux1-7</i>	aux1-7.F.OUT	AGAGTCGGTGGAGGAATATTATAGTTGG	65°	125	959	255	704
	aux1-7.R.OUT	AAGGAGTGAAAAACAAAATACAAAACCCC		125			
	aux1-7.F.IN	TTCGTCAAAGTCGACACTTTTGG		50			
	aux1-7.R.IN	GTAACACTTGGCAAAAGAGAT		350			
<i>axr1-3</i>	axr1-3.F.OUT	AAGGGCCCTTGGAAAGCGGAGTATCTG	65°	50	1141	468	673
	axr1-3.R.OUT	GGATTGAGCCCACTCTCAGCCATCTTTAG		50			
	axr1-3.F.IN	AITCAAATGTTGAAAACCTTGATAGAACTCG		250			
	axr1-3.R.IN	CAACTTAAAGTTTGGCATCTCCGAT		25			
<i>axr2-1</i>	axr2-1.F.OUT	AGCTCTCTGCTAAAGTAAGCTACAC	63°	125	1051	486	565
	axr2-1.R.OUT	CCTTGTGCTCCATAGTTTCCCTTGAAG		125			
	axr2-1.F.IN	CACAAAGTGGTGGGATGGC		100			
	axr2-1.R.IN	CTGTAGTTCCCTCACAGGTGA		300			
<i>ixr1-1</i>	ixr1-1.F.OUT	CGTCTGAACCAAGTGTGAGGTGGG	64°	125	1036	310	726
	ixr1-1.R.OUT	TGACACCAAGACAGAGAAGAACGACAGACAG		125			
	ixr1-1.F.IN	ATCATGGGGACCACTCTTTGG		20			
	ixr1-1.R.IN	CCTGAAGGCCAAAGAACCAACTTAT		150			
<i>ixr1-2</i>	ixr1-2.F.OUT	GCTTCCAGCTTTC AAGGGTCTGTCTCC	64°	125	1077	478	599
	ixr1-2.R.OUT	TGACACCAAGACAGAGAAGAACGACAGACAG		125			
	ixr1-2.F.IN	TATTGACACAAACTTCACAGTTAC		125			
	ixr1-2.R.IN	GGCATCTGAAGCITTTTGAGA		250			
<i>ixr2-1</i>	ixr2-1.F.OUT	TGCCACTGGGGGTTAAAAG	62°	125	1008	419	589
	ixr2-1.R.OUT	TCATAAAAAGATTACAGATTGAAAATGCTC		125			
	ixr2-1.F.IN	TCTTACACTCTTTGGGTCC		475			
	ixr2-1.R.IN	CCACAAAACGGATTAAACCCA		25			

TABLE 3
Effective class numbers and test for normal Mendelian segregation for the different resistant allele in each of the eight F₂ generations

Cross expected	Family	No. of plants				χ^2 ^a
		Total of 150	SS 37.5	RS 75	RR 37.5	
<i>csr1-1</i> × Ler	1	144	44	63	37	2.93
	2	141	41	66	34	1.27
<i>csr1-2</i> × Ler	1	147	55	79	13	24.82***
	2	150	34	83	33	1.72
<i>aux1-7</i> × Ler	1	143	37	78	28	2.31
	2	141	35	69	37	0.12
<i>axr1-3</i> × Ler	1	130	41	70	19	8.22*
	2	140	50	73	17	15.81***
<i>axr2-1</i> × Ler	1	99	40	44	15	13.85**
	2	105	35	53	17	6.18*
<i>ixr1-1</i> × Ler	1	145	39	66	40	1.18
	2	145	28	86	31	5.15
<i>ixr1-2</i> × Ler	1	138	38	64	36	0.78
	2	144	35	77	32	0.82
<i>ixr2-1</i> × Ler	1	141	40	69	32	0.97
	2	140	32	72	36	0.34
Col × Ler	1	140	140			
	2	147	147			

*0.05 > P > 0.01, **0.01 > P > 0.001, ***P < 0.001.

^a χ^2 was calculated on the basis of expected percentages of ¼ SS, ½ RS, and ¼ RR with 2 d.f.

their productivity. At this level, the cost of resistance increased for the two resistances that have unfavorable segregation distortion: from 78.2 to 91.4% for the *axr1-3* resistance and from 89 to 95.3% for the *axr2-1* resistance, respectively (Table 6). The *axr2-1* resistance allele remained dominant at the population level, whereas

the *axr1-3* resistance allele became slightly less recessive when taking into account the segregation distortion.

Plant morphology and phenology: PCA performed on the 12 morphological and phenological characters gave 40 and 22.4% of the total variance, respectively, to the first two factor axes (Figure 2a). The multivariate analy-

TABLE 4
Estimation of the fitness cost of EMS mutations other than those conferring resistance

Resistance source	<i>csr1-1</i>			<i>csr1-2</i>			<i>ixr1-1</i>			<i>ixr1-2</i>		
	d.f.	Sum of squares	F	d.f.	Sum of squares	F	d.f.	Sum of squares	F	d.f.	Sum of squares	F
Plot	29	7,987.49	1.586*	29	7,150.51	1.250	29	6,923.12	1.345	29	6,749.15	1.350
Cross	1	113.88	0.656	1	25.59	0.130	1	158.58	0.893	1	1,328.58	7.707**
Family (Cross)	2	388.68	1.119	2	378.70	0.960	2	752.75	2.120	2	761.34	2.208
Error	227	39,409.68		223	43,990.36		191	33,910.45		197	33,958.95	
Resistance source	<i>ixr2-1</i>			<i>aux1-7</i>			<i>axr1-3</i>			<i>axr2-1</i>		
	d.f.	Sum of squares	F	d.f.	Sum of squares	F	d.f.	Sum of squares	F	d.f.	Sum of squares	F
Plot	29	5,958.24	1.153	29	5,931.40	1.166	29	7,794.16	1.521	29	6,320.28	1.244
Cross	1	1,183.10	6.639*	1	1,550.17	8.841**	1	83.07	0.470	1	1,549.68	8.842**
Family (Cross)	2	739.02	2.074	2	446.82	1.274	2	823.92	2.331	2	421.07	1.201
Error	210	37,421.57		219	38,400.72		235	41,536.30		214	37,506.40	

For comparison of A and D genotypes, see Figure 1. Comparison of F₂ SS plants of each cross involving a resistance with F₂ SS plants of the control cross at the F₂ generation.

*Significant F-values at P < 0.05; **significance at P < 0.01.

TABLE 5
Analysis of fitness for the eight resistances at the F₂ generation

Resistance source	<i>csr1-1</i>			<i>csr1-2</i>			<i>ixr1-1</i>			<i>ixr1-2</i>		
	d.f.	SS	F	d.f.	SS	F	d.f.	SS	F	d.f.	SS	F
Plot	29	3,791.50	0.844	29	6,478.82	1.048	29	3,534.94	0.874	29	3,396.74	0.968
Family	1	123.39	0.796	1	57.51	0.270	1	576.14	4.131*	1	340.68	2.817
Genotype (family)	4	2,063.28	3.329*	4	758.70	0.889	4	106.98	0.192	4	2,633.76	5.444***
Error	149	23,088.60		166	35,402.21		152	21,197.76		152	18,383.16	

Resistance source	<i>ixr2-1</i>			<i>aux1-7</i>			<i>axr1-3</i>			<i>axr2-1</i>		
	d.f.	SS	F	d.f.	SS	F	d.f.	SS	F	d.f.	SS	F
Plot	29	2,195.60	0.628	29	2,205.74	0.528	29	4,786.65	1.450	29	3,908.59	1.482
Family	1	2,527.84	20.956***	1	89.71	0.623	1	2.47	0.022	1	115.84	1.274
Genotype (family)	4	962.98	1.996	4	2,144.14	3.724**	4	4,141.81	9.094***	4	12,745.60	35.033***
Error	161	19,420.48		153	22,022.16		130	14,801.63		84	7,640.16	

For comparison of A, B, and C genotypes, see Figure 1. SS, sum of squares. *Significant *F*-values at *P* < 0.05; **significance at *P* < 0.01; ***significance at *P* < 0.001.

sis performed on these two axes indicated a significant genotype effect on plant morphology for six resistances (Table 7): the resistance to chlorsulfuron (*csr1-1* line), two resistances to isoxaben (*ixr1-1* and *ixr1-2* lines), and the three resistances to 2-4D (*aux1-7*, *axr1-3*, and *axr2-1* lines). As no plot and family effect was detected for these resistances (except for the *axr2-1* line), comparisons among SS, RS, and RR classes were performed using multivariate analysis of variance (MANOVA) models. For the *axr2-1* line, comparisons were performed with a model that maintained plot and genotype effects. Results are illustrated in Figure 2, b–i. The morphology of the RR genotype differed from the morphology of the RS and SS genotypes in the *csr1-1*, the *ixr1-2*, and the *axr1-3* crosses. These resistance alleles were therefore recessive toward plant morphology. In the cross involving the *axr2-1* allele, all the three genotypes had different morphologies (Figure 2i). The RS plants were, however, morphologically closer to the RR plants than to the SS plants. The *axr2-1* allele was thus partially dominant toward plant morphology while being fully dominant

for the fitness cost. In the *aux1-7* cross, the RR and RS plants were morphologically similar but differed from the SS plants. The *aux1-7* resistance allele was thus dominant for plant morphology. This contrasts with results for fitness, which showed RR plants to be intermediate. Finally, the *ixr1-1* RR class exhibited morphological differences compared to the RS or SS classes while no difference could be detected among these classes for the fitness trait. Here, the *ixr1-1* resistance allele was recessive toward plant morphology. All differences in the degree of dominance according to the mutation and the trait considered are summarized in Table 8.

DISCUSSION

One difficulty in estimating a cost of resistance is to unambiguously attribute the reduction in fitness to the presence of the resistance gene. Using our genetic background randomization approach, we found that for four resistant lines (*ixr1-2*, *ixr2-1*, *aux1-7*, and *axr2-1*) there was an observable difference in fitness between the SS

TABLE 6
Estimation of fitness cost and dominance index at the individual and population level in the F₂ generation

Resistance	Mean fitness (±SE) at the F ₂ generation			Cost at the individual level (%)	Cost at the population level (%)	Dominance index at the individual level	Dominance index at the population level
	SS	SR	RR				
<i>csr1-1</i>	2047 ± 151*	2084 ± 137*	1292 ± 102 [†]	36.87	36.87	0.05	0.05
<i>ixr1-2</i>	1425 ± 154*	1343 ± 85*	809 ± 80 [†]	43.23	43.23	0.13	0.13
<i>aux1-7</i>	1348 ± 107*	1970 ± 100 [†]	1769 ± 164* [†]	None	None	1.48	1.48
<i>axr1-3</i>	2080 ± 154*	1923 ± 101*	454 ± 97 [†]	78.18	91.37	0.10	0.30
<i>axr2-1</i>	1294 ± 108*	259 ± 54 [†]	142 ± 51 [†]	89.01	95.31	0.90	0.91

Fitness corresponds to total silique length in millimeters (see MATERIALS AND METHODS). For each resistance, different symbols (*,†) indicate a significant difference in total silique length (fitness cost) at *P* = 0.05.

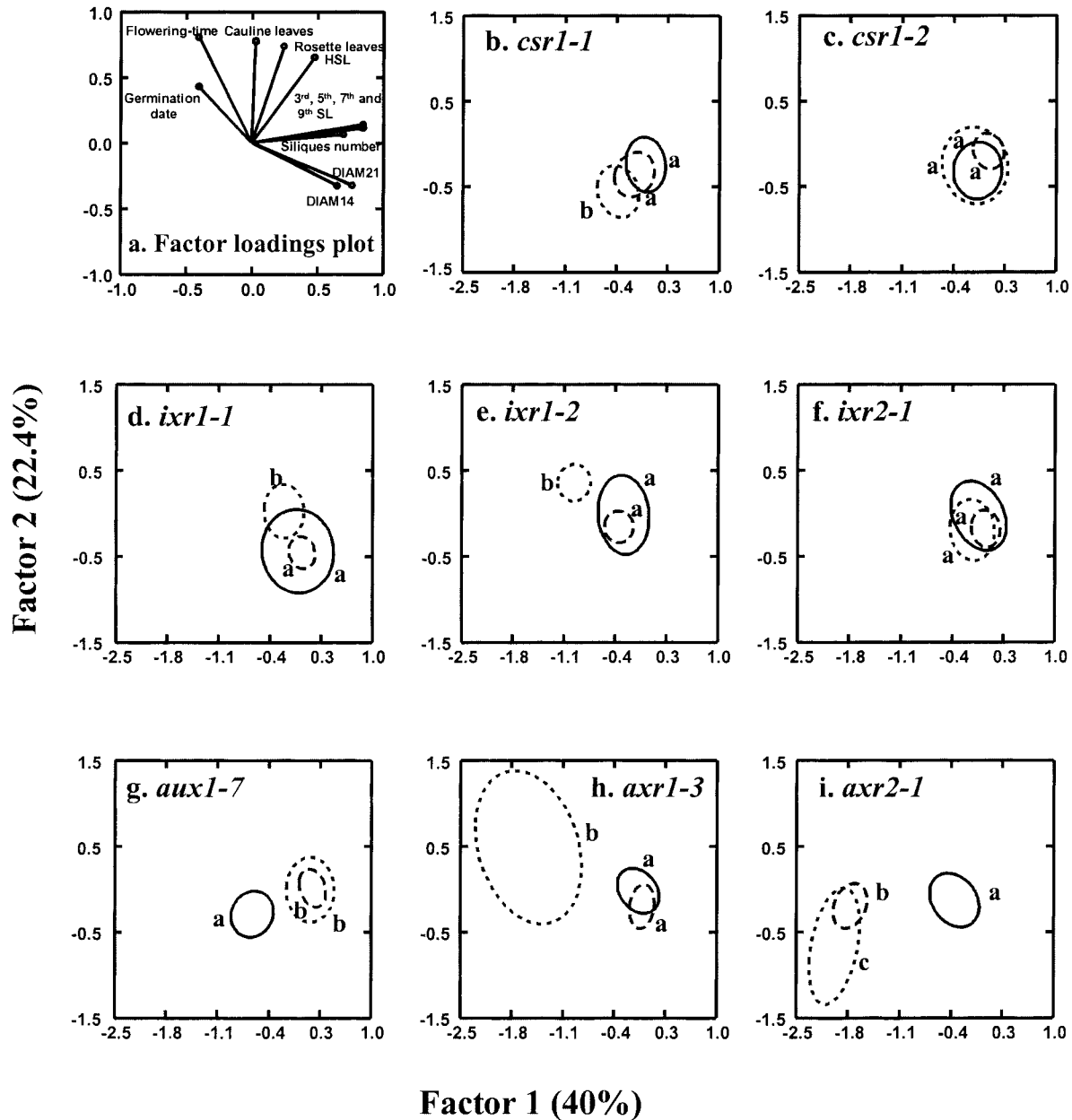


FIGURE 2.—Impact of each resistance trait on plant morphology: (a) Factor loading plot resulting from principal components analysis procedure. Total variance percentages of 40.106 and 22.390% were explained by factor 1 and factor 2, respectively. (b–i) Morphological space for each resistance. To illustrate the relative position of SS, RS, and RR genotypes, centroid confidence ellipses (ELM) centered on the sample means of the factors 1 and 2 were drawn for each genotype at a probability of 0.95. The size of the ELM is adjusted for the sample size. Results from MANOVA models (set up by general linear model; see RESULTS) are shown in each figure, different letters indicating different significant values at $P = 0.05$. DIAM14 and DIAM21: rosette diameter at the 14th and 21st day after sowing, respectively; HSL, height from soil to first flower at flowering; SL, silique length. —, SS plants; ---, SR plants; ···, RR plants.

individuals in the F_2 generation compared to the SS individuals from the control cross. These fitness effects resulting from segregation of EMS-derived deleterious mutations were substantial (23–34% reduction). This observation validates our experimental approach, which randomizes the genetic background in the F_2 generation by homogenizing the distribution of EMS-derived mutations. It does not, however, completely overcome linkage disequilibrium around the resistance gene. Although

this linkage block may be quite large (YOUNG and TANKSLEY 1989), it is important to remember that the genetic variation captured in these blocks should be relatively small (being due to EMS mutations and polymorphisms between Col and Ler). EMS-derived mutations that are closely linked to the resistance gene and are deleterious not only might exacerbate the observed resistance cost but also could modify the dominance level and measures of plant morphology. Use of transgenic engineering

TABLE 7
Analysis of the global morphology for the eight resistances in the F₂ generation

Resistance source	<i>csr1-1</i>			<i>csr1-2</i>			<i>ixr1-1</i>			<i>ixr1-2</i>		
	d.f.	Value	<i>F</i>	d.f.	Value	<i>F</i>	d.f.	Value	<i>F</i>	d.f.	Value	<i>F</i>
Plot	58.294	0.732	0.855	58.330	0.770	0.795	58.302	0.651	1.245	58.302	0.686	1.081
Family	2.147	0.984	1.180	2.165	0.937	5.563**	2.151	0.987	0.970	2.151	0.990	0.741
Genotype (family)	8.294	0.898	2.031*	8.330	0.939	1.319	8.302	0.891	2.248*	8.302	0.764	5.431***

Resistance source	<i>ixr2-1</i>			<i>aux1-7</i>			<i>axr1-3</i>			<i>axr2-1</i>		
	d.f.	Value	<i>F</i>	d.f.	Value	<i>F</i>	d.f.	Value	<i>F</i>	d.f.	Value	<i>F</i>
Plot	58.320	0.742	0.890	58.304	0.670	1.163	58.258	0.593	1.330	58.160	0.402	1.592*
Family	2.160	0.742	27.782***	2.152	0.988	0.935	2.129	0.999	0.033	2.80	0.969	1.275
Genotype (family)	8.320	0.937	1.323	8.304	0.709	7.139***	8.258	0.687	6.658***	8.160	0.322	15.270***

For comparison of A, B, and C genotypes, see Figure 1. Only Wilks' λ -test statistic values are described in this table, but are coherent with probability values associated with Pillai trace and Hotelling-Lawley trace test statistics. *Significant *F*-values at $P < 0.05$; **significance at $P < 0.01$; ***significance at $P < 0.001$.

technology could offer an alternative to precisely measure the fitness cost of resistance because it would introduce the resistance gene without any associated linkage block (BERGELSON *et al.* 1996). Such a transgenic approach, however, would require a thoughtful verification of the gene copy number and an extensive knowledge of the level of expression of the inserted gene.

Cost of resistance: Among the eight mutations conferring herbicide resistance tested here, four revealed a cost of resistance at the fitness level. The resistances associated with a fitness cost do not correspond to a restricted herbicide family group; on the contrary, they span the three herbicide classes. The chlorsulfuron-resistance fitness cost is found here to be 36.9%. This value is consistent with mean seed production of chlorsulfuron-resistant *A. thaliana* transgenic plants (Col genetic background), which were found to be 26–34% less productive than the corresponding susceptible *A. thaliana* segregants (PURRINGTON and BERGELSON 1997 and BERGELSON *et al.* 1996, respectively). This similarity in the cost estimates may suggest that chlorsulfuron resistance is not sensitive to the genetic background, the variance of which was increased in this study by crossing the two inbred lines Col and Ler. However, a literature review (BERGELSON and PURRINGTON 1996) revealed that in many systems, genetic background does have a profound effect on costs of resistance. In these instances, the fate of a resistance gene in the environment may indeed depend primarily on the genotype in which the resistance trait first appears.

The fitness effect of a gene mutation does not predict the fitness of another mutation in the same gene at a different base-pair position. This point is illustrated by the *IXRI* gene, which has two mutations conferring resistance to the same herbicide. One of these mutations has no cost, while the other has a 43.2% cost, even though both mutations are only 168 bases apart and in a highly conserved region (SCHEIBLE *et al.* 2001). In

such cases, natural selection would tend to favor the mutation that renders the plant more fit and thereby influence the molecular evolution of a gene.

With regard to plant morphology, significant differences between RR and SS plants were found in six crosses involving an herbicide-resistant line (including the four resistances conferring a fitness cost). Several morphological modifications could result in a fitness cost, and thus questions as to the origin of the cost remain: Is there an effect on the reproductive phase, an indirect pleiotropic effect that is expressed during the vegetative phase, or a combination of the two? This question is important because intra- or interspecific competition will act differently on vegetative and repro-

TABLE 8

A summary of the dominance of the herbicide resistance trait, its fitness cost, and its effect on vegetative plant morphology for each of the eight mutant lines

Mutant line	Dominance level		
	Herbicide	Fitness cost ^h	Morphology ^h
<i>csr1-1</i>	Dominant ^a	Recessive	Recessive
<i>csr1-2</i>	Dominant ^b	—	—
<i>aux1-7</i>	Recessive ^c	Underdominant	Dominant
<i>axr1-3</i>	Recessive ^d	Recessive	Recessive
<i>axr2-1</i>	Dominant ^e	Dominant	Dominant
<i>ixr1-1</i>	Recessive ^f	—	Recessive
<i>ixr1-2</i>	Recessive ^f	Recessive	Recessive
<i>ixr2-1</i>	Recessive ^g	—	—

^a HAUGHN *et al.* (1988).

^b SATHASIVAN *et al.* (1991).

^c PICKETT *et al.* (1990).

^d ESTELLE and SOMERVILLE (1987).

^e WILSON *et al.* (1990).

^f HEIM *et al.* (1989).

^g HEIM *et al.* (1990b).

^h This study.

ductive phases. When costs are expressed during the vegetative phase, they can be expected to decrease the competitive ability of RR genotypes at an earlier stage of development than costs that act during the reproductive phase. For example, in the presence of competition, a resistant plant with delayed rosette formation may not be able to accumulate enough resources to complete its life cycle, so that fitness would drop to zero. Conversely, a resistant plant with a cost expressed mainly during the reproductive phase could still complete its life cycle because of the resources accumulated during the vegetative phase. The overall cost of a resistance gene whose cost is expressed during the vegetative phase is therefore expected to be more sensitive to density than a fitness cost affecting a “reproductive” trait. Physiological investigations performed by PURRINGTON and BERGELSON (1999), for example, gave a more complete understanding of chlorsulfuron resistance. They demonstrated that the cost is closely associated with the expression level of ALS, which has its peak of activity in reproductive meristems. The cost of chlorsulfuron resistance would therefore affect mainly the reproductive phase. This explanation is supported in this study where differences in the length of siliques and the number of siliques are the main contributors to the difference between SS and RR plants (Figure 2b). Conversely, the fitness costs of *IXRI*, *AXRI*, and *AXR2* genes are expressed during many stages of plant growth and morphology from embryogenesis to senescence (NAGPAL *et al.* 2000; DHUGGA 2001). Resistance leads to alterations of plant development starting in the vegetative phase (as illustrated by Figure 2, d, e, h, and i), which can indirectly entail a decrease of seed production. Further investigations are needed to clarify whether the decrease in productivity is due to a shift of resource allocation toward the vegetative phase or due to morphological constraints that would compromise normal seed production. As BERGELSON and PURRINGTON (2002) reviewed that fitness cost for the *csr1-1* allele was enhanced under competition, it could be relevant to test how each of the costs described in this study would respond to various stressful conditions.

The two resistances *aux1-7* and *ixr1-1* that did not demonstrate a fitness cost in this study still lead to differences in morphology between RR and SS plants. Although these resistance genes do not affect seed set, they may still change traits affecting “weediness” (growth, dispersal, and persistence). For example, compared to SS plants, homozygous plants for the *ixr1-1* mutation have delayed flowering time (data not shown), a trait assumed to be well adjusted to the local environment (JOHANSON *et al.* 2000; LE CORRE *et al.* 2002) and of some importance when colonizing different habitats (LINDE *et al.* 2001). A delay in the flowering time of self-pollinating species would have no impact on the spread of resistance without a fitness cost. In the case of out-crossing species, different flowering times can prevent crosses between

RR and SS plants and thus delay the diffusion of resistance genes in the environment. This study of the cost paradigm provides examples in which the realization of a fitness penalty is highly dependent on the interaction between the physiological changes and the prevailing ecological conditions.

Dominance of resistance cost: In populations subject to natural selection that should increase adaptation, levels of dominance may be shaped by fixation of cost modifiers (OTTO and BOURGUET 1999). The resistances in this study provide powerful models to study dominance of costs that have not, as yet, been submitted to natural selection. All levels of dominance were observed on both fitness cost and morphology. All together, the *CSR*, *AXRI*, and *IXRI* genes coding for metabolic enzymes have a mean dominance (h) of 0.09, a value close to the one found by KORONA (1999) in the yeast *Saccharomyces cerevisiae* ($h = 0.08$). On the basis of gene function, the recessivity of cost at the *CSR*, *AXRI*, and *IXRI* enzymes is consistent with Wright’s physiological theory of dominance (WRIGHT 1977). The key element of this theory is that enzymes are seldom limiting factors and therefore have little influence on the flux through a metabolic pathway; *i.e.*, the chemical reaction rate is likely to be limited by substrate rather than by enzyme. In their detailed biochemical theory of dominance, KACSER and BURNS (1981) demonstrated that the activity of the wild-type enzymes is usually far in excess of that necessary as a consequence of the kinetic properties of metabolic pathways. For *CSR*, *AXRI*, and *IXRI* genes, a single copy of the sensitive allele would confer a sufficient enzymatic activity and thus an identical morphology between RS and SS plants. The absence of a cost for the *csr1-1* allele in a heterozygous state is also consistent with the observation of a positive correlation between the level of expression of this allele and the fitness cost (PURRINGTON and BERGELSON 1999). Conversely, the enzymatic activity associated with a single copy of the *axr1-3*, *ixr1-1*, or *ixr1-2* allele under herbicide treatment is probably not sufficient to prevent the lethal herbicide effect. In the presence of herbicide, the enzymatic activity of RS individuals would thus be out of the “safety margin,” a value used to describe the maximum decrease of the enzyme activity that can be tolerated without affecting the phenotype (WRIGHT 1929). As the *csr1-1* allele results in the overactivity of the ALS enzyme (PURRINGTON and BERGELSON 1999), a single copy of this allele would satisfy the safety margin and provide enough enzyme activity to confer resistance. The *csr1-1* allele illustrates how a single mutation could have dominant effect (in herbicide-treated environments) while being recessive for fitness cost and morphology (in herbicide-free environment). BERGELSON (1994a,b) reported, however, an absence of fitness cost in the *csr1-1* RS plants when grown without competition while a cost became apparent as plant density increased. The differences in dominance among different phenotypic traits (resis-

tance, fitness) thus also depend on environmental parameters.

Physiological requirements are variable within a plant and change over time. As a result, the safety margins for different enzymes are also expected to vary according to the plant tissues. Therefore, two morphological characters expressed in different parts of the plants or at different times could be affected differently by a mutant allele. A given allele could show varying dominance levels, depending on the morphological trait considered. Such a situation was observed in this study for the *ixr1-2* allele, which had a recessive fitness cost ($h = 0.13$) while being partially dominant for the number of rosette leaves ($h = 0.61$). But this particular case may be an exception as we otherwise found few differences between levels of dominance for fitness and for morphology (Table 8).

The other genes analyzed in our study encode structural proteins thought to act as homomeric dimers (*AUX1* and *AXR2*; REED 2001). To our knowledge, no theory of expected dominance level has been developed so far for this kind of gene. This issue would be interesting to explore since the *aux1-7* and *axr2-1* alleles show distinct patterns of dominance toward morphology. Unexpectedly, underdominance toward a fitness cost was found at the *aux1-7* allele. Both the restoration of normal hormone response by the wild-type allele (BENNETT *et al.* 1996) and the increased resource accumulation associated with root elongation conferred by the *aux1-7* allele (PICKETT *et al.* 1990) could explain this underdominance under our favorable growth conditions. Stability across generations and more thorough physiological analysis are required to support this hypothesis.

As far as we know, the parallel study of dominance levels in the presence or absence of a selection pressure has never been described in the resistance literature. Here, we have shown that the dominance level of a resistant allele in the presence of the herbicide for which it has been selected may still not always correctly predict the dominance level of a potential cost of resistance (Table 8). In habitats where selection is spatially or temporally heterogeneous, the best strategy for a resistant allele is to be dominant in pesticide-treated areas and cost recessive in untreated areas. The *csr1-1* and *csr1-2* alleles fit this strategy and could therefore be expected to spread faster under agronomic conditions than the other resistance alleles tested here. In other cases, locally disfavored alleles may still be maintained by migration from treated locations (LEVENE 1953; DEMPSTER 1955). Such conditions would indirectly favor the evolution of dominance in both areas by allowing substantial selection of dominance modifiers (reviewed in BOURGUET 1999). But evolutionary data are required to clearly evaluate how often the evolution of dominance toward pesticides is independent from the evolution of dominance toward an associated cost. Further research on dominance of resistance cost will thus allow

the management of pesticide resistance to be more accurately assessed.

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LITERATURE CITED

- BELL, C. J., and J. R. ECKER, 1994 Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. *Genomics* **19**: 137–144.
- BENNETT, M. J., A. MARCHANT, H. G. GREEN, S. T. MAY, S. P. WARD *et al.*, 1996 *Arabidopsis AUX1* gene: a permease-like regulator of root gravitropism. *Science* **273**: 948–950.
- BERGELSON, J., 1994a The effects of genotype and environment on costs of resistance in lettuce. *Am. Nat.* **143**: 349–359.
- BERGELSON, J., 1994b Changes in fecundity do not predict invasiveness: a model study of transgenic plants. *Ecology* **75**: 249–252.
- BERGELSON, J., and C. B. PURRINGTON, 1996 Surveying patterns in the cost of resistance in plants. *Am. Nat.* **148**: 536–558.
- BERGELSON, J., and C. B. PURRINGTON, 2002 Factors affecting the spread of resistant *Arabidopsis thaliana* populations, pp. 17–33 in *Genetically Engineered Organisms: Assessing Environmental and Human Health Effects*, edited by D. B. LETOURNEAU and B. E. BURROWS. CRC Press, Cleveland/Boca Raton, FL.
- BERGELSON, J., C. B. PURRINGTON, C. J. PALM and J. C. LOPEZ-GUITIERREZ, 1996 Costs of resistance: a test using transgenic *Arabidopsis thaliana*. *Proc. R. Soc. Lond. Ser. B* **263**: 1659–1663.
- BOURGUET, D., 1999 The evolution of dominance. *Heredity* **83**: 1–4.
- BOURGUET, D., M. PROUT and M. RAYMOND, 1996 Dominance of insecticide resistance presents a plastic response. *Genetics* **143**: 407–416.
- BOURGUET, D., T. LENORMAND, T. GUILLEMAUD, V. MARCEL, D. FOURNIER *et al.*, 1997 Variation of dominance of newly arisen adaptive genes. *Genetics* **147**: 1225–1234.
- CARRIÈRE, Y., and B. E. TABASHNIK, 2001 Reversing insect adaptation to transgenic plants. *Proc. R. Soc. Lond. Ser. B* **268**: 1475–1480.
- DÉLYE, C., F. LAIGRET and M.-F. CORIO-COSTET, 1997 A mutation in the 14 α -demethylase gene of *Uncinula necator* that correlates with high levels of resistance to a sterol biosynthesis inhibitor. *Appl. Environ. Microbiol.* **63**: 2960–2970.
- DEMPSTER, E. R., 1955 Maintenance of genetic heterogeneity. *Cold Spring Harbor Symp. Quant. Biol.* **20**: 25–32.
- DESPREZ, T., S. VERNHETTES, M. FAGARD, G. REFREGIER, T. DESNOS *et al.*, 2002 Resistance against herbicide isoxaben and cellulose deficiency caused by distinct mutations in same cellulose synthase isoform *CESA6*. *Plant Physiol.* **128**: 1–9.
- DHUGGA, K. S., 2001 Building the wall: genes and enzyme complexes for polysaccharide synthases. *Curr. Opin. Plant Biol.* **4**: 488–493.
- ESTELLE, M. A., and C. SOMERVILLE, 1987 Auxin-resistant mutants of *Arabidopsis* with an altered morphology. *Mol. Gen. Genet.* **206**: 200–206.
- HAUGHN, G. W., J. SMITH, B. MAZUR and C. SOMERVILLE, 1988 Transformation with a mutant *Arabidopsis* acetolactate synthase gene renders tobacco resistant to sulfonyleurea herbicides. *Mol. Gen. Genet.* **211**: 266–271.
- HEIL, M., and T. BALDWIN, 2002 Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends Plant Sci.* **7**: 61–67.
- HEIM, D. R., J. L. ROBERTS, P. D. PIKE and I. M. LARRINUA, 1989 Mutation of a locus of *Arabidopsis thaliana* confers resistance to the herbicide isoxaben. *Plant Physiol.* **90**: 146–150.
- HEIM, D. R., F. R. SKOMP, E. D. TSCHABOLD and I. M. LARRINUA, 1990a Isoxaben inhibits the synthesis of acid insoluble cell wall materials in *Arabidopsis thaliana*. *Plant Physiol.* **93**: 695–700.
- HEIM, D. R., J. L. ROBERTS, P. D. PIKE and I. M. LARRINUA, 1990b A second locus, *IxrB1* in *Arabidopsis thaliana*, that confers resistance to the herbicide isoxaben. *Plant Physiol.* **92**: 858–861.

- JANDER, G., S. R. BAERSON, J. A. HUDAK, K. A. GONZALEZ, K. J. GRUYS *et al.*, 2003 Ethylmethanesulfonate saturation mutagenesis in *Arabidopsis* to determine frequency of herbicide resistance. *Plant Physiol.* **131**: 139–146.
- JOHANSON, U., J. WEST, C. LISTER, M. SCOTT, R. AMASINO *et al.*, 2000 Molecular analysis of *FRL*, a major determinant of natural variation in *Arabidopsis* flowering time. *Science* **290**: 344–347.
- KACSER, H., and J. A. BURNS, 1981 The molecular basis of dominance. *Genetics* **97**: 639–666.
- KORONA, R., 1999 Unpredictable fitness transitions between haploid and diploid strains of the genetically loaded yeast *Saccharomyces cerevisiae*. *Genetics* **151**: 77–85.
- LAROSSA, R. A., and S. C. FALCO, 1984 Amino acid biosynthetic enzymes as targets of herbicide action. *Trends Biotechnol.* **2**: 158–161.
- LAROSSA, R. A., and J. V. SCHLOSS, 1984 The sulfonylurea herbicide sulfometuron methyl is an extremely potent and selective inhibitor of acetolactate synthase in *Salmonella typhimurium*. *J. Biol. Chem.* **259**: 8753–8757.
- LE CORRE, V., F. ROUX and X. REBOUD, 2002 DNA polymorphism at the *FRIGIDA* gene in *Arabidopsis thaliana*: nonsynonymous variation is consistent with local selection for flowering time. *Mol. Biol. Evol.* **19**: 1261–1271.
- LENORMAND, T., and M. RAYMOND, 1998 Resistance management: the stable zone strategy. *Proc. R. Soc. Lond. Ser. B* **265**: 1985–1990.
- LEVENE, H., 1953 Genetic equilibrium when more than one ecological niche is available. *Am. Nat.* **87**: 331–333.
- LEYSER, H. M. O., C. A. LINCOLN, C. TIMPTE, D. LAMMER, J. TURNER *et al.*, 1993 *Arabidopsis* auxin-resistance gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1. *Nature* **364**: 161–164.
- LINDE, M., S. DIEHL and B. NEUFFER, 2001 Flowering ecotypes of *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae) analysed by a cosegregation of phenotypic characters (QTL) and molecular markers. *Ann. Bot.* **87**: 91–99.
- MAXWELL, B. D., and A. M. MORTIMER, 1994 Selection for herbicide resistance, pp. 1–25 in *Herbicide Resistance in Plants: Biology and Biochemistry*, edited by S. B. POWLES and J. A. M. HOLTUM. Lewis Publishers, London.
- NAGPAL, P., L. N. WALKER, J. C. YOUNG, A. SONAWALA, C. TIMPTE *et al.*, 2000 *AXR2* encodes a member of the Aux/IAA protein family. *Plant Physiol.* **123**: 563–574.
- NORDBORG, M., J. O. BOREVITZ, J. BERGELSON, C. C. BERRY, J. CHORY *et al.*, 2002 The extent of linkage disequilibrium in *Arabidopsis thaliana*. *Nat. Genet.* **30**: 190–193.
- OTTO, S. P., and D. BOURGUET, 1999 Balanced polymorphisms and the evolution of dominance. *Am. Nat.* **153**: 561–574.
- PECK, S. L., 2001 Antibiotic and insecticide resistance modelling—Is it time to start talking? *Trends Microbiol.* **9**: 286–292.
- PICKETT, F. B., A. K. WILSON and M. ESTELLE, 1990 The *aux1* mutation of *Arabidopsis* confers both auxin and ethylene resistance. *Plant Physiol.* **94**: 1462–1466.
- PURRINGTON, C. B., 2000 Costs of resistance. *Curr. Opin. Plant Biol.* **3**: 305–308.
- PURRINGTON, C. B., and J. BERGELSON, 1997 Fitness consequences of genetically engineered herbicide and antibiotic resistance in *Arabidopsis thaliana*. *Genetics* **145**: 807–814.
- PURRINGTON, C. B., and J. BERGELSON, 1999 Exploring the physiological basis of costs of herbicide resistance in *Arabidopsis thaliana*. *Am. Nat.* **154**: S82–S91.
- REBOUD, X., H. MCKHANN, N. SCARCELLI, V. LE CORRE, F. ROUX *et al.*, 2004 Natural variation among accessions of *Arabidopsis thaliana*: Beyond the flowering date, what morphological traits are relevant to study adaptation?, in *Plant Adaptation: Molecular Biology and Ecology*, edited by Q. C. CRONK, J. WHITTON and I. E. P. TAYLOR. NRC Research Press, Ottawa, Canada (in press).
- REED, J. W., 2001 Roles and activities of Aux/IAA proteins in *Arabidopsis*. *Trends Plant Sci.* **6**: 420–425.
- SAINI, H. S., M. SHEPHERD and R. J. HENRY, 1999 Microwave extraction of total genomic DNA from barley grains for use in PCR. *J. Inst. Brew.* **105**: 185–190.
- SATHASIVAN, K., G. W. HAUGHN and N. MURAI, 1990 Nucleotide sequence of a mutant acetolactate synthase gene from an imidazolinone-resistant *Arabidopsis thaliana* var. Columbia. *Nucleic Acids Res.* **18**: 2188.
- SATHASIVAN, K., G. W. HAUGHN and N. MURAI, 1991 Molecular basis of imidazolinone herbicide resistance in *Arabidopsis thaliana* var. Columbia. *Plant Physiol.* **97**: 1044–1050.
- SCHEIBLE, W.-R., R. ESHED, T. RICHMOND, D. DELMER and C. SOMERVILLE, 2001 Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in *Arabidopsis* *Ixr1* mutants. *Proc. Natl. Acad. Sci. USA* **98**: 10079–10084.
- SNAPE, J. W., and M. J. LAWRENCE, 1971 The breeding system of *Arabidopsis thaliana*. *Heredity* **27**: 299–302.
- SOMMER, S. S., A. R. GROSZBAR and C. D. K. BOTTEMA, 1992 PCR amplification of specific alleles (PASA) is a general method for rapidly detecting known single base-pair changes. *Biotechniques* **12**: 82–87.
- STRAUSS, S. Y., J. A. RUDGERS, J. A. LAU and R. E. IRWIN, 2002 Direct and ecological costs of resistance to herbivory. *Trends Ecol. Evol.* **17**: 278–285.
- WILSON, A. K., F. B. PICKETT, J. C. TURNER and M. ESTELLE, 1990 A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. *Mol. Gen. Genet.* **222**: 377–383.
- WRIGHT, S., 1929 Fisher's theory of dominance. *Am. Nat.* **63**: 274–279.
- WRIGHT, S., 1977 The evolution of dominance, pp. 498–526 in *Evolution and the Genetics of Populations*, Vol. 3: *Experimental Results and Evolutionary Deductions*, edited by S. WRIGHT. University of Chicago Press, Chicago.
- YOUNG, N. D., and S. D. TANKSLEY, 1989 RFLP analysis of the size of the chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. *Theor. Appl. Genet.* **77**: 353–359.

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