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The effects of the genetic background on herbicide resistance fitness cost and its associated dominance in *Arabidopsis thaliana*

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The advantage of the resistance conferred by a mutation can sometimes be offset by a high fitness-cost penalty. This balance will affect possible fate of the resistance allele. Few studies have explored the impact of the genetic background on the expression of the resistance fitness cost and none has attempted to measure the variation in fitness-cost dominance. However, both the fitness penalty and its dominance may modify evolutionary trajectory and outcome. Here the impact of *Arabidopsis thaliana* intraspecific genetic diversity on fitness cost and its associated dominance was investigated by analysing 12 quantitative traits in crosses between a mutant conferring resistance to the herbicide 2,4-D and nine different natural genetic backgrounds. Fitness cost values were found to be more affected by intraspecific genetic diversity than fitness cost dominance, even though this effect depends on the quantitative trait measured. This observation has implications for the choice of the best strategy for preventing herbicide resistance development. In addition, our results pinpoint a potential compensatory improvement of the resistance fitness cost and its associated dominance by the genetic diversity locally present within a species.
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Introduction

Mirroring the previously observed trend for resistance to insecticides, fungicides (Holt and LeBaron, 1990) and antibiotics (Palumbi, 2001), the number of worldwide cases of herbicide resistance has increased at an exponential rate. This change has had a huge economical impact through a reduction of crops production (Heap, 2008). Herbicide resistance traits in local weed populations may originate from (1) spontaneous mutations conferring resistance in local plants, (2) migration of pollen or seeds from another population resistant to the same herbicide, or (3) introgression of transgenic herbicide resistance gene from herbicide-resistant crops and related weed species (Owen and Zelaya, 2005). In evolutionary terms, herbicide resistance could be considered as an adaptive response of a weed population to a sudden environmental change by using either new mutations (that is, spontaneous mutations) or alleles from the standing genetic variation (Orr and Betancourt, 2001). 'New mutations' means that herbicide resistance traits appear in a weed population after the first application of herbicide, whereas 'standing genetic variation' means that traits conferring herbicide resistance segregate in unexposed populations, that is, before the first application of herbicide.

According to a recent theory on adaptation, the evolutionary trajectories of 'new mutations' in a population depend on the net fitness effect associated with the adaptive allele (Orr, 1998; Barton and Keightley, 2002; also see Hastings, 2001 for a review of parasite drug resistance literature on the principal factors determining the rate at which resistance evolves). In the case of herbicide resistance, the net fitness effect results from the balance between the benefit to be resistant and the fitness cost of the resistance trait, as well as the dominances of these two variables (that is, the fitness of heterozygous RS individuals compared with resistant RR and sensitive SS homozygous individuals) (Roux *et al*., 2004; Roux and Reboud, 2005).

When adaptation originates from 'standing genetic variation', evolutionary trajectories of an adaptive mutation might primarily depend on its initial frequency in the population (Hermisson and Pennings, 2005). If a beneficial allele is originally neutral or weakly deleterious, its fixation probability becomes only weakly dependent on its selection coefficient after the environmental change. The reason is the high initial frequency reached by the adaptive allele before the environmental change. In weed populations, initial frequency of herbicide resistance allele in unexposed populations also depends on the fitness cost and its associated dominance (Preston and Powles, 2002).

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Although the genetic background is known to affect the fitness of the adaptive allele (Ungerer *et al*., 2003; Weinreich *et al*., 2005), and more precisely the fitness cost of herbicide resistance (Bergelson, 1994; Bergelson and Purrington, 1996), no previous experiment has been specifically designed to study the effects of the genetic background on cost dominance of herbicide resistance.

In this study, we focus on the effect of the genetic background on herbicide resistance fitness cost and its associated dominance. We used the *axr1-3* mutant line, developed in the model cruciferous plant species *Arabidopsis thaliana*. The *axr1-3* mutant line confers resistance to the 2,4-D herbicide (phenoxy-carboxylic acids group), from which different natural field resistances have been selected in weed populations (Heap, 2008). Using natural intraspecific genetic diversity in *A. thaliana*, we present results from an analysis of morphological and productivity-related traits in nine genetically different segregating R/S populations at the F_2 generation in the absence of herbicide treatment. Our main objective was to evaluate the relative sensitivity to the genetic background of the fitness cost and its associated dominance.

Materials and methods

Plant materials

The *axr1-3* 2,4-D resistance of the selfing species *A. thaliana* has been isolated from ethyl methanesulfonate (EMS) mutagenized populations of the wild-type Columbia (Col) ecotype by Estelle and Somerville (1987). The *axr1-3* mutant is resistant on account of a point mutation resulting in the Cys_{154} to Tyr substitution (Leyser *et al*., 1993). In addition to 2,4-D resistance status, the *axr1-3* mutant was found to affect different developmental traits, including a decrease in total seed production (Estelle and Somerville, 1987; Roux and Reboud, 2005).

To investigate the impact of *A. thaliana* intraspecific genetic diversity on the pleiotropic effects conferred by *axr1-3* herbicide resistance in both the homozygous and heterozygous state, the *axr1-3* resistance line was cross-pollinated by hand to eight natural accessions (Bur-0, Can-0, JEA, Mt-0, Mh-1, Oy-0, PYL-1 and Sah-0). These natural accessions were chosen according to two criteria to improve the possibility to detect a genetic background effect on the *axr1-3* resistance fitness cost and its associated dominance. First of all, they are present in the *A. thaliana* core-collection designed by McKhann *et al*. (2004). This core-collection was drawn to maximize the genetic diversity of *A. thaliana* in a reduced subset of natural accessions. Second, differences were found on a set of adaptive traits among the eight natural accessions (Reboud *et al*., 2004) as well as between each of the eight natural accessions and the *axr1-3* resistance line (Roux F, unpublished results). The *axr1-3* resistance was also crossed to the Ler genetic background, a common reference strain used in laboratory experiments. In each cross, the natural accessions and Ler strain were used as the maternal parent. Crossing success was checked by genotyping each resistance allele using an allele-specific PCR method (Roux *et al*., 2004). For each of the nine crosses, one resulting F1 plant was randomly chosen, then isolated and selfed to produce the F2 segregating generation with the expected resistant RR and sensitive SS homozygous individuals and RS heterozygous individuals. To limit maternal effects among crosses, all F1 plants were grown at the same time in a greenhouse, under natural light supplemented by artificial light to provide a 16-h photoperiod and temperature maintained between 20 and 25 °C. The EMS origin of the *axr1-3* line means that it may carry several mutations other than the ones conferring resistance (Jander *et al*., 2003). In a previous study, EMS mutations other than the one conferring *axr1-3* resistance were found not to induce a significant reduction of plant fitness (Roux *et al*., 2004). More, by our crossing protocol, any EMS mutations other than the one conferring resistance (except those closely linked to the resistance mutations) would contribute equally to the average fitness of each of the SS, RS and RR classes.

Growth and quantitative traits

To compare the relative sensitivity of the fitness cost and its associated dominance to the genetic background, an experiment involving 3120 plants was established. For each cross with a natural accession as well as with the Ler strain, 305 seeds of the F2 generation were included in the experiment. In addition, 40 Col SS seeds, 39 Ler SS seeds, 40 seeds of the *axr1-3* RR line and 32 seeds of each of the eight natural accessions were added as external controls for genotyping. Seeds were sown in 30 trays (44×28.5 cm) filled with a mix of 2/3 commercial soil (Terreau Semis Bouturage Repiquage, Composana, Roche-les-Beaupré, France) and 1/3 sand, and watered two times a week without supplementary nutrients. Each tray consisted of an 8×13 array of plants. All 3120 seeds were randomized among plots and grown in the absence of herbicides in a greenhouse, under natural light supplemented by artificial light to provide a 16-h photoperiod. The temperature was maintained between 20 and 25 °C. In each tray, the 104 seeds were regularly spaced 3 cm apart. To keep homogeneous density throughout plant development, ungerminated seeds were replaced by extra-seedlings that were further discarded from all statistical analysis other than the calculation of segregation distortion (see Results section). The edges of trays (46 positions) were sown with seeds from 23 natural accessions (two replicates per accession) of the core-collection described by McKhann *et al*. (2004) to buffer against possible border effects and were discarded from the analysis. The trays were daily rotated during the growing period to avoid micro-environmental effects. The experiment stopped after senescence of all the plants.

To assess the pleiotropic fitness costs associated with the *axr1-3* resistance, we extended the recorded information beyond solely seed production. Some characters (such as plant height) may still induce an ecological cost whereas they have no direct impact on seed production under optimal experimental conditions (Roux and Reboud, 2005; Wender *et al*., 2005). Four morphological characters were measured during the experiment: rosette diameter at the 15th and 21st day after sowing (DIAM15 and DIAM21, respectively), number of rosette leaves (LEAF) as a proxy for flowering time and height from the soil to the first flower at flowering (H1FL). The other morphological traits were measured on harvested plants:

height from the soil to the first silique at harvest (H1SIL); maximum plant height (HMAX); the number of primary branches on the primary shoot (RAM1F); the total number of flowering heads (HEADS) measured by summing the number of flowering axes and the number of primary and secondary branches on the primary and secondary axes; and the mean distance between siliques (LEN). As measuring fitness as seed production is appropriate for a self-pollinating species (Heil and Baldwin, 2002) like *A. thaliana*, total individual fitness was assessed by the total silique length (FITNESS), a derived trait closely matching the total seed production and corresponding to the multiplication of the mean silique size (SILSIZE; average of measures on the third, fifth, seventh and ninth siliques on the primary shoot) by the total number of siliques (TOTSIL). 'Early quantitative traits' (DIAM15, DIAM21, LEAF, H1FL, H1SIL, HMAX and RAM1F) were measured for all plants from the nine crosses as well as Col SS and *axr1-3* RR plants; whereas 'late quantitative traits' (HEADS, LEN, SILSIZE, TOTSIL and FITNESS) were only measured for Col SS and *axr1-3* RR plants as well as for plants from *axr1-3* × Oy-0 and *axr1-3* × PYL-1 crosses exhibiting contrasted results for early quantitative traits.

Genotyping the resistance status

DNA was extracted from a section of the rosette leaves that was cut during the last 3 days of the experiment. Each rosette leaf section was then placed in a micro-centrifuge tube containing 50 µl of the extraction buffer described by Saini *et al*. (1999). The leaf section was crushed using a mixer mill. Tubes were placed in a water bath at 95 °C for 6 min, transferred onto ice for 5 min and vortexed for 15 s. DNA extracts were kept at −20 °C before genotyping. The single nucleotide polymorphisms (SNP) conferring the *axr1-3* resistance were genotyped by using the fluorescence-based Amplifluor technology (Serological Corporation, Norcross, GA, USA). This technology is based on allele-specific PCR amplification combined with the use of universal energy-transfer-labeled Amplifluor primers (Giancola *et al*., 2006).

Statistical analyses

For each cross, the effect of *axr1-3* resistance mutation on each quantitative trait in the F2 generation was assessed by a one-way analysis of variance (ANOVA). These models treat genotype (SS, RS and RR) as a fixed effect. Because the planting of F2 seeds precluded having the same genetic composition among trays (genotypes of plants are known after the end of the greenhouse experiment), a 'tray' effect was not included in the ANOVA. Although a hypothetical 'tray' effect cannot be rejected, the daily rotation of trays within the greenhouse is thought to have buffered such a 'tray' effect. Statistical analyses were performed using Systat. LEAF, RAM1F were log-transformed to meet requirements of ANOVA; whereas H1FL, HEADS, TOTSIL, SILSIZE and FITNESS were square-root transformed. The remaining variables (DIAM15, DIAM21, H1SIL and LEN) did not require transformation.

Following Roux *et al*. (2005b), for each 'cross × quantitative trait' combination, a distribution of cost was generated by calculating a cost value for each value of the RR dataset with their respective SS mean traits. For each cross and each quantitative trait, using these generated distributions, cost values were grouped after a Tukey pairwise comparisons test.

The dominance index was taken as:

$$h = (\text{SS mean trait} - \text{RS mean trait})/(\text{SS mean trait} - \text{RR mean trait})$$

Following convention, the resistant allele is dominant toward cost when h equals 1, semi-dominant when h equals 0.5 and recessive when h approaches 0; over-dominant and underdominant when h is superior to 1 and inferior to 0, respectively. Overdominance and underdominance indicate identical and opposite effects of RS and RR plants compared with SS plants, respectively (Roux *et al*., 2005b). For each 'cross × quantitative trait' combination, a distribution of dominance was generated by calculating a dominance coefficient for each value of the RS dataset with their respective SS and RR mean traits. For each cross and each quantitative trait, using these generated distributions, dominance coefficients were grouped after a Tukey pairwise comparisons test.

Results

Genetic background effect on genotypic frequencies at the F2 generation

A number of 2680 F2 plants (out of a total of 2745 F2 plants) were successfully genotyped for the *axr1-3* resistance mutation. Significant segregation distortion was observed for two of the nine crosses (Table 1). For the *axr1-3* × Mh-1 and *axr1-3* × Oy-0 crosses, the RR class has fewer plants than expected and the SS class has more plants than expected. Because no reduction of survival rate was observed for the *axr1-3* × Mh-1 and *axr1-3* × Oy-0 crosses, gametes containing the *axr1-3* mutation are probably associated with a lower viability and/or fertility.

Herbicide resistance cost

The effects of the genetic background on estimates of cost associated with *axr1-3* resistance allele are depicted in Table 2. When comparing *axr1-3* RR plants (Col background) to wild-type Col SS plants, all quantitative traits (except H1FL and HEADS) measured in this study were

Table 1 Effective class numbers and test for normal Mendelian segregation for the *axr1-3* resistance allele in each of the nine crosses

Cross	*No. of plants*				$\chi^{2\,a}$
	Total	*SS*	*RS*	*RR*	
axr1-3 × Bur-0	302	68	144	90	3.85[b]
axr1-3 × Can-0	301	88	149	64	3.86[b]
axr1-3 × JEA	293	79	151	63	2.02[b]
axr1-3 × Ler	304	77	161	66	1.86[b]
axr1-3 × Mh-1	295	95	137	63	8.44*
axr1-3 × Mt-0	296	73	155	68	0.83[b]
axr1-3 × Oy-0	298	96	141	61	9.08*
axr1-3 × PYL-1	297	75	149	73	0.03[b]
axr1-3 × Sah-0	294	62	166	66	5.02[b]

[a]χ^2 was calculated on the basis of expected percentages of $\frac{1}{4}$ SS, $\frac{1}{2}$ RS and $\frac{1}{4}$ RR with d.f.

[b]Non-significant.

*$0.05 > P > 0.01$.

Table 2 Effect of genetic background and quantitative trait on mean estimates of cost associated with the *axr1-3* herbicide resistance allele

Cross	*Early quantitative trait*							*Late quantitative trait*				
	DIAM15	*DIAM21*	*LEAF*	*H1FL*	*H1SIL*	*HMAX*	*RAM1F*	*HEADS*	*LEN*	*TOTSIL*	*SILSIZE*	*FITNESS*
axr1-3 × Bur-0	$^{ab}27.2^{bc}$	$^{ab}27.7^{c}$	$^{c}47.9^{abc}$	—	$^{a}20.0^{abc}$	$^{c}41.2^{a}$	$^{ab}30.0^{a}$	NE	NE	NE	NE	NE
axr1-3 × Can-0	$^{a}21.0^{b}$	$^{a}22.1^{abc}$	$^{c}26.2^{ab}$	$^{ab}24.2^{ab}$	$^{ab}26.3^{abc}$	$^{bc}42.9^{a}$	—	NE	NE	NE	NE	NE
axr1-3 × JEA	$^{a}32.6^{c}$	$^{a}31.3^{c}$	$^{a}20.1^{a}$	—	$^{a}33.7^{bc}$	$^{a}48.3^{a}$	—	NE	NE	NE	NE	NE
axr1-3 × Ler	$^{a}24.7^{bc}$	$^{a}22.3^{ab}$	$^{c}51.4^{bc}$	—	$^{a}19.2^{ab}$	$^{b}41.3^{a}$	$^{bc}39.2^{ab}$	NE	NE	NE	NE	NE
axr1-3 × Mh-1	$^{ab}25.6^{bc}$	$^{a}23.6^{abc}$	$^{c}40.5^{abc}$	$^{ab}13.6^{a}$	$^{a}20.7^{abc}$	$^{bc}40.4^{a}$	$^{c}45.8^{ab}$	NE	NE	NE	NE	NE
axr1-3 × Mt-0	$^{a}31.4^{c}$	$^{a}33.3^{c}$	—	—	$^{a}26.4^{abc}$	$^{b}47.6^{a}$	$^{b}44.4^{ab}$	NE	NE	NE	NE	NE
axr1-3 × Oy-0	$^{ab}25.5^{bc}$	—	$^{c}59.2^{c}$	$^{b}18.5^{ab}$	$^{a}13.8^{a}$	$^{ab}36.5^{a}$	$^{cd}73.3^{b}$	$^{cd}58.7$	$^{ab}37.8^{a}$	—	$^{bc}50.2^{b}$	$^{d}61.6^{ab}$
axr1-3 × PYL-1	$^{a}24.0^{bc}$	$^{a}25.0^{bc}$	$^{ab}29.3^{abc}$	$^{ab}25.2^{ab}$	$^{ab}32.6^{c}$	$^{b}42.5^{a}$	$^{c}46.6^{ab}$	—	$^{ab}37.6^{a}$	—	$^{c}62.9^{b}$	$^{d}76.4^{b}$
axr1-3 × Sah-0	$^{a}21.4^{ab}$	$^{a}22.7^{abc}$	—	$^{b}43.0^{b}$	—	—	—	NE	NE	NE	NE	NE
Col SS vs *axr1-3* RR	$^{a}10.1^{a}$	$^{a}12.5^{a}$	$^{d}50.3^{abc}$	—	$^{ab}25.8^{abc}$	$^{bc}38.7^{a}$	$^{cd}48.3^{ab}$	—	$^{b}33.3^{a}$	$^{cd}48$	$^{b}25.5^{a}$	$^{d}60.0^{a}$

Abbreviation: NE, not estimated.
No value ('—') indicates no difference between SS and RR plants. Different superscript letters at the right and left of the mean estimates of cost indicate a significant cross (genetic background) effect and quantitative trait effect, respectively ($P>0.05$, with Tukey correction for multiple comparisons).

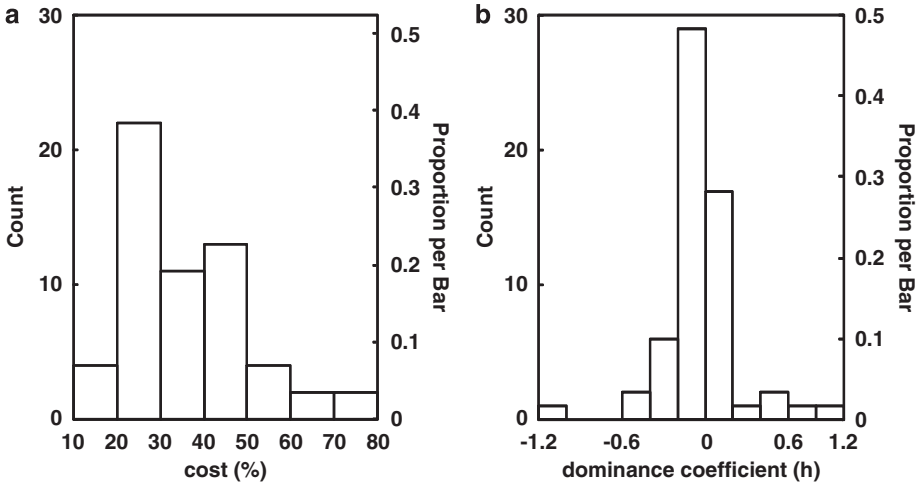

Figure 1 Overall distribution of significant cost (**a**) and dominance coefficient (**b**). The *axr1-3* allele is dominant for the fitness cost when h equals 1; semi-dominant when h equals 0.5; recessive when h approaches 0; overdominant and underdominant when h is superior to 1 and inferior to 0, respectively.

found to be affected by the *axr1-3* resistance mutation. This result confirms that the *axr1-3* resistance mutation affects several developmental traits, including a decrease in total seed production (Estelle and Somerville, 1987). Cost values associated with the *axr1-3* resistance allele in Col genetic background are significantly different among quantitative traits (Table 2). Cost values ranged from 10.1% (reduction of rosette diameter 15 days after sowing) to 60% (reduction in fitness). The number of siliques was reduced by 48%, whereas the mean silique length as a proxy of the number of seeds per silique was reduced by 25.5%, leading to a global fitness reduction of 60%.

Cost associated with *axr1-3* resistance allele was found to depend on both cross and quantitative trait. Cost associated with the *axr1-3* resistance allele was not statistically detected for 20.5% of all 'cross × quantitative trait' combinations (15 out of 73 combinations). For statistically significant costs (Figure 1a), estimates ranged from 13.6% (H1FL for *axr1-3* × Mh-1 cross) to 76.4% (FITNESS for *axr1-3* × PYL-1 cross), with a mean of 35.1% (s.d. = 14.4%). Because quantitative traits were not measured for all of the nine crosses, we distinguished an 'early quantitative trait' effect (nine crosses) on cost estimates from a 'late quantitative trait' effect (two crosses) on cost estimates. Over the nine crosses, 'early quantitative trait' has a significant effect on cost estimates (ANOVA, $F=8.17$, d.f. = 6, $P<10^{-3}$) with cost estimates for LEAF, HMAX and RAM1F being higher than cost estimates for DIAM15, DIAM21, H1FL and H1SIL. No 'late quantitative trait' effect was detected (ANOVA, $F=5.31$, d.f. = 3, $P=0.102$), certainly because of lack of statistical power (only two values for each late quantitative trait).

A significant quantitative trait effect was detected for each cross (Table 2), indicating trait-specific cost within the cross. A significant cross effect was detected for almost each quantitative trait (except HMAX and LEN; Table 2), indicating that *axr1-3* resistance cost expression detected between *axr1-3* RR plants and Col SS plants was modified by intra-species genetic diversity. Depending on both quantitative trait and cross, resistance cost detected between *axr1-3* RR plants and Col SS plants were found to be either enhanced or reduced by genetic background. No significant cost for the total number of siliques (TOTSIL) was statistically detected in *axr1-*

$3 \times$ Oy-0 and *axr1-3* $\times$ PYL-1 crosses; whereas a significant cost for TOTSIL was found when comparing *axr1-3* RR plants to Col SS plants. By contrast, cost for mean number of seeds per silique (SILSIZE) was at least two times enhanced in *axr1-3* $\times$ Oy-0 and *axr1-3* $\times$ PYL-1 crosses, in comparison to the cost detected between *axr1-3* RR and Col SS plants. Total seed production (FITNESS) cost was significantly higher for *axr1-3* $\times$ PYL-1 crosses than the one detected between *axr1-3* RR and Col SS plants. This result strongly suggests that both Oy-0 and PYL-1 accessions may have some compensatory alleles for TOTSIL and Col accession having compensatory allele for SILSIZE. For four early quantitative traits (LEAF, H1SIL, HMAX and RAM1F), costs detected between *axr1-3* RR and Col SS plants were mainly reduced in the *axr1-3* $\times$ Sah-0 cross. Sah-0 accession is thus suggested having either several compensatory alleles, each being specific to an early quantitative trait, or one general compensatory allele common to those four early quantitative traits.

Dominance level associated with *axr1-3* resistance cost

The effects of genetic background on estimates of cost dominance associated with *axr1-3* resistance allele are described in Table 3. For dominance coefficients h (Figure 1b), estimates ranged from -1.17 (DIAM21 for *axr1-3* $\times$ Oy-0 cross) to 0.81 (H1FL for *axr1-3* $\times$ Oy-0 cross), with a mean closed to 0 (mean $= -0.04$, s.d. $= 0.269$; without outlier '-1.17': mean $= -0.02$, s.d. $= 0.226$), indicating that *axr1-3* resistance allele is almost fully recessive whatever the quantitative trait or the genetic background. Negative dominance coefficients were found for FITNESS for *axr1-3* $\times$ Oy-0 and *axr1-3* $\times$ PYL-1 crosses, indicating that RS plants had a higher total seed production than SS and RR plants.

For two 'cross $\times$ quantitative trait' combinations, no cost was statistically detected (DIAM21 for *axr1-3* $\times$ Oy-0 cross, TOTSIL for *axr1-3* $\times$ PYL-1 cross), whereas dominance coefficient h was calculated, indicating that RS plants are statistically different from both SS and RR plants. For those two 'cross $\times$ quantitative trait' combinations, *axr1-3* resistance allele was found highly underdominant (Table 3).

Sign epistasis, referred as the conditionality on genetic background of the sign of the phenotype effect of a mutation (Weinreich *et al*., 2005), was detected for cost dominance associated with *axr1-3* resistance allele. For H1FL, *axr1-3* resistance allele is dominant and underdominant for *axr1-3* $\times$ Oy-0 and *axr1-3* $\times$ Mh-1 crosses, respectively (Figure 2). The sign of *axr1-3* resistance effect on dominance was also conditional on quantitative trait. For *axr1-3* $\times$ JEA cross, *axr1-3* resistance allele is codominant for LEAF whereas slightly underdominant for DIAM15 and DIAM21, indicating trait-specific dominance. Such a sign epistasis was not observed for cost estimates (Table 2).

Overall, dominance coefficients were less affected by genetic background and quantitative trait than cost estimates. Genetic background effect on dominance coefficients and cost estimates were found for three and six out of seven early quantitative traits, respectively. Quantitative trait effect on dominance coefficients and cost estimates was found for five and nine out of nine crosses, respectively.

We found no linear relationship between cost estimates and dominance coefficients (Pearson correlation, $n = 58$, $r = -0.144$, $P = 0.281$). This result is well illustrated by H1FL (Figure 2). For *axr1-3* $\times$ Mh-1 and *axr1-3* $\times$ Oy-0 crosses, the cost estimates were not significantly different whereas dominance coefficients were significantly different (Tables 2 and 3).

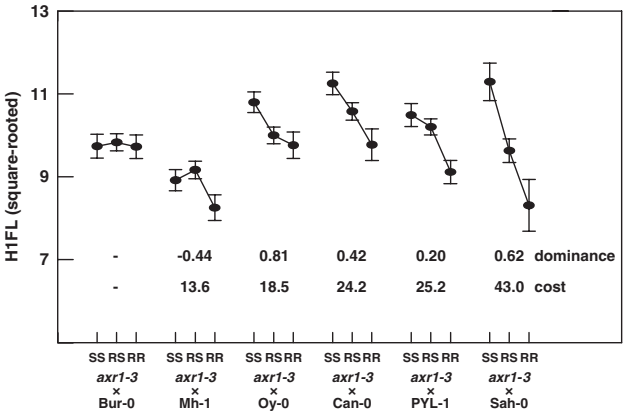

Figure 2 Effect of genetic background on estimates of cost and its associated dominance for the height to first flower at flowering (HFL). SS: *AXR1-3/AXR1-3*; RS: *AXR1-3/axr1-3*; RR: *axr1-3/axr1-3*. Filled circles and its associated errors bars correspond to the least-square mean values of the trait and its associated standard errors.

Table 3 Effect of genetic background and quantitative trait on estimates of dominance associated with the *axr1-3* herbicide resistance allele

Cross	*Early quantitative trait*							*Late quantitative trait*				
	DIAM15	*DIAM21*	*LEAF*	*H1FL*	*H1SIL*	*HMAX*	*RAM1F*	*HEADS*	*LEN*	*TOTSIL*	*SIZESIL*	*FITNESS*
axr1-3 $\times$ Bur-0	$^{a}0.02^{a}$	$^{a}-0.05^{b}$	$^{a}0.13^{a}$	—	$^{a}0.18^{a}$	$^{a}0.09^{a}$	$^{a}-0.10^{a}$	NE	NE	NE	NE	NE
axr1-3 $\times$ Can-0	$^{a}-0.09^{a}$	$^{a}-0.02^{b}$	$^{a}-0.24^{a}$	$^{b}0.42^{b}$	$^{ab}0.08^{a}$	$^{ab}-0.01^{a}$	—	NE	NE	NE	NE	NE
axr1-3 $\times$ JEA	$^{a}-0.15^{a}$	$^{a}-0.17^{b}$	$^{b}0.56^{b}$	—	$^{ab}-0.04^{a}$	$^{ab}0.12^{a}$	—	NE	NE	NE	NE	NE
axr1-3 $\times$ Ler	$^{a}0.04^{a}$	$^{a}0.00^{b}$	$^{a}0.09^{a}$	—	$^{a}-0.25^{a}$	$^{a}-0.19^{a}$	$^{a}-0.17^{a}$	NE	NE	NE	NE	NE
axr1-3 $\times$ Mh-1	$^{a}-0.15^{a}$	$^{a}-0.12^{b}$	$^{a}-0.14^{a}$	$^{a}-0.44^{a}$	$^{a}-0.10^{a}$	$^{a}0.13^{a}$	$^{a}-0.06^{a}$	NE	NE	NE	NE	NE
axr1-3 $\times$ Mt-0	$^{a}-0.01^{a}$	$^{a}-0.01^{b}$	—	—	$^{a}0.07^{a}$	$^{a}0.12^{a}$	$^{a}-0.02^{a}$	NE	NE	NE	NE	NE
axr1-3 $\times$ Oy-0	$^{bc}0.00^{a}$	$^{a}-1.17^{a}$	$^{bc}0.08^{a}$	$^{c}0.81^{b}$	$^{bc}-0.10^{a}$	$^{b}-0.06^{a}$	$^{bc}0.06^{a}$	$^{abc}-0.06$	$^{abc}-0.14^{a}$	—	$^{abc}-0.35^{a}$	$^{abc}-0.39^{a}$
axr1-3 $\times$ PYL-1	$^{ab}-0.14^{a}$	$^{ab}-0.27^{b}$	$^{ab}0.09^{a}$	$^{b}0.20^{ab}$	$^{ab}-0.13^{a}$	$^{ab}-0.13^{a}$	$^{ab}0.08^{a}$	—	$^{ab}-0.10^{a}$	$^{a}-0.49$	$^{ab}-0.03^{a}$	$^{ab}-0.25^{a}$
axr1-3 $\times$ Sah-0	$^{a}-0.01^{a}$	$^{a}-0.01^{b}$	—	$^{b}0.62^{b}$	—	—	—	NE	NE	NE	NE	NE

Abbreviation: NE, not estimated.
No value ('—') indicates no difference among SS, RS and RR plants. Different superscript letters at the right and left of the mean estimates of dominance indicate a significant cross (genetic background) effect and quantitative trait effect, respectively ($P > 0.05$, with Tukey correction for multiple comparisons).

Discussion

Genetic background may affect herbicide resistance dynamics

The fitness effect of a mutation, and its dominance determine the expected evolutionary trajectories of the corresponding trait. Previous empirical estimates have primarily been based on the observation of evolutionary equilibrium of resistance genes in natural populations (Purrington, 2000). Most models studying the dynamics of resistance in plant populations therefore incorporate the fitness costs associated with resistance (herbivores: Restif and Koella, 2004; pathogens: Stahl *et al*., 1999; Roy and Kirchner, 2000; Burdon and Thrall, 2003; herbicide: Neve *et al*., 2003; Roux *et al*., 2008). Most models have considered the resistance trait to be constant. However, by analyzing the same resistance mutation in eight different segregating genetic backgrounds, our results strongly suggest that the fitness cost of a resistant genotype and its associated dominance should rather be considered variable in natural populations, depending on local genetic composition. The effects of genetic background on cost of herbicide resistance have already been described in weed species (for a review, see Bergelson and Purrington, 1996). To our knowledge, no previous experiment was specifically designed to study the effects of genetic background on either distortion of segregation or cost dominance of herbicide resistance. Because distortion of segregation disadvantaged the *axr1-3* resistance allele in two genetic backgrounds, cost estimates and dominance coefficients are biased downward for *axr1-3* × Mh-1 and *axr1-3* × 0y-0 crosses. It is therefore important to include the analysis of meiotic drive when estimating cost and dominance (both at the individual level and the population level).

We have shown that the dominance coefficient is less sensitive to genetic background than the cost. The mean dominance coefficient associated with the *axr1-3* resistance allele was close to that previously found ($h = 0.07$) in an experiment investigating epistatic interactions among three herbicide resistance alleles in *A. thaliana* (including *axr1-3* resistance allele; Roux *et al*., 2005b). This low value is consistent with Wright's physiological theory of dominance (Wright, 1977), which proposes that enzymes activity is seldom limiting, because of excess activity of wild-type enzymes (Kacser and Burns, 1981). This type of explanation would be consistent with an absence of relationship between cost estimates and dominance coefficients. Nevertheless some genetic background effects on dominance were detected for several quantitative traits, suggesting that the 'safety margin' associated with the *AXR1* gene (a value describing the maximum decrease of the enzyme activity that can be tolerated without affecting the phenotype; Wright, 1934) might be trait and/or development dependent.

Because most spontaneous mutations initially appear in the heterozygous state, the initial dynamics in the absence of herbicide will depend on both the fitness cost and its dominance. However, the results suggest a relatively small effect of genetic background on the dominance; consequently, the main difference between populations in mutation-drift equilibrium will depend on the expressed within-population fitness cost.

Because the evolutionary trajectories of an adaptation originating from standing genetic variation depend on its initial frequency (Hermisson and Pennings, 2005), the initial allele frequency in population before the first herbicide treatment is a crucial parameter. This value is highly dependent on the genetic background, and so it is highly unpredictable. To date, only one study has estimated the initial frequency of the herbicide resistance allele in a one weed population of *Lolium rigidum* (Preston and Powles, 2002). Further experiments estimating initial resistance allele frequencies in different weed populations would be of great value to consider appropriate management strategies (Roux *et al*., 2008).

Ecological implications of trait-specific cost and dominance

In natural conditions, the outcome of selection might depend on trait-specific cost and dominance (van Dooren, 2006). In environments with no competition, one can expect genetic backgrounds conferring a cost for early traits but no cost for seed production to be selected for, whereas those conferring the converse (no cost for early traits but a cost for seed production) would be selected against. In competitive environments, the opposite result might well be expected as competition will tend to favour genotypes with the highest early vigour. For example, plants without cost on seed production but with reduced rosette diameter may strongly suffer of competition compared with plants without reduced rosette diameter but with cost on seed production. This pattern has been observed for the *csr1-1* herbicide resistance allele in experimental settings with *A. thaliana* in greenhouse experiments (Roux *et al*., 2005a, 2006).

Because genetic variation for trait-specific cost and dominance was observed in our study for *axr1-3* resistance allele, the timing at which the cost and its associated dominance are expressed might also be selected. In the same way *A. thaliana* lines associated with a high seed production in the fall because of adequate germination timing can be counter-selected in spring on account of a delay in germination timing (Donohue, 2002; Donohue *et al*., 2005). As a result, the dynamics of selection of an adaptive allele might, in turn, depend on the interactions between environment and timing of trait expression.

Compensatory evolution of herbicide resistance cost

Two modes of ameliorative evolution have been suggested to reduce the pleiotropic fitness costs. First, an adaptive mutation conferring a fitness cost could be replaced by another mutation conferring the same adaptation but with a lower cost (Cohan *et al*., 1994). This 'replacement' mode of amelioration may occur at a single gene (allelic replacement; Guillemaud *et al*., 1998) or at different genes (nonallelic replacement; Lenski, 1988; Cohan *et al*., 1994). Second and non-exclusively, the pleiotropic fitness costs may be shaped by natural selection of modifiers at other loci. That is, selection can occur at genes whose products interact with the product of the adaptive allele (Fisher, 1928). This 'compensatory' mode of amelioration could occur either by the accumulation of compensatory mutations in the genetic background containing the original adaptive mutation (Andersson, 2003) or by the contribution of

the genetic diversity already present within the local population of the corresponding species (Cohan *et al*., 1994). Factors affecting the accumulation of compensatory mutations have been extensively studied in organisms with a rapid life cycle: bacteria, yeast and fungi. These studies identified factors including the rate of compensatory mutation (Poon and Chao, 2005; Gagneux *et al*., 2006; Schoustra *et al*., 2006), the environment (Björkman *et al*., 2000; Reynolds, 2000) and the size of a population bottleneck (Levin *et al*., 2000; Maisnier-Patin *et al*., 2002). In organisms with longer generation time like weed species (often, only one generation per year), it is tempting to speculate that awaiting compensatory mutations might be less efficient than making use of the genetic diversity already present in the species. Although the accumulation of compensatory mutations in the genetic background containing the resistance allele seems hard to test in plant species, it is possible to survey intra-species genetic diversity to estimate the number of compensatory genes and their relative effects. Our results suggest that different compensatory genes are segregating in different genetic backgrounds.

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