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Is the cost of herbicide resistance expressed in the breakdown of the relationships between characters? A case study using synthetic-auxin-resistant *Arabidopsis thaliana* mutants

FABRICE ROUX AND XAVIER REBOUD*
UMR Biologie et Gestion des Adventices, 17 rue Sully, Institut National de la Recherche Agronomique, 21065 Dijon Cedex, France

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Summary

A mutation endowing herbicide resistance is often found to induce a parallel morphological or fitness penalty. To test whether such 'cost' of resistance to herbicides is expressed through lower resource acquisition, changes in resource allocation, or both, is of ecological significance. Here, we analysed 12 morphological traits in 900 plants covering three herbicide resistance mutations at genes *AUX1*, *AXR1* and *AXR2* in the model species *Arabidopsis thaliana*. Comparing these 2,4-D herbicide-resistant homozygous (RR) and heterozygous (RS) plants to homozygous susceptible (SS) plants, this analysis estimates the dominance level of the resistance allele on morphology. We also demonstrated that the herbicide resistance cost was primarily expressed as a change in resource acquisition (62·1–94% of the analysed traits). Although *AUX1*, *AXR1* and *AXR2* genes act in the same metabolic pathway of auxin response, each resistance factor was found to have its own unique signature in the way the cost was expressed. Furthermore, no link was observed between the absolute fitness penalty and the respective modifications of resource acquisition and/or resource allocation in the resistant plants. These results and their implications for herbicide resistance spread and establishment are discussed.

1. Introduction

Evolution of resistance in response to the use of herbicides generates practical problems with huge environmental, economic or health consequences. Herbicide resistance has therefore been the subject of intense attention (Heap & Lebaron, 2001) as well as empirical and theoretical attempts to determine and quantify the major factors underlying its evolution (reviewed in Diggle & Neve, 2001). For pesticide resistance in general, and herbicide resistance in plants, resistance expressed as a simple dominant inherited trait should be more rapidly and more efficiently selected under natural field conditions than a complex or recessive trait (Maxwell & Mortimer, 1994; Otto & Bourguet, 1999). Within the same dominance class, the success of a resistance trait depends primarily on the balance between its selective advantage in the presence of the pesticide and its disadvantage (fitness cost) in the absence of pesticide from the environment (Orr & Coyne, 1992; Carrière *et al.*, 1994; Lenormand & Raymond, 1998).

Genes conferring resistance are involved in very diverse aspects of metabolic or developmental processes (Davies *et al.*, 1996; Nagpal *et al.*, 2000). A mutation conferring resistance and incurring a fitness cost may affect the process of resource acquisition, i.e. mineral assimilation and photosynthetic efficiency in plants (Worley *et al.*, 2003) and/or the relationships between traits by changing the pattern of resource allocation (Uyenoyama, 1986). These contrasting possibilities are illustrated in Fig. 1 which illustrates the simple Y model of acquisition and allocation of resources of van Noordwijk & de Jong (1986). When a herbicide resistance cost is mainly a consequence of a decrease in the plant's ability to capture and fix resources without modification of their allocation among traits (Fig. 1 cases B and D), all plant traits are expected to suffer the cost. Conversely, when herbicide resistance results in changes in the way resources are allocated to different tissues (Fig. 1 cases C and D), then the cost of resistance results from the breakdown

* Corresponding author. Telephone: +33 3 80693184. Fax: +33 3 80693262. e-mail: reboud@dijon.inra.fr

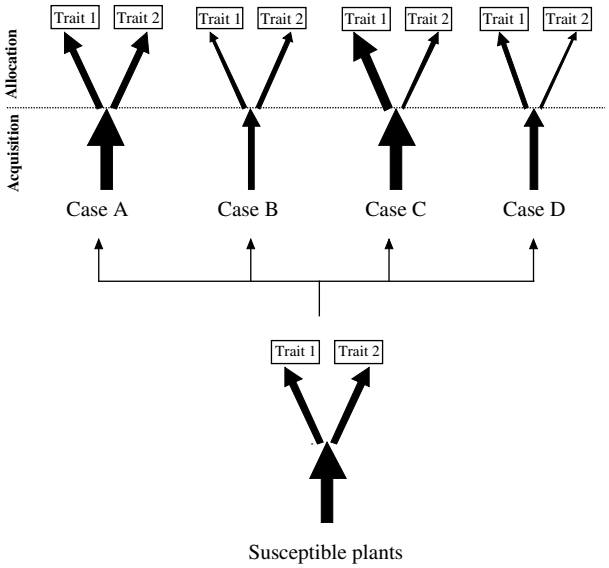

Fig. 1. Impact of a mutation on the resources acquisition and the pattern of resources allocation. A. The mutation leads to no change on the relationship between the two traits. B. The resources acquisition is modified by the mutation but the pattern of resources allocation is maintained unchanged. C. Only the pattern of resources allocation is modified by the mutation as the acquisition is unaffected. D. The mutation leads to a shift on both the phase of resources acquisition and the pattern of resources allocation.

of the relationships among traits without necessarily affecting the total plant biomass. These contrasting models may influence the selection and dynamics of genes conferring resistance. A resistance allele that changes resource acquisition (change in plant biomass) should be more susceptible to competition than one that only shifts resource allocation with no change in plant biomass (Worley *et al*., 2003). Furthermore, a shift in resource allocation can break down some genetic correlations, which can sometimes accelerate the adaptation to a new environment (Lynch & Walsh, 1997). The way the resistance cost is expressed via change in acquisition or allocation is therefore of ecological significance.

Bergelson & Purrington (1996) have reviewed experimental evidence for fitness costs associated with different herbicide-resistant mutations. They concluded that significant fitness costs occurred in some 60% of the analysed cases and varied greatly. Analysing eight different herbicide resistance mutations in the same genetic background of a standard line of *Arabidopsis thaliana*, we found that the fitness cost may vary from no observable fitness penalty to nearly 90% fitness reduction (Roux *et al*., 2004). In that study, the costs did not depend on the herbicide chemical family. Here we have extended this analysis in order to partition observed costs of resistance to a single herbicide chemical family between changes in resource acquisition and changes in the pattern of resource allocation. For a subset of three discrete mutations conferring resistance to the auxin-inhibiting herbicide 2,4-D, we analysed the relationship between 12 quantitative traits involving flowering phenology and architecture. A two-step statistical analysis permitted us to classify the response into one of the four hypothetical categories described in Fig. 1, and to assess the impact of each resistance mutation on resource acquisition and/or patterns of resource allocation. This analysis was performed on the F2 generation of segregating populations in the absence of herbicide treatment. Such a cross allows comparison of the impacts on the phenotypic traits of the three mutations in both the homozygous and heterozygous states. The implications of these results are then discussed in relation to the spread and establishment of herbicide resistance.

2. Materials and methods

(i) *Plant materials and genotyping the resistance status*

Our study focuses on three mutations conferring-resistance to the herbicide 2,4-D, each of which is a mutation at a different target gene involved in the auxin response: the *AUX1* (*aux1-7* mutation), *AXR1* (*axr1-3* mutation) and *AXR2* (*axr2-1* mutation) genes (Leyser *et al*., 1993; Bennett *et al*., 1996; Nagpal *et al*., 2000; respectively). The *AXR1* and *AXR2* proteins are required for rapid auxin-mediated changes in gene expression in most organs of the plant (Timpte *et al*., 1994, 1995). The *AUX1* protein is expressed predominantly within root epidermal cells, reflecting the close association between *AUX1* expression and basipetal auxin transport (Bennett *et al*., 1996). This subset of three mutations obtained from the Nottingham and INRA Versailles *A. thaliana* stock Centres, was chosen based on both their implication to a single chemical family and their contrasting patterns of fitness effects as observed in a previous comparative analysis of eight different herbicide resistance mutations in *A. thaliana* (Roux *et al*., 2004).

Plant materials and genotyping of resistance status are thoroughly described in Roux *et al*. (2004). Briefly, the three herbicide resistance mutations in *A. thaliana* studied here were all generated from the wild-type Columbia (Col) strain. The ethyl-methanesulfonate (EMS) mutation induction process may have caused other mutations in the genetic background of each resistance (Jander *et al*., 2003), so that one cannot test for cost of resistance by simply testing plants with the mutation (as an apparent cost might be due to deleterious mutations in the genetic background). To test for a cost of resistance, each RR mutant resistant line was thus crossed-pollinated by hand with the male sterile SS susceptible line NW77

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(Ler genetic background). A segregating F2 generation was made in each case by selfing the resulting F1. Clearly, deleterious mutations in the genetic background will be partially, but not completely, separated in the F2 generation, depending on their linkage to the resistance mutations. Plants with each of the three possible genotypes at a resistance locus will have roughly the same distribution of genotypes at the other loci in the genetic background, except for loci linked to the resistance locus. Only repeated backcrosses to the parental line could ensure complete removal of these other mutations (Bergelson & Purrington, 1996; Purrington, 2000). So, although this process of 'genetic background randomization' will not remove all the undesirable side-effects of the mutation induction, this simple design is the best possible to correctly approach the pleiotropic effects of each resistance mutation. Other reasons for choosing this crossing scheme involving the Ler and Col genetic backgrounds can be advanced: (i) the pleiotropic effects of a resistant mutation are influenced by the genetic background (Bergelson, 1994). A cross between Col and Ler genetic backgrounds yields an F2 generation with a high genetic variance which provides an average cost of resistance over a number of genetic backgrounds. (ii) Although a comparison of several *A. thaliana* strains did not demonstrate extensive linkage disequilibrium (Nordborg *et al.*, 2002), the high selfing rate of *A. thaliana* may temporarily create and maintain large linkage disequilibria through the entire genome that a crossing process between two inbred lines will break (Cheverud, 1988). (iii) Other genes modifying the expression of its pleiotropic effects could be responsible for an artificial increase or decrease in these pleiotropic effects (reviewed in Strauss *et al.*, 2002). The strategy of crossing inbred lines (Col and Ler) breaks such associations and allows the estimation of the pleiotropic effects due solely to the resistance mutation.

For each mutation, an allele-specific PCR method (Roux *et al.*, 2004) was used to discriminate the segregating SS, RS and RR genotypes at the F2 generation.

(ii) *Growth, morphological traits and fitness components*

For each resistance mutation, 300 F2 plants were grown. The experimental design and the greenhouse conditions were as described in Roux *et al.* (2004). Seeds were randomized among trays and grown in the absence of herbicides in the greenhouse from 10 April to 25 June 2002. In each tray, the seeds were regularly spaced (3 cm apart). The edges of trays were sown with Ler SS seeds to buffer against possible border effects. These plants were not included in the analysis. To avoid micro-environmental effects, the plots were regularly rotated during the growing period.

Up to 12 phenological and morphological traits were measured during the experiment: germination date (time from sowing to cotyledon emergence, GERM), rosette diameter at the 14th and 21st days after sowing (DIAM14 and DIAM21), flowering time (FLOR), number of rosette (LEAF) and cauline (CAULIN) leaves, and height from soil to first flower at flowering (H1FL). All other traits were measured on harvested plants: maximum plant height (HMAX); height from soil to first silique (H1SIL); the total silique length (TOTSIL), a derived trait closely matching the total seed production and corresponding to the multiplication of the mean silique size (measured on the 3rd, 5th, 7th and 9th siliques on the main stem) by the total number of mature siliques; 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). These variables describe the height and volume occupied by the inflorescence, and its degree of ramification and compactness (Reboud *et al.*, 2004). Most of these traits have been shown to have values of heritability above 0·2 when analysed for a worldwide collection of wild-type *A. thaliana* accessions (McKhann *et al.*, 2004).

(iii) *Statistical analysis*

For each resistance mutation in both the heterozygous and homozygous states, two sets of analyses were performed on all combinations of traits to sort them into the four categories in Fig. 1. First, based on a reduced major axis (RMA) regression, the mutual slope estimates describe the joint variation of two random variables. As there are no 'dependent' and 'independent' variables under RMA regression, the slope between the pair (trait X, trait Y) is the same as that of the reverse pair (trait Y, trait X). Following Sokal & Rohlf (2003), this Model II regression is expected to be a suitable measure of the functional relationship between variables in morphometric work. A comparison between mutual slopes between SS and either RR or RS genotypes was thus performed to test the impact of each resistance on the pattern of resources allocation. A significant difference in slopes indicates a shift in the pattern of resource allocation (cases C and D), while absence of a difference indicates that the pattern of resources allocation between traits is not modified (cases A and B). To overcome the scale dependence of the mutual slope to the different units of measurement, variables were standardized before the slope was computed (Sokal & Rohlf, 2003). Second, a multivariate analysis (MANOVA) using the two traits of each combination

Table 1. *Two-step analyses allowing the discrimination into one of the four theoretical cases described in Fig. 1*

	Case A	Case B	Case C	Case D
Resources allocation: SLOPE CHANGE	Non-significant	Non-significant	Significant	Significant
Resources acquisition: MANOVA	Non-significant	Significant	Non-significant	Significant

Case A, no change; case B, shift in resources acquisition; case C, shift in the pattern of resources allocation; case D, shift in both resources acquisition and pattern of resources allocation.

as response variables was then performed using the following model: traits(x,y) = plot + genotype + error, in which x and y are the first and second traits, respectively. A significant genotype effect indicates a shift in trait value(s), indicating a change in resource acquisition (Fig. 1, cases B and D), while absence of a genotype effect would primarily indicate no modification of the resources acquisition over that pair of traits (Fig. 1, cases A and C). GERM, DIAM14, DIAM21, H1SIL and LEN were not transformed for this multivariate analysis. HMAX was square transformed to homogenize variance. All other variables were square-root transformed. All transformations succeeded in restoring variance homogeneity as confirmed by non-significant Levene's test results (not shown).

Integrating the information from this two step analysis (see Table 1) allows the discrimination into one of the four theoretical cases described in Fig. 1. In all cases, significance was assigned at the $P<0{\cdot}05$ level. Because the number of degrees of freedom when comparing linked traits is not straightforward to determine, no Bonferroni correction was applied. In our study, the number of comparisons is therefore expected with 95% confidence to have generated up to six cases of potentially false significance.

3. Results

After genotyping, the resistance mutations could each be compared as RS heterozygotes with the wild-type SS homozygote, and also as RR homozygotes (a total of 72 tests). The quantitative effects of each resistance mutation in both the heterozygous and homozygous states on the 12 quantitative traits are given in Table 2. The use of the Ryan–Einot–Gabriel–Welsch (REGWQ) multiple range test procedure allowed us to maintain an overall significance at $P=0{\cdot}05$ for each quantitative trait. In the homozygous state, the *aux1-7*, *axr1-3* and *axr2-1* mutations had significant effects on five, six and nine quantitative traits, respectively. In the heterozygous state, the *aux1-7* and *axr2-1* mutations significantly affected nine and eight quantitative traits, respectively, while no trait was affected by the *axr1-3* mutation.

Examples of each of cases A, B, C or D are illustrated in Fig. 2. The relationship between the total number of flowering heads (HEADS) and the total silique length (TOTSIL) was not affected by the *aux1-7* mutation when comparing SS and RR plants (Fig. 2, case A). Less resource was available for both the height from soil to first silique (H1SIL) and the total silique length (TOTSIL) in the presence of the *axr2-1* mutation (Fig. 2, case B). The amount of available resources was thus modified by the *axr2-1* allele in the homozygous state. A shift in the pattern of resource allocation was detected in the relationship between the number of cauline leaves (CAULIN) and the height from soil to first flower at flowering (H1FL) when comparing SS and RR plants for the *axr1-3* mutation (Fig. 2, case C). More resources were allocated to CAULIN with the *axr1-3* mutation in the homozygous state. Last, the combination of date to flowering time (FLOR) with height from soil to first flower at flowering (H1FL) in the *axr2-1* mutation in the homozygous state illustrates a case where both the resource acquisition and the pattern of resource allocation are affected (Fig. 2, case D). Less total resource was available for these two traits but was also preferentially allocated to FLOR when compared with the pattern of the SS reference.

The effects of each resistance mutation on the total quantity of resources available and the pattern of resource allocation are detailed in Table 3 and summarized in Table 4. Overall, differences in inclusive fitness measured as total silique length were not reflected in the respective proportion of cases A, B, C or D. Compared with SS plants, 15·2%, 28·8% and 3·0% of the relationships between traits remained unaffected (case A) in the homozygous state by the *aux1-7*, *axr1-3* and *axr2-1* mutations, respectively; while only the *axr1-3* and *axr2-1* mutations led to a difference in fitness. When present, altered relationships between traits resulted predominantly from a shift in the quantity of allocated resources (case B or D). In the heterozygous state, the patterns observed for the *aux1-7* and *axr1-3* mutations contrasted with the ones observed in the homozygous state. Only 4·5% of the relationships between traits were unaffected by the *aux1-7* mutation in RS plants, the remaining relationships being affected by a shift of the

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Table 2. *Pleiotropic effects in both the heterozygous and homozygous states of the aux1-7, axr1-3 and axr2-1 mutations for 12 quantitative traits*

		GERM	DIAM14	DIAM21	FLOR	LEAF	CAULIN	H1FL	HMAX	H1SIL	LEN	HEADS	TOTSIL
aux1-7	SS	$6{\cdot}5 \pm 0{\cdot}3^{a}$	$3{\cdot}6 \pm 0{\cdot}7^{a}$	$20{\cdot}7 \pm 1{\cdot}1^{a}$	$32{\cdot}7 \pm 0{\cdot}5^{a}$	$9{\cdot}2 \pm 0{\cdot}2^{a}$	$3{\cdot}2 \pm 0{\cdot}1^{a}$	$60{\cdot}5 \pm 4{\cdot}7^{a}$	$29{\cdot}2 \pm 1{\cdot}3^{a}$	$14{\cdot}0 \pm 0{\cdot}6^{a}$	$2{\cdot}5 \pm 0{\cdot}1^{a}$	$20{\cdot}6 \pm 1{\cdot}4^{a}$	$1347{\cdot}9 \pm 107{\cdot}2^{a}$
	RS	$6{\cdot}4 \pm 0{\cdot}2^{a}$	$6{\cdot}5 \pm 0{\cdot}5^{b}$	$25{\cdot}0 \pm 0{\cdot}9^{b}$	$33{\cdot}7 \pm 0{\cdot}4^{a}$	$10{\cdot}5 \pm 0{\cdot}3^{b}$	$3{\cdot}4 \pm 0{\cdot}1^{a}$	$101{\cdot}8 \pm 4{\cdot}2^{b}$	$39{\cdot}6 \pm 0{\cdot}9^{b}$	$17{\cdot}5 \pm 0{\cdot}5^{b}$	$1{\cdot}8 \pm 0{\cdot}1^{b}$	$27{\cdot}5 \pm 1{\cdot}3^{b}$	$1970{\cdot}1 \pm 100{\cdot}3^{b}$
	RR	$6{\cdot}1 \pm 0{\cdot}2^{a}$	$4{\cdot}9 \pm 0{\cdot}7^{ab}$	$22{\cdot}5 \pm 1{\cdot}1^{ab}$	$34{\cdot}6 \pm 0{\cdot}6^{a}$	$11{\cdot}1 \pm 0{\cdot}4^{b}$	$3{\cdot}4 \pm 0{\cdot}2^{a}$	$89{\cdot}7 \pm 5{\cdot}4^{b}$	$37{\cdot}1 \pm 1{\cdot}3^{b}$	$17{\cdot}2 \pm 0{\cdot}7^{b}$	$1{\cdot}8 \pm 0{\cdot}1^{b}$	$25{\cdot}6 \pm 1{\cdot}8^{ab}$	$1768{\cdot}6 \pm 163{\cdot}7^{ab}$
axr1-3	SS	$6{\cdot}2 \pm 0{\cdot}2^{a}$	$5{\cdot}0 \pm 0{\cdot}6^{a}$	$24{\cdot}7 \pm 1{\cdot}0^{a}$	$33{\cdot}5 \pm 0{\cdot}5^{a}$	$10{\cdot}3 \pm 0{\cdot}3^{a}$	$3{\cdot}5 \pm 0{\cdot}2^{ab}$	$85{\cdot}5 \pm 4{\cdot}7^{a}$	$38{\cdot}3 \pm 1{\cdot}4^{a}$	$16{\cdot}5 \pm 0{\cdot}7^{a}$	$2{\cdot}0 \pm 0{\cdot}1^{a}$	$26{\cdot}3 \pm 1{\cdot}8^{a}$	$2079{\cdot}7 \pm 154{\cdot}1^{a}$
	RS	$6{\cdot}1 \pm 0{\cdot}2^{a}$	$6{\cdot}7 \pm 0{\cdot}5^{a}$	$25{\cdot}6 \pm 0{\cdot}8^{a}$	$33{\cdot}2 \pm 0{\cdot}4^{a}$	$10{\cdot}3 \pm 0{\cdot}3^{a}$	$3{\cdot}4 \pm 0{\cdot}1^{a}$	$92{\cdot}3 \pm 4{\cdot}3^{a}$	$37{\cdot}8 \pm 0{\cdot}9^{a}$	$17{\cdot}0 \pm 0{\cdot}5^{a}$	$2{\cdot}0 \pm 0{\cdot}1^{a}$	$27{\cdot}1 \pm 1{\cdot}3^{a}$	$1922{\cdot}9 \pm 100{\cdot}7^{a}$
	RR	$6{\cdot}5 \pm 0{\cdot}4^{a}$	$4{\cdot}6 \pm 1{\cdot}1^{a}$	$18{\cdot}2 \pm 1{\cdot}4^{b}$	$37{\cdot}2 \pm 1{\cdot}6^{b}$	$8{\cdot}4 \pm 0{\cdot}4^{b}$	$4{\cdot}4 \pm 0{\cdot}5^{b}$	$97{\cdot}6 \pm 7{\cdot}9^{a}$	$25{\cdot}2 \pm 1{\cdot}3^{b}$	$13{\cdot}8 \pm 0{\cdot}8^{a}$	$2{\cdot}4 \pm 0{\cdot}4^{a}$	$23{\cdot}1 \pm 4{\cdot}4^{a}$	$453{\cdot}8 \pm 96{\cdot}6^{b}$
axr2-1	SS	$7{\cdot}1 \pm 0{\cdot}3^{a}$	$2{\cdot}5 \pm 0{\cdot}5^{a}$	$18{\cdot}5 \pm 1{\cdot}2^{a}$	$35{\cdot}4 \pm 0{\cdot}9^{a}$	$9{\cdot}1 \pm 0{\cdot}3^{a}$	$3{\cdot}0 \pm 0{\cdot}1^{a}$	$83{\cdot}3 \pm 5{\cdot}1^{a}$	$32{\cdot}3 \pm 1{\cdot}6^{a}$	$14{\cdot}0 \pm 0{\cdot}7^{a}$	$2{\cdot}0 \pm 0{\cdot}1^{a}$	$23{\cdot}3 \pm 1{\cdot}9^{a}$	$1293{\cdot}6 \pm 107{\cdot}5^{a}$
	RS	$7{\cdot}9 \pm 0{\cdot}2^{b}$	$0{\cdot}7 \pm 0{\cdot}4^{b}$	$6{\cdot}9 \pm 0{\cdot}6^{b}$	$42{\cdot}6 \pm 0{\cdot}9^{b}$	$8{\cdot}6 \pm 0{\cdot}2^{ab}$	$2{\cdot}8 \pm 0{\cdot}1^{a}$	$19{\cdot}2 \pm 0{\cdot}9^{b}$	$9{\cdot}0 \pm 1{\cdot}2^{b}$	$3{\cdot}6 \pm 0{\cdot}5^{b}$	$3{\cdot}7 \pm 0{\cdot}8^{a}$	$9{\cdot}5 \pm 0{\cdot}7^{b}$	$259{\cdot}1 \pm 53{\cdot}6^{b}$
	RR	$7{\cdot}2 \pm 0{\cdot}3^{ab}$	$0{\cdot}8 \pm 0{\cdot}5^{b}$	$5{\cdot}6 \pm 0{\cdot}9^{b}$	$40{\cdot}8 \pm 1{\cdot}4^{b}$	$8{\cdot}1 \pm 0{\cdot}2^{b}$	$2{\cdot}7 \pm 0{\cdot}1^{a}$	$14{\cdot}6 \pm 0{\cdot}9^{b}$	$5{\cdot}5 \pm 0{\cdot}9^{b}$	$2{\cdot}2 \pm 0{\cdot}3^{b}$	$4{\cdot}2 \pm 2{\cdot}0^{a}$	$8{\cdot}3 \pm 0{\cdot}7^{b}$	$142{\cdot}2 \pm 51{\cdot}3^{b}$

For each mutation and each quantitative trait, different letters indicate different groups according to the genotypes after a Ryan–Einot–Gabriel–Welsch (REGWQ) multiple range test at $P = 0{\cdot}05$.
GERM and FLOR are expressed in days after sowing; DIAM14, DIAM21, H1FL, LEN and TOTSIL in millimetres; HMAX and H1SIL in centimetres. LEAF, CAULIN and HEADS correspond to the number of rosette and cauline leaves and the total number of flowering heads, respectively. All traits are presented as the mean value $\pm$SE.

total quantity of resources available. In RS plants, 97·0% of the relationships between traits were unchanged by the *axr1-3* mutation. For the *axr2-1* mutation, a similar pattern is observed in the heterozygous and homozygous states. Overall, there was no detectable relationship between total fitness difference and ABCD partitioning proportions.

4. Discussion

The effects of plant hormones on development are very complex, because a given substance and concentration can affect various traits in ways that depend not only on the cell or tissue involved but also on environmental conditions and plant age (e.g. Sultan, 2000). Assuming the measure of total silique length correctly approximates fitness, homozygotes for the *axr1-3* mutation exhibited a fitness cost of 78% when compared with SS plants, whereas no fitness difference was observed between RS and SS plants (see Table 4). For the *axr2-1* mutation, homozygotes showed an average 89% reduction in fitness relative to SS plants, while again no difference was observed between RS and RR plants. Finally, we detected no cost of resistance between RR and SS plants in the cross involving the *aux1-7* resistant line, while RS plants exhibited a slightly higher fitness relative to SS plants. Although the *AUX1*, *AXR1* and *AXR2* genes are all involved in the auxin-response pathway, fitness penalties expressed by each mutant line were thus highly contrasting. Mutations in auxin pathways therefore provide a powerful experimental model to link adaptation to a selective pressure (here the herbicide 2,4-D) and its impact on resource acquisition and/or patterns of resource allocation.

It is important to stress that some resistant mutant lines used here could have EMS mutations affecting phenotypes other than the one conferring resistance to the herbicide 2,4-D. This point is well illustrated by the difference of the mean trait values between SS plants from the different crosses that share the Col × Ler cross origin (see Table 2), differing only in the EMS mutations specific to each resistant line. Within each segregating F2 family, these additional mutations should be distributed similarly in each of the three genotype classes (SS, RS and RR) by our process of 'genetic background randomization' and were therefore expected to cause no overall bias. Only EMS mutations in linkage disequilibrium with the resistance gene would remain imperfectly homogenized over the three classes. Although Young & Tanksley (1989) suggest that such linkage may be quite large, it is important to remember that the variation due to EMS mutations and polymorphisms between Col and Ler in these blocks is not necessarily important.

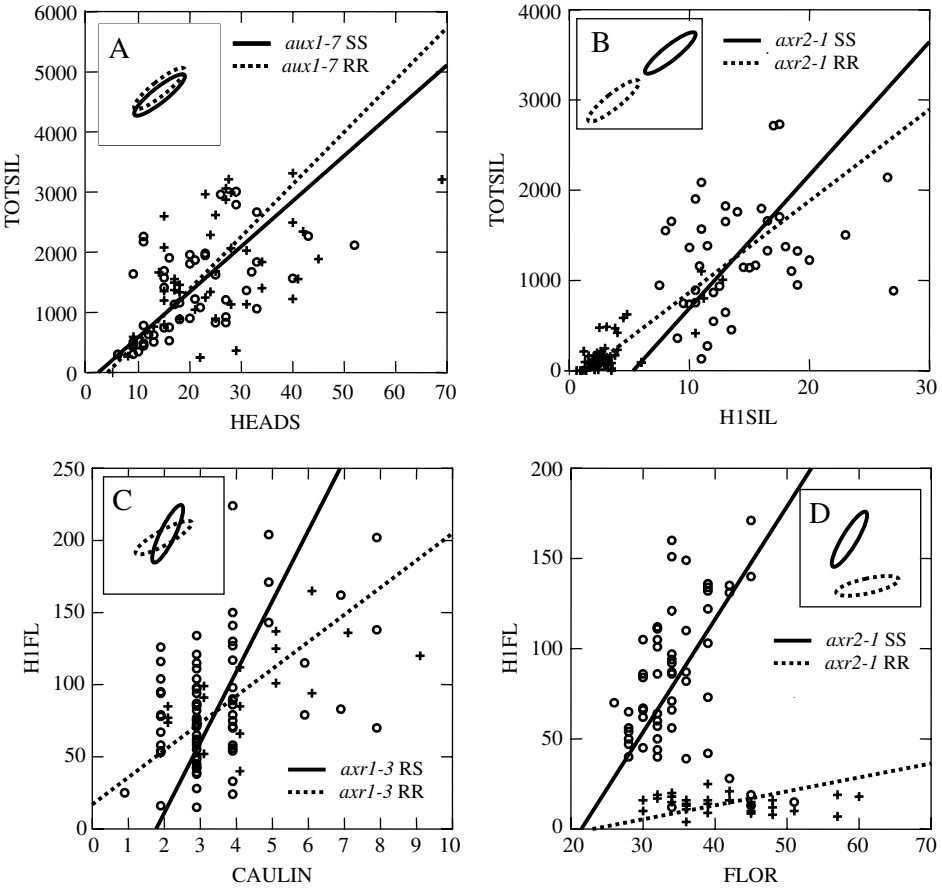

Fig. 2. Illustration of the impact of a mutation on the relationship between two traits. A. No change; B. Shift in resources acquisition; C. Shift in the pattern of resources allocation; D. Shift in both resources acquisition and pattern of resources allocation. Circles and crosses indicate experimental data for SS and RR plants, respectively. Relationships among traits are represented by midrange smoother. H1FL, H1SIL and TOTSIL are expressed in millimetres; FLOR in days from sowing. CAULIN and HEADS correspond to the number of cauline leaves and the total number of flowering heads, respectively.

(i) *Divergent pleiotropic effects among herbicide resistance genotypes*

The literature on fitness costs associated with adaptive changes reports few comparisons of distinct mutations which confer the same phenotypic adaptation within a single species, especially where the difference in the nature and origin of the adaptive genes is known. Analysing numerous phenological and morphological traits, we have shown that three 2,4-D resistant mutations in the homozygous state result in different plant morphologies. No association was observed between difference in fitness and the impact of each resistant mutation on either the acquisition or the allocation of resources. In the homozygous state, both the *axr1-3* allele, which expressed a fitness cost and the *aux1-7* allele, which did not, had an effect on plant morphology that resulted from a shift in the acquisition of resources. The *aux1-7* mutation is an illustrative case where the same fitness can be attained by different morphologies. The predominance of case A when comparing RS and RR for *aux1-7* and *axr2-1* mutations further indicates that these resistant mutations are expressed mostly as a dominant trait on morphology. For the *axr1-3* mutation, the greatest similarity is observed when comparing SS with RS plants confirming the recessive character of this resistant mutation on morphology (Table 4).

Other studies presenting morphological and ecological divergences have already been published with insecticide-resistant genotypes. For *Culex pipiens*, the A4-B4 overproducing esterase genotype is at no disadvantage during the winter, whereas the *ace-1*^{R} acetylcholinesterase-resistant allele incurs a constant, severe and dominant survival cost (Chevillon *et al.*, 1997). Two resistant strains of *Cydia pomonella* display different spring emergence relative to a sensitive strain (Boivin *et al.*, 2003). Two different insecticide-resistant genotypes (target-site mutation and gene amplification) resulted in similar pleiotropic effects in the peach–potato aphid *Myzus persicae* (Foster *et al.*, 2003). In our study, divergent morphological trajectories lead to differences between RR and SS plants, even though genes conferring resistance act in the

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Table 3. *Effects on resources acquisition and allocation among the SS, RS and RR plants of the aux1-7, axr1-3 and axr2-1 mutations*

		SS-RR comparison											
		GERM	DIAM14	DIAM21	FLOR	LEAF	CAULIN	H1FL	HMAX	H1SIL	LEN	HEADS	TOTSIL
aux1-7	GERM												
	DIAM 14	A											
	DIAM 2 1	A	A										
	FLOR	D	B	B									
	LEAF	D	D	D	D								
	CAULIN	C	C	C	B	B							
	H1FL	B	B	B	B	D	B						
	HMAX	B	B	B	D	D	D	D					
	H1SIL	B	B	B	B	D	D	B	B				
	LEN	B	D	D	D	D	C	D	B	D			
	HEADS	A	A	A	B	D	D	B	B	B	D		
	TOTSIL	C	A	A	B	B	A	B	D	B	D	A	
axr1-3	GERM												
	DIAM 14	A											
	DIAM 2 1	D	D										
	FLOR	B	B	D									
	LEAF	C	C	B	D								
	CAULIN	A	A	B	B	D							
	H1FL	A	A	A	D	D	C						
	HMAX	D	D	B	D	D	D	D					
	H1SIL	C	C	A	D	A	D	D	B				
	LEN	A	A	A	B	A	B	B	B	A			
	HEADS	A	A	A	D	C	B	A	B	A	A		
	TOTSIL	D	D	B	D	D	D	D	B	B	D	D	
axr2-1	GERM												
	DIAM 14	B											
	DIAM 2 1	B	D										
	FLOR	B	D	B									
	LEAF	B	D	B	D								
	CAULIN	A	D	B	D	A							
	H1FL	D	D	D	D	D	D						
	HMAX	B	D	B	D	B	B	D					
	H1SIL	B	D	B	D	B	B	D	B				
	LEN	C	D	D	D	D	C	D	D	D			
	HEADS	D	D	D	D	D	D	D	D	D	D		
	TOTSIL	D	D	B	D	B	B	D	D	B	D	D	

		SS-RS comparison											
		GERM	DIAM14	DIAM21	FLOR	LEAF	CAULIN	H1FL	HMAX	H1SIL	LEN	HEADS	TOTSIL
aux1-7	GERM												
	DIAM 14	B											
	DIAM 2 1	B	B										
	FLOR	C	B	B									
	LEAF	C	D	D	A								
	CAULIN	C	D	A	A	B							
	H1FL	B	B	B	B	B	B						
	HMAX	B	B	B	B	D	D	D					
	H1SIL	B	B	B	B	D	B	B	D				
	LEN	B	B	D	D	D	D	D	B	D			
	HEADS	B	B	B	B	B	B	B	B	B	D		
	TOTSIL	B	B	B	B	B	B	B	D	B	D	B	
axr1-3	GERM												
	DIAM 14	A											
	DIAM 2 1	A	B										
	FLOR	A	A	A									
	LEAF	A	A	A	A								
	CAULIN	A	A	A	A	A							
	H1FL	A	A	A	A	A	A						
	HMAX	A	A	A	A	A	A	A					
	H1SIL	A	A	A	A	A	A	A	A				
	LEN	A	A	A	A	A	A	A	C	A			
	HEADS	A	A	A	A	A	A	A	A	A	A		
	TOTSIL	A	A	A	A	A	A	A	A	A	A	A	
axr2-1	GERM												
	DIAM 14	D											
	DIAM 2 1	B	D										
	FLOR	D	D	D									
	LEAF	A	D	B	D								
	CAULIN	A	D	B	B	A							
	H1FL	D	D	D	D	D	D						
	HMAX	B	D	B	B	B	B	D					
	H1SIL	B	D	B	B	B	B	D	B				
	LEN	C	D	D	D	C	C	D	D	D			
	HEADS	D	D	B	D	D	D	B	D	D	D		
	TOTSIL	B	D	B	D	B	B	D	D	B	D	B	

		RS-RR comparison											
		GERM	DIAM14	DIAM21	FLOR	LEAF	CAULIN	H1FL	HMAX	H1SIL	LEN	HEADS	TOTSIL
aux1-7	GERM												
	DIAM 14	A											
	DIAM 2 1	A	A										
	FLOR	A	A	A									
	LEAF	A	A	A	A								
	CAULIN	A	A	A	A	A							
	H1FL	A	A	A	A	A	C						
	HMAX	A	B	A	A	A	A	A					
	H1SIL	A	A	A	A	A	A	A	A				
	LEN	A	A	A	A	A	A	A	A	A			
	HEADS	A	A	A	A	A	A	A	A	A	A		
	TOTSIL	A	A	A	A	A	A	A	A	A	A	A	
axr1-3	GERM												
	DIAM 14	A											
	DIAM 2 1	D	D										
	FLOR	A	B	D									
	LEAF	D	D	B	D								
	CAULIN	A	B	B	B	D							
	H1FL	A	A	B	D	D	C						
	HMAX	D	D	B	D	D	D	B					
	H1SIL	C	C	B	D	A	D	D	B				
	LEN	A	A	B	B	A	B	A	B	A			
	HEADS	A	A	A	B	C	B	A	B	A	A		
	TOTSIL	D	D	B	D	D	D	D	B	B	D	D	
axr2-1	GERM												
	DIAM 14	A											
	DIAM 2 1	A	A										
	FLOR	A	A	A									
	LEAF	C	A	A	A								
	CAULIN	D	A	A	A	A							
	H1FL	D	A	C	C	A	A						
	HMAX	D	A	A	A	A	A	A					
	H1SIL	A	A	A	A	A	A	A	A				
	LEN	B	A	A	A	A	A	A	A	A			
	HEADS	A	A	A	A	A	A	A	A	A	C		
	TOTSIL	A	A	A	A	A	A	A	A	C	C	A	

A, no significant change; B, significant shift in resources acquisition; C, significant shift in the pattern of resources allocation; D, significant shift in both resources acquisition and pattern of allocation.

Table 4. *Summary of the impact on the relationships among traits for the aux1-7, axr1-3 and axr2-1 mutations*

Mutations	Comparison	A (%)	B (%)	C (%)	D (%)	Fitness difference
aux1-7	SS-RR	15·2	42·4	7·6	34·8	no difference
	SS-RS	4·5	65·2	4·5	25·8	46%
	RS-RR	97·0	1·5	1·5	0·0	no difference
axr1-3	SS-RR	28·8	24·2	9·1	37·9	78%
	SS-RS	97·0	1·5	1·5	0·0	no difference
	RS-RR	25·7	30·3	6·1	37·9	76%
axr2-1	SS-RR	3·0	30·3	3·0	63·7	89%
	SS-RS	4·5	34·9	4·5	56·1	80%
	RS-RR	84·8	1·5	9·1	4·6	no difference

A, no significant change; B, shift in resources acquisition; C, shift in the pattern of resources allocation; D, shift in both resources acquisition and pattern of resources allocation. Fitness differences among SS, RS and RR plants were calculated from mean of total silique length and are expressed as percentage of SS fitness.

same metabolic pathway, i.e. the auxin response. Such patterns could imply that the fitness and dynamics under selection of a resistance gene would therefore depend on both the genetic background where the mutation first occurs and the local environmental conditions. Weed species such as *A. thaliana* inhabit rather disturbed environments (Alonso-Blanco & Koornneef, 2000), i.e. a heterogeneous environment with various optima. Flowering time is usually assumed to be well adjusted to the local environment (Le Corre *et al*., 2002) and of some importance when colonizing different habitats (Linde *et al*., 2001). A resistant mutation that would delay flowering time could be easily counter-selected in its native habitat but, conversely, spread over other environments where a late flowering fits the local selection pressure. Such conditions may exist as the late flowering trait is rather commonly encountered among accessions of the worldwide collection of *A. thaliana* wild-types (McKhann *et al*., 2004; Reboud *et al*., 2004). In such habitats, the *axr1-3* or *axr2-1* resistant mutations with a delay in flowering time (of 3·7 and 5·4 days, respectively) would become more efficiently selected than the *aux1-7* allele whose precocity may prevent crosses between RR and SS plants and delay the diffusion of the resistance gene. Depending on the trait affected by the resistant genotype, some mutations conferring the resistance status could be beneficial in specific environmental conditions.

(ii) *Origin of the cost*

When studying the relationships among traits, consideration is generally limited to the relative allocation of resources to competing traits without considering any variation in the total acquisition of resources. Because many traits affect an organism's ability to acquire resources, loci affecting acquisition are likely to outnumber those influencing allocation between any pair of traits, so that genetic variation for acquisition may exceed that for allocation (Charlesworth, 1990; Houle, 1991). Our data support suggestions that deleterious mutations, i.e. resistance mutations in the absence of herbicide in our study, may more often influence the quantity of resources to allocate than the way in which these resources are partitioned among traits (Lynch & Walsh, 1997). This part of our results may, however, suffer from three potential limitations and should therefore be interpreted with caution. First, traits more directly involved in resource acquisition such as the rosette diameter, could have a higher probability of being involved in cases B and D than A and C. Although the analysis conducted trait by trait did not demonstrate such a trend in our study, the choice of the measured traits could theoretically affect ABCD partitioning. Second, in their genetic Y model van Noordwijk & de Jong (1986) assumed that the two traits are competing for the resource at the same time. In our study, the effect of each resistant mutation was conducted on every pair of traits even if the two considered traits were not expressed at the same time during the *A. thaliana* life-cycle. This point might also affect our ability to correctly partition the cost into the B, C or D cases in a statistical manner. Third, because the two statistics used may be of varying significance power, the measure of a shift in either allocation or acquisition could be biased towards the most powerful test. If MANOVA is a much more powerful test than the comparison between mutual slopes, it would overestimate cases B and D. An unbiased analysis would be one that partitions between shifts in acquisition and shifts in allocation using a single statistical test. But, to our knowledge, such a test does not exist. If statistical biases are present in our study, it does not, however, alter our ability to compare the pleiotropic effects of the *aux1-7*, *axr1-3* and *axr2-1* mutations.

A corollary that a cost of resistance is originating mainly from a reduction in the resource acquisition would thus be that the resistant plants (primarily

affected by a change in plant biomass) will usually express higher fitness cost when under severe competition (Heil & Baldwin, 2002). Reboud & Till-Botteraud (1991) found a much higher cost of resistance in photosynthesis-pathway-affected resistant *Setaria* plants when they were grown under increased competition. An expectation could follow that the spread of herbicide resistance would be more delayed under high competing conditions (as expressed by the crop for example). Long-term experiments in fields would be relevant to test this hypothesis further.

This study provides some evidence that a resistant mutation can be associated with no general reproductive cost but with an ecological cost depending on the balance of local selective pressures. In these situations, resistance evolution may be quite unpredictable and therefore may most freely appear and develop. This further emphasizes the need for studies which estimate fitness costs in heterogeneous environments in order to reconsider management strategies at a large geographic scale or gain insights into the ecological conditions that would have prevailed to favour resistance in the early stages of its spread.

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