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IDEA AND PERSPECTIVE

Ontogenetic switches from plant resistance to tolerance: minimizing costs with age?

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Abstract

Changes in herbivory and resource availability during a plant's development should promote ontogenetic shifts in resistance and tolerance, if the costs and benefits of these basic strategies also change as plants develop. We proposed and tested a general model to detect the expression of ontogenetic tradeoffs for these two alternative anti-herbivory strategies in *Raphanus sativus*. We found that ontogenetic trajectories occur in both resistance and tolerance but in opposite directions. The juvenile stage was more resistant but less tolerant than the reproductive stage. The ontogenetic switch from resistance to tolerance was consistent with the greater vulnerability of young plants to leaf damage and with the costs of resistance and tolerance found at each stage. We posit that the ontogenetic perspective presented here will be helpful in resolving the current debate on the existence and detection of a general resistance–tolerance tradeoff.

Keywords

Costs, herbivory, ontogenetic trajectories, resistance, tolerance, tradeoffs.

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INTRODUCTION

As individuals grow and develop, they face different abiotic and biotic challenges. As a result, they are generally expected to change their ecological niche with respect to resource utilization and their interaction with other species (Hjelm *et al.* 2000). Changes in ecological niche are likely to promote the evolution of ontogenetic changes in behaviour and morphology of many organisms (Werner & Guillam 1984). For example, in the tropical herbaceous climber *Monstera gigantea*, ontogenetic changes in leaf shape and physiology during its three defined ontogenetic stages (leafless skototropic seedlings, round-leafed phototropic juveniles and adults with aroid leaves growing perpendicular to the trunk) are triggered by changes in light availability as plants climb their host trees (Strong & Ray 1975). Ontogenetic changes in the magnitude and correlation among traits resulting in different morphologies have been defined as ontogenetic tradeoffs (Hjelm *et al.* 2000). Here we specifically define ontogenetic tradeoffs as the occurrence of simultaneous ontogenetic trajectories of at least two quantitative traits in opposite directions, as a result of changes in resource availability and/or in fitness optima associated with the expression of such traits during plant development.

Plants eaten by herbivores often face temporal variation in the intensity of damage and risk of attack as they develop (reviewed by Boege & Marquis 2005), which coincides with physiological and morphological changes throughout their life-cycle. Additional changes in resource availability, associated with extrinsic factors (e.g. seasonality) or intrinsic properties of the plants influencing resource acquisition and allocation at each stage (e.g. shoot : root ratio, stored reserves, etc.), modify the abiotic environment in which plants harvest resources as they develop (Bryant *et al.* 1991). Such heterogeneous 'ontogenetic environments' should exert strong selection on the developmental plasticity of those characters that best maximize plant fitness components of each stage (Pigliucci & Schlichting 1995; Wright & McConnaughay 2002). In particular, plants should be expected to produce ontogenetic trajectories (i.e. ontogenetic changes) that optimize plant defence. Plants deploy two basic types of alternative defences: resistance traits that reduce herbivore damage and tolerance traits that minimize the negative fitness effects of damage (Stowe *et al.* 2000). One approach to assess whether ontogenetic trajectories are indeed targets of natural selection is to quantify their costs and benefits throughout ontogeny.

If changes in both the abiotic and biotic environments occur during plant development, the costs and benefits of

expressing resistance and/or tolerance are also likely to change as plants develop (Stowe *et al.* 2000). Changes in defence costs and benefits may be caused by ontogenetic variation in resource availability (Bergelson 1994; Hochwender *et al.* 2000; Stowe *et al.* 2000), stored reserves, growth rates, availability of meristems and tradeoffs with other functions (e.g. resistance or tolerance, growth and reproduction, Stowe *et al.* 2000). Furthermore, the benefits of either strategy also can be influenced by seasonal or ontogenetic changes in risk of herbivore attack (Boege & Marquis 2005). Numerous studies have documented the existence of and variation in resistance and tolerance costs at a single ontogenetic stage (Simms & Rausher 1989; Fineblum & Rausher 1995; Stinchcombe 2002; Fornoni *et al.* 2004), and only one study has suggested the presence of ontogenetic changes in the costs of resistance (Briggs & Schultz 1990). However, no studies have assessed the evolutionary response to such age-dependent costs via the expression of ontogenetic trajectories in resistance and tolerance.

Under the assumptions that the expression of resistance and tolerance traits are costly for plants and that they are alternative evolutionary solutions to the same selective pressure, previous models have proposed that the maximization of their benefit : cost ratio should promote a negative genetic correlation between the two functions with two adaptive peaks, representing the maximum levels of either strategy (Fineblum & Rausher 1995; Mauricio *et al.* 1997; Tiffin 2000). This negative correlation has been explained using the rationale that as plants become more tolerant, the benefits of resistance decrease, whereas in plants with high levels of resistance, fitness benefits of tolerance should be lower than its costs (Simms & Triplett 1994; Stowe *et al.* 2000). However, maximum levels of either strategy could be prevented if opposing selective agents (e.g. multiple herbivore species) operate simultaneously on the same attribute (Jokela *et al.* 2000; Strauss & Irwin 2004), and/or if environmental heterogeneity influences fitness costs and benefits, promoting the simultaneous expression of both resistance and tolerance (Hochwender *et al.* 2000; Stinchcombe 2002; Fornoni *et al.* 2004).

This same reasoning could be used to incorporate ontogeny into the predictions of the evolution and expression of tolerance and resistance. Under the assumptions that: (a) herbivore pressure is constant and has significant impacts on plant fitness throughout ontogeny; (b) tolerance and resistance are costly and redundant strategies during plant development; and (c) costs of both tolerance and resistance are equivalent throughout ontogeny, we should expect to see that when the ontogenetic trajectory in one strategy is expressed as a function of its benefits on fitness, an ontogenetic trajectory with the opposite trend is expressed in the second strategy. This would promote a negative genetic correlation between ontogenetic trajectories in tolerance and resistance throughout plant development (i.e. ontogenetic tradeoff). However, alterations of the assumptions stated above could promote different ontogenetic patterns in the simultaneous expression of tolerance and resistance. For example, if the risk of herbivore attack is concentrated only at a particular ontogenetic stage [violating assumption (a)] and plants can afford the costs of both defensive strategies, an efficient defence would be to express tolerance and resistance simultaneously at the stage with higher risk of attack or with the greatest fitness losses due to herbivore damage. Under this scenario, rather than an ontogenetic tradeoff we would find a positive correlation between ontogenetic trajectories of each strategy. Similarly, if the identity and diversity of herbivores attacking the plant modify the efficiency of resistance throughout ontogeny, the redundancy between both strategies [assumption (b)] would disappear, or at least not be maintained as plants develop. Thus, their ontogenetic trajectories would be selected independently as a function of their benefits, and not of the expression of the alternative strategy. In this case, both strategies could be redundant at one stage, but complementary strategies at a different stage, not necessarily producing an ontogenetic tradeoff. Finally, if the costs of both strategies change throughout ontogeny [violating assumption (c)] as a consequence of changes in resource availability (both in the environment and in storage organs), this could either generate an ontogenetic tradeoff, if the costs of each strategy change to the same degree but in opposite directions during ontogeny, or no tradeoff, if such ontogenetic changes occur independently and/or with different magnitudes for each strategy.

To test for the existence of an ontogenetic tradeoff between tolerance and resistance we constructed a model based on the mean genotypic difference in the expression of resistance and tolerance between ontogenetic stages (Fig. 1). The main assumption of this model is that both resistance and tolerance are costly and that fitness optima associated with the expression of these traits are different between ontogenetic stages. In this model, the correlation between ontogenetic differences in resistance and ontogenetic differences in tolerance allows the assessment of the magnitude and direction of ontogenetic change in one strategy relative to the degree of change in the alternative strategy (Fig. 1). A lack of any relationship between ontogenetic differences in resistance and tolerance would be interpreted as the non-existence of ontogenetic trajectories in at least one strategy. A negative relationship between ontogenetic differences in tolerance and resistance would indicate the presence of an ontogenetic tradeoff. In this case, we should expect to find genotypes highly resistant but poorly tolerant at one stage (Fig. 1a), and genotypes highly tolerant but poorly resistant at the other stage

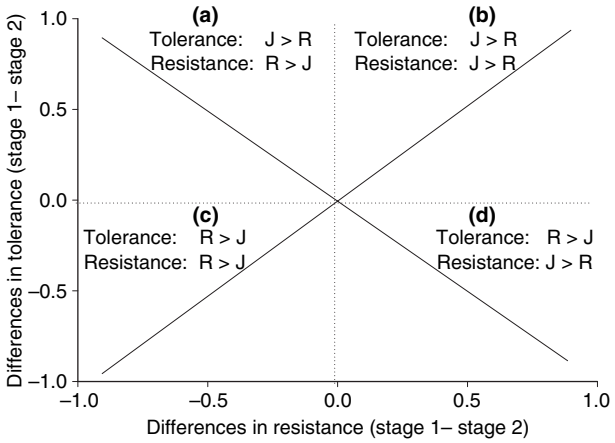

Figure 1 Proposed graphic model to interpret the simultaneous ontogenetic changes in tolerance and resistance at two ontogenetic stages (e.g. J = Juvenile, R = Reproductive). For interpretation of correlations between the mean genotypic ontogenetic differences in tolerance and resistance see text.

(Fig. 1d). This scenario could be expected for plants with limited storage abilities (e.g. annual plants) that cannot afford the simultaneous expression of both costly and redundant strategies at a given ontogenetic stage. In contrast, a positive relationship would indicate that both strategies follow the same ontogenetic trajectory but with variable directions among genotypes. Thus, we should find genotypes more resistant and tolerant at one stage (Fig. 1b) and genotypes more resistant and tolerant at the other stage (Fig. 1c). Because of their lifespan, perennial plants are more likely to follow this trend than annual plants, given the increased storage abilities to afford the simultaneous expression of both defensive strategies at a given stage, even when both are costly. In addition, the diversity of herbivores is likely to be greater in perennial than in annual plants (Futuyma 1976), thus reducing the efficiency of a given resistance trait for long-lived species and promoting the simultaneous expression of tolerance and multiple resistance traits (Jokela *et al.* 2000). Finally, because damage is likely to be less predictable during development of perennial than of annual plants (Futuyma 1976), some genotypes would benefit by expressing both tolerance and resistance at one stage, whereas others would benefit to express both strategies at a different stage, generating the positive relationship between ontogenetic trajectories of tolerance and resistance described previously (Fig. 1).

Although a recent review has found that ontogenetic changes in the expression of resistance and tolerance are rather common in nature (Boege & Marquis 2005), limited information is available regarding their simultaneous expression (Simms & Triplett 1994), costs (Baldwin *et al.* 1990) and tradeoffs throughout plant ontogeny. Thus, the aim of this study was to assess: (i) ontogenetic differences of vulnerability to leaf damage; (ii) the presence of ontogenetic trajectories in plant resistance and tolerance; (iii) whether fitness costs of resistance and tolerance are maintained throughout plant ontogeny; and (iv) whether there are ontogenetic tradeoffs in the expression of both strategies, using the proposed model.

MATERIALS AND METHODS

Study system

Raphanus sativus L. (Brassicaceae) is a self-incompatible annual weed that commonly grows in open and disturbed areas. In California, USA, seeds germinate in winter, at the beginning of the rainy season (November to December) and plants bloom in March, producing flowers during the next 3–4 months (Strauss *et al.* 2004). Plants from *R. sativus* are fed upon by a large diversity of generalist and specialist herbivores, including aphids, snails, slugs, flea beetles, caterpillars, rabbits and deer (Karban & Nagasaka 2004). Glucosinolates have been described as effective resistance traits for *R. sativus* against generalist herbivores, although such compounds can also attract specialist herbivores (Agrawal & Sherriffs 2001).

Seed collection and growing conditions

Raphanus sativus seeds were collected from seven localities across the Bodega Bay area in California, USA (seeds were kindly provided by R. Karban, University of California-Davis, USA). On 1 February 2005, we planted 40 seeds from each of 22 maternal families (880 seeds) in germination flats within the greenhouse of the Biology Plant Growth Facility at Stanford University. We used a soil mix of 75% peat moss (Orchard Supply Hardware, San Jose, CA, USA), 20% of potting soil and 5% of fine white sand. Because maternal effects in wild radish appear to be exceedingly shortlived (Agrawal 2002), no attempt was made to minimize or eliminate these effects. Variation among the full-sib families thus estimated total genetic variation. Some caution should therefore be exercised in making inferences regarding the heritability of traits herein, which would be derived from half-sib analyses. Seeds germinated on February 4 and after 2 weeks seedlings were transplanted to 0.8-L pots. Each plant received 0.3 g of slow release fertilizer (17 : 9 : 13 N : P : K, Scotts-Sierra Horticultural Products Co., Scotslawn, OH, USA). Plants were maintained at a 26 °C/21 °C, 12 h/12 h cycle and watered once a day. Their location in the greenhouse was randomly rotated every other day to avoid biases due to microclimatic differences in temperature and light within the greenhouse.

Impact of damage and tolerance

To assess ontogenetic differences in vulnerability to leaf area loss, 10 plants from each family were randomly assigned to one of two defoliation treatments with two levels each (0, 1) : damage when juvenile and/or damage at the reproductive stage. Using a 2×2 factorial design, we produced four different groups: control (C), in which no leaf damage was applied; juvenile (J), in which 50% of each leaf area was removed when plants had four fully expanded leaves and started to expand their fifth leaf; reproductive (R), in which 50% of each leaf area was removed when the plants had produced their first five to 10 flowers, and both (J × R), in which plants were defoliated by removing 50% of each leaf area at the juvenile stage and 50% of subsequently produced leaves when plants reached maturity (i.e. every leaf was damaged only once). Thus, this last treatment had both greater frequency and intensity of defoliation. Leaf damage was applied with scissors avoiding the mid-rib, as natural damage occurs in the field (Strauss *et al.* 2004). As we wanted to detect the impact of damage associated exclusively with the production of constitutive resistance, we intentionally did not use herbivore damage to avoid the induction of resistance previously found in *R. sativus* (Agrawal *et al.* 1999, 2002). Leaf tissue from treatments J and R was collected when damage treatments were applied and was immediately flash-frozen in liquid nitrogen for the assessment of glucosinolate concentrations (see below).

Because *R. sativus* is self-incompatible, hand pollination was necessary for the plants to set seeds in the greenhouse. Hand pollination was performed by loading a make-up brush with pollen from multiple donors, and then gently touching every flower of all flowering plants. This procedure was repeated every third day form the onset of flowering and until the plants stopped flower production, to assure that all flowers were pollinated. Fruits were allowed to mature on the plants and were collected when yellow and dry. To assess the impact of defoliation on plant fitness we quantified the number of flowers and fruits produced by plants from each treatment. We estimated the total number of seeds from the mean seed number of 20 randomly chosen fruits per plant multiplied by the total number of fruits. Finally, we calculated seed set (W) for each plant as $W =$ seeds/flowers, and we considered this variable as the expression of one component of plant fitness.

Glucosinolate analysis

To quantify glucosinolate concentration at each ontogenetic stage, leaf material was collected from plants of treatments (J) and (R) from the experiment described above (i.e. each plant was harvested only once, either at the juvenile or at the reproductive stage). A cork borer was used to cut 2.7-cm discs of the third and fifth fully expanded leaves, which were immediately frozen in liquid nitrogen. In the case of late-flowering plants, these leaves were starting to show senescence symptoms, in which case we proceeded to collect the oldest non-senescing leaves. Leaf material was flash-frozen with liquid nitrogen, freeze-dried and stored at −80°C until chemical assays were performed.

Glucosinolates were extracted in methanol, isolated on Sephadex ion-exchange columns (BioRad, Hercules, CA, USA), and analysed using high-performance liquid chromatography (HPLC) (Prestera *et al.* 1996; Brown *et al.* 2003). We used a Shimadzu HPLC system (Shimadzu Corporation, Columbia, MD, USA) with a Lichroshpere (RP-C18, endcapped) 250 × 4 mm analytical column. Glucosinolates were eluted using a water–acetonitrile gradient programme and monitored by UV absorption at 229 nm. Injection volume was 20 μL and flow rate was 1 mL min^{-1}. Chromatograms generated were quantified by integrating peak areas of sample compounds in comparison with an internal standard of allyl glucosinolate (sinigrin). Identification of compounds was achieved using an Agilent 1100 HPLC system (Agilent Company, Santa Clara, CA, USA) equipped with a diode-array UV detector. Retention times and UV absorption spectra (190–360 nm) were compared with standard UV spectra from materials identified previously. Concentration of glucosinolates was expressed as mg g^{-1} of dry mass.

Statistical analyses

To assess the impact of treatments on fitness we used a mixed model with maximum likelihood estimates (PROC MIXED, SAS Institute 1999), considering damage at the juvenile stage (J), at the reproductive stage (R) and their interaction (J × R) as fixed factors, and family and interactions with each treatment as random factors. The model was simplified eliminating the three-factor random term (family × juvenile × reproductive) which was not significant. The response variable W was Box–Cox transformed to meet assumptions of normality and homogeneity of variances.

Tolerance when plants were damaged at the juvenile (T_J) and reproductive (T_R) stages was estimated for each family as the difference in fitness between the damaged group (either J or R) and the control (non-damaged) group (C) ($T_J = W_J - W_C$, $T_R = W_R - W_C$), thus positive values indicate greater tolerance than smaller and negative values (Strauss & Agrawal 1999). Differences in tolerance between ontogenetic stages were assessed using a paired t-test, with family as the unit of replication ($N = 20$; due to mortality and flowering delays, we had to eliminate two families from the analyses). Genetic variation in tolerance was estimated from the mixed-model analysis previously described, based on the significance of the interaction terms. When genetic

variation was found, cost of tolerance was estimated using a regression model (JMP, SAS Institute 2001) to assess the covariance between tolerance expressed by each family and mean fitness of the same family from the undamaged group. Because this covariance was not different from zero (i.e. no cost was detected), we did not correct for the correlation between the estimate of tolerance and fitness in the undamaged group, following the recommendations of Tiffin & Rausher (1999).

To assess ontogenetic differences in the concentrations of the five glucosinolates found, we used multivariate mixed models on arcsin-transformed data (PROC GLM, SAS Institute 1999). The model included developmental stage as a fixed effect, and family and its interaction with stage as random effects. We also included leaf disk weight as a covariate to control for developmental effects associated with leaf mass (Siemens *et al.* 2002). In addition, we used univariate mixed models with maximum likelihood estimates to assess the influence of ontogeny on each individual glucosinolate concentration. To detect costs of resistance family averages of total glucosinolate concentration (the sum of the concentrations of all five glucosinolates found) were regressed against fitness values of the same families in the undamaged group. Significant negative relationships were interpreted as a cost in the expression of these resistance traits in the absence of herbivory. To assess the correlations between tolerance and resistance at each stage we calculated Pearson correlation coefficients (JMP, SAS Institute 2001) between total glucosinolate concentration and tolerance levels of the same families at each ontogenetic stage.

To describe the ontogenetic trajectories in resistance and tolerance and their degree of correlation, we calculated the mean genotypic ontogenetic change (Δ) in total glucosinolate concentration (G) as $\Delta G =$ (Family average G_J) − (Family average G_R), and the degree of ontogenetic change in tolerance (T) as $\Delta T =$ (Family average T_J) − (Family average T_R). We then used a regression analysis to describe the joint pattern of ontogenetic variation in tolerance and resistance (JMP, SAS Institute 2001).

RESULTS

Clipping reduced seed set when plants were damaged at the juvenile stage ($F_{1,610} = 14.01$, $P = 0.0002$) but not when they were damaged at the reproductive stage ($F_{1,610} = 0.67$, $P = 0.41$). The interaction term was not significant ($F_{1,610} = 2.08$, $P = 0.14$, Fig. 2). We found seed set to be variable across families ($\chi^2 = 77.5$, $P < 0.0001$) and a marginally significant evidence of genetic variation of tolerance at the reproductive stage (family × reproductive: $\chi^2 = 2.1$, $P = 0.07$).

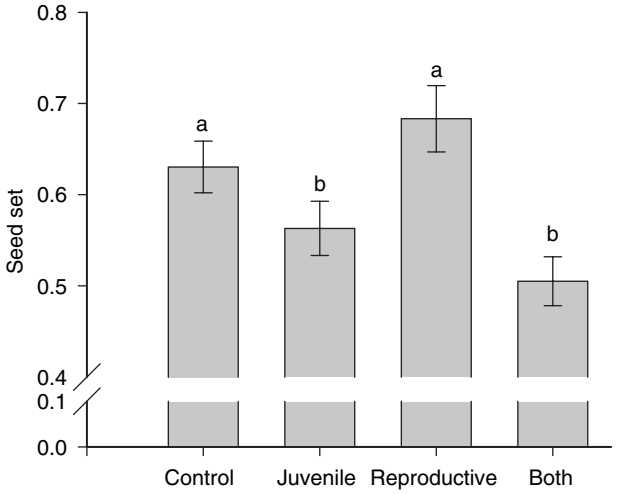

Figure 2 Vulnerability of plants to defoliation when damaged at the juvenile or reproductive stage, and consecutively during both stages. Different letters represent significant differences among treatments ($P < 0.05$).

Figure 3 Ontogenetic changes in: (a) plant tolerance to defoliation; (b) resistance. Asterisks represent significant differences between ontogenetic stages ($P < 0.05$).

Table 1 Variance components (VC) with significance tests for the effects of ontogeny and family on the concentration of five glucosinolates in *Raphanus sativus*

	A		B		C		D		E	
Source	VC	F or χ^2	VC	F or χ^2	VC	F or χ^2	VC	F or χ^2	VC	F or χ^2
Stage	–	4.51*	–	4.58*	–	40.56****	–	0.01	–	0.04
Family	0.000	0.9	0.000	2.7*	0.001	6.8**	0.000	2.2	0	0
F × S	0.000	1.3	0.000	0.1	0.000	0.9	0.000	0	0.000	0.4
Disk weight	–	3.39**	–	3.10****	–	8.22****	–	2.48**	–	6.11****
Residuals	0.001	3.39**	0.021	3.10****	0.004	8.22****	0.000	2.48**	0.001	6.11****

(A) 4-hydroxy-3-indolylmethyl, (B) 4-methylthiobutyl, (C) 4-methylthio-3-butenyl, (D) indolyl -3-methyl, (E) unknown.
*$P \leq 0.05$, **$P \leq 0.01$, ****$P \leq 0.0001$. Except for the family-by-stage interaction, MANOVA tests were significant; therefore, these P-values are protected from Type I errors.

Significant ontogenetic changes were found for both tolerance and resistance. Plants at the reproductive stage were more tolerant than juvenile plants ($t_{19} = -3.07$, $P = 0.006$, Fig. 3a). In contrast, we found that overall concentration of glucosinolates was 26% greater in the juvenile stage than when plants reached maturity ($F_{5,198} = 12.37$, $P < 0.0001$, Fig. 3b). Univariate analyses revealed that the compounds 4-hydroxy-3-indolylmethyl, 4-methylthiobutyl and 4-methylthio-3-butenyl exhibited this ontogenetic variation (Table 1). Although there were significant main effects of family on glucosinolate concentration ($F_{100,970} = 2.16$, $P < 0.0001$), there were no significant effects of the interaction between family and developmental stage ($F_{100,970} = 1.17$, $P = 0.14$), indicating that the ontogenetic trajectories in plant resistance did not differ among families. Nevertheless, *post hoc* contrast analyses performed within each stage demonstrated genetic variation in the expression of glucosinolates both at the juvenile ($F_{100,585} = 2.26$, $P < 0.0001$) and at the reproductive stage ($F_{100,341} = 1.29$, $P = 0.05$).

The cost of glucosinolates was found to change throughout ontogeny. Whereas for the juvenile stage lifetime fitness in the undamaged control group was unrelated to mean concentration of glucosinolates ($F_{19} = 0.01$, $P = 0.93$, Fig. 4a), we found a strong negative relationship between these variables at the reproductive stage ($R^2 = 0.36$, $F_{19} = 9.66$, $P = 0.006$), suggesting that resistance becomes costly as plants mature (Fig. 4b). In contrast, we found that the expression of tolerance at the reproductive stage had no costs in terms of fitness expressed in undamaged plants of the same family ($R^2 = 0.04$, $F_{19} = 0.75$, $P = 0.39$, Fig. 4c). Costs of tolerance were not calculated for the juvenile stage, as we found no genetic variation for the impacts of damage when applied at this stage. For this same reason, we estimated the correlation between tolerance and resistance only for the reproductive stage, which we found to be not significant ($r = -0.3$, $F_{19} = 1.8$, $P = 0.19$).

Finally, using our model we found a significant negative correlation between the degree of ontogenetic change in tolerance and the degree of ontogenetic change in resistance (i.e. ontogenetic tradeoff) ($r = 0.22$, $F_{19} = 5.28$, $P = 0.03$, Fig. 5). However, a bias towards one strategy was evident: most of the families showed greater resistance when juvenile and greater tolerance when reproductive, whereas only two families were found to be more tolerant when juvenile and more resistant when reproductive (Fig. 5).

DISCUSSION

There is a current call to integrate development and evolution into a coherent theory to understand how natural selection acts on integrated phenotypes (Pigliucci & Schlichting 1995; Wright & McConnaughay 2002). Here, we have incorporated plant development into the context of the evolutionary ecology of plant–herbivore interactions, considering the different environments the plants face as they develop and the changes in costs and benefits in the strategies expressed to deal with herbivory. The most relevant finding of our study is that *R. sativus* can simultaneously express ontogenetic trajectories of resistance and tolerance with an apparent tradeoff. We suggest that the ontogenetic trajectories in opposite directions may have ultimately evolved in response to ontogenetic changes found in the costs and benefits of resistance and tolerance.

Ontogenetic trajectories in resistance and tolerance

As previous studies have described only the independent ontogenetic change of both resistance and tolerance (reviewed by Boege & Marquis 2005), this is the first study to report the simultaneous ontogenetic change in both strategies in an apparent switch: for most of the families, the concentration of glucosinolates decreased as plants matured, while tolerance to defoliation increased. A decrease in plant resistance during ontogeny has been described for other

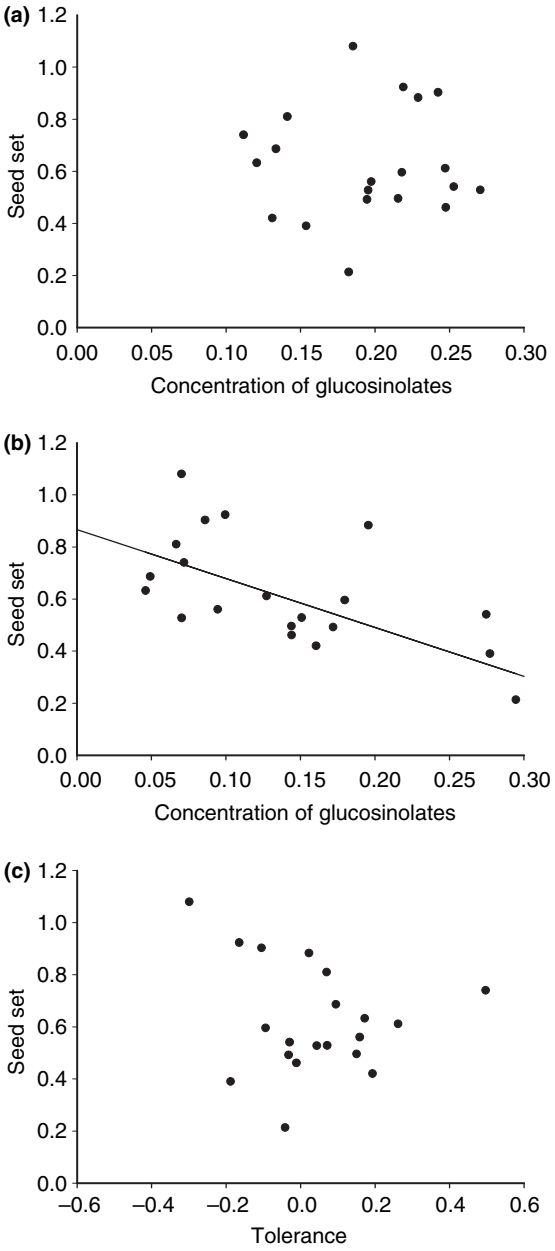

Figure 4 Costs of resistance at the juvenile stage (a) and at the reproductive stage (b) and cost of tolerance at the reproductive stage (c) of *Raphanus sativus*.

herbaceous species, such as *Nicotiana sylvestris* (Baldwin & Schmelz 1996), *Nicotiana attenuata* (Van Dam *et al.* 2001) and *Lycopersicon esculentum* (Wolfson & Murdock 1990). In the case of annual plants, these ontogenetic changes have been associated with the onset of reproduction, when most resources should be allocated to the production of offspring and not to functions that are not directly involved in fitness (e.g. resistance). In addition, resistance should have greater benefits at juvenile stages, when herbivory can greatly

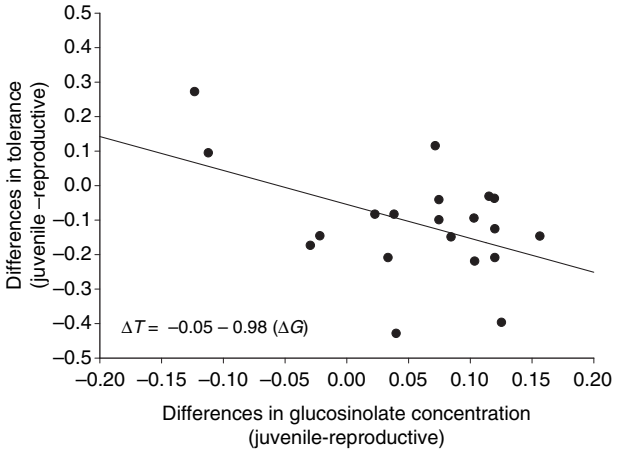

Figure 5 Ontogenetic tradeoff between resistance and tolerance in *Raphanus sativus*. Positive values along the axes represent greater levels of resistance and/or tolerance at the juvenile stage relative to the reproductive stages, whereas negative values are interpreted as greater tolerance and/or resistance at the reproductive than at the juvenile stage. Note that the ordinate axis (ΔT) is shifted to the −0.2 value of the (ΔG) axis.

reduce the performance of young seedlings (Moles & Westoby 2004), and increase their mortality (Shaw *et al.* 2002). Indeed, we found that the juvenile stage of *R. sativus* was more vulnerable to damage than the reproductive stage, which provides a proximate explanation for why the juvenile stage would benefit more by increased levels of resistance. Similarly, an increase in tolerance to damage with plant development has also been found in some species (Brandt & Lamb 1994; Warner & Cushman 2002), although not always in a linear fashion (del-Val & Crawley 2005). These changes have been associated with the release of resource limitation and availability of meristems to produce compensatory responses and with increased benefits of tolerance as plants develop (Haukioja & Koricheva 2000).

Ontogenetic changes in the costs of resistance and tolerance

The simultaneous decrease in resistance and increase in tolerance as *R. sativus* plants mature is consistent with the fitness cost found for the production of glucosinolates and the lack of costs of tolerance at the reproductive stage. The difference in costs of both strategies at this stage could be explained by the life history of *R. sativus*. As an annual species, *R. sativus* plants should allocate most of their resources to reproduction when reaching the end of their life cycle. Thus, investment in resistance to protect low-value leaves that will soon senesce should be more costly than tolerating damage. Fitness costs of resistance have been detected previously in several systems (Fineblum & Rausher

1995; Mauricio 1998; Agrawal *et al.* 1999) although not in others (Simms & Rausher 1987; Ågren & Schemske 1993). Similarly, the assessment of the fitness cost of tolerance has provided both positive (Tiffin & Rausher 1999; Pilson 2000; Stinchcombe 2002; Fornoni *et al.* 2004) and negative (Mauricio *et al.* 1997; Agrawal *et al.* 1999) evidence. This apparent inconsistency has been attributed to the presence of genetic correlations between tolerance and resistance (Mauricio *et al.* 1997; Tiffin & Rausher 1999), environmental variation (Bergelson 1994), multiple functions of secondary metabolites (Siemens *et al.* 2002, 2003;) and with allocation or ecological costs of both strategies (Pilson 2000; Strauss *et al.* 2002). We suggest that discrepancies among studies on the cost of resistance and tolerance also are likely to be caused by plant development, when such costs become evident only at some ontogenetic stages (see Briggs & Schultz 1990).

Ontogenetic differences in the costs of resistance could be caused by changes in the priority of plant functions (i.e. resistance, growth and storage to reproduction) (Weiner 2004), or in the patterns of resource allocation to shoots and roots (Bryant *et al.* 1991; Boege & Marquis 2005). We suggest that such changes could be also due to the highly seasonal climate where *R. sativus* grows in California (Karban & Nagasaka 2004), where resources such as water and nutrients become temporarily restricted when *R. sativus* plants start to bloom (Hooper & Vitousek 1998). Thus, as plants grow and mature, resources are depleted and plant stress is likely to increase with ontogeny. It is well known that plant stress may affect the expression (Price 1991) and the cost (Bergelson 1994) of resistance. Although we conducted the present study in a greenhouse, such temporal depletion of resources is likely to have occurred as well in our system given that we fertilized the plants only once, at the beginning of the experiment, and we stopped watering plants when they finished flowering. This warrants consideration to explain the decrease in resistance and the increase in the cost of resistance as plants matured, because the effects of ontogeny are confounded with those of plant stress.

The lack of genetic variation in tolerance when damage was applied at the juvenile stage, could have to do with an insufficient number of maternal families to detect such variation, but also could be explained by the low levels of tolerance at the juvenile stage, which probably all families were able to reach (i.e. thus no genetic variation is expressed). Furthermore, this study was aimed at assessing ontogenetic tradeoffs between tolerance and constitutive resistance, thus controlling for the possible effects of inducible resistance traits, which have additional costs and impact on plant fitness (Agrawal *et al.* 1999). Previous studies have demonstrated both genetic variation in tolerance when induced resistance is expressed (Agrawal *et al.* 1999), and a negative correlation between tolerance and induced glucosinolates (Strauss *et al.* 2003). Thus, the results of this study should consider this caveat. Further research, including more genetic families, should investigate the role of induced resistance in ontogenetic tradeoffs.

Ontogenetic tradeoff in the expression of tolerance and resistance

The negative correlation between mean genotypic differences in tolerance and resistance suggests the presence of ontogenetic tradeoffs between these strategies: the genotypes more resistant when juveniles were the most tolerant when reproductive, and those genotypes most tolerant when juvenile were the most resistant when reproductive. Ontogenetic tradeoffs between resistance and tolerance in annual plants could evolve under at least three different scenarios. First, if differences exist in herbivore species attacking the plant (e.g. generalists vs. specialists) throughout ontogeny (i.e. throughout the season) plants could be effectively resistant against one type (e.g. generalists attacking one stage) but not against the other (e.g. specialists attacking the other stage). Therefore, tolerance would be a better strategy to deal with herbivory at the stage that is not effectively resistant. However, variation in seasonality of attack by specialist and generalist herbivores could maintain genetic variation in ontogenetic trajectories of both strategies. Second, tradeoffs could occur when ontogenetic changes in resource acquisition and availability, together with changes in storage and regrowth organs (meristems) determine the costs and benefits of one strategy over the other at a given ontogenetic stage. For example, reduced photosynthetic tissue and meristem availability at young stages could constrain the ability of plants to tolerate damage compared to older stages. Variation in the availability of resources throughout the season could maintain the genetic variation of such benefits during plant development. Finally, a third scenario invokes a genetic constraint: when damage is constant throughout ontogeny, but the expression of either strategy at one stage has pleiotropic effects on the expression of the other strategy at a different ontogenetic stage.

In the case of *R. sativus*, we found that all but two families followed the strategy to resist more at the juvenile stage and to express greater tolerance at the reproductive stage. The more common strategy we observed is unlikely to be promoted by the first proposed scenario, given that both generalists (e.g. snails, slugs, aphids, rabbits, deer and noctuid caterpillars) and specialists of the Brassicaceae family (pierid caterpillars) attack *R. sativus* plants early and late in the season (Agrawal & Sherriffs 2001; Karban & Nagasaka 2004). However, because herbivore identities and damage can differ from one population to the other

(Karban & Nagasaka 2004), a more detailed study of herbivore seasonality would be required to discard this possibility. The second scenario seems to better explain the switch in strategies that we found if one considers the patterns of resource acquisition, storage and allocation characteristic of annual weeds. When young, and after depletion of resources stored in cotyledons, plants may not have enough resources and available meristems to produce compensatory responses after herbivore damage (del Val & Crawley 2005). Thus, the protection of the few tissues they have is likely to be less costly than the replacement of tissue lost to herbivores. In contrast, once the plants start to reproduce they have enough stored resources to allocate as much as they can to reproduction (Bloom *et al.* 1985). Thus, at this stage plants would be able to express a great degree of tolerance for damage on their less-valued leaves and benefit less from resistance. Finally, although pleiotropic effects between resistance and tolerance have been previously suggested in other species (Roy & Kirchner 2000), we currently have no information whether such a genetic correlation occurs in *R. sativus*.

The model we have proposed detected an ontogenetic tradeoff in the expression of resistance and tolerance, which we suggest is related to changes in costs of resistance and tolerance and vulnerability to damage throughout ontogeny. However, this model is certainly a simplified version of the trends that the simultaneous expression of tolerance and resistance can follow throughout plant ontogeny, which can be more complex if factors such as resource availability, plant vigour, and the simultaneous expression of different resistance traits are considered. In particular, predictions of the model should differ for annual and perennial plants, given the differences in their apparency and probability of damage, the diversity of herbivore species attacking them, storage abilities and on the impacts of damage at the mature stage as a function of: (i) their reproductive strategy (i.e. iteroparous or semelparous); and (ii) the proportion of their biomass allocated to tissues attacked by herbivores (Haukioja & Koricheva 2000). A future challenge is to incorporate more of these variables in theoretical and experimental studies to better predict and understand the simultaneous expression of resistance and tolerance throughout ontogeny. Nevertheless, our simplified model underscores the fact that an understanding of the evolutionary ecology of plant–herbivore interactions must incorporate the dynamics that define organisms as integrated phenotypes throughout development. We posit that in the current resistance–tolerance debate, an ontogenetic perspective could help to clarify inconsistent patterns in the relationship between these strategies (Leimu & Koricheva 2006). This perspective requires an understanding of the natural history of plant development and attack, ontogenetic switches of phenotypes, and quantitative genetic studies of costs and benefits of alternate strategies at different life stages. This approach would be relevant not only in the context of plant–herbivore interactions but also in the more general situation where, throughout development, organisms possess alternative strategies and inevitably deal with contrasting environmental challenges.

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