

# Introduced *Brassica nigra* populations exhibit greater growth and herbivore resistance but less tolerance than native populations in the native range

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## Summary

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- Rapid post-introduction evolution has been found in many invasive plant species, and includes changes in defence (resistance and tolerance) and competitive ability traits. Here, we explored the post-introduction evolution of a trade-off between resistance to and tolerance of herbivory, which has received little attention.
- In a common garden experiment in a native range, nine invasive and 16 native populations of *Brassica nigra* were compared for growth and defence traits.
- Invasive populations had higher resistance to, but lower tolerance of, herbivore damage than native populations. Invasive populations survived better and produced more seeds than native ones when released from herbivores; but fitness was equivalent between the regions under ambient herbivory. The invasive populations grew taller, and produced more biomass and lighter seeds than natives, irrespective of insecticide treatment.
- In addition to supporting the idea of post-introduction rapid evolution of plant traits, our results also contribute to an emerging pattern of both increasing resistance and growth in invasive populations, contrary to the predictions of earlier theories of resistance–growth trade-offs.

## Introduction

Rapid, post-introduction evolution has been found in many invasive plant species, and includes changes in the following traits: defence, stature, biomass, reproductive output, competitive, and dispersal abilities (e.g. Blossey & Nötzold, 1995; Buckley *et al.*, 2003; Leger & Rice, 2003; Bossdorf *et al.*, 2004a; Maron *et al.*, 2004; Wolfe *et al.*, 2004; Meyer *et al.*, 2005; Müller & Martens, 2005; Stastny *et al.*, 2005; see review by Whitney & Gabler, 2008; Williams *et al.*, 2008; Zou *et al.*, 2008; Caño *et al.*, 2009; Cripps *et al.*, 2009; Moloney *et al.*, 2009; Monty & Mahy, 2010; Rapo *et al.*, 2010). Three theories are considered as fundamental in explaining plant invasion success (Doorduyn & Vrieling, 2011): the enemy release hypothesis (ERH) (Keane & Crawley, 2002), the evolution of increased competitive ability (EICA) (Blossey & Nötzold, 1995), and the shifting defence hypothesis (SDH) (Müller-Schärer *et al.*, 2004; Joshi & Vrieling, 2005).

According to the ERH, plants that are introduced to new ranges leave behind their specialized herbivores, and are therefore released from harmful herbivore pressure exerted by those specialist herbivores. The EICA hypothesis, for its part, predicts that release from specialist herbivore pressures will cause plant defences against specialists to decline in exotic ranges over evolutionary time (Blossey & Nötzold, 1995). The EICA hypothesis assumes that secondary metabolites defend plants against specialist herbivores. Genetically based intraspecific variations in concentrations of secondary metabolites that are thought to defend plants against herbivory have been shown (Müller & Martens, 2005; Stastny *et al.*, 2005; Arany *et al.*, 2009; Caño *et al.*, 2009; Poelman *et al.*, 2009). As a consequence of an absence of specialist herbivores in the invasive ranges, selection may favour plant genotypes that have low concentrations of defence compounds because these compounds are thought to be costly to produce. It is thought that plants that reduce their resource investment in defence

compounds, and afterwards reallocate the freed resources to growth and reproduction, will have a competitive edge over local plants in the introduced ranges. EICA, therefore, predicts a post-introduction evolutionary change such that invasive populations of a given plant species have lower concentrations of chemical defence compounds than their native conspecifics in the native ranges (Blossey & Nötzold, 1995).

The SDH is an extension of the EICA hypothesis (Doorduyn & Vrieling, 2011). The SDH classifies defence traits into two categories: quantitative and qualitative, depending on whether they are effective against specialist or generalist herbivores, respectively (Müller-Schärer *et al.*, 2004; Joshi & Vrieling, 2005). Quantitative defence traits are based on digestibility reducers (e.g. cellulose, tannins and trichomes) that usually occur in high concentrations and act in a dosage-dependent manner. In contrast, qualitative defence traits include secondary plant metabolites, such as glucosinolates and alkaloids, which usually occur at low concentrations and are toxic to many generalist herbivores (Doorduyn & Vrieling, 2011). Adapted specialized herbivores might use these chemicals to locate their host plants for feeding, for egg deposition, and to sequester them for their own defence (Agrawal & Kurashige, 2003; Müller-Schärer *et al.*, 2004).

Rather than escaping completely from herbivory, invasive plants often only undergo a change in the composition of the herbivore assemblages with which they interact (Müller-Schärer *et al.*, 2004; Joshi & Vrieling, 2005; Liu & Stiling, 2006). In the native ranges, the invasive plants are attacked by both generalist and specialist herbivores, while in the invaded ranges, the plants are much more likely to interact with generalists than with specialists (Müller-Schärer *et al.*, 2004). Therefore, according to the SDH, herbivore pressure on introduced plants is expected to come mainly from generalist herbivores. This may in turn select for plant genotypes that have high concentrations of qualitative defence compounds and low concentrations of quantitative defence compounds (Müller-Schärer *et al.*, 2004; Joshi & Vrieling, 2005).

Plant defence against herbivory assumes two forms: resistance (plant traits that minimize damage from herbivores, e.g. defence compounds) and tolerance (traits that allow a plant to maintain fitness after damage has occurred) (Strauss & Agrawal, 1999; Stowe *et al.*, 2000). Resistance and tolerance are alternative, but not mutually exclusive defence traits; an individual plant may have low tolerance but high resistance or vice versa (Rosenthal & Kotanen, 1994; Mauricio *et al.*, 1997; Fineblum & Rausher, 2002; Weinig *et al.*, 2003; Leimu & Koricheva, 2006). This trade-off between resistance and tolerance is thought to occur because of a limitation on the amount of resources available for allocation to defence (Leimu & Koricheva, 2006). Alternatively or additionally, generalist and specialist

herbivores may impose differential selection pressures on plant defence strategies, resulting in the maintenance of variation in both tolerance and resistance within a population (Stowe, 1998; Tiffin, 2000; Muola *et al.*, 2010). For example, tolerance traits are likely to decrease detrimental effects of specialists adapted to the chemical defences of their host plants (Bowers & Puttick, 1988; Jokela *et al.*, 2000). Resistance traits such as toxic chemical compounds function, in turn, especially against generalist herbivores. Therefore, the higher species diversity of both generalist and specialist herbivores in the native ranges vs invasive ranges might select for plant genotypes that have intermediate levels of both resistance and tolerance (Weinig *et al.*, 2003), while the herbivore community in the invasive ranges dominated by generalists might select for plant genotypes that have high levels of resistance and low tolerance.

We know of only a few studies that have investigated a trade-off between herbivory resistance and tolerance in the context of biological invasions. Such studies have produced mixed results, with some plants showing a trade-off and others not (Bossdorf *et al.*, 2004b; Joshi & Vrieling, 2005; Stastny *et al.*, 2005; Zou *et al.*, 2008). Therefore, more studies of post-introduction evolutionary changes in invaders that take into account both resistance to and tolerance of herbivory are needed.

We conducted a common garden experiment in the native range of *Brassica nigra* (Cadiz, southwestern Spain) to compare survival, a trade-off between resistance and tolerance, vegetative growth, and reproductive output between invasive and native populations. On the basis of the SDH and theory concerning a trade-off between resistance and tolerance, we made the following predictions. Because they come from ranges dominated by generalist herbivores, invasive populations will have greater investment in qualitative defence and lower investment in quantitative defence than native populations. Invasive populations will have higher resistance to, and lower tolerance of, herbivore damage than native populations. Invasive populations will exhibit lower survival, growth (total plant biomass), and reproductive output than native populations when exposed to damage from herbivores in the native range (or higher survival, growth, and reproductive output when protected from the herbivores).

## Materials and Methods

### Study system

*Brassica nigra* (L.) W. D. J. Koch is an annual herb native to the Mediterranean region, and other parts of North Africa and Europe that has spread widely across the globe (Bell & Muller, 1973; Feeny & Rosenberry, 1982; Westman & Kresovich, 1999). Seeds of *B. nigra* have long been used in southern Europe, Asia and North Africa for cooking oil,

condiment mustard and medicine (Westman & Kresovich, 1999). In temperate regions, *B. nigra* was a major mustard crop until the 1950s when it was replaced by its close relative, *Brassica juncea*, in commercial production. Presently, *B. nigra* is a widespread weed (Westman & Kresovich, 1999). In North America, *B. nigra* weed populations may have arisen from multiple sources: crop seed, commercial mustard from Europe and India, or weed seed introduced with European colonists c. 200 yr ago (Westman & Kresovich, 1999). In North America, *B. nigra* can form thick monospecific stands, although generally in disturbed areas (Lankau & Strauss, 2008). *Brassica nigra* defends itself from herbivores by synthesizing glucosinolates, a class of secondary compounds derived from several amino acids (Feeny & Rosenberry, 1982). In *B. nigra*, sinigrin (allyl-glucosinolate) represents 90–99% of the total glucosinolate concentration and has a heritable basis (Feeny & Rosenberry, 1982; Traw, 2002). Upon coming into contact with an enzyme known as myrosinase, glucosinolates break down into various toxic by-products involved in resistance to herbivores and pathogens (e.g. Agrawal & Kurashige, 2003; Kliebenstein, 2004) and competition against other plants (Bell & Muller, 1973; Lankau & Strauss, 2008; Müller, 2009). In addition to the glucosinolate-based resistance, *B. nigra* employs nonglandular trichomes to resist herbivore damage (Traw & Dawson, 2002).

### Plant material

Seeds of nine invasive (North American) and 16 native (Mediterranean region, parts of Africa, Europe, and Asia) populations (Westman & Kresovich, 1999) of *B. nigra* were obtained from the United States Department of Agriculture (USDA) germplasm resources information network (GRIN). USDA GRIN germplasm collection and botanical gardens, or were field-collected by the authors or their collaborators (see Supporting Information Table S1). Seeds obtained from germplasm collections or botanical gardens had been collected directly from wild populations in the field and were then grown for only one generation in common glasshouses to remove maternal effects. Plants raised from seeds collected from distinct populations were grown in controlled pollination environments inside screened cages with added pollinators in order to preserve the genetic integrity of the populations.

### Experimental design

Twenty seedlings (2 wk old) of each of the invasive and native populations of *B. nigra* were transplanted to a plot at Pedralera La Loca, Cadiz, southwestern Spain (36°31'N, 6°11'W) on 30 and 31 December 2007. Pedralera La Loca is part of a large agro-ecosystem where *B. nigra* grows naturally. However, the presence of *B. nigra* has not been

reported for the last 20 yr at the site where we carried out the experiment. In wild populations of *B. nigra* closest to the experimental site, seeds usually germinate in the months of December and January, plants start flowering in March/April and fruits reach physiological maturity in June/July. The experimental site we used was one where plant species other than *B. nigra* had been growing naturally. The site was cleared and planting holes dug before transplanting seedlings. A randomized complete block design was employed. The plot was divided into two blocks, with each block having five rows of equal length. Each row was divided into two equal parts. Each row was planted with two seedlings from each of the 25 populations, making sure that each of the two parts of every row had all the 25 populations randomly arranged. The seedlings were planted 30 cm apart within a row, and the rows were spaced 75 cm apart. The transplanted seedlings were watered once a week until they established, after which the watering frequency was scaled up to twice a week as temperatures rose through the growing season to reach 40°C. Watering throughout the season was necessary for survival of the experimental plants because in southern Spain where the plants were grown, spring and summer seasons are characterized by higher temperatures and lower soil moisture than other parts of Europe, Asia, and North America from which we had obtained seeds for the experiment.

Half of the plants were sprayed weekly with an insecticide to compare survival, growth, reproductive output, and resistance to herbivory between the invasive and native populations of *B. nigra* under very low herbivory and under natural levels of herbivory. The insecticide was applied alternately per row. That is, if the first part of row number one was sprayed, then the first part of row number two was jumped, spraying only the second part of that row. This zig-zag pattern of insecticide application ensured that all the five rows in each of the two blocks received both insecticide treatments (insecticide applied or not). A sprayer with a nozzle that produces a narrow swath was used to ensure that the insecticide could easily be directed at plant leaves only, thereby avoiding interference with pollinators that visited flowers of the same plants. The insecticide used was CHAS® 48 EC (Cheminova, Madrid, Spain) at the rate of 0.5 ml per litre of water. The plots were weeded regularly to remove any other unwanted plants.

### Measurements of resistance to herbivory, growth and reproductive output

We measured actual resistance to herbivory as the inverse of damage by herbivores (i.e. 100 minus per cent leaf damage or seed predation) (Leimu & Koricheva, 2006). A low percentage of leaf damage or seed predation indicates a high level of resistance. Leaf concentrations of sinigrin and trichome density, which are putative defence traits, were also

used as indicators of resistance to herbivory; high values of these traits indicate high investments in resistance. Per cent leaf damage on 5-wk-old plants (i.e. 3 wk after transplanting) was estimated by visually assessing the proportion of the total leaf area damaged on the plant. Plants had *c.* 6 leaves at the time of scoring leaf damage. Trichome count was carried out *in situ* by observing the upper surface of intact leaves (whose lengths and widths had been measured) using a magnifying glass, and counting all the trichomes that fell under the area covered by the glass. The number of trichomes resulting from the count was then multiplied by the leaf area to obtain the trichome density per unit of upper leaf surface area. Leaf area was estimated by multiplying leaf length and width measured earlier. Leaf tissue for sinigrin analysis was obtained from 4-month-old plants by punching four leaf discs from the youngest fully expanded leaf in a rosette with a paper hole punch (5 mm in diameter). The leaf discs were placed immediately in 95% methanol in 1.2-ml Eppendorf tubes, which were stored at 4°C until the sinigrin analysis was performed. Seed predation was determined by randomly picking 10 dry fruits per plant (after the plants had been harvested from the field), and observing them under a dissection microscope to see how many fruits had holes made by insect seed predators.

Above- and below-ground portions of plants were harvested as individual plants matured (from the fifth month following the date of transplanting). Growth and fitness were then estimated as follows: the proportion of plants that survived to reproduce was obtained by dividing up the number of plants with fruits by the number of seedlings transplanted for each population; the maximum height of the plants was obtained by using a tape measure to take the length from root collar to the tip of the tallest tiller, and the fruit yield per plant (determined by dividing plants down the middle, and then counting all fruits that occurred in the entire one half of the plant; the resulting fruit count was then multiplied by 2 to obtain the total fruit yield per plant). The average seed yield per fruit was determined by counting the number of individual seeds per fruit for two fruits and obtaining the mean. Multiplying the average number of seeds per fruit by the total number of fruits per plant allowed us to determine the total seed yield per plant. The mean biomass of individual seeds was determined by averaging seed biomass from six randomly picked fruits from each plant. This was then multiplied by the total number of seeds per plant to obtain the seed biomass per plant. The total plant biomass was obtained by oven-drying the shoots and roots of each plant at 65°C for 12 h and then weighing them together.

#### Analysis for leaf sinigrin concentrations

Sinigrin identity and concentration were determined by high-performance liquid chromatography. A ball-bearing

was added to each 1.2-ml Eppendorf tube, and tubes were shaken for 1 min in a Qiagen FastPrep-24 tissue homogenizer. After shaking, the tubes sat for 1 h, and then were centrifuged and 300 µl of the supernatant was passed through a DEAE-Sephadex column (Pharmacia Biotech, Uppsala, Sweden), using 96-well microtitre plates. Columns were washed twice with 70% methanol and twice with water. Desulphoglucosinolates were extracted from the column by adding 100 µl of a 5% sulfatase enzyme solution and incubated overnight. The resulting solution was transferred to a new 96-well plate and stored at 4°C until analysis on a high-performance liquid chromatograph equipped with an auto-sampler and a diode array detector. Sinigrin was identified by comparison of retention times and absorbance spectra with a standard, and peak areas were converted to concentrations using published response factors (Kliebenstein *et al.*, 2001).

#### Statistical analyses

Permutational multivariate analysis of variance was performed to compare the whole set of traits between invasive and native populations of *B. nigra*. For this, we used the ADONIS function in library VEGAN in R (R Development Core Team 2009). We then compared population means between invasive and native populations by means of linear mixed-effects models (LMMs) or generalized linear mixed-effects models (GLMMs), including, as main-effect variables, the invasive status of *B. nigra* (invasive or native), insecticide treatment (insecticide applied or not), and their interaction. Population was treated as a random-effect variable and nested within invasive status. Block was also treated as a random-effect variable. The following continuous data were analysed using LMMs with restricted maximum likelihood (REML) approximation: concentration of leaf sinigrin, seed biomass, plant height, plant biomass, and actual resistance (i.e. 100 minus the percentage of leaf damage or 100 minus the percentage of fruits predated upon). Percentage data were arc-sin square-root-transformed. Count data (number of seeds, trichomes, and plants that survived) were fitted to a poisson with link = log using the Laplace approximation method (GLMM).

Tolerance of herbivory was estimated by regressing log<sub>10</sub>-transformed lifetime seed yield (i.e. seed biomass) against arc-sin square-root-transformed per cent leaf damage for each of the 25 populations. Lifetime seed yield was used as a fitness measure, because *B. nigra* is an annual plant. Tolerance is defined as the slope of the resulting regression (Strauss & Agrawal, 1999). The slopes of the regression are interpreted as follows: a zero slope means that the plant is tolerant of damage as its reproductive output remains unaffected by herbivory (i.e. the plant achieves full compensation for damage). A negative slope means that a plant is not tolerant (i.e. the plant undercompensates for



damage), while a positive slope means that the plant benefits from herbivory because it produces more seeds when damaged than when undamaged (i.e. the plant overcompensates for damage) (Strauss & Agrawal, 1999). Because leaf damage occurred under both insecticide treatments, mean percent leaf damage per population was computed across both insecticide treatments. Seed biomass was log<sub>10</sub>-transformed to avoid a problem of regressing additive measures (in our case seed biomass) on multiplicative measure (in our case percent leaf damage) (see Wise & Carr, 2008).

A nested analysis of variance (ANOVA) was used to compare tolerance between invasive and native populations in which we included, as a dependent variable, the 25 slopes that resulted from the regressions cited above. Invasive status was specified as a fixed-effect independent variable. Population was treated as a random-effect independent variable and nested within invasive status. Block was also treated as a random-effect independent variable. Additionally, the slopes were regressed against the measured actual resistance (i.e. 100 minus the percentage of leaf damage) to herbivores. This enabled us to directly test the trade-off between tolerance and resistance. Leaf sinigrin concentration and trichome density were also regressed against the percentage of leaf damage to test for correlations between the putative resistance traits and the percentage of leaf damage experienced.

In all the analyses above, populations were treated as the experimental units. Thus scores for individual plants for the various traits listed above were averaged for each population according to the treatments. R version 2.9.1 was employed in the GLMM analyses (using the lme4 library) while the linear regressions and LMMs were performed using JMP 7.0 (SAS Institute Inc, 2007).

## Results

Invasive status had a significant effect on plant traits when all traits were analysed together (Table 1). When each trait was analysed independently, invasive status had a significant effect on leaf sinigrin concentration, trichome density, plant height, plant biomass, and the biomass of individual seeds (Table 2). Invasive populations were composed of plants with higher leaf sinigrin concentration, leaf trichome density and biomass, greater height, and lighter seeds than

**Table 1** Permutational multivariate analysis of variance (ADONIS) comparing the whole set of plant traits between invasive and native populations of *Brassica nigra*

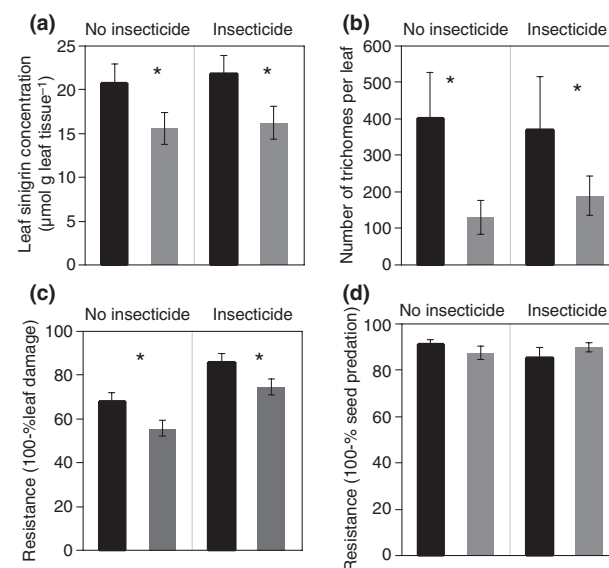
Source	df	F	P
Invasive status	1	4.635	0.009
Insecticide	1	0.853	0.386
Invasive status × insecticide	1	0.531	0.643

**Table 2** Linear and generalized linear mixed-effect models (LMMs and GLMMs) testing effects of invasive status, insecticide and their interactions on resistance and growth performance traits of invasive and native populations of *Brassica nigra*

Traits	Invasive status	Insecticide	Invasive status × insecticide
Sinigrin	$F = 23.55^{***}$	$F = 0.21$	$F = 0.018$
Actual resistance (100 – % leaf damage)	$F = 11.12^{**}$	$F = 23.82^{***}$	$F = 0.026$
Trichome	$ z  = 2.78^{**}$	$ z  = 0.104$	$ z  = 0.24$
Seed predation	$F = 0.0026$	$F = 0.4443$	$F = 2.49$
Seed number/plant	$ z  = 1.18$	$ z  = 56.36^{***}$	$ z  = 2.79^{**}$
Biomass of individual seed	$F = 15.53^{***}$	$F = 1.86$	$F = 1.59$
Height	$F = 48.56^{***}$	$F = 2.29$	$F = 3.82$
Plant biomass	$F = 23.5^{***}$	$F = 0.36$	$F = 0.65$
Survival	$ z  = 1.15$	$ z  = 2.18^*$	$ z  = 1.99^*$
df	1, 23	1, 72	1, 72

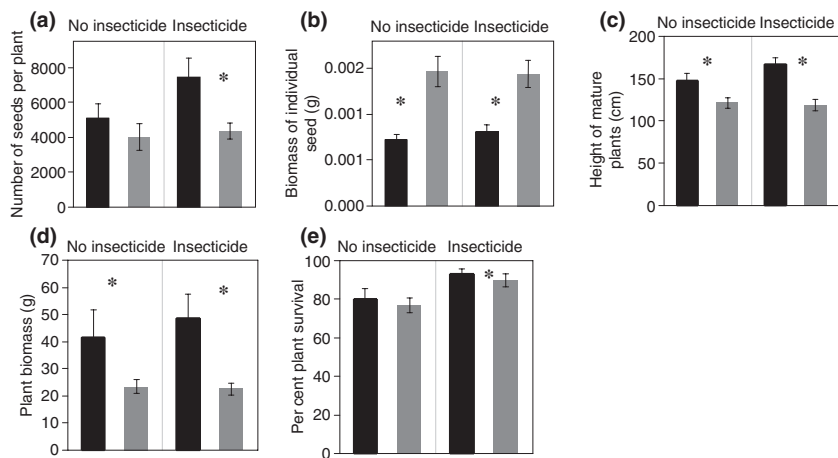
\*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ .

Populations and block were treated as random-effect variables in the models (results not shown); hence the  $F/Z$  statistics and degrees of freedom given are for fixed-effect variables only.



**Fig. 1** Resistance traits (mean ± 1 SE) of invasive (black bars) and native (grey bars) populations of *Brassica nigra* grown in a common garden with(out) insecticide treatment. Asterisks (\*) represent statistically significant differences ( $P < 0.05$ ). (a) Concentration of sinigrin in leaf tissues; (b) leaf trichome density; (c) resistance expressed as 100-percent leaf damage; (d) resistance expressed as 100-percent seed predation.

native populations (Figs 1a,b, 2b–d). Percentages of seeds preyed upon were similar for invasive and native populations under both insecticide treatments (Table 2; Fig. 1d). Leaf damage was influenced by both invasive status and insecticide treatment (Table 2); insecticide treatment resulted in less damage in both invasive and native populations,



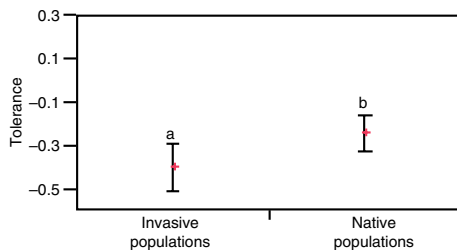
**Fig. 2** Growth performance traits (mean  $\pm$  1 SE) of invasive (black bars) and native (grey bars) populations of *Brassica nigra* grown in a common garden with(out) insecticide treatment. Asterisks (\*) represent statistically significant differences ( $P < 0.05$ ). (a) Seed yield per plant; (b) individual seed biomass; (c) height of mature plants; (d) biomass yield of plants; (e) percentage plant survival.

and, contrary to our expectations, damage in invasive populations was less than that in native populations under either insecticide treatment; that is, the invasive populations had higher resistance to leaf damage than native populations (Fig. 1c). The number of seeds per plant was affected by both insecticide treatment and the interaction between insecticide treatment and invasive status (Table 2). Invasive and native populations produced similar numbers of seeds per plant under no insecticide treatment, while under insecticide treatment, the invasive populations produced more seeds than native ones (Fig. 2a).

The number of plants that survived was also influenced by insecticide treatment and an interaction between insecticide and invasive status (Table 2); application of insecticide resulted in higher survival for both invasive and native populations, although it provided an even greater benefit to invaders than to native plants (Fig. 2e). Neither leaf trichome density nor sinigrin concentration was correlated with damage levels (results not shown).

### Plant tolerance of herbivory

There was a significant difference between invasive and native populations with regard to tolerance of leaf damage (ANOVA:  $F_{1, 23} = 5.634$ ,  $P = 0.026$ ) (Fig. 3). The native populations were more tolerant than the invasive popula-

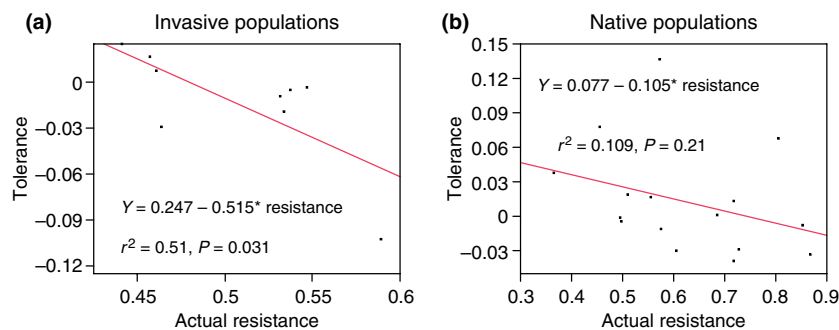


**Fig. 3** Mean ( $\pm$  1 SE) tolerance of herbivore damage in invasive (a) and native populations (b) of *Brassica nigra*. Tolerance was computed by regressing  $\log_{10}$ -transformed seed biomass against arc-sin square-root-transformed per cent leaf damage.

tions as they had a lower mean negative slope ( $-0.243$ ) than invasive populations ( $-0.4032$ ) (Fig. 3). However, both groups of plants undercompensated for damage, as indicated by the negative slopes (Fig. 3). Both invasive and native populations had a trade-off between tolerance and resistance, as indicated by negative slopes resulting from regressing tolerance against actual resistance (Fig. 4). Nevertheless, while the trade-off was statistically significant for invasive populations ( $r^2 = 0.51$ ,  $P = 0.031$ ), it was not for native populations ( $r^2 = 0.109$ ,  $P = 0.21$ ) (Fig. 4). This indicates that the trade-off was strong among invasive populations, and intermediate among the native populations.

### Discussion

Our finding that invasive populations invested more in a putative qualitative defence compound (i.e. sinigrin) is consistent with a prediction of the SDH that herbivore communities dominated by generalists in the invasive ranges select for plants that have a high concentration of that compound (Doorduyn & Vrieling, 2011). With a reduced importance of specialist herbivores in the invasive ranges, increased production of a secondary metabolite such as sinigrin that is toxic to generalists may have an overall positive effect on plant performance. Other studies have also reported significant increases in qualitative chemical defences in invasive populations. Significantly higher concentrations of pyrrolizidine alkaloids in invasive populations relative to native populations have been reported for *Senecio jacobaea* (Stastny *et al.*, 2005), *Senecio pterophorus* and *Senecio inaequidens* (Caño *et al.*, 2009). Invasive populations of *Lepidium draba* produced higher concentrations of p-hydroxybenzyl glucosinolate than native populations (Müller & Martens, 2005). However, our finding of a greater leaf trichome density among invasive populations is not consistent with a prediction of the SDH that quantitative defence traits will be selected against among invasive populations (Doorduyn & Vrieling, 2011).



**Fig. 4** Linear regressions showing trade-offs (i.e. negative correlations) between tolerance of and resistance to herbivore damage in invasive (a) and native (b) populations of *Brassica nigra* ( $n = 9$  for invasive populations;  $n = 16$  for native populations).

True to our prediction, invasive populations of *B. nigra* had higher actual resistance (i.e. a lower percentage of leaf damage) than native populations when exposed to natural levels of herbivory in the native range, and also in the insecticide treatment, which significantly reduced but did not eliminate damage. The literature is filled with conflicting results in similar studies. For example, invasive and native populations of *Solidago gigantea* received similar levels of herbivore damage under natural herbivory in the native range (Meyer *et al.*, 2005). However, invasive *Sapium sebiferum*, *Silene latifolia* and *Senecio jacobaea* populations experienced higher herbivore damage than native populations (Wolfe *et al.*, 2004; Stastny *et al.*, 2005; Zou *et al.*, 2008). Invasive genotypes of *S. jacobaea* experienced less herbivore damage by a generalist herbivore than native genotypes (Joshi & Vrieling, 2005).

In our study, neither leaf sinigrin concentration nor trichome density was correlated with leaf damage level. However, it should be noted that the damage we recorded was that caused by leaf-chewing herbivores only. Other types of herbivores (floral chewers and phloem feeders) were noted on the experimental plants. It was beyond the scope of our study to quantify the amount of damage caused by such herbivores. Perhaps a significant correlation between sinigrin concentration and damage on plants would have been found had damage by all types of herbivores been quantified. It is also possible that sinigrin and trichome play other ecological roles. For example, higher sinigrin content is linked to greater competition between *B. nigra* and other plants (Lankau & Strauss, 2008). Trichomes play significant roles in regulating leaf temperature and light reflection (Smith & Nobel, 1977) and leaf evaporation (Brewer *et al.*, 1991). It has, nevertheless, been shown that a high leaf concentration of sinigrin can deter feeding damage by generalists, while at the same time inducing damage by specialists in a field experiment in California using some of the populations of *B. nigra* we used in the current study (Lankau, 2007). Hence it is possible that most of the damage in the current study was caused by specialist herbivores whose feeding activities were not quantified (e.g. the phloem feeders).

Our results support the idea of post-introduction evolution of a trade-off between resistance and tolerance, as both

invasive and native populations had a trade-off, although it was stronger among the invasive populations (Figs 3, 4). Among the invasive populations, every unit increase in resistance resulted in a large, significant decline in tolerance, while among the native populations, every unit increase in resistance resulted in only a small non-significant decline in tolerance (Fig. 4). This supports the idea that high species diversities of both generalists and specialists within native ranges exert diffuse selective pressures, thus causing maintenance of intermediate levels of both resistance and tolerance (Weinig *et al.*, 2003). However, these results also support the idea that herbivore communities dominated by generalists in the invasive ranges exert a strong directional selection pressure so that plant genotypes in those ranges have high resistance and low tolerance. In another similar study, it was reported that invasive *Alliaria petiolata* ecotypes had lower resistance than native ecotypes, but there were no significant differences in herbivory tolerance between the invasive and native populations (Bossdorf *et al.*, 2004b). It was demonstrated that invasive ecotypes of *S. jacobaea* had both greater resistance to and tolerance of herbivory than native ecotypes of the same species (Stastny *et al.*, 2005). Invasive populations of *S. sebiferum* experienced more damage but produced more biomass than native populations (Zou *et al.*, 2008), indicating a trade-off between resistance to and tolerance of herbivore damage.

Invasive and native populations of *B. nigra* had similar survival rates under natural levels of herbivory, but with insecticide treatment, invasive populations had greater survival than native populations, suggesting perhaps greater vulnerability to cryptic seedling herbivory. Under natural levels of herbivory, invasive and native populations produced similar numbers of seeds per plant, but when protected from herbivory, invasive populations had significantly greater reproductive success than natives. This finding partly supports our prediction that the invasive populations would suffer reduced vigour and reproduction when they were grown in their native range where they were exposed to herbivory. However, the invasive populations grew taller and produced more biomass than native populations regardless of insecticide treatment, a result that contradicts that prediction. Seeds were consistently lighter for plants from invasive populations, irrespective of insecticide

treatment. As invaders and natives were grown in the same environment, these results suggest that all the differences in traits noted above are heritable.

Our results support the idea of post-introduction rapid evolution of plant traits leading to invasion success. Dates of seed collections from wild populations of *B. nigra* in its native ranges varied from 1942 to as recently as 1996. However, seeds of invasive populations of *B. nigra* used in the current study were field-collected in 2005 (see Table S1). This means that the invasive populations were separated from their native conspecifics by anywhere between  $\leq 70$  and 200 yr. That the invasive and native populations were separated only for those years, yet differed markedly in the various traits above, supports the idea of rapid evolution of plant traits after introduction.

Our results also contribute to an emerging pattern of both increasing defence and growth in invasive populations, in contrast to the predictions of earlier theories of defence–growth trade-offs. We found rapid changes in concentrations of sinigrin, levels of tolerance of herbivory, stature and seed ‘packaging’ (more, lighter seeds). These changes may come about in response to natural selection pressures exerted by changes in herbivore regimes (Lankau, 2007) and competition from other plants (Lankau & Strauss, 2008; Lankau *et al.*, 2009). Furthermore, as in other invading organisms, selection might have resulted in increased seed dispersal ability among invasive populations of *B. nigra*; that is, selection for tall plants that produce light seeds in high amounts (Muller-Landau *et al.*, 2008; Whitney & Gabler, 2008). The possibility that natural selection might have led to the differences in traits between invasive and native populations of *B. nigra* as discussed herein is supported by results of our molecular phylogeography study, in which all the populations employed in the current study were also used (A. M. O. Oduor *et al.*, unpublished data). The phylogeography study showed that multiple introductions of *B. nigra* genotypes took place from its native to invasive ranges (A. M. O. Oduor *et al.*, unpublished data). Through multiple introductions of genetic variation, founder effects and inbreeding after bottlenecks can be mitigated, resulting in invasive populations having the same genetic diversity as, or even higher diversity than native populations (Kolbe *et al.*, 2004; Durka *et al.*, 2005; Dlugosch & Parker, 2008). However, with the passage of time, natural selection in the novel invasive ranges might have caused genetic heterogeneity between invasive and native ranges in the various traits discussed herein.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Table S1** Invasive and native populations of *Brassica nigra* used in the current experiment

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