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DISSECTING ECOLOGICAL AND EVOLUTIONARY MECHANISMS UNDERLYING INVASIVENESS OF PLANTS: USING *BRASSICA NIGRA* AS A MODEL PLANT

PhD THESIS

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DISSECTING ECOLOGICAL AND EVOLUTIONARY MECHANISMS UNDERLYING INVASIVENESS OF PLANTS: USING *BRASSICA NIGRA* AS A MODEL PLANT

A thesis submitted in partial fulfillment of the
requirements for the degree of Doctor of philosophy of University of

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Hereby certify that:

This thesis is an original piece of work that has not been previously submitted for any other degree or qualification. The work of which it is a record has been performed by the PhD candidate and all sources of information have been specifically acknowledged.

Dr. José María Gómez   Dr. Sharon Y. Strauss   Dr. Francisco Perfectti

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RESUMEN

RESUMEN

Son numerosas las hipótesis que explican los mecanismos que afectan al éxito y establecimiento de las plantas invasoras. Esas hipótesis incluyen: 1) hibridación como consecuencia de introducciones de distintos genotipos de diferente procedencia, 2) alelopatía, 3) “novel weapons” (la ventaja de tener un sistema novedoso de ataque y/o defensa), 4) escape de enemigos especialistas, 5) evolución para incrementar la capacidad competidora, 6) “invasional meltdown” (sinergia entre invasoras) y 7) cambio en el sistema defensivo. A pesar de que la biología de la invasión ha sido objeto de numerosas investigaciones, algunas de estas hipótesis no han sido rigurosamente testadas. Durante los capítulos que componen esta tesis doctoral, se han puesto a prueba alguna de estas hipótesis, utilizando *Brassica nigra* como modelo de estudio. *B. nigra* es una crucífera precedente del Norte de África, Europa y Asia, siendo invasora en América del Norte. Para empezar, en el **capítulo 1** se presenta una introducción general de las investigacioens realizadas. En el **capítulo 2**, se realiza un estudio filogeográfico sobre esta planta, para desarrollar los siguientes objetivos: (1) identificar la procedencia de las poblaciones invasoras de América del Norte, (2) determinar si hubo introducciones múltiples o procede de un único evento de introducción, y (3) comparar la diversidad genética entre poblaciones invasoras y autóctonas. En el **capítulo 3** fue testada la hipótesis 5, de las mencionadas anteriormente. Específicamente, se estudió la evolución y diversidad de los compuestos químicos defensivos de *B. nigra* como adaptación a la invasión. Para ello se partió de dos predicciones, la primera, que las poblaciones invasoras presentan baja diversidad de glucosinolatos (un grupo de compuestos químicos para la defensa ante depredadores). La segunda, dada la baja diversidad de glucosinolatos mencionados anteriormente, las poblaciones invasoras suelen sufrir más ataques por herbívoros cuando son sembradas en las zonas nativas. En el **capítulo 4**, se testó la hipótesis del cambio en el sistema defensivo de especies invasoras, específicamente se estudiaron las siguientes predicciones: (a) las poblaciones invasoras, al encontrase en zonas con depredadores generalistas, suelen tener más defensas cualitativas y menos defensas cuantitativas, ya que las defensas cualitativas son más eficaces frente a los depredadores generalistas; (b) las poblaciones invasoras presentan alta resistencia y baja tolerancia al daño por herbívoros en comparación con las poblaciones nativas; y (c) las poblaciones invasoras suelen presentar una baja supervivencia, crecimiento y tasa reproductiva cuando son dañadas por herbívoros presentes en las zonas nativas. Por otra parte, en el **capítulo 5**, se testó si las poblaciones invasoras de *B. nigra* evolucionaron para incrementar su capacidad competidora con presencia y ausencia de herbívoros. Para terminar, en el **capítulo 6**, ser realizó un meta-análisis para comparar los efectos de los herbívoros vertebrados e invertebrados sobre el crecimiento y desarrollo de las plantas procedentes de poblaciones invasoras y nativas. En este último capítulo se testó la hipótesis de la sinergia entre especies invasoras. Finalmente, en el **capítulo 7** se ha realizado una discusión general, resumiendo y destacando los resultados más importantes obtenidos, comparándolo con otras investigaciones similares.

CHAPTER 1:

GENERAL INTRODUCTION

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Many plant species have been introduced to ranges where they are not native. However, only a small fraction of introduced plant species become invasive (i.e. superabundant in the introduced ranges) and have dramatic detrimental economic and ecological effects (D'Antonio and Vitousek 1992; Vitousek et al. 1996; Mack et al. 2000; Pimentel et al. 2005). Invasive plants modify species distributions, ecosystem processes, and community structure, and are a key threat to native biodiversity (e.g. D'Antonio and Vitousek 1992; Vitousek et al. 1996; Mack et al. 2000; Pimentel et al. 2000; 2005). Economic impacts of invasive plants are felt when the invasive plants cause drastic reductions in crop yields (Pimentel et al. 2005). Furthermore, a lot of costs are incurred when controlling the invasive plants (Pimentel et al. 2005). Consequently, a copious amount of research has been directed towards a better understanding of the mechanisms underlying invasion success of certain plant species (Reichard and Hamilton 1997; Mack et al. 2000; Daehler 2003).

A number of hypotheses have been put forth and tested in a bid to explain mechanisms that underlie invasion success of plants. These include: hybridization resulting from multiple introductions of distinct genotypes (Ellstrand and Schierenbeck 2000; Dlugosch and Parker 2008), allelopathy and novel weapons hypothesis (NWH) (Callaway and Aschehoug 2000; Vivanco et al. 2004), enemy release hypothesis (ERH) (Keane and Crawley 2002), evolution of increased competitive ability (EICA) (Blossey and Nötzold 1995), invasional meltdown

(Simberloff and Von Holle 1999), and shifting defence hypothesis (Doorduin and Vrieling 2011).

Multiple introductions of diverse genetic material are thought to contribute to invasion success of certain plants. Multiple introductions may precipitate evolutionary changes when previously isolated genotypes are mixed and subsequently interbreed thereby creating new, aggressively invading genotypes (Ellstrand and Schierenbeck 2000; Maron et al. 2004; Dlugosch and Parker 2008).

Allelopathy refers to the production and release of chemicals that harm or otherwise decrease fitness of other plants (Hierro and Callaway 2003). Allelopathy occurs in two forms: direct and indirect. Direct allelopathy occurs when a phytotoxic chemical is released, often into the soil through the roots but also through other means such as toxins in decomposing leaf matter, that negatively impacts other plants (Vivanco et al. 2004). Indirect allelopathy is manifested when a phytotoxin released into the soil affects negatively microbial mutualists of other plants (Callaway et al. 2008). Allelopathy is thought to aid invasion success of such plants, as for example, *Centaurea maculosa* (He et al. 2009), *C. diffusa* (Callaway and Aschehoug 2000; Vivanco et al. 2004), and *Alliaria petiolata* (Callaway et al. 2008; Prati and Bossdorf 2004). Allelopathy and NWH are related in the sense that allelopathic phytotoxins are often novel to their target organisms (Callaway et al. 2008). NWH posits that within native ranges, plant communities co-evolve in species- and population-specific ways, so that allelopathy mounted by a given plant species is countered by chemical resistances by other plant species in the native range. However, when a plant is introduced to a new range, the vegetation is naïve to the chemical weapons of the invaders, enabling the invasive plants to outgrow native plants (Callaway et al. 2008).

Enemy release hypothesis (ERH) predicts that introduced plant species often undergo a release from intense damage from herbivores and other natural enemies, resulting in a rapid increase in growth, reproduction, distribution and abundance (Keane and Crawley 2002). In support of ERH, a meta-analysis by Liu and Stiling (2006) found that indeed invasive populations of plants harbour lower diversities of herbivores than native populations of such plants, and that invasive populations experience lower herbivore damage than native populations.

Evolution of increased competitive ability (EICA) is one of the most extensively tested hypotheses regarding invasion success of plants (see Bossdorf et al. 2005 for review). EICA hypothesis posits that escape from specialized natural enemies enable certain introduced plant species to re-allocate resources away from defence and invest them in growth and reproduction because herbivory pressure is low in the exotic ranges (Blossey and Nötzold 1995). Various tests of EICA hypotheses have shown that invasive populations of certain plant species have undergone post-introduction evolutionary changes to have higher mean values of such traits, as for example, stature, biomass, and reproductive output than native populations of the same plant species (e.g. Blossey and Nötzold 1995; Buckley et al. 2003; Leger and Rice 2003; Maron et al. 2004; Wolfe et al. 2004; Bossdorf et al. 2005 for see review; Stastny et al. 2005; see Whitney and Gabler 2008 for review; Williams et al. 2008; Zou et al. 2008a,b; Caño et al. 2009; Moloney et al. 2009; Oduor et al. 2011).

SDH is an extension of the EICA hypothesis (Doorduin and Vrieling 2011). SDH classifies plant defence traits against herbivores into two categories: quantitative and qualitative, depending on whether they are effective against specialist or generalist herbivores, respectively (Müller-Schärer et al. 2004*;* Joshi and Vrieling 2005). Quantitative defence traits are based on digestibility-reducers (e.g. cellulose, tannins, and trichomes) that usually occur in high concentration and act in a dosage-dependent manner. On the other hand, 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 (Doorduin and Vrieling 2011). SDH predicts that invasive populations of plants have undergone post-introduction evolutionary change to express higher concentration of qualitative defence compounds because most of the herbivores that invasive plants interact with are generalists (Doorduin and Vrieling 2011). SDH assumes that expression of high concentrations of qualitative defence compounds does not have much physiological costs to plants (Doorduin and Vrieling 2011).

According to invasional meltdown hypothesis, mutualistic interactions between exotic plants and other exotic organisms (for example between an exotic plant and exotic pollinator) may aid invasion success of certain exotic plants (Simberloff and Von Holle 1999). Positive feedbacks resulting from interactions involving exotic herbivores and plants have also been reported. Infact a meta-analysis carried out by Parker et al. (2006) found that exotic herbivores generally aided invasion success of exotic plants. The exotic herbivores preferred feeding on the native plants while avoiding the exotic plants thereby suppressing abundances and species richness of the native plants, while at the same time abundance and species richness of exotic plants increased (Parker et al. 2006). Native plants were preferentially fed on by exotic herbivores because the plants were evolutionarily naïve to the exotic herbivores; hence the plants had not evolved defences against such herbivores (Parker et al. 2006). However, exotic plants were less preferred by exotic herbivores because the plants had co-evolved with the exotic herbivores, thus the plants had evolved defences that enable them to co-exist with such herbivores (Parker et al. 2006).

While some of the hypotheses stated above, such as for example, EICA, have been extensively tested, others like multiple introductions and SDH have not received much attention. Nevertheless, even EICA that has itself been extensively explored has not been exhaustively tested in certain aspects in an ecological setting. For example, very few studies to date have been documented regarding post-introduction evolution of higher competitive abilities of invasive populations of plants relative to their non-introduced, native conspecifics. Therefore, work on which this thesis is based involved testing some hypotheses and addressing some questions that have received little attention in plant invasion. *Brassica nigra* (Brassicaceae) (L.) W. D. J. Koch was used as model plant for testing those hypotheses.

OUTLINE OF THIS THESIS

In **chapter 2**, I report on phylogeographical relationships of invasive (North American) and native (North African, Mediteranean region and Eurasian) populations of *Brassica nigra* that were studied using chloroplast DNA intron (*trnF*-*trnL*) sequences. The main objectives here were to: (1) Identify sources of introductions of invasive populations of *B. nigra* from native ranges; (2) find out whether or not multiple introductions of *B. nigra* took place from native to invasive range; (3) compare diversity of chloroplast DNA between invasive and native populations of *B. nigra*.

In **chapter 3** EICA hypothesis regarding evolution of diversity of plant defence compounds was tested. To date, only a handful of studies have compared expressions of two or more defence compounds between invasive and native populations of the same plant (Cipollini et al. 2005; Joshi and Vrieling 2005; Hull-Sanders et al. 2007; Wheeler et al. 2007; Franks et al. 2008). However, none of those studies used any of the known diversity indices to compare diversities of defence compounds between invasive and native populations of any given plant. Here, it was predicted that invasive populations of *B. nigra* express a lower diversity of glucosinolates because they interact with a less diverse herbivore community than native populations of *B. nigra*. Because they express a lower diversity of glucosinolates, invasive populations should harbour a higher diversity of arthropod herbivores than native populations of the same plant when they are grown in a common garden within a native range.

Results of tests of the recently formulated shifting defence hypothesis (SDH) are presented in **chapter 4.** On the basis of SDH and theory on a trade-off between resistance to, and tolerance of herbivore damage , the following predictions were made:

1. Because they come from ranges dominated by generalist herbivores, invasive populations of *B. nigra* will have greater investment in qualitative defence and lower investment in quantitative defence than native populations of *B. nigra*.
2. Invasive populations will have higher resistance, and lower tolerance of herbivore damage than native populations.
3. 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).

In **chapter 5**, I present results of a test of EICA hypothesis regarding post-introduction evolution of competitive abilities of invasive plants under conspecific and heterospecific competitive environments as well as in the presence or absence of insect herbivores. In most ecosystems, competition from neighbouring plants and herbivore damage determine growth, survival and reproduction of plant individuals, and consequently the abundance of plant populations (Hämback and Beckerman 2003). Nevertheless, little is known about the potential interactive effects of herbivore damage and competition on plant performance with regard to invasive plants (Suwa et al. 2010). Here, I experimentally compared competitive abilities of invasive and native populations of *B. nigra* under natural and reduced (or zero) levels of insect herbivory within a native range of the plant. Previous tests of competitive abilities of invasive and native populations of the same plant species have not manipulated presence or absence of herbivores to see what effect herbivores have on competitive interactions of plants. In the experiment, I asked whether or not invasive populations of *B. nigra* are better competitors than native populations when they are exposed to natural or reduced (zero) level of insect herbivory in the native range.

In **chapter 6**, I report on results of a meta-analysis that was done to compare effects of exotic vertebrate and invertebrate herbivores on exotic and native plants. A previous meta-analysis by Parker et al. (2006) found that exotic herbivores generally caused an increase in abundance and species richness of exotic plants, while at the same time suppressed abundance and species richness of native plants. Hence the exotic herbivores promote invasion success of exotic plants, in accordance with invasional meltdown hypothesis. However, it remained unknown whether exotic vertebrate and invertebrate herbivores have similar effects on native and exotic plants. Using two distinct data sets, meta-analyses were run to compare exotic vertebrate and invertebrate herbivore preferences for, and effects on performance and population sizes of native and exotic plants. We also tested an evolutionary logic that posits that herbivores with similar evolutionary history as plants will affect the plants less negatively than plants with which they have not co-evolved.

Finally in **chapter 7**, I give a general discussion summarizing the main findings of this thesis and compare them with findings from other previous studies of invasive plants. I also give recommendations for future research.

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CHAPTER 2:

INVASION OF *BRASSICA NIGRA* (BRASSICACEAE) IN NORTH AMERICA: DISTRIBUTIONS AND ORIGINS OF CHLOROPLAST DNA HAPLOTYPES.

Ayub M.O. Oduor, María Belen Herrador, Sharon Y. Strauss, José M. Gómez and Francisco Perfectti.

ABSTRACT

Deciphering origin of invasive genotypes, whether or not there have been multiple introductions and genetic differentiations between invasive and native ranges is crucial in testing hypotheses underlying biological invasions. Here, we studied phylogeographical relationships of invasive (North American) and native (North African, Mediteranean region and Eurasian) populations of *Brassica nigra* using chloroplast DNA intron (*trnF-trnL*) sequences. We sequenced 302 individuals representing a total of 53 native and invasive populations of *B. nigra*. Each of the 53 populations was represented by six individuals except for two native populations represented by two individuals each, and one invasive population represented by four individuals. Thirty seven haplotypes were found. Native populations had higher number and diversity of haplotypes than invasive populations. Twenty five haplotypes occurred among the native populations while 15 haplotypes occurred among the invasive populations. Three haplotypes were shared between invasive and native populations. Four haplotypes likely underwent multiple introductions from native to invasive ranges of *B. nigra*. The other 34 haplotypes were private, occurring exclusively in invasive or native populations. A nested analysis of molecular variance (AMOVA) revealed a lack of genetic differentiation between invasive and native range populations. However, there were genetic differentiations among populations within both invasive and native ranges. A Mantel test showed a lack of correlation between pairwise genetic distances (F_{ST}) and geographical distances when data were analyzed for native and invasive populations together or separately for the native populations. However, a Mantel test for invasive populations only revealed a significant positive correlation between pairwise genetic distances (F_{ST}) and geographical distances.

Keywords**:** Exotic plants, biological invasions, *Brassica nigra*, cpDNA, AMOVA, phylogeography, multiple introductions.

INTRODUCTION

Exotic invasive plants modify species distributions, ecosystem processes, and community structure, and are a key threat to native biodiversity (e.g. Mack et al. 2000; Pimentel et al. 2000). Identification of geographical origins of exotic invasive plants is becoming increasingly important in order to: 1) understand how climatic and biotic selective pressures in the native range translate into success in the novel range; 2) understand their routes of spread (Scheffer and Grissell 2003); 3) identify enemies in the native range (Alberti et al. 2008; Hufbauer and Sforza 2008); and 4) detect cryptic hybridization in the novel range that might increase invasiveness. Phylogeography, the study of geographical origin, distribution of genetic lineages, and genealogical histories of organisms (Avise 2000), is an excellent way of identifying source populations of exotic invasive plants (Geller et al. 1997; Slade and Moritz 1998; Scheffer 2000).

Molecular genetic markers are a powerful tool for phylogeography due to their ability to resolve low levels of intraspecific lineage sorting (Avise 2009; Freeland 2005). Furthermore, the molecular markers may aid in drawing conclusions regarding relative levels of variations among and within exotic and native populations of invasive species (Hall and Muralidharan 1989; Gasparich et al. 1997; Hufbauer and Sforza 2008). Molecular genetic markers have also been used to provide evidence that many invasions are the result of multiple introductions from multiple sources to multiple locations (e.g. Kolbe et al. 2004; Genton et al. 2005; Williams et al. 2005; Durka et al. 2005; Hufbauer and Sforza, 2008; Rosenthal et al. 2008; Pairon et al. 2010).

Multiple introductions bearing diverse genetic material appear to contribute to invasion success in various ways. 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 (e.g. Allendorf and Lundquist 2003; Walker et al. 2003; Kolbe et al. 2004; Durka et al. 2005; Dlugosch and Parker 2008). Multiple introductions may also precipitate evolutionary changes when previously isolated genotypes are mixed and subsequently interbreed thereby creating new, aggressively invading genotypes (Ellstrand and Schierenbeck 2000; Maron et al. 2004; Dlugosch and Parker 2008). Multiple introductions of pre-adapted genotypes from areas similar in climate to novel range can also occur (Dlugosch and Parker 2008).

Here, we used a molecular marker (chloroplast DNA, cpDNA) to conduct a phylogeographical structure analysis of *Brassica nigra* (Brassicaceae). The uniparental inheritance of cpDNA (i.e. via seeds) makes it a suitable marker for phylogeography because colonization of new habitats often occurs through seeds (Freeland 2005). We addressed the following questions:

1. Do our Mediteranean region and Eurasian samples represent possible sources of introduction of the invasive populations?
2. Are multiple introductions likely to have occurred in *B. nigra*?
3. Is genetic diversity among the invasive populations of *B. nigra* reduced or increased relative to the native range populations?
4. Is genetic variation structured similarly between the two ranges?

5. What are the genealogical relationships among haplotypes of the invasive and native populations of *B. nigra*?

MATERIALS AND METHODS

STUDY SYSTEM

Brassica nigra (L.) W. D. J. Koch is an annual herb native to the Mediteranean region, North Africa, and Europe that has spread widely across the globe (Bell and Muller 1973; Feeny and Rosenberry 1982; Westman and 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 and Kresovich 1999). In temperate regions, *B. nigra* was a major mustard crop until the 1950s when it was replaced by its close relative, *B. juncea* in commercial production. Presently, *B. nigra* is a widespread weed (Westman and Kresovich 1999). In North America, *B. nigra* weed populations may have arisen from multiple sources: crop seed, commercial mustard seeds from Europe and India, or weed seed introduced with European colonists approximately 200 years ago (Westman and Kresovich 1999). In North America, *B. nigra* can form thick monospecific stands, although generally in disturbed areas (Lankau and Strauss 2008). To date, however, no information is available on molecular phylogeography of *B.nigra*.

DNA SAMPLING, POLYMERASE CHAIN REACTIONS AND SEQUENCING

Seeds of 38 invasive and 15 native populations of *Brassica nigra* were obtained from United States Department of Agriculture (USDA) germplasm resources information network (GRIN), botanical gardens across Europe or field collected by the authors or their collaborators (see Table 1 for details). Seeds from each of the invasive and native populations of *B. nigra* were then germinated and leaf tissues were harvested separately from resulting six individual seedlings per population. The leaf tissues were stored in silica desiccant until DNA could be extracted.

Total DNA was extracted according to the protocol of GenElute^{TM} Plant Genomic DNA kit (SIGMA). One non-coding region of cpDNA (*trnF-trnL*) was amplified using the universal primer pair Tab F and Tab C. Polymerase chain reaction (PCR) products were purified as follows: 17.6 µl H_2O_d, 3 µl of 3M sodium acetate at pH of 4.6, and 59.4 µl of pure ethanol at 4^o C were added to 25 µl of PCR product. The mix was then incubated for 15 minutes at room temperature after which it was centrifuged for 20 minutes at 14, 000 revolutions per minute (rpm). The resulting pellet was washed by adding to it 250 µl of 70 % ethanol and centrifuging for 20 minutes at 14, 000 rpm. The pellet was then dried in an oven at 37^oC to eliminate all the ethanol. The pellet was resuspended in ultra pure water at a concentration of 50 ng/µl and then held in a 1.5 ml eppendorf tube in readiness for sequencing. The purified PCR products were then sequenced commercially by Macrogen Sequencing Service (Macrogen, Korea).

Table 1: *Fifty three populations of Brassica nigra that were used in the phylogeography analyses. Populations marked with asterisks (*) are those whose accession numbers were not provided by the botanic gardens from which they were sourced. Geographical coordinates of populations marked by † were not recorded. However, they were spaced at least 30 km away from the nearest population of B. nigra.*

Population	Abbreviation	Accession number/Collector's name	Country of seed germplasm collection	Latitude	Longitude	Invasive status
Afghanistan	AF	PI274284	USA	34° 0' 0'' N	69° 0' 0'' E	Native
Afghanistan	AF	CR 2744	Germany	†	†	Native
Germany	GE	PI 633142	Germany	51° 25' 0'' N	12° 1' 0'' E	Native
Germany	GE	PI 633143	Germany	51° 49' 0'' N	11° 17' 0'' E	Native
California	CA	Richard Lankau	USA	†	†	Invasive
California	CA	Richard Lankau	USA	†	†	Invasive
California	CA	Richard Lankau	USA	†	†	Invasive
California	CA	Richard Lankau	USA	†	†	Invasive
California	CA	Richard Lankau	USA	†	†	Invasive
California	CA	Richard Lankau	USA	†	†	Invasive
California (Napa county)	CA	Richard Lankau	USA	†	†	Invasive
California (Davis, CA)	CA	Sharon Y. Strauss	USA	38°53' 14'' N	121°78'6''W	Invasive
Canada	CAN	PI649154	USA	†	†	Invasive
Canada	CAN	Ames 25399	USA	43° 40' 0'' N	79° 25' 0'' W	Invasive
Denmark	DEN	CR 2710	Germany	†	†	Invasive
Denmark	DEN	CR 2762	Germany	†	†	Invasive
Spain1	SP1	PL597829	Spain	36° 0' 0'' N	6° 0' 0'' E	Native
Spain1	SP1	*	Spain	†	†	Native
Spain1	SP1	PI 597829	USA	36° 0' 0'' N	6° 0' 0'' W	Native
Spain2	SP2	J.M. Gómez	Cádiz	36° 25' 39'' N	6° 3' 77'' W	Native
Spain2	SP2	J.M. Gómez	Cádiz	36° 28' 85'' N	6° 0' 99''W	Native
Ethiopia	ET	CR 2117	Germany	†	†	Native
Ethiopia	ET	CR 2119	Germany	†	†	Native
France	FR	CSFR CR 77	Germany	†	†	Native

Table 1 *continued*

France	FR	2113	Germany	†	†	Native
Great Britain	GB	CR 2618	Germany	†	†	Native
Greece	GR	PI 633147	USA	37° 20' 5" N	22° 21'08" E	Native
Greece	GR	CR 2735	Germany	†	†	Native
Greece	GR	CR 2093	Germany	†	†	Native
Greece	GR	PI 263866	USA	37° 58' 0" N	23° 43' 0" E	Native
Hungary	HU	*	Hungary	†	†	Native
Illinois	IL	Jeff Conner	USA	†	†	Invasive
Illinois	IL	Jeff Conner	USA	†	†	Invasive
Illinois	IL	Jeff Conner	USA	†	†	Invasive
India	IND	CR 2743	Germany	†	†	Native
India	IND	CR 75	Germany	†	†	Native
Italy	IT	PL 633148	USDA	40° 10' 0" N	16° 31' 0"E	Native
New York	NY	Jeff Conner	USA	†	†	Invasive
New York	NY	Jeff Conner	USA	†	†	Invasive
The Netherlands	NL	CR 480	Germany	†	†	Native
The Netherlands (Afferden)	NL	Mirka Macel	The Netherlands	51° 53' 0" N	5° 38' 0"E	Native
The Netherlands(Doorweth)	NL	Mirka Macel	The Netherlands	51° 38' 41" N	5° 30' 23"E	Native
The Netherlands	NL	CR 480	Germany	†	†	Native
Pakistan	PAK	CR 2620	Germany	†	†	Native
Poland	POL	*	Poland	†	†	Native
Czech Republic	CZE	*	Czech Republic	†	†	Native
Turkey	TU	PI 169066	USA	40° 2' 47" N	27° 58'12" E	Native
Turkey	TU	PI 592737	USA	39° 38' 5" N	27° 53' 6"E	Native
Turkey	TU	PI 176881	USA	39° 52' 0" N	32° 52' 0" E	Native
Soviet Union	SOV	CR 2700	Germany	†	†	Native
Soviet Union	SOV	CR 1206	Germany	†	†	Native
Yugoslavia	YU	PI 379102	USA	43° 52' 0" N	18° 25' E	Native
Yugoslavia	YU	CR 2758	Germany	†	†	Native

DATA ANALYSIS

Sequences were aligned using ClustalW Sequence Alignment Editor version 7.0 (@Tom Hall, Ibis Therapeutics, Carlsbad, California). First and final parts of the sequences were then trimmed to 705bp after which they were subjected to further analyses as detailed here below. Computations of number and diversity of haplotypes were carried out using DnaSP version 5.0 (Librado and Rozas 2009). Phylogeographical structure analysis was done by quantifying the degree of genetic differentiation between and within the invasive and native ranges of *B. nigra* by an analysis of molecular variance (AMOVA) using the package ARLEQUIN version 3.1(Excoffier et al. 1992). Three separate AMOVAs were carried out to: 1) compare genetic diversities between invasive and native ranges; 2) compare genetic diversities among and within populations from the native range; and 3) compare genetic diversities among and within populations from the invasive range. The first type of AMOVA involved lumping together all *B. nigra* populations from Europe/Africa/Asia as native while treating all North American populations as invasive. For the second AMOVA, populations from the native range were divided into three groups: African and European countries surrounding the Mediteranean sea, Western/Central/Northen Europe, and Asia. The third AMOVA involved grouping North American populations of *B.nigra* into four according to the state from which they were sourced: California (USA), Illinois (USA), New York (USA), and Ontario (Canada). In each AMOVA, statistical significance was determined by a permutation process with 10,000 runs and the method used to determine variation in the dataset was based on genetic distances between DNA sequences of haplotypes. We further used a Mantel test to examine whether genetic distances between population pairs increased as linear functions of the corresponding geographical distance. This was done by regressing pairwise genetic distances of the haplotypes (F_{ST}) on geographic distances of the populations from which the haplotypes were identified. The pairwise F_{ST} values for haplotypes were calculated using ARLEQUIN, while the pairwise geographic distances were computed based on geographical coordinates of seed sources. When carrying out Mantel tests, all individual populations coming from the same country/state were grouped together. For example, if there were eight different populations from California, then they were all lumped together into one group (see Table 3). This resulted in 53 individual populations being categorized into 23 groups of invasive and native populations (Table 3). Such groupings were necessitated due to lack of information on geographical references of seed collection points for many of the 53 individual populations (see Table 1). Therefore, the geographical coordinates we used for the Mantel tests were actually those of capital cities of those countries/ states where all the 53 individual populations had been sampled. There was only one exception where geographic coordinates were used for two different cities for the same country (i.e. Spain). This is because two different sets of seeds were obtained: one from a botanic garden in the capital city (Madrid) and another from South West of the capital city (Cádiz) (see Tables 1 & 3). Three different Mantel tests were run: 1) using a whole data set combining both native and invasive populations; 2) using data for native populations only; 3) using data for invasive populations only. The Mantel tests were performed using SAM v4.0 (Rangel et al. 2010) and statistical significances of the tests were examined with 200 permutations. Geographical distribution of haplotypes was plotted using ArcMap 8.3 (ESRI, Inc.). Genealogical relationships among haplotypes were represented by a statistical parsimony network using genetic distances among haplotypes generated by ARLEQUIN. A haplotype network representing genealogical relationships among the haplotypes was constructed using a program HapStar v0.5 (Teacher and Griffiths 2011).

RESULTS

cpDNA was sequenced for a total of 302 individuals representing all the 53 invasive and native populations of *B. nigra*. Out of the 53 populations, two native populations were represented by two individuals each, while one invasive population was represented by four individuals. The rest of 49 populations (i.e. 35 native and 14 invasive populations) were represented by six individuals each. A total of 37 haplotypes (H1-H37) were detected among all the 302 individuals from invasive and native populations. Fifteen haplotypes occurred in invasive populations while 25 haplotypes occurred in native populations (Table 2). Native populations of *B.nigra* had higher diversity of haplotypes than invasive populations (Table 2).

Table 2: *Summary statistics for polymorphism in TrnL-TrnF region of chloroplast DNA among invasive and native populations of Brassica nigra.*

Invasive status	Sample Size (n)	Number of haplotypes	Haplotype diversity
Invasive	90	15	0.3069
Native	212	25	0.4704

Haplotype H1 was the most abundant (detected in 228 individuals) followed by haplotype H3 (detected in 16 individuals), with haplotypes H4 and H2 detected in 13 and 8 individuals, respectively. Four haplotypes (H5, H9, H12, and H21) were detected in two individuals each, while the remaining 29 haplotypes occurred only once (Table 3; Fig. 1). Three haplotypes (H1, H2, and H5) were shared between the invasive and native populations of *B. nigra* (Table 3; Fig. 4). The rest of the haplotypes were private, i.e., they occurred exclusively in native or invasive range populations (Table 3; Fig. 4).

Table 3: *Frequency distribution of 37 haplotypes detected based on TrnL-TrnF region of cpDNA among invasive and native populations of Brassica nigra. The first two or three letters refer to countries/states where populations were obtained from (see Table 1 for population abbreviations). Numbers in brackets refer to numbers of populations per country/state. Bold font indicates invasive populations.*

	Haplotypes																		
Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AF (2)	8	1													1	1			
GE (2)	9	2							1										
CA (8)	41					1	1	1						1			1		
CAN (2)	9	1																1	
CZE (1)	1			5															
DEN (2)	12																		
ET (2)	9											1	1						
FR (2)	11																		
GB (1)	6																		
GR (4)	22	1																	
HU (1)			6																
IL (3)	15	1			1														
IND (2)	10																		
IT (1)	3				1														
NL (4)	13	1																	1
NY (2)	10										1								
PAK (1)	6																		
POL (1)	1			5															
SOV (2)	6		6																
SP1 (3)	15		1							1									
SP2 (2)				3															
TU (3)	10	1	3						1										
YU (2)	11											1							

Table 3 ***continued***.

	Haplotypes																	
Population	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
AF (2)						1												
GE (2)																		
CA (8)							1	1										
CAN (2)									1									
CZE (1)																		
DEN (2)																		
ET (2)										1								
FR (2)			1															
GB (1)																		
GR (4)											1							
HU (1)																		
IL (3)												1						
IND (2)	1	1																
IT (1)													1	1				
NL (3)		1		1											1			
NY (2)																		1
PAK (1)																		
POL (1)																		
SOV (2)																		
SP1 (3)																1		
SP2 (2)					1													
TU (3)																	1	
YU (2)																		

Twenty one haplotypes (H23, H19, H22, H29, H15, H2, H13, H21, H14, H16, H33, H31, H30, H4, H34, H17, H9, H20, H35, H25, and H27) are different from the most abundant haplotype H1 by one mutational change only while haplotype H32 differs from haplotype H1 by 49 mutational changes (Fig. 1). Other haplotypes differ from haplotype H1 by various mutational changes ranging from two (e.g. H12) to 21 (H36) (Fig. 1).

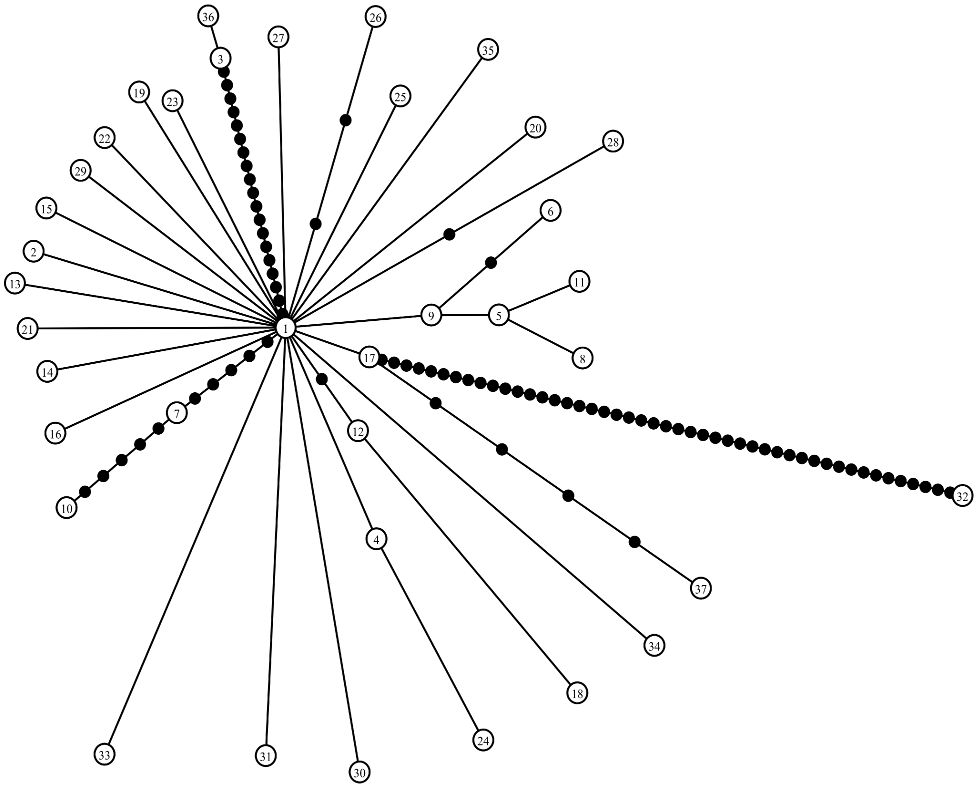

Figure 1: *Statistical parsimony networks showing genealogical relationships among 37 cpDNA haplotypes detected among invasive and native populations of Brassica nigra. Haplotypes H1, H3, H2, and H4 were the most abundant. Haplotypes H5, H9, H12, and H21 were each harboured by two individuals. The rest of the haplotypes occurred only once. Each line connecting the ellipses represents a mutational change between haplotypes. Empty ellipses represent nonsampled or extinct haplotypes. There were 49 mutational changes between H1 (likely ancestral haplotype) and H32 (likely the most recent haplotype to diverge). Some steps between H1, H21 and H36 have been truncated for purposes of clarity.*

AMOVA tests showed that there is no genetic differentiation in cpDNA between invasive and native range populations (Table 4). However, there is a significant genetic differentiation among and within populations when data for invasive and native populations were combined (Table 4). When AMOVAs were carried out on native and invasive populations separately, the three regions within the native range (i.e. African and European countries surrounding the Mediteranean sea, Western/Central/Northen Europe, and Asia) did not differ statistically in cpDNA haplotypes (Table 4). Nevertheless, there was a significant cpDNA variation among and within populations in those three regions (Table 4). AMOVA on data for invasive populations did not detect a significant differentiation in cpDNA among the four states (Fig. 4). However, there was a significant differentiation among populations within those states (Fig. 4).

Table 4: *Analysis of molecular variance (AMOVA) using genetic variation in TrnL-TrnF region of chloroplast DNA among invasive and native populations of Brassica nigra. Significant values are marked in bold font.*

Source of variation	*df*	Sums of squares	Variance components	Percentage of variation	P
Native + invasive ranges					
Between invasive ranges	1	8.643	-0.08610	-5.69	0.52102
Among populations	21	197.074	0.69553	46.00	**<0.001**
Within populations	279	251.826	0.90260	59.69	**<0.001**
Total	301	457.543	1.51203		
Native range					
Among regions	2	11.248	-0.11697	-5.91	0.52786
Among populations	16	184.453	0.96351	48.64	**<0.001**
Within populations	192	217.792	1.13434	57.26	**<0.001**
Total	210	413.493	1.98087		
Invasive range					
Among states	3	0.797	-0.01371	-4.18	0.80
Among populations within states	9	4.411	0.02873	8.77	**0.026**
Within populations	74	23.125	0.31251	95.41	0.13881
Total	86	28.333	0.32753		

Mantel tests showed that there was no correlation between pairwise genetic distances (F_{ST}) and geographic distances among the native and invasive populations combined nor among the native populations alone (Figs. 2a & b, respectively). Nevertheless, there was a significant positive correlation among pairwise genetic distances (F_{ST}) and geographic distances among invasive populations (Fig. 3).

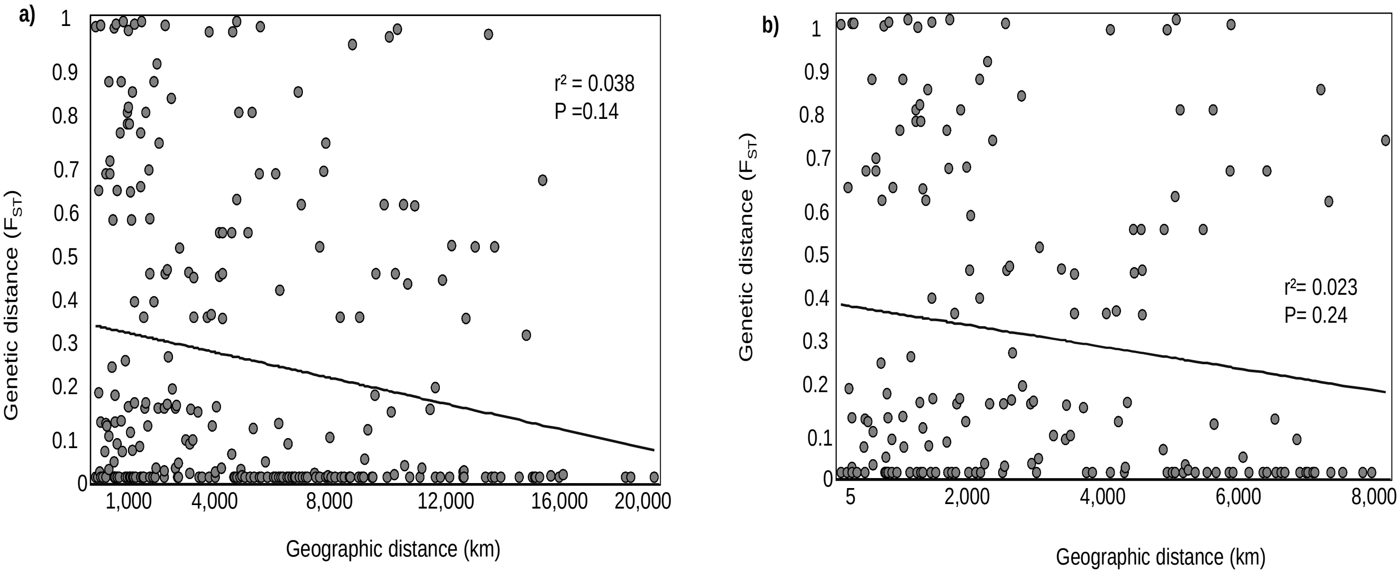

Figure 2: *Correlation between pairwise genetic (F_{ST}) and geographic distances among invasive and native populations combined (**a**) and native populations only (**b**) of Brassica nigra. All F_{ST} values <0 were changed to zero for plotting.*

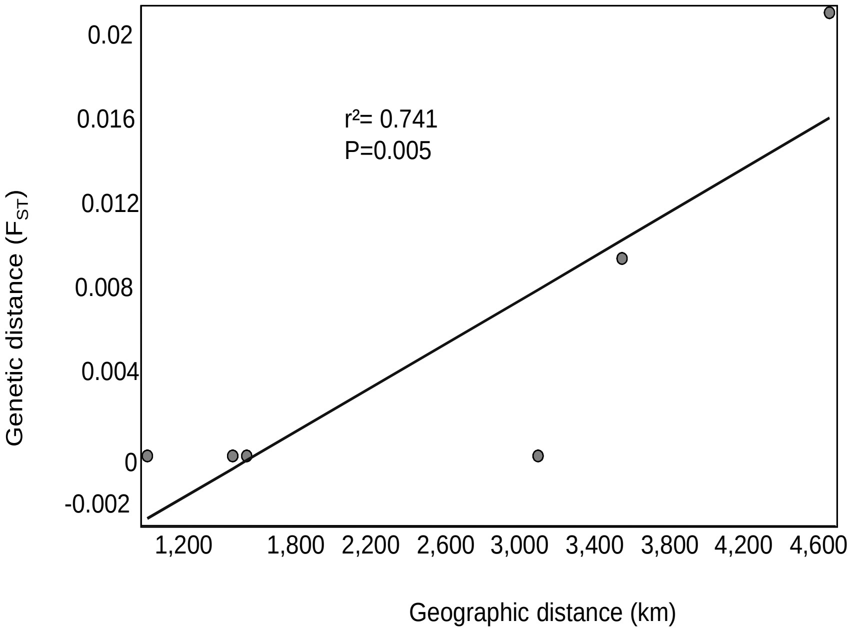

Figure 3: *Correlation between pairwise genetic (F_{ST}) and geographic distances among invasive populations of Brassica nigra. All F_{ST} values <0 were changed to zero for plotting*

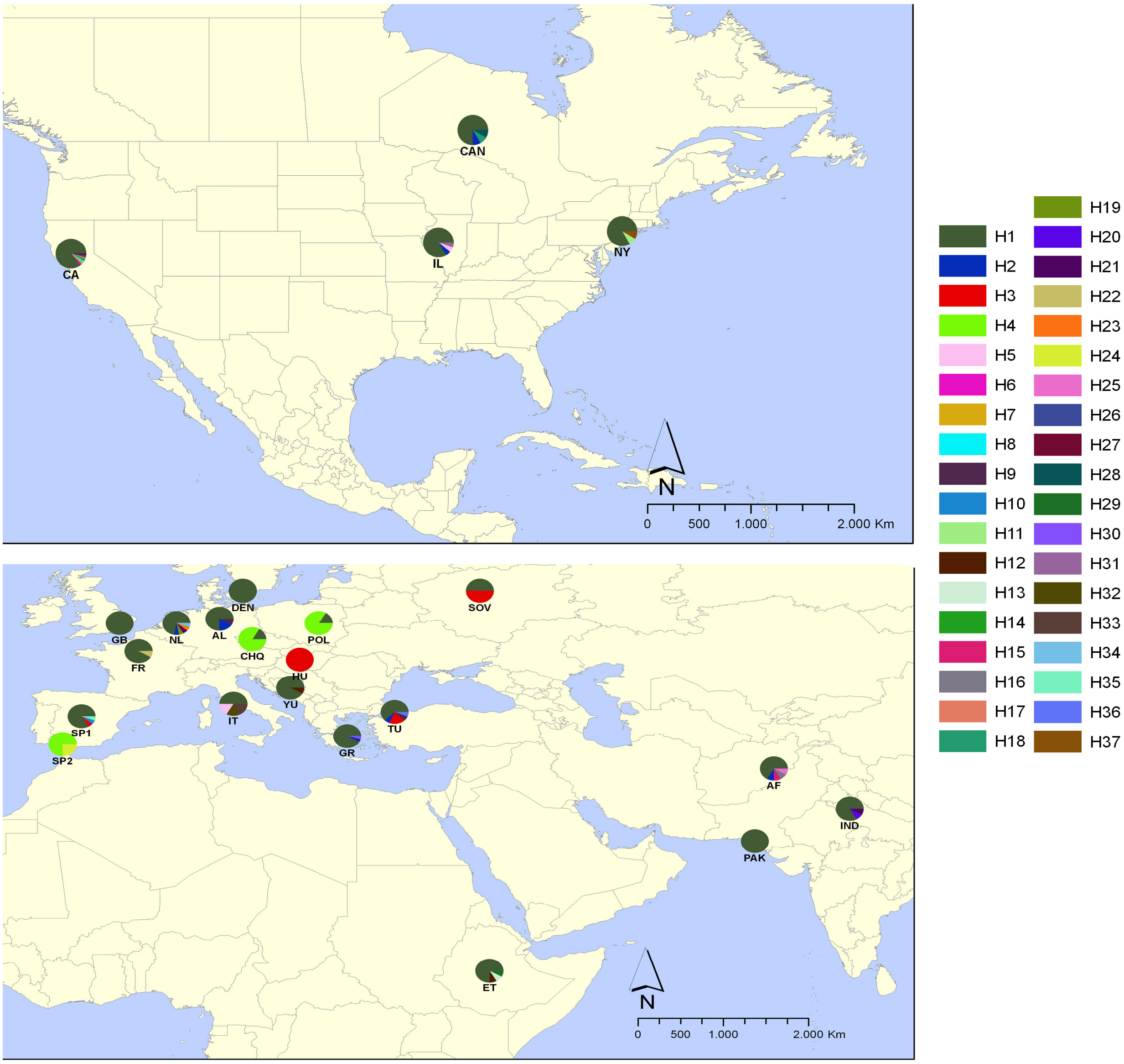

Figure 4: *Geographical distributions of chloroplast DNA haplotypes identified in invasive (North American) (top) and native (European, Asian and African) populations of Brassica nigra (bottom). Circle sizes are proportional to the number of samples per population. Colour bars on the right represent each of the individual haplotypes (H1-H37).*

DISCUSSION

Molecular evidence for multiple introductions of invasive genotypes from native to invasive ranges has been reported before. For example, Kolbe et al. (2004)

found two pieces of evidence for multiple introductions of an invasive Cuban lizard into Florida: all of the haplotypes identified in 8 different clades co-occurred in Florida, while they did not co-occur in the native range in Cuba. Furthermore, haplotypes whose distributions were distantly separated in Cuba occurred together in Florida. Two distinct haplotypes of invasive Brazilian peppertree that co-occurred in Florida came from spatially distant sources in South America (Williams et al. 2005). Three distinct European regions to which *Alliaria petiolata* is native shared common alleles with invasive North American populations of the plant (Durka et al. 2005). Our current results also support the idea of multiple introductions particularly with respect to haplotypes H2 and H5 (Table 3; Fig. 4). Within the native range, haplotype H2 occurs in populations from Afghanistan, Germany, Turkey, Greece and Netherlands, while in the invasive range H2 occurs in populations from Illinois and Canada (Table 3; Fig. 4). Haplotype H5 is shared between a native range population from Italy and an invasive range population from Illinois (Table 3; Fig. 4). Thus the various native populations that share haplotypes with various invasive populations could be sources of introductions of the invasive populations. A genealogical relationship among haplotypes presented in Fig. 1 shows that haplotypes H8 and H11 are derived from haplotype H5. Because haplotype H5 is found within an Italian population in a native range (Table 3; Fig. 4), it is possible that haplotypes H8 and H11 were introduced from Italy to California and New York, respectively (Table 3; Fig. 4). Haplotype H6 derived directly from haplotype H9 (Fig. 1). Hence a Californian population harbouring H6 was possibly introduced from

German/Turkish populations harbouring H9 (Table 3; Figs. 1& 4). Similarly, haplotype H18 found in a Canadian population descended directly from H12 found in populations from Yugoslavia and Ethiopia (Table 3; Figs. 1 & 4). Therefore, the Canadian populations having H18 was likely introduced from Yugoslavia and Ethiopia. Therefore, haplotypes H6 and H18, probably also underwent multiple introductions into California and Canada, respectively. This latter kind of inference of possible multiple introductions of descendant haplotypes into invasive ranges derived from ancenstral haplotypes in distinct locations in the native ranges is based on an interpretation of similar data as done by Hufbauer and Sforza (2008). The occurrence of private haplotypes (H6-H8, H11, H14, H17-H18, H26-H28, and H31 & H37) among invasive populations (Table 3; Fig. 4) suggests that some native populations that were sources of introductions of the private haplotypes remained unsampled.

GENETIC DIVERSITY AND STRUCTURE

The observed absence of genetic structuring between invasive and native range populations as revealed by the AMOVA test may be explained by sharing of three haplotypes (H1, H2, and H5) between the invasive and native populations. All the three haplotypes occur in more than 75 % all of the 302 individuals genotyped and are widely distributed both among the invasive and native populations (Table 3; Fig. 4). Interestingly, haplotypes H2 and H5 are themselves likely products of multiple introductions as discussed in the preceding paragraph. Multiple introductions of genotypes into invasive ranges may lead to invasive populations having similar genetic diversity as native populations (e.g. Allendorf and Lundquist 2003; Walker et al. 2003; Kolbe et al. 2004; Durka et al. 2005; Dlugosch and Parker 2008). Because all the AMOVA analyses were based on genetic distances among the 37 haplotypes, haplotypes H6 and H18 that also possibly underwent multiple introductions could also have contributed to the lack of significant genetic structuring between invasive and native populations of *B. nigra*. This is because there were small genetic distances between H6, H18 and ancestral haplotypes from which they descended (Fig. 1). The lack of genetic structuring among three regions within the native range (Table 4) could be due to highly variable patterns of migration and flow of seeds harboring various haplotypes among the regions. This finding is similar to those of Sakai et al. (2001) and Tsy et al. (2009). On the other hand, the genetic structure noted among and within populations (Table 4) within the native range may indicate restricted dispersal of seeds containing certain haplotypes, also similar to results of Tsy et al. (2009). The same explanation may apply to the results of AMOVA on invasive populations (Table 4).

Various studies have reported significantly positive (e.g. Durka et al. 2005; Genton et al. 2005; Williams et al. 2005; Memberg et al. 2006; Blum et al. 2007; Hufbauer and Sforza 2008; Ward et al. 2008; Alexander et al. 2009; Chun et al. 2009; Tang et al. 2009; Lachmuth et al. 2010; Le Roux et al. 2010) or zero (Dewalt and Hamrick 2004; Durka et al. 2005; Okada et al. 2007; Wang et al. 2008; Marrs et al. 2008; Ward et al. 2008) pairwise correlations between genetic differentiation and geographical distance either within the invasive or native ranges or both, of different invasive plant species. In our study, we found no significant correlation between pairwise genetic differentiation and geographic distances when data on native and invasive populations were combined (Fig. 2a), and also when data on native populations were treated separately (Fig. 2b). Such lack of significant correlation between pairwise genetic differentiation and geographic distances could result from range expansion (Slatkin 1993), multiple introductions of the same haplotypes into different areas, introductions of unrelated haplotypes to the same area, or any combination of the three factors (Schlaepfer et al. 2008). Our results support the idea of multiple introductions of haplotypes H2, H5, H6, and H18 that could have led to lack of significant correlation between pairwise genetic differentiation and geographic distances when data on native and invasive of *B. nigra* populations were analyzed together (Fig. 2a). Furthermore, widespread distribution of the dominant haplotype H1 in both invasive and native ranges could have caused such lack of significant correlation between pairwise genetic differentiation and geographic distances. On the other hand, range expansion (i.e. seed dispersal) among regions within the native range could have eliminated genetic structuring as our data show (Fig. 2b).

GENEALOGICAL RELATIONSHIPS AMONG THE HAPLOTYPES

Based on coalescent theory, the most common ancestral haplotype from which others evolved has the following features: a high frequency of occurrence, occupies a central position in the haplotype network, has more connections and a wider geographic distribution than other haplotypes (Avise 2000; Clement et al.

2000; Freeland 2005). Haplotype H1 seems to be ancestral, as it fulfills all the criteria above (Table 3; Figs.1& 4). Based on the same coalescent theory, haplotype H32 seems to be the most recent as it occupies the farthest position from the most frequent haplotype H1 (Fig. 1). As H32 was found in an Italian population, it seems that population was the most recent to diverge from ancestral population.

CONCLUSIONS

The most common and likely ancestral haplotype, H1, was widely distributed, occurring in many populations in both native and invasive ranges of *B. nigra*. Haplotypes H2, H5, H6, and H18 likely underwent multiple introductions from native to invasive ranges. Significant correlation between pairwise genetic differentiation and geographic distances existed in the invasive range only where there was also genetic structure among populations. Many private haplotypes occurred in both invasive and native ranges. This means that our sampling was not exhaustive enough and that a more thorough sampling of native populations would identify sources of introductions of the private haplotypes that occur among invasive populations.

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Appendix 1. A matrix of pairwise genetic distances (F_{ST}) among and within invasive and native populations of *Brassica nigra* .

	AF	GE	CA	CAN	CHQ	DEN	ET	FR	GB	GR	HU	IL	IND	IT	NL	NY	PAK	POL	SOV	SP1	SP2	TU	YU
AF	0.00																						
GE	0.00	0.00																					
CA	0.00	0.01	0.00																				
CAN	0.00	0.00	0.01	0.00																			
CHQ	0.54	0.63	0.50	0.45	0.00																		
DEN	0.00	0.12	0.00	0.00	0.87	0.00																	
ET	0.00	0.05	0.00	0.00	0.54	0.00	0.00																
FR	0.00	0.09	0.00	0.00	0.75	0.00	0.00	0.00															
GB	0.00	0.03	0.00	0.00	0.80	0.00	0.00	0.00	0.00														
GR	0.01	0.07	0.00	0.02	0.78	0.00	0.03	0.00	0.00	0.00													
HU	0.98	0.99	0.97	0.97	0.99	1.00	0.98	0.99	1.00	0.99	0.00												
IL	0.00	0.00	0.00	0.00	0.60	0.00	0.01	0.00	0.00	0.00	0.98	0.00											
IND	0.00	0.07	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.02	0.99	0.00	0.00										
IT	0.11	0.11	0.35	0.10	0.06	0.12	0.11	0.12	0.00	0.25	0.69	0.18	0.12	0.00									
NL	0.00	0.01	0.00	0.00	0.56	0.00	0.00	0.00	0.00	0.00	0.98	0.00	0.00	0.18	0.00								
NY	0.00	0.00	0.02	0.00	0.35	0.00	0.00	0.00	0.00	0.04	0.95	0.00	0.00	0.09	0.01	0.00							
PAK	0.00	0.03	0.00	0.00	0.80	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00						
POL	0.54	0.63	0.50	0.45	0.00	0.87	0.54	0.75	0.80	0.78	0.99	0.60	0.67	0.06	0.56	0.35	0.80	0.00					
SOV	0.44	0.45	0.65	0.43	0.38	0.45	0.44	0.45	0.35	0.57	0.35	0.51	0.45	0.27	0.50	0.42	0.35	0.38	0.00				
SP1	0.00	0.00	0.02	0.00	0.15	0.00	0.00	0.00	0.00	0.01	0.87	0.00	0.00	0.10	0.00	0.00	0.00	0.15	0.36	0.00			
SP2	0.60	0.67	0.60	0.51	0.02	0.91	0.61	0.81	0.85	0.83	0.99	0.67	0.73	0.00	0.64	0.41	0.85	0.02	0.35	0.19	0.00		
TU	0.15	0.15	0.31	0.15	0.16	0.16	0.15	0.16	0.08	0.24	0.63	0.20	0.16	0.11	0.20	0.14	0.08	0.16	0.06	0.07	0.14	0.00	
YU	0.00	0.07	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.02	0.99	0.00	0.00	0.12	0.00	0.00	0.00	0.67	0.45	0.00	0.73	0.16	0.00

CHAPTER 3:

INVADER POPULATIONS OF *BRASSICA NIGRA* HAVE LOWER GLUCOSINOLATE DIVERSITY THAN NATIVE POPULATIONS

Ayub M.O. Oduor, Richard A. Lankau, Sharon Y. Strauss, and José M. Gómez

ABSTRACT

Evolution of increased competitive ability (EICA) hypothesis predicts that escape from natural enemies allow certain exotic plants to be successful invaders because they have evolved into highly vigorous and poorly defended genotypes. However, invasive plants often undergo only a change in herbivore community with which they interact. In the exotic ranges, most of the herbivores that attack invasive plants are generalists, while in the native ranges, the plants are commonly attacked by both generalist and specialist herbivores. Under such circumstances, it should be expected that the invasive plants have undergone selection to express a smaller diversity of chemical defence compounds than their native conspecifics that need to maintain a higher diversity of such compounds against a more diverse assemblage of herbivores. Thus far, no reports exist of any study that explicitly compared diversities of defence compounds between invasive and native populations of any plant. Here, using 11 invasive and 14 native populations of *Brassica nigra* grown in a common garden in its native range (Spain), we found that invasive populations of *B. nigra* had significantly lower diversity of glucosinolates than native populations of the same plant. Invasive and native populations of *B. nigra* expressed similar concentrations of total glucosinolates. Nevertheless, invasive populations expressed higher concentrations of sinigrin (the main glucosinolate compound in *B. nigra*). A field survey showed that the invasive and native populations of *B. nigra* harboured similar species richness and diversities of generalist and specialists herbivores. Nevertheless, concentrations of leaf sinigrin and total glucosinolates were significantly positively correlated with species richness of specialist herbivores for invasive populations of *B. nigra*.

Key words: *Brassica nigra,* EICA hypothesis, diversity of defence compounds, glucosinolates, invasive populations, native populations, common garden experiment, generalist/specialist herbivores.

INTRODUCTION

According to the evolution of increased competitive ability (EICA) hypothesis, certain exotic plants are successful invaders because they left behind their co-evolved natural enemies in their native ranges (Blossey and Nötzold 1995). However, escape from co-evolved natural enemies does not mean that the plants are free from herbivory given that generalist herbivores are frequent and widespread in most both terrestrial and aquatic ecosystems (Cyr and Pace 1993; Keane and Crawley 2002). As such, invasive plants should not be expected to undergo a complete escape from herbivory, but rather, a change in herbivore community with which they interact. In their native ranges, the invasive plants are subjected to herbivory pressure by both generalist and specialist herbivores, while in the invasive ranges, the plants suffer herbivory mainly from generalists, and no or only very few specialists (Fenner and Lee 2001; Cripps et al. 2006; see Liu and Stiling 2006 for meta-analysis; Puliafico et al. 2008). To cope with herbivore damage, plants produce a suite of chemical defences that have been shown to vary at the intraspecific level (Schoonhoven et al. 2005; Hopkins et al. 2009; Poelman et al. 2009). As an enormous diversity of related defence compounds can be synthesized within a plant species (Schoonhoven et al. 2005; Hopkins et al. 2009; Poelman et al. 2009; Kleine and Müller 2010), a mix of such different individual compounds (i.e. chemical diversity) within a plant species may structure the herbivore community with which the plant interacts (Mithen et al. 1995; Johnson and Agrawal 2005; Bidart-Bouzart and Kliebenstein 2008; Poelman et al. 2009, Newton et al. 2009; Kleine and Müller 2010). Conversely, herbivores may differ in their preferences for plant genotypes that have different chemical profiles; these differential preferences may result in herbivores exerting selective pressures on plants to produce diverse chemical defence compounds depending on the species identity of herbivores at hand (Agrawal and Kurashige 2003; Mithen et al. 1995; Van der Meijden 1996; Shonle and Bergelson 2000; Lankau 2007; Hopkins et al. 2009; Poelman et al 2009; Newton et al. 2009; Kleine and Müller 2010).

Differences in herbivore species richness and diversity between exotic and native ranges may result in invasive plants facing different selective pressures on their chemical defences (Müller-Schärer et al 2004; Joshi and Vrieling 2005). Certain defence compounds such as, for example, glucosinolates and terpenoids, act in a species-specific manner against herbivores (Agrawal and Kurashige 2003; Hopkins et al. 2009; Kleine and Müller 2010). This means that a single glucosinolate/terpenoid compound may not be effective against all types of herbivores with which a plant interacts (Agrawal and Kurashige 2003; Hopkins et al. 2009; Kleine and Müller 2010). Therefore, a higher diversity of both generalist and specialist herbivores in the native ranges may select for maintenance of plant genotypes that express high diversity of defence compounds. On the other hand, the relatively lower diversity of herbivores in the invasive ranges may select for plant genotypes that have a lower diversity of such defence compounds. Because production of defence compounds is thought to have both ecological and physiological costs (Strauss et al. 2002; Cipollini et al. 2003; Koricheva et al. 2004), invasive plants in the exotic ranges may undergo a post-introduction evolutionary change driven by a low diversity of herbivore community to re-allocate resources from production of a high diversity of defence compounds to growth and reproduction, hence conferring upon the invasive plants a competitive edge over local plants in the exotic ranges. The post-introduction evolutionary change in chemical diversity may occur after multiple introductions of various genotypes expressing different defence compounds have occurred from native to invasive ranges. Multiple introductions of genetic variation may dilute or eliminate founder effects 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 and Parker 2008). Alternatively, invasive populations may be selected to invest heavily in a reduced set of the chemical defences that are most effective against the simplified herbivore communities they face in their new range.

Only a few studies to date have been undertaken to compare expressions of two or more chemical defence compounds between invasive and native populations of plants (Cipollini et al. 2005; Joshi and Vrieling 2005; Hull-Sanders et al. 2007; Wheeler et al. 2007; Franks et al. 2008). Of those studies, only two (Cipollini et al. 2005 and Hull-Sanders et al. 2007) compared variations in concentrations of defence compounds between invasive and native populations. However, none of those studies employed any one of the known indices of diversity to explicitly compare diversities of chemical defences between invasive and native populations of the plants. As such, it is still unclear whether or not invasive and native populations of various plants differ significantly in the diversity of chemical defences they express. Here, we made two predictions regarding post-introduction evolutionary changes in chemical defences in *Brassica nigra* (Brassicaceae):

1. Invasive populations of *B. nigra* express a lower diversity of glucosinolates because they interact with a less diverse herbivore community than native populations of *B. nigra*.
2. Because they express a lower diversity of glucosinolates, invasive populations should harbour a higher diversity of arthropod herbivores than native populations of the same plant when they are grown in a common garden within a native range.

MATERIALS AND METHODS

STUDY SYSTEM

Brassica nigra (L.) W. D. J. Koch is a dicotyledonous annual plant native to the Mediteranean region, North Africa, Asia, and Europe and introduced into North America (Bell and Muller 1973; Feeny and Rosenberry 1982). In the United States of America, *B. nigra* is an invader, where it can form thick monospecific stands, although in generally disturbed areas (Lankau and Strauss 2008). *B. nigra* defends from herbivores by producing glucosinolates, a class of secondary compounds derived from several amino acids (Feeny and Rosenberry 1982). Glucosinolates compounds are synthesized and expressed by members of the Brassicaceae family to which *B. nigra* belongs (Fahey et al. 2001). Over 120 different glucosinolate compounds are known to exist, although a given plant species contains just a limited number of such compounds (Hopkins et al. 2009). There are also variations within a plant species with regard to identity and concentrations of various glucosinolate compounds they express (Poelman et al. 2009). In *B. nigra,* sinigrin (allyl-glucosinolate) may represent upto 90%–99% of the total glucosinolate concentration, and has a heritable basis (Feeny and 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 (e.g. Agrawal and Kurashige 2003; Kliebenstein 2004).

PLANT MATERIAL

Seeds of 11 invasive (North American) and 14 native (Mediteranean region, European, African and Asian) populations of *B. nigra* were field-collected by the authors and their colleagues or obtained from the United States Department of Agriculture (USDA) germplasm resources information network (GRIN) (Table 1).

Table 1: *Invasive and native populations of Brassica nigra used in the current experiment. Asterisks (*) indicate populations whose accession numbers and collection sites were not provided by the botanical gardens from which the seeds were sourced. Geographical coordinates of populations marked by † were not noted down. However, those populations were spaced at least 30 km apart.*

Country/State of origin	Accession number or collector's name	Geographic region	Latitude	Longitude	Invasive status
Hungary	*	Europe			Native
Germany	PL 633142	Europe	51° 25' 0" N	12° 1' 0" E	Native
Germany	PL 633143	Europe	51° 49' 0" N	11° 17' 0" E	Native
Poland	PL 358590	Europe	52°13'56" N	21°0 '30" E	Native
Poland	*	Europe	49° 28'43"N	17° 7' 20"E	Native
Turkey	PL176881	Mediteranean	39° 52' 0" N	32° 52' 0" E	Native
Turkey	PL592737	Mediteranean	39° 38' 5" N	27° 53' 6"E	Native
Turkey	PL169066	Mediteranean	40° 2' 47" N	27° 58'12" E	Native
Ethiopia	PL597830	Africa	9° 1' 48 " N	38° 44' 24"E	Native
Afghanistan	PL274284	Asia	34° 0' 0" N	69° 0' 0" E	Native
Italy	PL633148	Mediteranean	40° 10' 0" N	16° 31' 0"E	Native
Ireland	PL 649155	Europe	52° 03' N	009° 30' W	Native
Canada	Ames 25399	North America	43° 40' 0" N	79° 25' 0" W	Invasive
Greece	PL263866	Mediteranean	37° 58' 0" N	23° 43' 0" E	Native
Greece	PL633147	Mediteranean	37° 20' 5" N	22° 21' 08" E	Native
Spain	PL597829	Mediteranean	36° 0' 0" N	6° 0' 0" E	Native
Spain	*	Mediteranean			Native
Illinois	R. Lankau	North America	†		Invasive
New York	J. Conner	North America	†		Invasive
New York	J. Conner	North America	†		Invasive
California	R. Lankau	North America	†		Invasive
California	R. Lankau	North America	†		Invasive
California	R. Lankau	North America	†		Invasive
California	R. Lankau	North America	†		Invasive
California	R. Lankau	North America	†		Invasive

EXPERIMENTAL DESIGN

Seeds from all populations were sown in a commercial potting substrate (dry matter: 60-62%, total organic matter:74-76%, pH:5.2-5.5, electrical conductivity:0.4-0.5, Nitrogen: 100-150 mg/L, P_2O_5: 150-200 mg/L, K_2O:200-250 mg/L, total pore space:85-90 %) in organic trays (with each cubicle measuring 5 cm x 5 cm x 6 cm) in a glasshouse in April 2008. The resulting seedlings were raised under glasshouse conditions for two and a half weeks (under temperatures that varied from 6°C at night to 25° C during the day), after which 20 seedlings per population were transplanted to a field plot in Granada, Spain (37° 10' 30" N, 03° 38' 10" W) from 25th April to 2nd May 2008. The plot was divided into 20 rows. Each row was planted with one seedling from each of the 25 populations (resulting in 25 plants per row), with the population arrangements being randomized per row. Thus each row was a complete replicate (resulting in a completely randomized design). The seedlings were planted 50 cm apart within a row, and the rows were spaced 150 cm apart. The transplanted seedlings were watered regularly until they established. This experiment had a broad objective that also included comparing growth and reproductive performance between invasive and native populations of *B. nigra* when exposed to natural level of herbivory and when protected from herbivores. Therefore, an insecticide and a molluscicide were applied on a weekly basis to release half of the plants from herbivory. The remaining half of the plants was treated with water from the same source as that used for insecticide spray (i.e. this served as a control treatment). The insecticide used was KB® (active ingredients: Bifentrin

10% EC) at a rate of 2g/L. The molluscicide used was Carmort® (active ingredient: metaldehyde 5%, p/p (50g/Kg) at a rate of 5Kg/Ha.

COLLECTION OF LEAF MATERIAL FOR GLUCOSINOLATE ANALYSIS

Leaf tissue for glucosinolate analysis was obtained on the 4th week following transplanting by punching five leaf discs from the youngest fully expanded leaf with a paper hole punch (5 mm in diameter). The leaf discs were then held immediately in 95 % methanol in 1.2 mL eppendorf tubes that were then stored at 4°C until glucosinolate analysis was done.

GLUCOSINOLATE ANALYSIS

Glucosinolate contents 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 minute in a Qiagen FastPrep-24 tissue homogenizer. After shaking, the tubes sat for 1 hour, were centrifuged and 300 ml of the supernatant passed through a DEAE-Sephadex column, using 96-well microtiter plates. Columns were washed twice with 70% methanol and twice with water. Desulphoglucosinolates were extracted from the column by adding 100 ml of a 5% sulfatase enzyme solution and incubating overnight. The resulting solution was transferred to a new 96 well plate and stored at 4 ºC until analysis on a HPLC equipped with an auto sampler and a diode array detector. Glucosinolate compounds were identified by comparison of retention times and absorbance spectra with standards or mass spectroscopy (Kliebenstein et al. 2001).

SCORING FOR HERBIVORES THAT OCCURRED ON PLANTS

Arthropods that occurred on plants in rows not treated with insecticide/ molluscicide were noted every two weeks by visiting each plant and inspecting the leaves and flowers. Arthropod identity and abundance was noted. Aphids were the most abundant arthropods, they abounded particularly on flowers. As each *B. nigra* plant had several bunches of flowers, it was difficult to count individually all of the aphids that occurred on each plant. Thus only an estimate of the number of aphids per plant was done. To do this, all aphids occurring in four bunches of flower were counted after which an average number of aphids per flower bunch was obtained from the four bunches. The average number of aphids per flower bunch was multiplied by all flower bunches per plant, and this gave us an estimate of total number of aphids per plant.

STATISTICAL ANALYSES

Glucosinolate diversities for invasive and native populations of *B. nigra* were determined using Shannon-Wiener (H´) diversity index employing the software EcoSim 7.0 (Gotelli and Colwell 2005). Thereafter, the diversity indices were compared using a linear mixed-effects model (LMM) in which invasive status of *B. nigra* (invasive or native), insecticide and molluscicide treatment (insecticide and molluscicide applied or not), and interactions between them were treated as fixed-effect independent variables. Populations were treated as a random-effect variable and nested within invasive status. In the analysis, tube weight was also included as a fixed-effect independent variable because spillage of methanol in which leaf tissues were preserved occurred in some tubes during transportation. Tube weight acted as a proxy measure for the amount of spillage and evaporation, which could affect chemical concentrations. The same model was run to compare invasive and native populations of *B. nigra* for concentrations of total glucosinolates and sinigrin (i.e. the most abundant glucosinolate that was expressed by all plants sampled) in particular. Diversity and species richness of all herbivores were also compared for invasive and native populations of *B. nigra*. Species richness and two diversity indices, Shannon-Wiener (H´) and Hulbert´s PIE, were calculated for each population using EcoSim 7.0 (Gotelli and Colwell 2005). The resulting species richness and diversity indices were then compared using linear and generalized linear mixed effects models (LMM and GLMM, respectively). Invasive status of *B. nigra* (i.e. invasive or native) was specified as a fixed-effect independent variable while populations were treated as a random-effect variable and nested within invasive status. Species richness was fitted to a Poisson (GLMM), whereas diversity indices were fitted to a Normal distribution (LMM). The LMM and GLMM models of herbivore species richness and diversity indices were run for generalist and specialist herbivores combined, and also separately. Furthermore, an analysis of covariance (ANCOVA) was used to test for possible correlations between leaf concentrations of sinigrin/ total glucosinolates, glucosinolate diversity and species richness, abundances and diversity indices (Shannon-Wiener, H´, and Hulbert´s PIE) of generalist and specialist herbivores combined. In the ANCOVA, dependent variables were: species richness, abundances and diversity of herbivores, while fixed-effect independent variables were: invasive status of *B. nigra* and concentrations of sinigrin/ total glucosinolates, and glucosinolate diversity. Populations were treated as a random-effect independent variable and nested within invasive status. A significant interaction between invasive status of *B. nigra* and diversity/concentrations of glucosinolates/sinigrin was treated as a significant correlation between the glucosinolates/sinigrin and abundances, diversity, and species richness of the herbivores for invasive or native populations of *B. nigra*. All GLMM and LMM models were run on R version 2.9.1 (R Development Core Team 2009). The ANCOVA was done by JMP 7.0 (SAS Institute Inc. 2007).

RESULTS

A total of seven glucosinolate compounds were detected between invasive and native populations of *B. nigra* (Fig. 1). Sinigrin was the most dominant compound, representing 97.28 % and 76.72% of total glucosinolates for invasive and native populations, respectively (Fig. 1). Invasive status of *B. nigra* and tube weight had significant main effects on glucosinolate diversities (Table 2). Invasive populations of *B. nigra* had significantly lower diversities of glucosinolates under both types of insecticide/ molluscicide treatments (Fig. 2). Neither main effect of insecticide / molluscicide treatment nor interaction between insecticide / molluscicide treatment and invasive status of *B. nigra* influenced glucosinolate diversities (Table 2). Only tube weight had a significant main effect on concentration of total glucosinolate accumulation (Table 2). Invasive status of *B. nigra* and insecticide / molluscicide treatments did not have main or interactive effects on concentration of total glucosinolates (Table 2). Invasive and native populations of *B. nigra* had similar concentrations of total glucosinolates (Fig. 3a). Invasive status and tube weight had significant main effects on concentrations of sinigrin, with invasive populations expressing higher concentrations of sinigrin than native populations under both insecticide/molluscicide treatments (Table 2; Fig. 3b). However, there was no significant main effect of insecticide /molluscicide treatment or its interaction with invasive status of *B. nigra* on concentration of sinigrin (Table 2).

Table 2: *Linear mixed-effects model (LMM) showing effects of invasive status of Brassica nigra, insecticide/molluscicide treatment, interaction between them, and tube weight on glucosinolate diversity (based on Shannon-Wiener (H´) diversity index) and concentrations of total glucosinolates and sinigrin. Populations were treated as a random-effect independent variable and nested within invasive status*

		Glucosinolate diversities		Concentration of total glucosinolates		Concentration of sinigrin	
Source	df	t	P	t	P	t	P
Invasive status	23	2.31	0.029	-0.71	0.48	-2.19	0.038
Insecticide/molluscicide	314	0.89	0.37	0.24	0.81	0.039	0.96
Invasive status * insecticide/molluscicide	314	-1.65	0.099	-1.67	0.08	-1.48	0.13
Tube weight	314	7.190	0.000	18.45	0.000	17.59	0.000

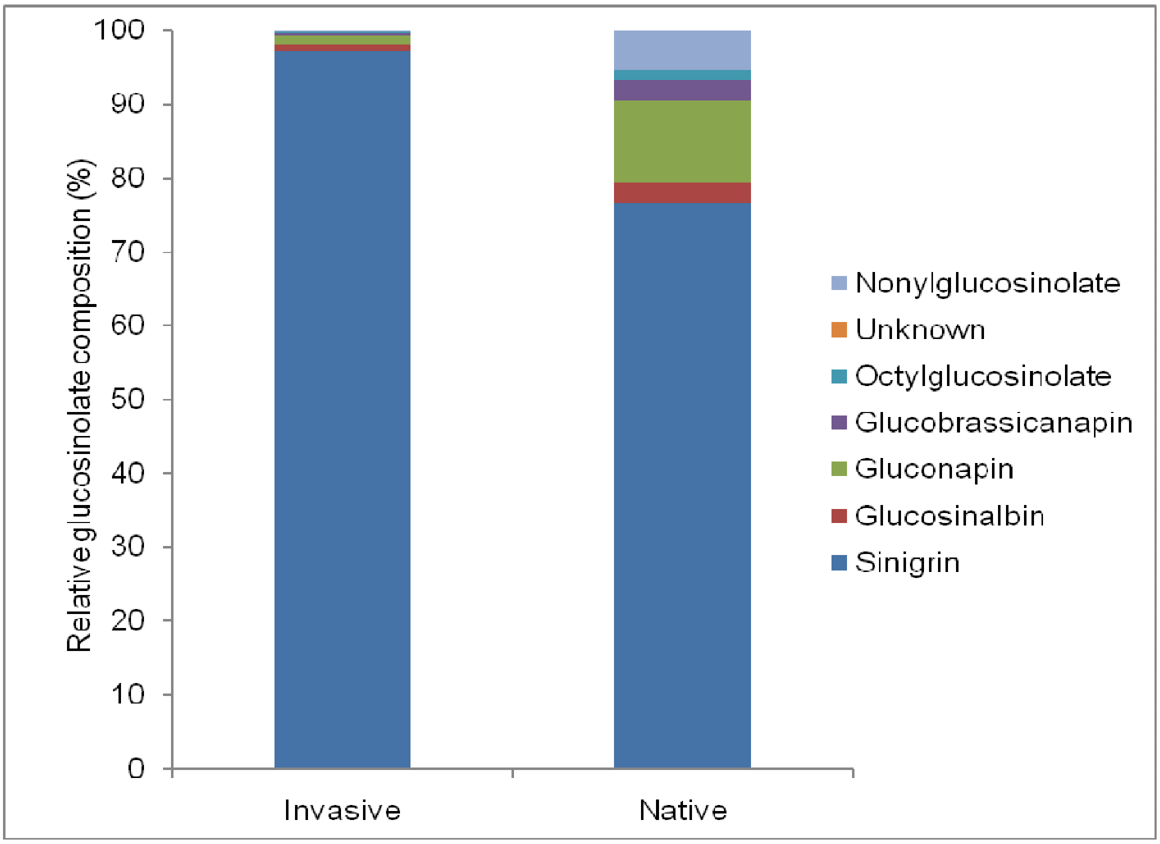

Figure 1: *Relative glucosinolate composition in invasive and native populations of Brassica nigra*

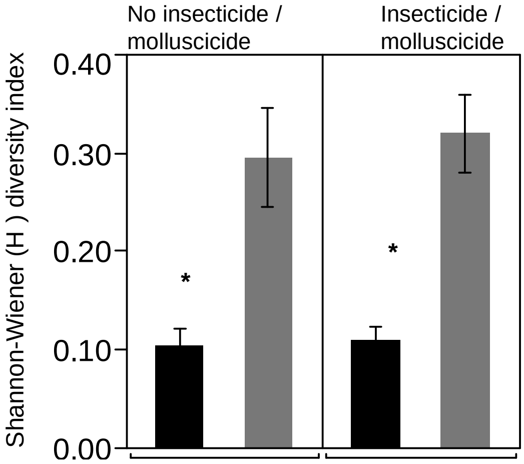

Figure 2: *Mean (± 1 SE) Shannon-Wiener (H´) diversity index of glucosinolates for invasive (black bars) and native (gray bars) populations of Brassica nigra under insecticide/molluscicide or no insecticide/molluscicide treatment. Asterisks (*) indicate statistically-significant differences.*

A total of 14 generalist and specialist herbivores were noted on invasive and native populations of *B. nigra* (see Appendix 1). Invasive and native populations of *B. nigra* had similar species richness and diversities (based on Shannon-Wiener, H´, or Hulbert´s PIE) of the generalist and specialist herbivores (Table 4; Fig. 4). Results of ANCOVA show that there was a significant positive correlation between glucosinolate diversity and generalist herbivore species richness for invasive populations (Table 3; Fig. 5). Concentrations of total glucosinolates and sinigrin were also positively correlated with species richness of specialist herbivores for invasive populations (Table 3; Fig. 6 a & c). There were no significant correlations between diversity and concentrations of glucosinolates/sinigrin and diversity indices of specialist and generalist herbivores combined or separately (results not shown).

Table 3: *Analysis of covariance (ANCOVA) showing effects of invasive status, diversity and concentration of glucosinolates and sinigrin, and interactions between them on species richness of generalist and specialist herbivores. Significant interactions show that there are significant correlations between diversities and concentrations of glucosinolates/sinigrin and species richness. P<0.01** *, P<0.01**, P<0.05*.*

	Glucosinolate diversity			Glucosinolate concentration			Sinigrin concentration		
Herbivore	Invasive status	Glucosinolate diversity	Invasive status * Glucosinolate diversity	Invasive status	Glucosinolate concentratiom	Invasive status* Glucosinolate concentratiom	Invasive status	Sinigrin concentration	Invasive status * Sinigrin concentration
Generalist	F=1.98	F=3.45	F=4.01*	F=0.028	F=0.0018	F=0.02	F=0.74	F=0.76	F=0.74
Specialist	F=2.04	F=3.51	F=0.48	F=2.13	F=5.14*	F=6.74*	F=5.7*	F=8.5**	F=15.39***

Table 4: *Linear and generalized linear mixed-effects models (LMM and GLMM) showing effect of invasive status of Brassica nigra on species richness and diversity indices (Shannon-Wiener, H´, and Hulbert's PIE) of herbivores noted on invasive and native populations of Brassica nigra. Total herbivores = generalist + specialist herbivores. Populationswere treated as a random-effect independent variable and nested within invasive status.*

Source	df	Total herbivores			Generalist Herbivores			Specialist Herbivores		
		Shannon-Wiener	Hulbert's PIE	Species richness	Shannon-Wiener	Hulbert's PIE	Species richness	Shannon-Wiener	Hulbert's PIE	Species richness
Invasive status	1	t= -0.65	t=0.208	z= -0.79	t= -1.28	t= -1.22	t= -0.55	t= -0.58	t= -0.6	z= -0.07
		P=0.52	P=0.83	P=0.43	P=0.211	P=0.24	P=0.58	P=0.56	P=0.55	P=0.51

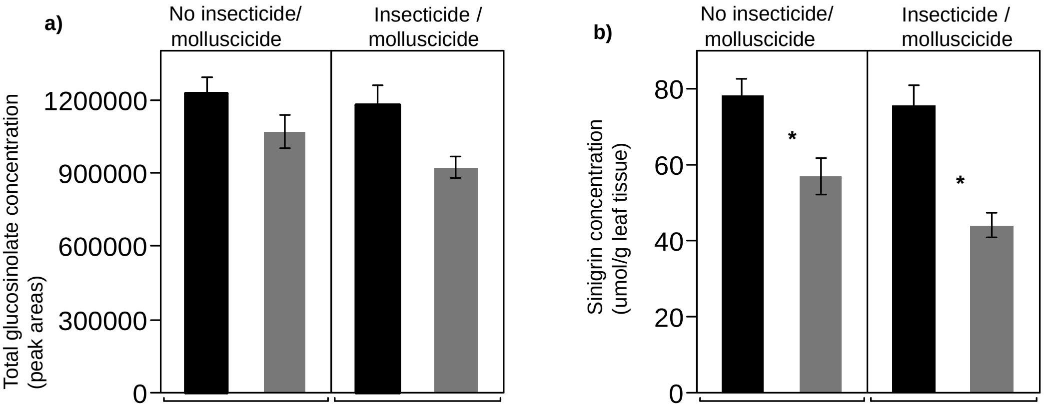

Figure 3: *Mean (± 1 SE) leaf concentrations of total glucosinolates (**a**) and sinigrin (**b**) for invasive (black bars) and native (gray bars) populations of Brassica nigra under insecticide/molluscicide or no insecticide/molluscicide treatment. Peak areas for total glucosnilates were not converted to concentrations as was done for sinigrin. However, peak area is directly proportional to concentration. Asterisks (*) indicate statistically-significant differences.*

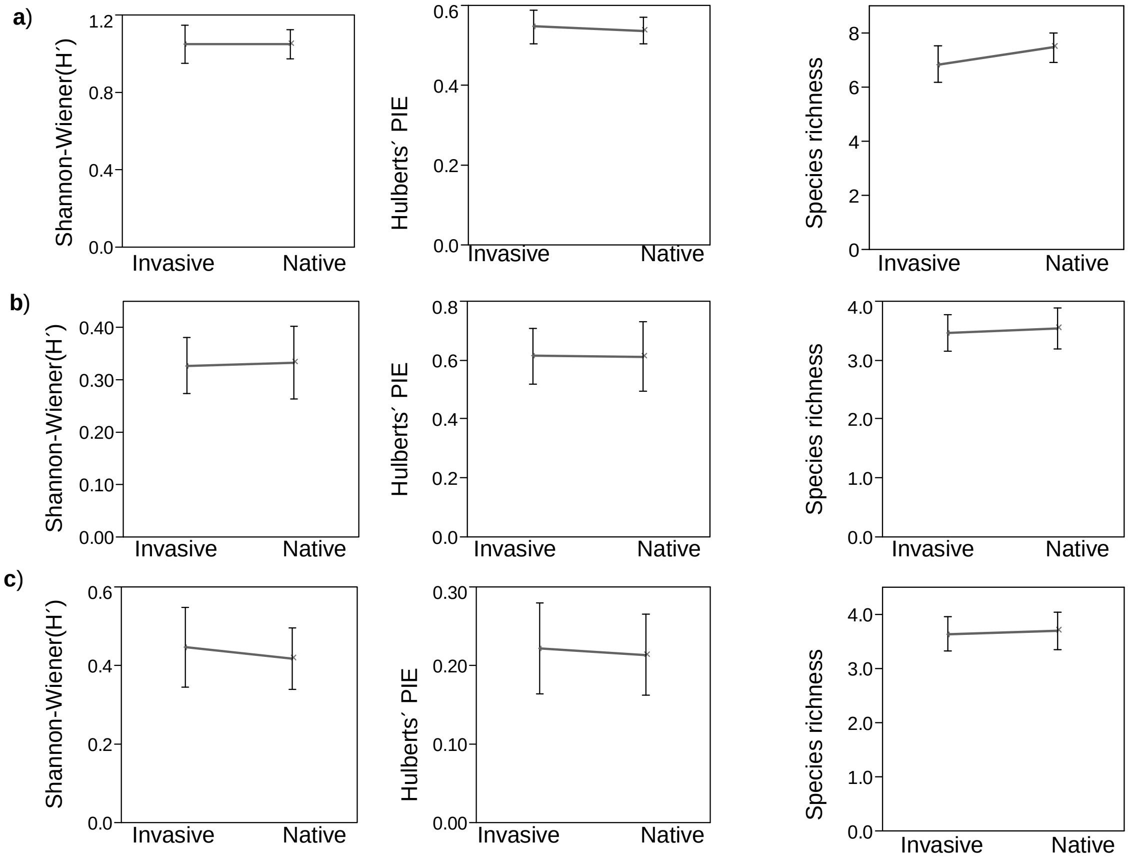

Figure 4: *Mean (± 1 SE) diversity indices (Shannon-Wiener (H´) and Hulberts´ PIE) and species richness of total herbivores (**a**), generalist herbivores (**b**), and specialist herbivores (**c**) noted on invasive (black bars) and native (gray) populations of Brassica nigra.*

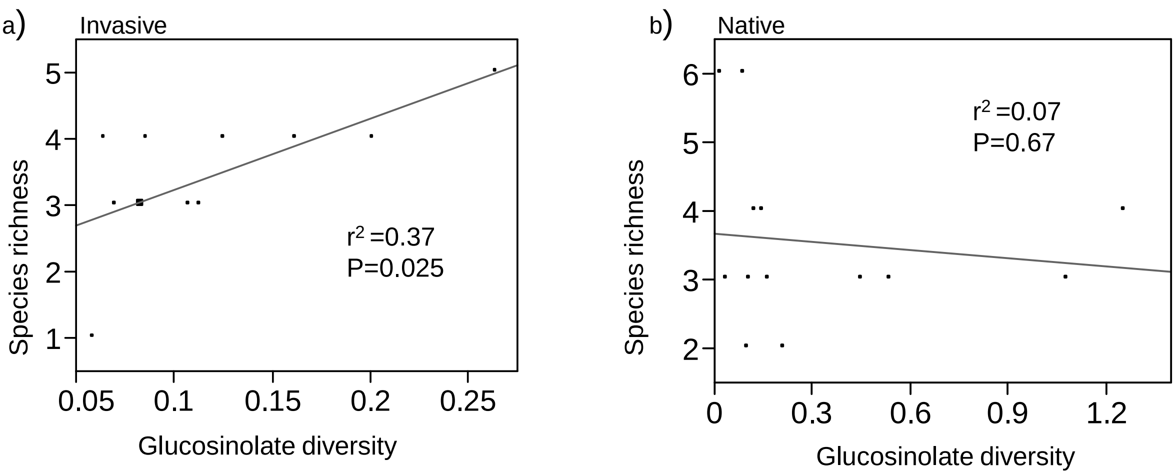

Figure 5: *Linear regressions showing correlations between glucosinolate diversity and species richness of generalist herbivores for invasive (**a**) and native (**b**) populations of Brassica nigra.*

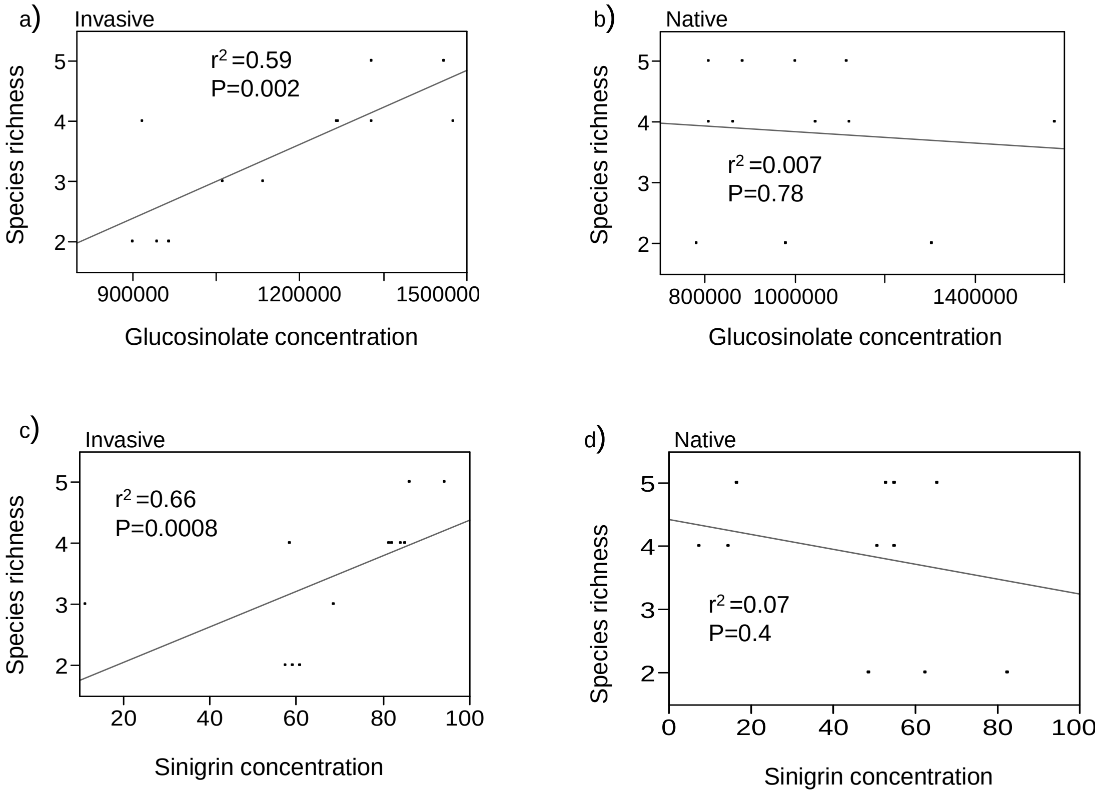

Figure 6: *Linear regressions showing correlations between glucosinolate and sinigrin concentrations and species richness of specialist herbivores for invasive (**a** & **c**) and native (**b** & **d**) populations of Brassica nigra.*

DISCUSSION

True to our prediction, invasive populations of *B. nigra* had a lower diversity of glucosinolates than native populations. Majority of studies summarized in a meta-analysis by Liu and Stiling (2006) have found that insect fauna richness is far less in invasive ranges than in native ranges of exotic invasive plants. This may explain why invasive populations of *B. nigra* express a lower diversity of glucosinolates than native populations. Invasive and native populations of *B. nigra* expressed similar concentrations of total glucosinolates. Nevertheless, invasive populations expressed higher concentrations of sinigrin than native populations. Whether it is the total amount of an expressed class of defence compounds (e.g. total glucosinolates in our case) or specific compound(s) within that class (e.g. sinigrin in our case) determine(s) herbivore resistance remains open to debate (Bidart-Bouzart and Kliebenstein 2008). A recent study that used the same populations of *B. nigra* as used here to compare growth/reproductive performance and a trade-off between resistance and herbivory tolerance also found that invasive populations of *B. nigra* expressed consistently higher leaf concentration of sinigrin than native populations (Oduor et al. 2011). That finding was in accordance with a prediction of a shifting defence hypothesis (SDH), according to which invasive populations should express higher concentration of a qualitative defence compound such as sinigrin than native populations of the same plant species (Doorduin and Vrieling 2011). SDH assumes that presence of generalist and a near complete absence of specialist herbivores in exotic ranges of invasive plants impose a selective pressure on the invasive plants to express higher concentrations of a qualitative defence compound that are effective against generalist herbivores (Doorduin and Vrieling 2011). In fact Liu and Stiling (2006) found that invasive plants do experience damage inflicted mainly by generalist herbivores. Thus, our findings that invasive populations of *B. nigra* express consistently higher leaf concentrations of sinigrin than native populations of *B. nigra* (Oduor et al. 2011 and this study) are in agreement with SDH. Most of the herbivores that *B.nigra* interacts with in the invasive range (at least in California) are generalists (Strauss et al. unpublished data), thus it is possible that such generalist herbivores have selected for *B. nigra* genotypes that express higher sinigrin concentrations than their native conspecifics. Using Californian populations of *B. nigra* that were also used in the present study, Lankau (2007) indeed found that genotypes of *B. nigra* that expressed high concentrations of sinigrin were less attacked by generalist herbivores than those that expressed low concentrations of sinigrin.

Even though invasive and native populations differed significantly in glucosinolate diversities, they haboured similar diversities and species richness of generalist and specialist herbivores (Table 4; Fig. 4). The reason for this lack of differences in herbivore diversity and richness remains unknown to us. Perhaps the method of arthropod survey we used was not adequate to detect the differences in arthropod assemblages between the invasive and native populations. Not all plants were surveyed at the same time due to the high number of plants involved in the experiment (arthropod survey on all the plants lasted three days during each round of survey). Furthermore, some species of herbivores (e.g. *Piezodorus lituratus* and *Rhynocoris iracundus*) were newly cited on some of the same *B.nigra* populations as used here in a separate competition experiment undertaken at the same site a year later (A.M.O Oduor et al. unpublished data). There is a generally high temporal variation in diversity and richness of herbivore communities that interact with plants (Skippari et al. 2009 and references therein), hence it is sometimes difficult to identify all the herbivores that a plant interacts with, especially when a survey is conducted over a short time span as we did here. Therefore, the possibility still remains that the invasive and native populations of *B. nigra* we used in this study genuinely differed in the assemblages of herbivores they interacted with.

Our finding that sinigrin and total glucosinolate concentrations were positively correlated with species richness of specialist herbivores for invasive populations of *B. nigra* (Fig. 6c) supports the idea that adapted specialized herbivores might use glucosinolates to locate their host plants for feeding, egg deposition, and to sequester them for their own defence (Agrawal and Kurashige 2003). Hence *B. nigra* genotypes that express high concentrations of glucosinolates are likely to be attacked by specialist herbivores more than genotypes expressing low concentrations of glucosinolates. Interestingly, neither sinigrin nor totall glucosinolate concentration was positively correlated with species richness of specialist herbivores for native populations of *B. nigra* (Fig.6b &d).

Only a few studies to date have correlated diversities of plant defence compounds and herbivore communities interacting with the same plant species.

However, such studies have not been conducted in the context of biological invasions, that is, comparing invasive and native populations of the same plant for diversities in defence compounds and associated herbivore communities. Nevertheless, those studies found that plant genotypes that differed in profiles of defence compounds also harboured different herbivore species (Macel and Klinkhamer 2009; Newton et al. 2009; Poelman et al. 2009; Kleine and Müller 2010; Lason et al. 2011). For instance, genotypes of *Brassica oleracea* that differed in glucosinolate profiles also differed greatly in herbivore species richness, composition and density (Newton et al. 2009; Poelman et al. 2009). All the examples cited here together with our current results indicate that different herbivores can exert selective pressures on defence compounds of plants, though the studies were only correlational in nature. Direct, field- based experimental evidence is still generally lacking. Nevertheless, Lankau (2007) demonstrated through a manipulative field experiment that generalist and specialist herbivores can indeed exert opposing selective pressure on leaf concentration of a plant chemical defence.

That invasive populations of *B. nigra* express a lower diversity of glucosinolates and a higher concentration of sinigrin than native populations of the same plant raises the possibility that the invasive populations experience generally consistent selection pressure by generalist herbivores in the invasive ranges to express a lower diversity of glucosinolates and a higher level of sinigrin. Nevertheless, founder effect could have played a role in the differences between invasive and native populations of *B. nigra* we find here; it may well be the case that invasive populations of *B. nigra* were founded by genotypes that express a lower diversity and higher concentrations of sinigrin than other genotypes. It should, however, be noted that results of our molecular phylogeography study (A.M. O. Oduor et al. unpublished data) support the idea of selection pressure, rather than founder effect being responsible for the differences in glucosinolate diversities and sinigrin concentration we report here. All the populations employed in the current study were also used in the molecular phylogeography study. The phylogeography study shows that multiple introductions of *B. nigra* genotypes took place from its native to invasive ranges (Oduor et al. unpublished data). Multiple introductions of various genotypes of the same plant may neutralize founder effects and result 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 and Parker 2008). However, through evolutionary time, natural selection in the novel invasive ranges might have led to a difference in glucosinolate diversities and sinigrin concentrations between invasive and native ranges as discussed here.

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Appendix S1: *Herbivores noted on invasive and native populations of Brassica nigra when grown in a common garden in Granada, Spain.*

Species	Order	Family	Feeding type	Host specificity
Myzus persicae	Hemiptera	Aphididae	Phloem feeder	Generalist
Helix aspersa	Pulmonata	Helicidae	Leaf chewer	Generalist
Whitefly	Hemiptera	Aleyrodidae	Phloem feeder	Generalist
Thrips tabaci	Thysanoptera	Thrripidae	Cell contents	Generalist
Dolycoris baccarum	Hemiptera	Pentatomoidea	Phloem feeder	Generalist
Porrostoma rhipidium	Coleoptera	Lycidae	Phloem feeder	Generalist
Palomena prasina	Hemiptera	Pentatomoidea	Phloem feeder	Generalist
Eurydema ornatum	Hemiptera	Pentatomoidea	Phloem feeder	Specialist
Brevicoryne brassicae	Hemiptera	Aphididae	Phloem feeder	Specialist
Pieris rapae	Lepidoptera	Pieridae	Leaf chewer	Specialist
Pieris brassicae	Lepidoptera	Pieridae	Leaf chewer	Specialist
Plutella xylostella	Lepidoptera	Yponomeutidae	Leaf chewer	Specialist
Phyllotreta nemorum	Coleoptera	Chrysomelidae	Leaf chewer	Specialist
Phyllotreta cruciferae	Coleoptera	Chrysomelidae	Leaf chewer	Specialist

CHAPTER 4:

INTRODUCED *BRASSICA NIGRA* POPULATIONS EXHIBIT GREATER GROWTH AND HERBIVORE RESISTANCE BUT LESS TOLERANCE THAN NATIVE POPULATIONS IN THE NATIVE RANGE

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ABSTRACT

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 post-introduction evolution of a trade-off between resistance to and tolerance of herbivory, which has received little attention. Through a common garden experiment in a native range, nine invasive and sixteen native populations of *B.nigra* were compared for growth and defence traits. Invasive populations had higher resistance 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, produced more biomass, and lighter seeds than natives irrespective of insecticide treatment. Besides 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.

Key words: *Brassica nigra*, enemy release hypothesis (ERH), EICA, shifting defence hypothesis (SDH), invasive species, glucosinolates, defence, tolerance

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 and Nötzold 1995; Buckley et al. 2003; Leger and Rice 2003; Bossdorf et al. 2004a; Maron et al. 2004; Wolfe et al. 2004; Meyer et al. 2005; Müller and Martens 2005; Stastny et al. 2005; see Whitney and Gabler 2008 for review; Williams et al. 2008; Zou et al. 2008; Caño et al. 2009; Cripps et al. 2009; Moloney et al. 2009; Monty and Mahy 2010; Rapo et al. 2010). Three theories are considered as fundamental in explaining plant invasion success (Doorduin and Vrieling 2011): enemy release hypothesis (ERH) (Keane and Crawley 2002), evolution of increased competitive ability (EICA) (Blossey and Nötzold 1995), and shifting defence hypothesis (SDH) (Müller-Schärer et al. 2004; Joshi and Vrieling 2005).

According to ERH, plants that are introduced to new ranges leave behind their specialized herbivores, and are, therefore, released from harmful herbivore pressure by those specialist herbivores. EICA hypothesis, on its part, predicts that release from specialist herbivore pressures will cause plant defences against specialists to decline in exotic ranges over evolutionary time (Blossey and Nötzold 1995). EICA hypothesis assumes that secondary metabolites defend plants against specialist herbivores. Genetically-based intra-specific variations in concentrations of secondary metabolites that are thought to defend plants against herbivory have been shown (Stastny et al. 2005; Müller and Martens 2005; Arany et al. 2009; Caño et al. 2009; Poelman et al. 2009). Due to 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 re-allocate 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 and Nötzold 1995).

SDH is an extension of the EICA hypothesis (Doorduin and Vrieling 2011). 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 and Vrieling 2005). Quantitative defence traits are based on digestibility-reducers (e.g. cellulose, tannins, and trichomes) that usually occur in high concentration and act in a dosage-dependent manner. On the other hand, 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 (Doorduin and Vrieling 2011). Adapted specialized herbivores might use these chemicals to locate their host plants for feeding, egg deposition, and to sequester them for their own defence (Agrawal and 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 and Vrieling 2005; Liu and 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 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 and 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 and 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 and Kotanen 1994; Mauricio et al. 1997; Fineblum and Rausher 2002; Weinig et al. 2003; Leimu and Koricheva 2006). This trade-off between resistance and tolerance is thought to occur due to a limitation on amount of resources available for allocation to defence (Leimu and 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 chemical defences of their host plants (Bowers and 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 given mixed results with some plants showing a trade-off and others not (Bossdorf et al. 2004b; Joshi and 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, SW-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 SDH and theory on a trade-off between resistance and tolerance, we made the following predictions:

1. 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.
2. Invasive populations will have higher resistance, and lower tolerance of herbivore damage than native populations.
3. 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 Mediteranean region and other parts of North Africa and Europe that has spread widely across the globe (Bell and Muller 1973; Feeny and Rosenberry 1982; Westman and 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 and Kresovich 1999). In temperate regions, *B. nigra* was a major mustard crop until the 1950s when it was replaced by its close relative, *B. juncea* in commercial production. Presently, *B. nigra* is a widespread weed (Westman and 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 approximately 200 years ago (Westman and Kresovich 1999). In North America, *B. nigra* can form thick monospecific stands, although generally in disturbed areas (Lankau and Strauss 2008). *B. nigra* defends itself from herbivores by synthesizing glucosinolates, a class of secondary compounds derived from several amino acids (Feeny and Rosenberry 1982). In *B. nigra*, sinigrin (allyl-glucosinolate) represents 90%–99% of the total glucosinolate concentration and has a heritable basis (Feeny and 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 and Kurashige 2003; Kliebenstein 2004) and competition against other plants (Bell and Muller 1973; Lankau and Strauss 2008; Müller 2009). In addition to the glucosinolate-based resistance, *B. nigra* employs non-glandular trichomes to resist herbivore damage (Traw and Dawson 2002).

PLANT MATERIAL

Seeds of nine invasive (North American) and sixteen native (Mediteranean region, European, African, and Asian) populations (Westman and Kresovich 1999) of *B. nigra* were obtained from the United States Department of Agricultre (USDA) Germplasm Resource Information Network (GRIN), botanical gardens, field- collected by the authors or their collaborators (see table 1). Seeds obtained from germplasm collections/ botanical gardens had been collected directly from wild populations in the field and then grown for only one generation in common green houses 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 genetic integrity of the populations.

EXPERIMENTAL DESIGN

Twenty seedlings (two-weeks old) of each of the invasive and native populations of *B. nigra* were transplanted to a plot at Pedralera La Loca, Cadiz, SW Spain (36º 31' N, 6º 11' W) on 30th and 31st December 2007. Pedralera La Loca is part of a large agro-ecosystem where *B. nigra* grows naturally. However, *B. nigra* presence has not been reported over the last 20 years in 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 lengths. 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 watering frequency was scaled up to twice a week as temperatures rose

Table 1: *Invasive and native populations of Brassica nigra used in the current experiment. Asterisks (*) indicate populations whose accession numbers and collection sites were not provided by the botanical gardens from which the seeds were sourced. Geographical coordinates of populations marked by † were not noted down. However, those populations were spaced at least 30 km apart.*

Country/State of origin	Accession number or collector’s name	Geographic region	Latitude	Longitude	Year of field-collection from wild populations	Invasive status
Hungary	*	Europe				Native
Germany	PL 633142	Europe	51° 25' 0" N	12° 1' 0" E	1994	Native
Germany	PL 633143	Europe	51° 49' 0" N	11° 17' 0" E	1996	Native
Poland	PL 358590	Europe	52°13'56" N	21°0 '30" E	1971	Native
Poland	*	Europe	49° 28'43"N	17° 7' 20"E	1992	Native
Turkey	PL176881	Mediteranean	39° 52' 0" N	32° 52' 0" E	1948	Native
Turkey	PL592737	Mediteranean	39° 38' 5" N	27° 53' 6"E	1948	Native
Turkey	PL169066	Mediteranean	40° 2' 47" N	27° 58'12" E	1948	Native
Ethiopia	PL597830	Africa	9° 1' 48 " N	38° 44' 24"E	1993	Native
Afghanistan	PL274284	Asia	34° 0' 0" N	69° 0' 0" E	1961	Native
Italy	PL633148	Mediteranean	40° 10' 0" N	16° 31' 0"E	1980	Native

Table 1 *continued*

Ireland	PL 649155	Europe	52° 03' N	009° 30' W	1992	Native
Canada	Ames 25399	North America	43° 40' 0" N	79° 25' 0" W	1991	Invasive
Greece	PL263866	Mediteranean	37° 58' 0" N	23° 43' 0" E	1960	Native
Greece	PL633147	Mediteranean	37° 20' 5" N	22° 21' 08" E	1942	Native
Spain	PL597829	Mediteranean	36° 0' 0" N	6° 0' 0" E	1993	Native
Spain	*	Mediteranean			1993	Native
Illinois	R. Lankau	North America	†		2005	Invasive
New York	J. Conner	North America	†		2005	Invasive
New York	J. Conner	North America	†		2005	Invasive
California	R. Lankau	North America	†		2005	Invasive
California	R. Lankau	North America	†		2005	Invasive
California	R. Lankau	North America	†		2005	Invasive
California	R. Lankau	North America	†		2005	Invasive
California	R. Lankau	North America	†		2005	Invasive

through the growing season to reach 40ºC. 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 *ziz-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 inverse of damage by herbivores (i.e. 100 minus percent leaf damage or seed predation) (Leimu and Koricheva

2006). Low percent leaf damage or seed predation indicates a high level of resistance. Leaf concentrations of sinigrin and trichome density that are putative defence traits were also used as indicators of resistance to herbivory; high levels of these traits indicate high investments in resistance. Percent leaf damage on five-week old plants (i.e. three weeks after transplanting) was estimated by visually assessing proportion of the total leaf area damaged on the plant. Plants had *ca*. six leaves at the time of scoring leaf damage. Trichome count was done *in situ* by observing 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 get 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 four-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 that were stored at 4^{o}C until sinigrin analysis was done. Seed predation was determined by randomly picking ten 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 date of transplanting). Growth and fitness were then estimated as follows: 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. 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. Fruit yield per plant was determined by dividing plants down the middle, and then counting all fruits that occured in the entire one half of the plant. The resulting fruit count was then multiplied by two to get total fruit yield per plant. Average seed yield per fruit was determined by counting individual seeds per fruit for two fruits and getting their mean number. Multiplying average number of seeds per fruit by total number of fruits per plant allowed us to determine total seed yield per plant. 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 get seed biomass per plant. Total plant biomass was obtained by oven drying shoot and root of each plant at 65ºC for 12 hours and then weighing them together.

ANALYSIS FOR LEAF SINIGRIN CONCENTRATIONS

Sinigrin identity and concentration was determined by high performance liquid chromatography. A ball bearing was added to each 1.2 mL Eppendorf tube, and tubes were shaken for 1 minute in a Qiagen FastPrep-24 tissue homogenizer. After shaking, the tubes sat for 1 hour, then were centrifuged and 300 ml of the supernatant passed through a DEAE-Sephadex column (Pharmacia Biotech, Uppsala, Sweden), using 96-well microtiter plates. Columns were washed twice with 70% methanol and twice with water. Desulphoglucosinolates were extracted from the column by adding 100 ml of a 5% sulfatase enzyme solution and incubated overnight. The resulting solution was transferred to a new 96 well plate and stored at 4 ºC until analyzed on a HPLC 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). Afterward, we 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. Populations were treated as a random-effect variable and nested within invasive status. Blocks were also treated as a random-effect variable. The following continuous data were analyzed 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 percent leaf damage or 100 minus 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 $\log_{10}$-transformed lifetime seed yield (i.e. seed biomass) against arc-sin square root –transformed percent 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 and 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 and 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 $\log_{10}$-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 and 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. Populations were treated as a random-effect independent variable and nested within invasive status. Blocks were also treated as a random-effect independent variable. Additionally, the slopes were regressed against the measured actual resistance (i.e. 100 minus percent leaf damage) against herbivores. This enabled us to directly test a trade-off between tolerance and resistance. Leaf sinigrin concentration and trichome density were also regressed against percent leaf damage to test for correlations between the putative resistance traits and percent leaf damage experienced.

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

RESULTS

Invasive status had a significant effect on plant traits when all traits were analyzed together (Table 2). When analyzing each trait independently, invasive status had a significant effect on leaf sinigrin concentration, trichome density, plant height, plant biomass, and biomass of individual seeds (Table 3). Invasive populations were composed of plants with higher leaf sinigrin concentration, leaf trichome density, biomass, greater height, and lighter seeds than native populations (Figs. 1a, 1b, 2b, 2c & 2d). Percentages of seeds preyed upon were similar for invasive and native populations under both insecticide treatments

(Table 3; Fig. 1d). Leaf damage was influenced by both invasive status and insecticide treatment (Table 2); insecticide treatment resulted in less damage on both invasive and native populations, and, contrary to our expectations, damage on invasive populations was less than that on native populations under either insecticide treatment, that is, the invasive populations had higher resistance to leaf damage than native populations (Fig.1c).

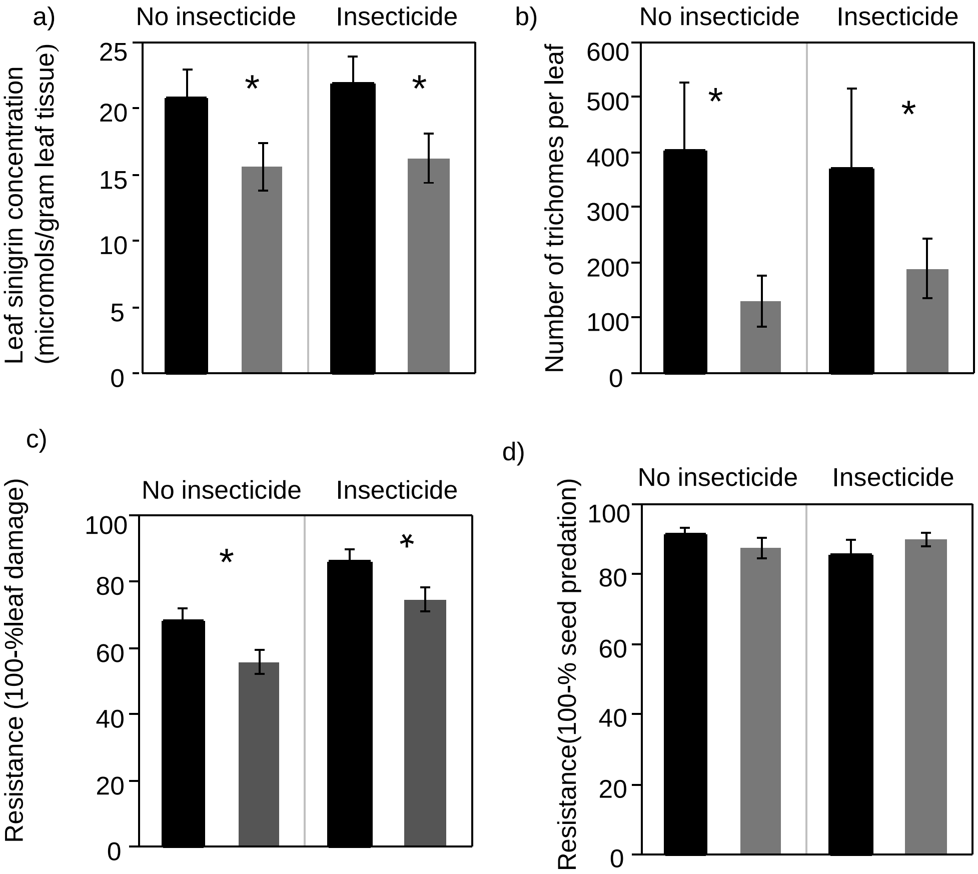

Figure 1: *Mean ± 1 SE resistance traits of invasive (black bars) and native (gray bars) populations of Brassica nigra grown in a common garden with (out) insecticide treatment. Asterisks (*) represent statistically significant differences (P<0.05). (**a**) Concentration of sinigrin in leaf tissues,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

Table 2: *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

Number of seeds per plant was affected by both insecticide treatment and interaction between insecticide treatment and invasive status (Table 3). 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 3); application of insecticide resulted in higher survival for both invasive and native populations, though provided an even greater benefit to invaders than to native plants (Fig. 2e). Neither leaf trichome density nor sinigrin concentration was correlated damage levels (results not shown).

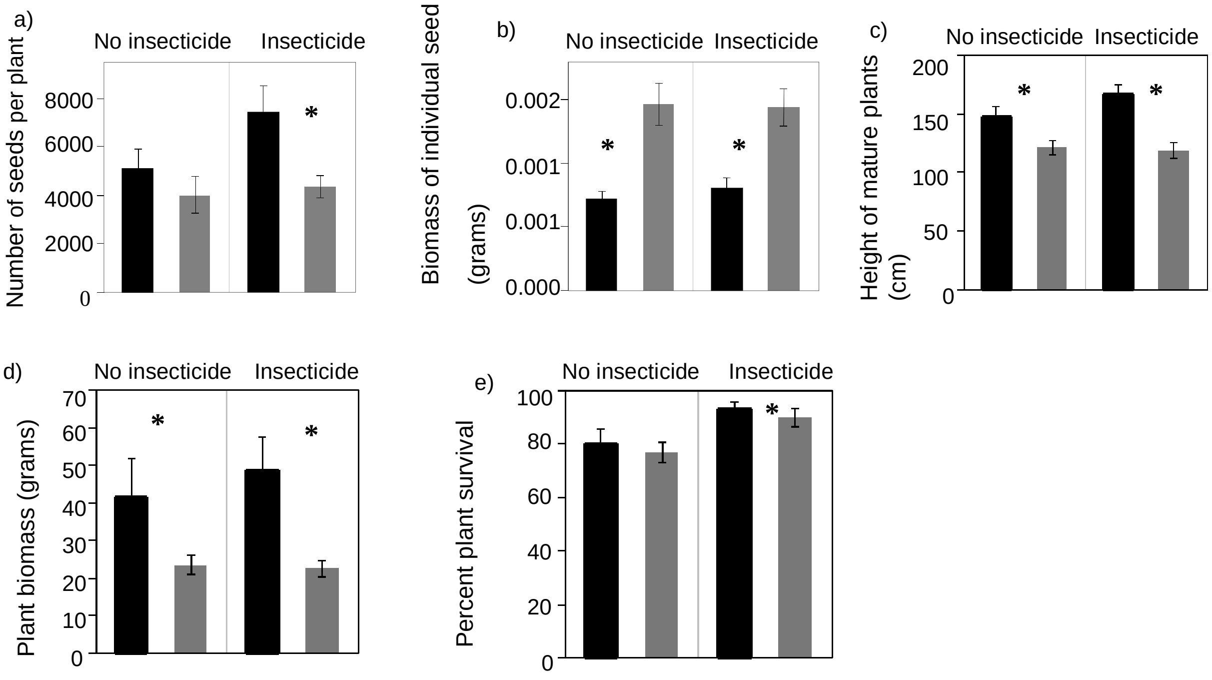

Figure 2: *Mean ± 1 SE growth performance traits of invasive (black bars) and native (gray bars) populations of Brassica nigra when grown in a common garden with (out) insecticide treatment. Asterisks (*) represent statistically significant differences (P<0.05).*

Table 3: *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. Populations and block were treated as random-effect variables in the models; hence the F/Z statistics and degrees of freedom given are for fixed-effect variables only.*

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

$P < 0.001$***, $P<0.01$**, $P<0.05$*

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 populations as they had a lower mean negative slope (-0.243) than that of 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.

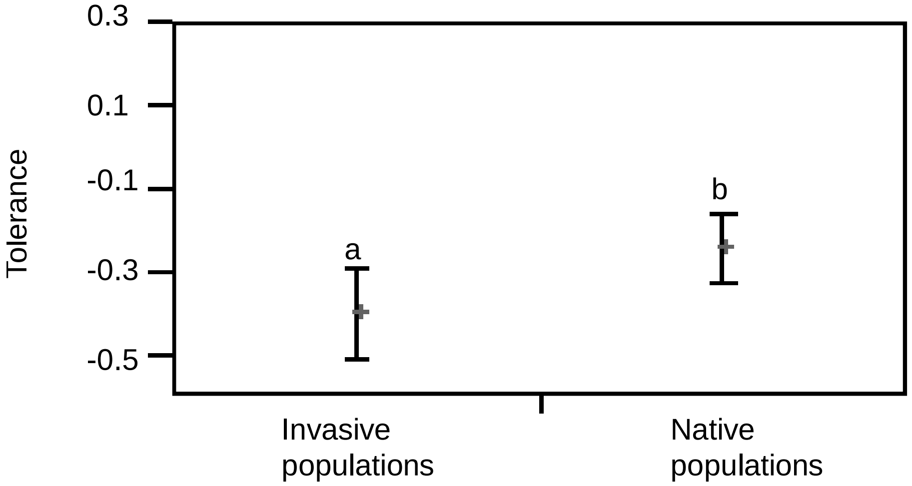

Figure 3: *Mean (± 1 SE) tolerance of herbivore damage by invasive and native populations of Brassica nigra. Tolerance was computed by regressing* log_{10}*-tranformed-seed biomass against arc-sin square root –transformed percent leaf damage.*

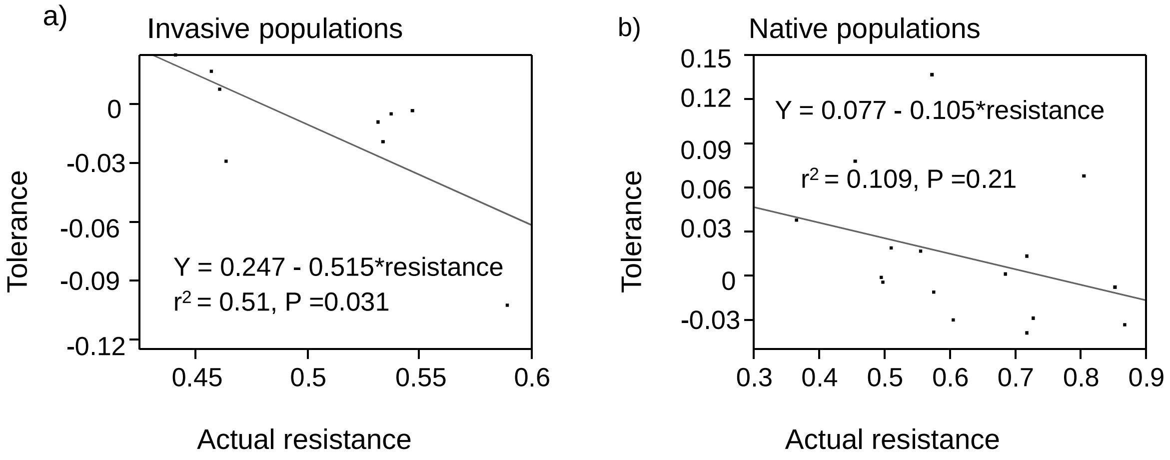

Figure 4: *Linear regressions showing trade-offs (i.e. negative correlations) between tolerance of, and resistance to herbivore damage by invasive (a) and native (b) populations of Brassica nigra (n=9 for invasive populations; n=16 for native populations).*

DISCUSSION

Our finding that invasive populations invest more in a putative qualitative defence compound (i.e. sinigrin) is consistent with a prediction of SDH that herbivore communities dominated by generalists in the invasive ranges select for plants that have high concentration of that compound (Doorduin and Vrieling 2011). With reduced importance of specialist herbivores in the invasive ranges, increased production of a secondary metabolite like sinigrin that is toxic to generalists may have an overall positive effect on plant performance. Other studies have also reported significant increase 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 and Martens 2005). However, our finding of a greater leaf trichome density among invasive populations as our data show is not consistent with a prediction of SDH that quantitative defence traits will be selected against among invasive populations (Doorduin and Vrieling 2011).

True to our prediction, invasive populations of *B.nigra* had higher actual resistance (i.e. lower percent 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 *Senecio jacobaea* experienced less herbivore damage by a generalist herbivore than native genotypes (Joshi and 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 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 and Strauss 2008). Trichomes play significant roles in regulating leaf temperature and light reflection (Smith and Nobel 1977) and leaf evaporation (Brewer et al. 1991). It has, nevertheless, been shown that 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 our 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, though it was stronger among 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 amount of 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. Compared to other similar studies, 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 *Senecio jacobaea* had both greater resistance to, and tolerance of herbivory than native ecotypes of the same species (Stastny et al. 2005). Invasive populations of *Sapium 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 a reduced vigour and reproduction when they are grown in their native range where they are exposed to herbivory. On the other hand, 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. Since 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. On the other hand, seeds of invasive populations of *B.nigra* we used in the current study were field-collected in 2005 (see table 1). This means that the invasive populations were separated from their native conspecifics anywhere between ≤ 70-200 years. That the invasive and native populations were separated only for those years, yet they differ markedly in the various traits above, support 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 find rapid changes in concentrations of sinigrin, levels of tolerance of herbivory, stature and seed ‘packaging’ (more, lighter seeds). These changes may come in response to natural selection pressures exerted by changes in herbivore regimes (Lankau 2007) and competition from other plants (Lankau and 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 and 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 shows 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 and Parker 2008). However, with 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 above.

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CHAPTER 5:

HERBIVORES MEDIATE DIFFERENCES IN COMPETITIVE ABILITY OF NATIVE AND INVASIVE POPULATIONS OF *BRASSICA NIGRA*

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ABSTRACT

Post-introduction evolution has been found in many invasive plant species, and includes changes in various traits: stature, biomass, and reproductive output. However, these traits have largely been compared for invasive and native populations of various plants in the absence of competition with other plants and without manipulation of herbivore presence/absence. Here, we explored a post-introduction evolution of increased competitive ability under conspecific or heterospecific competitive environment and under natural or reduced level of insect herbivory. Through a common garden experiment in a native range, three invasive and three native populations of *Brassica nigra* were compared for the following traits when grown with conspecific or heterospecific competitors and under natural or reduced levels of insect herbivory: plant height, biomass, fruit number, and number and weight of seeds per plant. Insecticide spray was used to reduce level of damage on half of the plants. There was a three-way interactive effect of invasive status of *B.nigra,* insecticide treatment, and type of competition on final plant height at maturity. Main effect of insecticide treatment and interaction between invasive status and type of competition also had statistically significant effects on plant height. The rest of the traits were not affected either by main or interactive effects of invasive status of *B.nigra*, insecticide treatment, and type of competition. Using relative interaction intensity (RII) as ameasure of competition, we found that invasive populations of *B.nigra* benefited from protection from herbivory under heterospecific competition, while insect damage actually conferred benefits to native populations when competiting with heterospecifics. These results suggest that herbivory interacts with competitive ability, and that native populations are better competitiors in the presence of herbivory, while invasive populations, which may esacpe many herbivores, are better competitiors in low herbivory regimes.

Key words: EICA, *Brassica nigra*, invasive species, native and introduced populations, common garden experiment, competitive ability, herbivory, post-introduction evolution.

INTRODUCTION

Many plant species have been introduced to ranges where they are not native. A small fraction of introduced plant species become invasive (i.e. superabundant in the introduced ranges) and have dramatic detrimental economic and ecological effects (D'Antonio and Vitousek 1992; Vitousek et al. 1996; Mack et al. 2000; Pimentel et al. 2005). Consequently, a copious amount of research has been directed towards a better understanding of the factors driving plant invasion (Reichard and Hamilton 1997; Mack et al. 2000; Daehler 2003). Various reports from such studies indicate rapid post- introduction evolution of increases in the following plant traits: stature, biomass, and reproductive output (e.g. Blossey and Nötzold 1995; Buckley et al. 2003; Leger and Rice 2003; Maron et al. 2004; Wolfe et al. 2004; see Bossdorf et al. 2005 for review; Stastny et al. 2005; see Whitney and Gabler 2008 for review; Williams et al. 2008; Zou et al. 2008a; Caño et al. 2009; Moloney et al. 2009). The post-introduction evolution of an increase in these traits is in line with predictions of evolution of increased competitive ability (EICA) hypothesis. EICA predicts that release from specialist herbivores will cause plant defences against specialists to decline in exotic ranges over evolutionary time (Blossey and Nötzold 1995). Consequently, introduced plants in the exotic ranges are expected to evolve higher competitive abilities (i.e. higher growth and reproductive output) relative to their non-introduced conspecifics in the native ranges.

However, in most experimental tests of the EICA hypothesis, invasive and native populations of various plants were grown in competition-free environments and protected from herbivore damage or not (as was the case in the studies cited above), and are thus not likely to provide useful information about post-introduction evolution of increased competitive abilities (Goldberg 1996; Bossdorf et al. 2005). In effect, most of those studies can be interpreted to have only compared invasive and native populations for changes in traits when released from herbivore damage; this means that their competitive abilities were not compared *per se*. Many plant species invade natural communities where they experience both conspecific and heterospecific competition (e.g. Weir et al. 2003, 2006; DeWalt et al. 2004; Lankau 2008; Ni et al. 2010). Therefore, ideal tests of evolution of increased competitive abilities of invasive plant species would require comparing performances of invasive and native populations of a plant when they are grown in conspecific and heterospecific competitive environments in a common garden setting (Bossdorf et al. 2005). To date only a few studies have directly compared competitive abilities between invasive and native populations of plants in common garden settings (Leger and Rice 2003; Vilà et al. 2003; Blair and Wolfe 2004; Bossdorf et al. 2004; McKenney et al. 2007; Ridenour et al. 2008; Zou et al. 2008a; He et al. 2009). However, presence or absence of herbivores was not manipulated in those studies to see the effect herbivores could have on the outcome of competition by invasive and native populations. In most ecosystems, competition from neighbouring plants and herbivore damage determine growth, survival and reproduction of plant individuals, and consequently the abundance of plant populations (Hämback and Beckerman 2003). Nevertheless, little is known about the potential interactive effects of herbivore damage and competition on plant performance with regard to invasive plants (Suwa et al. 2010). While initial thoughts were that invasive plants might escape enemies in the novel range, a more nuanced understanding of changing relationships with enemies is that the composition of the herbivore assemblages changes in novel habitats (Müller-Schärer et al. 2004; Joshi and Vrieling 2005; Liu and Stiling 2006). Specifically, generalist herbivores are more important in the invaded range (Liu and Stiling 2006), while both generalists and specialists in the native range may exert conflicting selection on traits in the native range, and might favor tolerance over resistance to herbivore damage (e.g. Zou et al. 2008b; Oduor et al. 2011). On the other hand, dominance of generalist herbivores in the invasive ranges may select for plant genotypes that invest more in resistance and less in tolerance (Oduor et al. 2011).

Plant as well as herbivore communities may change with invasion. In some cases, as for *Brassica nigra* studied here, traits that are favored in heterospecific competition are selected against in conspecific competition, and vice versa (Lankau and Strauss 2008). When a plant invades a new area, its competitors are heterospecific. As these plants become more abundant in the invaded range, the selective regime may become one that is dominated by strong competition from conspecifics. Studies are, therefore, needed to test post-introduction evolution of competitive abilities of invasive plants under conspecific and heterospecific competitive environments as well as in the presence or absence of herbivores. In the current study, we experimentally compared competitive abilities of invasive and native populations of *Brassica* *nigra* under natural and reduced levels of insect herbivory within a native range of the plant. We asked: Are invasive populations of *B. nigra* better hetero- or conspecific competitors than native populations when they are exposed to natural or reduced level of insect herbivory in the native range?

MATERIALS AND METHODS

STUDY SYSTEM

Brassica nigra (L.) W. D. J. Koch is an annual herb native to the Mediteranean region and some parts of North Africa and Europe that has spread widely across the globe (Bell and Muller 1973; Feeny and Rosenberry 1982; Westman and 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 approximately 200 years ago (Westman and Kresovich 1999). In North America, *B. nigra* can form thick monospecific stands, although generally in disturbed areas (Lankau and Strauss 2008). *B. nigra* defends itself from some herbivores by synthesizing glucosinolates, a class of secondary compounds derived from several amino acids (Feeny and Rosenberry 1982). In *B. nigra*, sinigrin (allyl-glucosinolate) represents 90%–99% of the total glucosinolate concentration and has a heritable basis (Feeny and Rosenberry 1982; Traw 2002, Lankau and Strauss 2008). 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 and Kurashige 2003; Kliebenstein 2004; Hopkins et al.

2009) and competition against other plants (Bell and Muller 1973; Lankau and Strauss 2008; Müller 2009).

We used both conspecific and heterospecific competitor plants. Seeds from three invasive (Californian) populations and three native (Mediteranean region and European) populations of *B. nigra* were field-collected by the authors, their collaborators or obtained from a seed germplasm collection (see Table 1 for details). Seeds of four other species (i.e. heterospecific competitors): *Vicia sativa*, *Medicago sativa*, *Lolium perenne*, and *Dactylis glomerata* were bought from a commercial seed dealer, Rocalba, SA (Barcelona, Spain) (www.rocalba.com). All these heterospecific competitors are widely distributed, covering places where *B.nigra* occurs naturally in its native as well as in its introduced ranges.

Table 1: *Invasive and native populations of Brassica nigra used in this study.*

Country/State of origin	Accession number or	Geographic region	Invasive status
McLaughlin, California, USA	R. Lankau	North America	Invasive
Napa county, California, USA	R. Lankau	North America	Invasive
Davis airport (Yolo County), California, USA	S.Y. Strauss	North America	Invasive
Puerto Real, Cádiz, Spain	J.M. Gómez	Mediteranean region	Native
France	CR 2113	Mediteranean region	Native
Doorweth, Gelderland, The Netherlands	M. Macel	Central Europe	Native

EXPERIMENTAL DESIGN

Seeds of all six *B.nigra* populations and the four other species were sown in a commercial potting substrate (dry matter : 60-62%, total organic matter: 74-76% , pH: 5.2-5.5, electrical conductivity: 0.4-0.5, Nitrogen: 100-150 mg/L, P2O5: 150-200 mg/L, K2O: 200-250 mg/L, total pore space: 85-90 %) in plastic plug-trays with a volume of 16.7 cm^3 per cell in a glasshouse at the end of March 2009. The resulting seedlings were raised under glasshouse conditions for two weeks (under natural lighting and temperature conditions). At two weeks, seedlings were transplanted into a field plot in Granada, SW Spain (37º 10' 30" N, 03º 38' 10" W). The experimental set up consisted of growing seedlings of the six populations of *B. nigra* with conspecific or heterospecific competition. Under conspecific competition, a seedling from each of the three native populations was grown in a pair -wise combination with a randomly selected seedling from each of the three other invasive populations of *B. nigra*. This resulted in nine treatment combinations for conspecific competition. Heterospecific competition involved growing a seedling from each of the six populations of *B.nigra* in pair-wise combination with a seedling from each of the four other species, yielding a total of 24 treatment combinations for heterospecific competition. The experimental design employed was a completely randomized design, in which all the 33 treatment combinations above were represented once in each of 20 planting rows. In total, there were 20 replicates per treatment, with the treatments allocated randomly within and among rows. The treatments were spaced 70 cm apart within a row while the rows were spaced 100 cm apart. The transplanted seedlings were watered regularly until they established, after which watering frequency was fixed at twice a week as temperatures rose through the growing season to reach 40ºC or above. Half of the plants (i.e. plants in 10 out of 20 rows) were released from natural level of herbivory by treating them weekly with an insecticide; rows treated with insecticide were chosen alternately. This enabled us to test the competitive abilities of invasive and native populations under natural or reduced levels of herbivory. The insecticide used was KB® (active ingredients: Bifentrin 10% EC) at a rate of 2g/L. The plants were weeded regularly to remove any unwanted competitor species.

MEASUREMENT OF PLANT PERFORMANCE

Plants were harvested individually as they matured (from approximately fourth month following date of transplanting) after which the following performance traits were quantified for each plant: maximum plant height (in cm), plant biomass (shoot + root biomasses), individual seed biomass, seed biomass per plant, and number of fruits and seeds per plant. Mean number of seeds per fruit was obtained from six fruits randomly picked per plant. Multiplying mean number of seeds per fruit by total number of fruits per plant allowed us to determine number of seeds per plant. Mean biomass of individual seeds was determined from the same six fruits. This was then multiplied by the number of seeds per plant to get seed biomass per plant. Plant biomass was obtained by oven drying shoots and roots of each plant at 65ºC for 15 hours and then weighing.

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). Afterward, we employed Markov Chain Monte Carlo Generalized Linear Mixed Models (MCMCglmm) using library MCMCglmm to compare invasive and native populations for each of the six traits named above. Invasive status of *B.nigra* (invasive or native), insecticide treatment (insecticide applied or not), type of competition (conspecific or heterospecific), and interactions between them were treated as fixed-effect independent variables. The heterospecific competitors were further divided into two functional groups: legumes and grasses. Populations were nested within invasive status and treated as a random-effect variable. Species identity of the four heterospecific competitors was nested within functional group status (i.e. legume or grass) and treated as a random-effect variable. Functional group status was treated as a random-effect independent variable. For conspecific competition, we used scores of traits for only half the plants involved in pair- wise competitions. For example, if 20 plants from an invasive population were made to compete against 20 plants from a native population, then scores of only 10 plants from that invasive population (that we denominated focal plants) were used in the MCMCglmm analyses, and scores of plants from native population that were in those pair-wise competitions were discarded. Similarly, for native plants, the MCMCglmm were computed using only 10 plants (that we also denominated focal plants) and the values of invasive plants that were in those pair- wise competitions were discarded. This kind of computation enabled us to avoid a problem of lack of statistical independence of scores of traits obtained from plants in pair- wise competitions. For heterospecific competition, we included in the MCMCglmm data of all plants from each of the six *B. nigra* populations. Data from heterospecific competitors were discarded. We also analyzed for effect of competitive interactions on fitness of invasive and native populations of *B. nigra* using relative interaction intensity (RII) index. RII measures the effect of interactions between plants (Armas et al. 2004). RII values range from -1 to 1, with negative values indicating competitive interactions and positive values indicating facilitative interactions (Armas et al. 2004). A zero value indicates a net effect of no interaction between plants (Armas et al. 2004). RII is calculated as follows: RII = (Fw/c –Fwo/c) / (Tw/c + Two/c), where F= Focal plant; w/c = performance with competition; wo/c = performance without ompetition). For RII values, we compared the invasive and native populations of *B. nigra* using number and biomass of seeds produced per plant as they are a reliable measure of fitness of an annual plant (Boalt and Lehtilä 2007). The RII values were also compared using MCMCglmm running the same model as for absolute values as is explained above. R version 2.9.1 was employed in all of the analyses.

RESULTS

Invasive and native populations differed statistically when all traits were analyzed together (Table 2). When analyzing each trait independently, there was a main effect of insecticide as well as a three-way interactive effect of invasive status of *B. nigra,* insecticide treatment, and type of competition on final plant height at maturity (Table 3). The rest of the traits were not affected either by main or interactive effects of invasive status of *B. nigra*, insecticide treatment, and type of competition (Table 3).

Table 2: *Permutational multivariate analysis of variance (ADONIS) comparing the whole set of plant traits between invasive and native populations of Brassica nigra. Significant values are marked in bold font.*

Source	df	F	P
Invasive status	1	29.53	0.009
Insecticide treatment	1	1.94	0.12
Type of competition	1	3.15	0.06
Invasive status * Insecticide treatment	1	0.51	0.61
Invasive status * Type of competition	1	0.40	0.71
Type of Competition * Insecticide treatment	1	1.08	0.32
Invasive Status * Type of competition* Insecticide treatment	1	0.52	0.71

Invasive and native populations of *B. nigra* sprayed with insecticide grew taller than unsprayed controls (Fig. 1a). Plants were generally smaller under conspecific competition than under competition with grasses or legumes (Fig. 1a). There was a three-way interactive effect of invasive status of *B. nigra*, insecticide treatment, and type of competition on RII based on plant fitness (Table 4). Invasive populations had mean positive RII values for both seed number and biomass per plant under insecticide spray when in competition with grass or legumes (Fig. 2). Native populations, on the other hand, had mean positive RII values for both traits when competing with legumes or grasses under natural level of insect damage (Fig. 2). These results suggest that plants in both native and invasive populations have traits shaped by the joint effects of competition and herbivory.

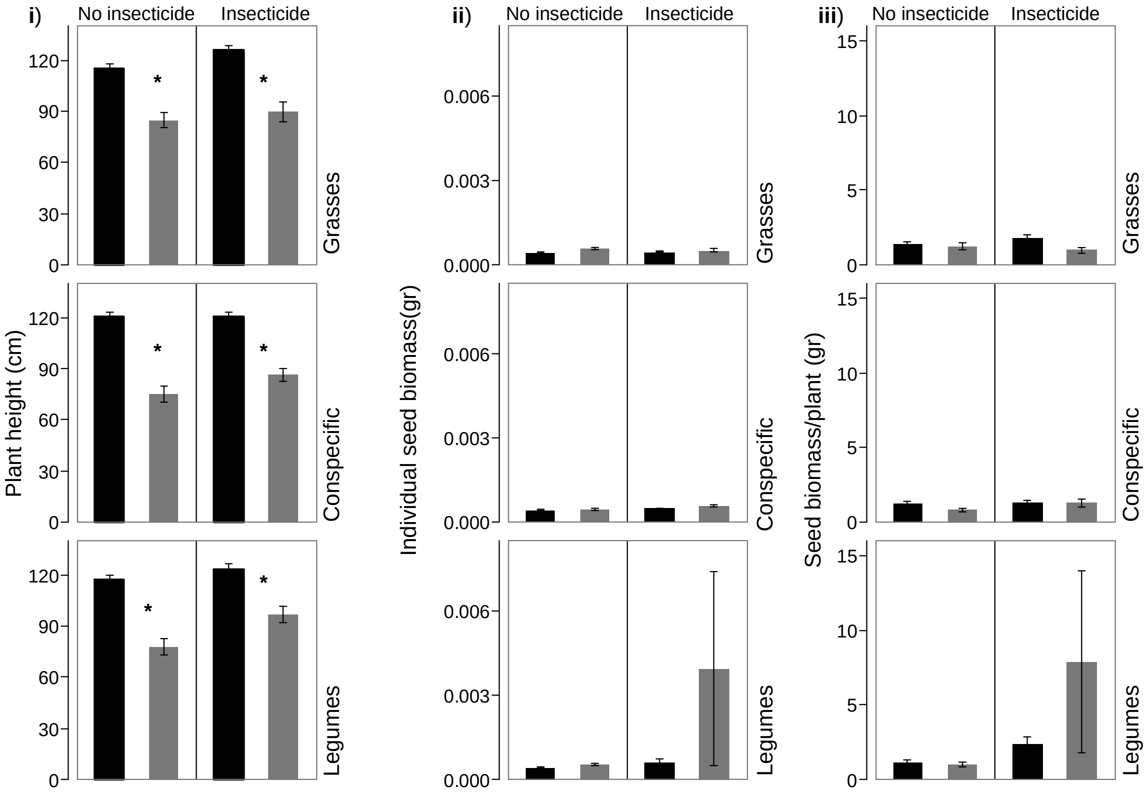

Figure 1a: *Mean (± 1SE) performance traits of invasive (black bars) and native (gray bars) populations of Brassica nigra when grown with conspecific or heterospecific (Legumes/Grasses) competition and under insecticide treatment or not. Asterisks (*) indicate statistically significant differences (P<0.05).*

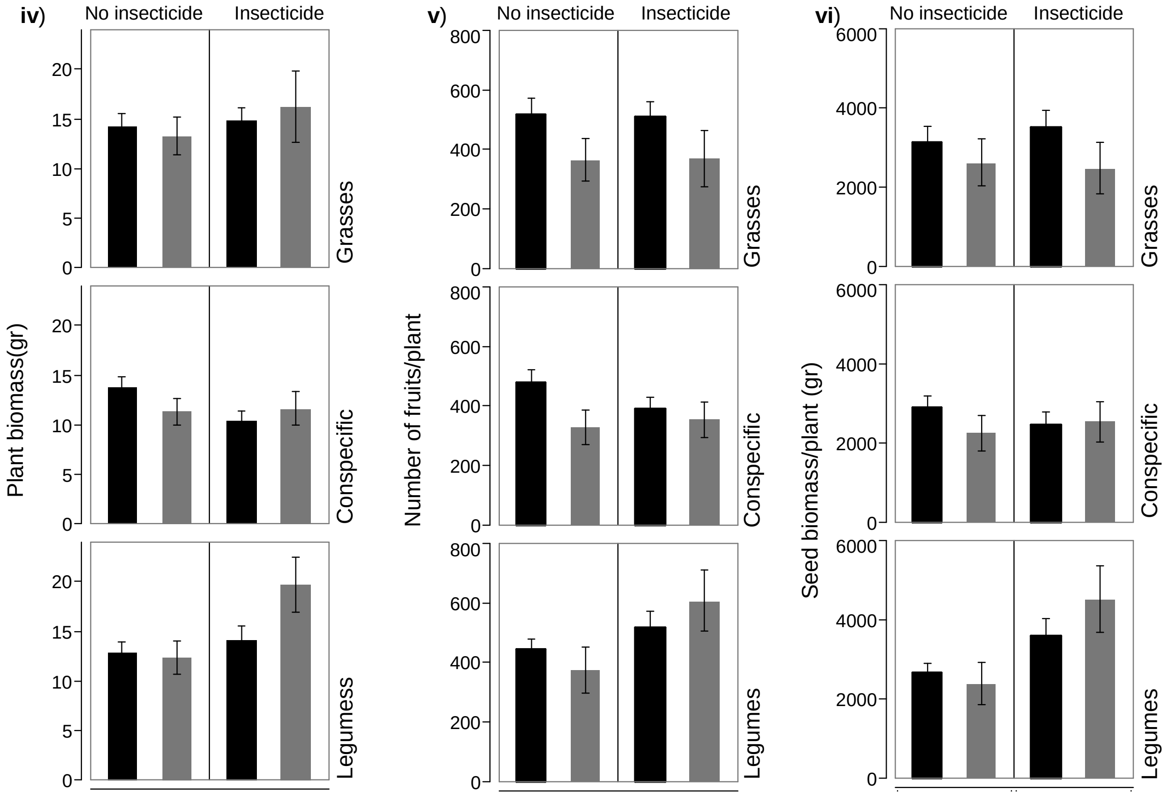

Figure 1b: *Mean (± 1SE) performance traits of invasive (black bars) and native (gray bars) populations of Brassica nigra when grown with conspecific or heterospecific (Legumes/Grasses) competition and under insecticide treatment or not.*

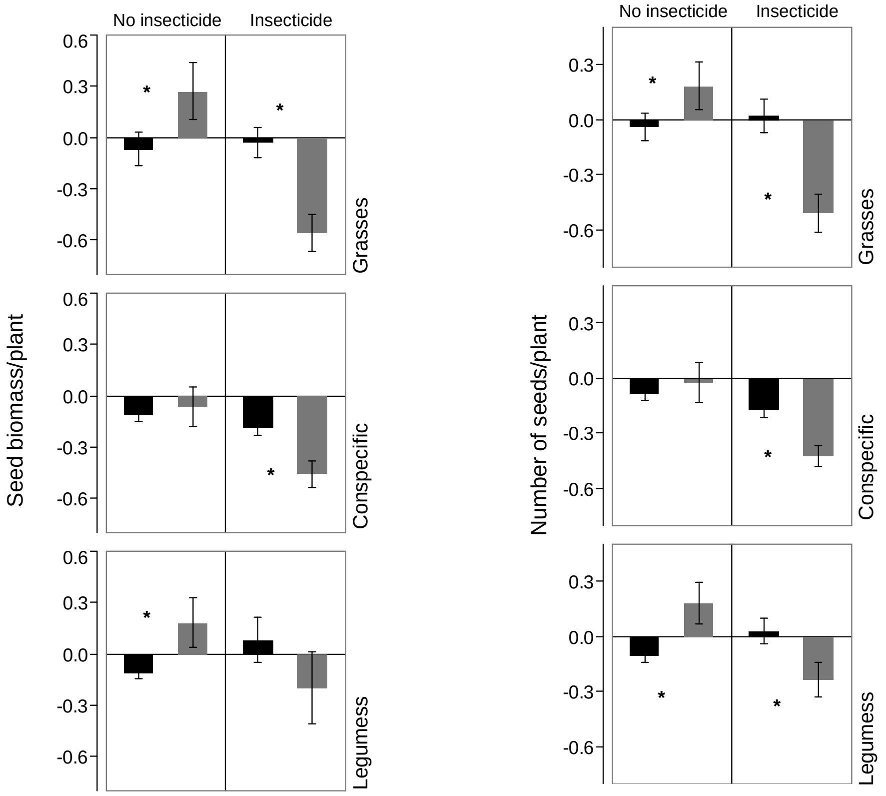

Figure 2: *Mean (± 1SE) relative interaction intensity (RII) of invasive (black bars) and native (gray bars) populations of Brassica nigra when grown with conspecific or heterospecific competition and under insecticide treatment or not. A positive RII value indicates that neighbours facilitate growth and negative RII values indicate that neighbours inhibit growth. Asterisks (*) indicate statistically significant difference (P<0.05).*

Table 3: *Markov Chain Monte Carlo Generalized Linear Mixed Models (MCMCglmm) showing posterior mean parameter estimates and 95 per cent credible intervals [95% CI] for fixed effects of invasive status, insecticide, type of competition, and their interactions on growth performance traits of invasive and native populations of Brassica nigra. Populations were nested within invasive status and treated as a random-effect variable in the models. Species identity of the four heterospefic competitors was also nested within functional group (i.e. legume or grass) and treated as a random-effect variable. P<0.01**, P<0.05*. Last three rows are random-effect variables. Parameter estimates whose 95% CI do not cross zero are statistically significant. Significant values are marked in bold font.*

Source of variation	Plant height	Individual seed biomass	Seed biomass/ plant	Plant biomass	Fruit number/plant	Seed number/plant
Invasive status	-32.03[-76.4 13.4]	-0.93[-2.4 0.7]	0.012[-4.2 4.0]	-0.99[-15.8 12.4]	-154[-530 260]	-504.6[-2,949 2,072]
Type of competition	19.02[-17, 108 15, 177]	-30.3[-131 125]	14.5[-9 190 16, 600]	461[-23,000 15,000]	494[-3,600 3, 400]	-305[-6,924 7,884]
Invasive status * Type of competition	-16.67[-28.8 - 2.67]*	-0.7[-0.88 0.55]	-0.44[-5.5 5.0]	-1.75[-6.8 4.4]	-14.8[-230 190]	-247[-2,086 1,426]
Insecticide treatment	**10.6[1.47 19.3]***	0.09[-0.5 0.56]	0.35[-3.5 4.4]	0.56[-3.4 4.6]	-7.1[-159 170]	363[-974 1,563]
Invasive status * Insecticide treatment	-6.4[-20.0 5.8]	-0.42[-1.21 1.2]	-0.66[-6.7 5.07]	1.57[-5.3 7.08]	-0.013[-210 290]	-545[-2,346 1,344]
Type of Competition * Insecticide treatment	-10.6[-21.3 3.6]	0.29[-0.6 0.88]	-0.34[-4.9 5.7]	-4.03[-9.7 1.49]	-85[-310 133]	-812[-2,349 974]
Invasive Status * Type of competition* Insecticide treatment	**20.6[4.7 40.4]***	0.9[-0.59 2.01]	1.05[-6.7 9.4]	2.7[-5.9 9.8]	138[-180 450]	1,381[-1,110 3,864]
Population[Invasive Status]	**758.6[84.3 1959]***	**0.06[0.008 0.18]***	**0.03[3.1e-17 0.03]***	**73.3[6.5 214.3]***	**4.9e4[4.8e3 1.5e5]***	**1.8e6[5.4e4 5.1e6]***
Species ID of heterospecific competitor [Grass/Legume functional group]	**3.4[2.5e-17 9.9]***	**0.0004[4.5e-17 0.0005]***	**0.18[6.9e-17 0.45]**	**0.67[5.1e-17 0.49]***	**1,025[5.8e-17 3563]***	**9.0e4[2.8e-17 5.7e4]***
Grass/Legume functional group	**3.5e8[2.9e-17 3.2e8]***	**3.8e+08[4.0e-17 9.6e8]***	**5.4e8[4.8e-17 4.3e8]***	**3.6e8[3.3e-17 4.7e8]***	**1.1e8[4.8e-17 2.2e7]***	**9.8e7[5.3e-17 1.3e8]***

Table 4. *Markov Chain Monte Carlo Generalized Linear Mixed Models (MCMCglmm) showing posterior mean parameter estimates and 95 per cent credible intervals [95% CI] for fixed effects of invasive status, insecticide, type of competition, and their interactions on relative interaction intensity (RII) based on fitness of invasive and native populations of Brassica nigra. Populations were nested within invasive status and treated as a random-effect variable in the models. Species identity of the four heterospefic competitors was also nested within functional group (i.e. legume or grass) and treated as a random-effect variable. P<0.001***, P<0.01**, P<0.05*. Last three rows are random-effect variables. Parameter estimates whose 95% CI do not cross zero are statistically significant. Significant values are marked in bold font*

Source of variation	Seed biomass/ plant	Seed number/plant
Invasive status	0.33[-0.14, 0.82]	0.24[-0.16, 0.64]
Type of competition	-90.6[-18.10, 19 400]	-210[-2 400, 3 610]
Invasive status * Type of competition	-0.29[-0.63, 0.09]	-0.16[-0.43, 0.12]
Insecticide treatment	0.036[-0.28, 0.33]	0.81[-0.19, 0.29]
Invasive status * Insecticide treatment	**-0.86[-1.27, -0.42]****	**-0.76[-1.10, -0.43]*****
Type of Competition * Insecticide treatment	0.15[-0.25, 0.61]	-0.15[-0.4, 0.12]
Invasive Status * Type of competition* Insecticide treatment	**0.54[0.11, 1.14]***	**0.44[0.008, 0.81]***
Population[Invasive Status]	**0.06[0.0004, 0.19]***	**0.05[0.003, 0.14]***
Species ID of heterospecific competitor [Grass/Legume functional group	**0.005[3.6e-17, 0.002]***	**0.0006[3.6e-17, 0.0015]***
Grass/Legume functional group	**8.5e7[1.9e-17, 5.8e8]***	**0.043[0.03, 0.05]***

DISCUSSION

Our data based on absolute mean trait values show that invasive and native populations of *B. nigra* had similar performances regardless of the type of competition they were subjected to and the kind of insecticide treatment they received (Table 3 ; Fig.1). Only plant height was higher for invasive populations than for native populations (Fig.1a). Of the few studies that have compared performances of invasive and native populations when grown with competition, there have been mixed results. Similar performances for invasive and native populations have been detected in several plants such as *Eschscholzia californica* (Leger and Rice 2003), *Hypericum perforatum* (Vilá et al. 2003), *Silene latifolia* (Blair and Wolfe 2004), *Lepidium draba* (Mckenney et al. 2007), *Centaurea maculosa* (He et al. 2009). However, other studies have found higher performances among invasive populations of *S. sebiferum* (Zou et al. 2008a) and *C. maculosa* (Ridenour et al. 2008) relative to their native conspecifics. Native populations of *Alliaria petiolata* (Bossdorf et al. 2004) had higher performance than invasive populations of the same plant.

Both theoretical and empirical studies on plant species coexistence show that conspecific competition is more intense than heterospecific competition given that a greater similarity in requirements should occur between conspecific than heterospecific neighbours (MacArthur and Levins 1967; Berendse 1983; Johansson and Keddy 1991; Rees et al. 1996; Chesson 2000). However, our data are not in agreement with the prediction of coexistence theory as both invasive and native populations of *B. nigra* had similar performances regardless of whether or not they had conspecific or heterospecific competitors (Table 3; Fig.1).

RII data indicate that invasive populations of *B. nigra* benefited from protection from herbivory when made to compete with legumes and grasses. This is indicated by the positive mean RII values for invasive populations under insecticide spray (Fig. 2). The legumes/grasses seemingly facilitated fitness of the invasive populations in the absence of, or under low level of insect damage only but did not do so under natural level of insect damage (Fig. 2). On the contrary, native populations benefited from insect damage because they had mean positive RII values when not protected from insect damage (Fig. 2). This indicates that fitness of the native populations was facilitated when interacting with the legumes/grasses under natural level of insect damage. However, when protected from insect damage, fitness of the native populations was not facilitated by the legumes/grasses (Fig. 2). These data indicate that the native populations of *B. nigra* were more tolerant of insect damage (i.e. compensated for herbivore damage by increasing fitness) than invasive populations because performances of the native populations were enhanced under natural level of herbivory while those of invasive populations were suppressed (Fig. 2). In fact, a study that used the same populations of *B. nigra* as used here found that native populations of *B.nigra* were more tolerant of herbivore damage than invasive populations (Oduor et al. 2011). A meta-analysis by Liu and Stiling (2006) found that invasive populations experience lower herbivore damage in exotic ranges than native populations of the same plants (Liu and Stiling 2006).The reduction in herbivore damage in the invasive ranges could have selected for plant genotypes that have high competitive abilities but have low tolerance of insect herbivore damage. Therefore, our finding that invasive populations of *B. nigra* were better competitors than native populations of the same plant (based on RII data) when treated with insecticide suggest that the invasive populations of *B. nigra* outcompete plants native to the exotic ranges because while being there, *B. nigra* experience low level of damage. On the other hand, high level of herbivore damage in the native ranges could have selected for plant genotypes that have high levels of tolerance to herbivory. This could explain why our results show that the native populations of *B. nigra* were more competitive than invasive populations when they were exposed to ambient levels of insect damge. The negative mean RII values for both invasive and native populations when grown with conspecific neighbours regardless of insecticide treatment indicates that there were only competitive interactions (and no facilitation) pitting the invasive populations of *B. nigra* versus native populations of the same plant species (Fig.2).

While it is important to test an invasive plant’s competitive ability, a major challenge still exists when it comes to choosing appropriate competitors (Bossdorf et al. 2005). Exotic invasive plants encounter different sets of competitors in their native and introduced ranges (i.e. species-specific interactions in either ranges) (Callaway and Aschehoug 2000). This means that invasive and native populations of the same plant species may be adapted to competing with different plant species leading to different co-evolutionary trajectories for both invasive and native populations. Various tests of post-introduction evolution in defence traits against natural enemies have been done by growing plants from both invasive and native populations in a common garden setting within native ranges of invasive plants (e.g. Wolfe et al. 2004; Meyer et al. 2005; Joshi and Vrieling 2005; Stastny et al. 2005; Franks et al. 2008; Zou et al. 2008a, b; Cripps et al. 2009; Huang et al. 2010; Oduor et al.2011). Any observed differences (or lack therefore of) between invasive and native populations in damage caused by herbivores in the native range were interpreted as post-introduction evolution in defence traits (or lack thereof) (e.g. Wolfe et al. 2004; Meyer et al. 2005; Joshi and Vrieling 2005; Stastny et al. 2005; Franks et al. 2008; Zou et al. 2008a, b; Cripps et al. 2009; Huang et al. 2010; Oduor et al. 2011). Similarly, use of plant species that co-occur with, and presumably have adapted to competing with invasive plants in their native ranges to compare competitive abilities of invasive and native populations may be justified. Any observed significant difference (or lack thereof) in traits between invasive and native populations due to competition with those heterospecific competitors would indicate post-introduction evolutionary changes (or lack thereof) in competitive abilities. Nevertheless, Zou et al. (2008a) propose that use of conspecific competitors should be preferred over heterospecific competitors when comparing competitive abilities of invasive and native populations. This is based on an argument that using heterospecific competitors from only one distributional range may give biased results (Bossdorf et al. 2004, 2005). However, our results show that use of both conspecific and heterospecific competitors may give consistent results that all point in the same direction. As it has been reported that various invasive plant species including *B. nigra* grow in dense monospecific stands as well as in heterospecific stands with other plant species in the exotic ranges (e.g. Weir et al. 2003, 2006; DeWalt et al. 2004; Lankau 2008; Ni et al. 2010), it should be expected that such plants experience both conspecific and heterospecific competition. We, therefore, do believe that using both conspecific and heterospecific competitors may give more robust results than using only either.

In conclusion, our data based on absolute mean trait values show that invasive and native populations of *B. nigra* had similar performances under both natural and reduced levels of insect damage and with conspecific or heterospecific competition. These results are not consistent with predictions of a theory on plant species coexistence according to which conspecific competition should be stiffer than heterospecific competition. RII data, nevertheless show that invasive populations of *B. nigra* were more competitive than native populations of *B. nigra* when they were all protected from insect herbivore damage.

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CHAPTER 6:

EXOTIC VERTEBRATE AND INVERTEBRATE HERBIVORES DIFFER IN THEIR IMPACTS ON NATIVE AND EXOTIC PLANTS: A META-ANALYSIS

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ABSTRACT

Herbivores modify various ecological processes including interactions between native and exotic plants that may affect invasion success by the exotic plants. It is unknown whether different types of exotic herbivores have similar effects on native and exotic plants. Using two distinct data sets, we ran meta-analyses to compare exotic vertebrate and invertebrate herbivore preferences for, and effects on performance and population sizes of native and exotic plants. We found that exotic vertebrate herbivores have positive effects on exotic plant performance and population sizes, and no significant effects on native plants. Exotic invertebrates have significant negative effects on performance and population sizes of both exotic and native plants. Vertebrates prefer to feed on native plants relative to exotic plants, while invertebrates prefer the exotic plants to native plants. Thus the exotic vertebrate herbivores may aid invasiveness of exotic plants, in accordance with the invasional meltdown hypothesis, while exotic invertebrate herbivores probably have no net effect on invasion process of the exotic plants. Invertebrate herbivore preferences for exotic plants support the biotic resistance hypothesis, as the native plants probably resist the invertebrate herbivory. We also tested an evolutionary logic that posits that herbivores with similar evolutionary history as plants will affect the plants less negatively than plants with which they have not co-evolved. Our results indicate that there is no consistent pattern in effects of exotic vertebrate and invertebrate herbivores on exotic plants with or without which they have co-evolved.

Key words: Exotic herbivores, invasional meltdown, biotic resistance, native plants, exotic plants, Meta-analysis.

INTRODUCTION

The effects of herbivores on plant individuals, populations, communities, and ecosystems have been widely investigated (e.g. Bergelson 1990; Hulme 1994,1996a, b; Karban and Baldwin 1997; Adler et al. 2001; Donlan et al. 2002; Vázquez 2002; Bisigato et al. 2005; Focardi and Tinelli 2005; Tierney and Cushman 2006; Baraza et al. 2007; Branson and Sword 2008; Egan and Irwin 2008; Jasmin et al. 2008; Joe and Daehler 2008; Nuñez et al. 2008; Veen et al. 2008). One important role of herbivores may be as mediators of the interaction between native and exotic plants. Herbivores may affect the outcome of competition between native and exotic plants through such mechanisms as direct feeding on the plants, soil disturbance, and indirect effects of feeding on plant competitors (Hulme 1996b; Stohlgren et al. 1999; Yates et al. 2000; Holmgren 2002; Cushman et al. 2004). Through any of these mechanisms, resident herbivores can reduce the invasion success of alien plants, and can thus contribute to biotic resistance to invasion (Maron and Vilá 2001; Levine et al. 2004). Alternatively, exotic herbivores, through the same mechanisms above, may facilitate the invasion of exotic plants, and this synergistic interaction between exotic organisms is termed ‘invasional meltdown’ (Simberloff 2006; Nuñez et al. 2008).

Exotic herbivore effects on various attributes of exotic and native plants have been widely studied by various researchers. In a meta-analysis of some of those studies, Parker et al. (2006) found that exotic herbivores generally caused an increase in abundance and species richness of exotic plants, while at the same time suppressed native plant abundance and species richness. What remains unresolved, however, is whether different types of exotic herbivores (i.e. vertebrate and invertebrate) can be expected to have similar impacts on native and exotic plants. Vertebrates differ from invertebrates in various ways. For example, vertebrates are larger, can consume more biomass per individual herbivore, have higher metabolic rates and bite sizes than invertebrates. Furthermore, vertebrates are more polyphagous and less affected by declines in food quality than invertebrates (Crawley 1989a; Gordon 1989; Maron and Crone 2006). Large vertebrate herbivores consume their host plant through browsing or grazing on foliage, or bark stripping (Gill 1992; Vázquez 2002). Arthropods employ various strategies to feed on different parts of a plant and these include sap sucking from phloem and xylem, leaf mining, chewing of roots, shoots, and reproductive plant parts (Bezemer and Jones 1998; Waldbauer 2003; Schoonhoven et al. 2005). These fundamental differences place the vertebrate and invertebrate herbivores into different feeding guilds characterized by different feeding strategies and patterns (Gordon 1989; Hulme 1994, 1996a). For example, small vertebrates like rodents may consume a grass seedling entirely, while molluscs, when presented with a similar seedling as the one presented to a rodent, would only graze on a certain part of the seedling, avoiding other plant parts that contain phytochemicals that challenge physiology of the herbivore (Hulme 1994). Strauss (1991) noted that whitetail deer, *Odocoileus virginianus*, browses woody stems of its host plant (*Rhus glabra*), while two beetles that use the same plant as a host, limit their feeding to the green tissue. Given these different feeding strategies and patterns between vertebrate and invertebrate herbivores, we asked first: Do exotic vertebrate and invertebrate herbivores affect performance and population sizes of native and exotic plants in the same way? As direct feeding is one way through which herbivores can affect plant performance and population sizes, we further asked: Do exotic vertebrate and invertebrate herbivores have the same preferences for native or exotic plants? Finally, evolutionary logic predicts that herbivores with similar evolutionary history with host plants will affect those plants less negatively than plants with which they have not co-evolved (Colautti et al. 2004; Parker et al. 2006). The meta-analysis by Parker et al. (2006) revealed an overall positive effect of exotic herbivores on exotic plants (i.e. invasional meltdown). However, it is still not known whether the general positive effects of exotic herbivores on exotic plants occurs regardless of whether or not the exotic vertebrate and invertebrate herbivores have co-evolved with the exotic plants. This knowledge gap led us to the third question: Does the impact of exotic vertebrate and invertebrate herbivores on performance and population sizes of exotic plants vary with co-evolutionary history of the herbivore with plant?

In the current paper, we attempted to resolve the three questions above by doing meta-analyses. We quantified the relative importance of herbivore types in affecting various attributes of native and exotic plants and also the preferences of the herbivores for native or exotic plants. We divided the herbivores into two types: exotic vertebrate and invertebrate herbivores. The exotic herbivores (vertebrate and invertebrate) and plants were further grouped as having similar evolutionary history or not, based on the information available regarding their (dis) similarities in sources of origins. This grouping enabled us to analyze whether the impacts of exotic herbivores on exotic plant performance and population sizes depended on whether they shared an evolutionary history.

MATERIALS AND METHODS

THE DATABASE

Two different databases were used in the analysis of exotic herbivore preferences for, and effects on performance and population sizes of native or exotic plants. The data were obtained from published papers by using various keyword searches on online databases. These were: introduced herbivore*, exotic herbivore*, exotic herbivore* and (enclos* or fence*), herbivore* and exotic*, herbivore* and (introduced or invasion*), exotic herbivore* and (plant invasion*), introduced herbivore* and (plant invasion*), introduced herbivore* and (native plant*), and introduced herbivore* and (exotic plant*) in the online database Web of Science. More studies were found by searching the reference lists of empirical studies and review articles. In addition, data were obtained from one manuscript that was in press and one master’s thesis. All the papers from which the data were obtained came from peer-reviewed journals.

Most of the studies *experimentally* assessed the effects of exotic herbivores on biomass or performance (e.g. survival, flower and seed production, canopy cover and height) of exotic or native plants. That is, herbivores had to be experimentally allowed access into, or excluded from field studies. A few studies, however, were observational in which the authors collected data in fields where the exotic herbivores had been active or not. The authors confirmed presence or absence of activities of target herbivores using various criteria, for example, looking for fecal deposits, soil disturbance and plant damage typical of the herbivore concerned. Areas of origin of the exotic vertebrate and invertebrate herbivores and plants were determined for the two different datasets used in the analysis. When the origin of a plant or herbivore was not stated by the authors, we determined the plant origin using an online database of the United States Department of Agriculture (USDA) PLANTS Database http://plants.usda.gov., and for the herbivore, we used Wikipedia (http://www.wikipedia.org/). We assume a shared evolutionary history between plants and herbivores when they originate from the same geographic areas (e.g. a continent). Hence, to analyze whether the effects of exotic herbivores on exotic plants depended on their shared history, we classified the plants and herbivores as co-evolved when they came from the same continent. For example, if an herbivore and a plant that are both native to Europe are both introduced to North or South America, or Australia, then we classified them as exotics that share an evolutionary history.

Herbivores intentionally introduced as biological control agents were not considered in the analyses, except in one case where an exotic biological control agent was reported attacking both target exotic and non-target native plants. This case involved a flowerhead weevil, *Rhinocyllus conicus*, which had been introduced to control exotic Eurasian thistles of the genus *Carduus* L. but has evolved post-introduction to attack native *Cirsium* thistles (Louda et al. 1997). Biological control agents are introduced on a general assumption that they have narrow host ranges (Louda et al. 2003), therefore, we did not include them in our analysis as including them would bias the results since it is highly likely that they would prefer the exotic plants to native plants.

In the analysis of effects of exotic herbivores on plant performance and population sizes, most of the studies involving vertebrates were of cattle (74 studies), with other studies considering effects of hare (20 studies), horses (18 studies), feral pigs (13 studies), sheep (12 studies), rats (7 studies), goats (6 studies), and wombats (3 studies). Studies on invertebrates included five species of slugs (*Arion* reticulate**)** ***(***Arionidae***),*** *Deroceras laeve* (Agriolimacidae), *Limacus flavus* (Limacidae), *L. maximus* (Limacidae) and *Meghimatium striatum*) (Philomycidae) (17 studies for them all), one snail (*Lymnaea stagnalis*) (Lymnaeidae**)** (1 study), and two insect species: *Brachypterolus pulicarius* (Nitidulidae) and *Hypera brunneipennis* (Curculionidae) (4 studies for them both) (See appendix 1). Treatments involved presence of herbivores while controls constituted exclusion of herbivores. Vertebrate herbivore exclusions were achieved by use of fences. In some studies, the focus was on excluding the large herbivores only; hence it was possible that non-target small herbivores could access the excluded areas. Under such circumstances, we regarded as valid, only studies in which the authors reported that the observed effects were due only to the activity of target herbivores using the criteria mentioned in the previous paragraph. Presence or absence of exotic invertebrate herbivores was manipulated by application or not, of broad-based molluscicides or insecticides that were equally effective on native invertebrates. All studies were done in the field. We omitted studies of invasions into agricultural crop systems, but included data from studies on pasture systems. Artificial selection of crops plants may have altered their defenses against natural enemies (Rosenthal and Dirzo 1997). Therefore, including studies involving interactions between exotic herbivores and cultivated crop plants in our analysis would not allow us to understand how herbivores may influence plant invasion.

Data on feeding preferences of exotic vertebrate and invertebrate herbivores were obtained from field-based or greenhouse studies. The greenhouse studies used cages to exclude unwanted invertebrate herbivores. In all, 64 studies involved invertebrates (five species of slugs, three of snails and one of insect). The slugs were: *Arion ater* (1 study), *A. subfuscus* (1 study), *A. fasciatus* (1 study) (all of them members of Arionidae family), *Milax gagates* (Milacidae) and *Deroceras reticulatum* (Agriolimacidae) (10 studies).The snails were *Pomacea canaliculata* (5 studies), *P. insularum* (both from the

Ampullariidae family) (32 studies) *and Otala lactea* (Helicidae) (7 studies). Seven studies used the insect *Rhinocyllus conicus* (Curculionidae). Seven vertebrate species were involved. These were fish (*Ctenopharyngodon idella*) (Cyprinidae) (1 study), red deer (*Cervus elaphus*) (Cervidae) and fallow deer (*Dama dama*) (Cervidae) (2 studies), cattle and horses (2 studies), European rabbit (*Oryctolagus cuniculus*) (Leporidae) (1 study) and goats (1 study). In all the studies, amount of plant tissue eaten, or change in plant canopy cover following herbivory, was measured with respect to the presence or absence of herbivores (see appendix 2).

Data (mean response values, standard deviations and sample sizes of control and treatment groups) from each source were extracted from the graphs, tables, or digitized from figures. Measurements of standard errors (SE) of the means were all converted to standard deviations (SD). Experiments in which the effect of herbivory was measured for different aspects of plant performance, for example, % canopy cover, maximum plant height, and flower production, mean values of effect size, *d*, were computed from a combination of all of them to avoid a problem of pseudoreplication. To compute the mean values of effect size, *d*, we computed individual effect sizes, *d*, (see the paragraph below on how to compute effect size, *d*,) for each aspect of plant performance measured, summed up the different values of *d* and divided the sum by total number of aspects of plant performance that contributed to the summed up value of *d*. In certain studies, the authors reported all the variables they had measured over different time and spatial scales. For example, they measured plant canopy cover per plot, with the plots ranging in sizes. Furthermore, they used new transects every time they took measurements. Because of spatial and temporal variabilities of such outcomes, we treated them as independent studies. Consequently, 174 studies from 26 papers were used in the analysis of effects of exotic herbivores on performance and population sizes of native and exotic plants (Appendix 1), while 71 studies from 11 papers and one master's thesis were used in the analysis of exotic herbivore preference for native or exotic plants (Appendix 2).

STATISTICAL ANALYSES

Three different meta-analyses were run as detailed below.

ANALYSIS OF EFFECTS OF EXOTIC VERTEBRATE AND INVERTEBRATE HERBIVORES ON PERFORMANCE AND POPULATION SIZES OF NATIVE AND EXOTIC PLANTS

For each study, we calculated an effect size, *d*, (Appendices 1 & 2) (Rosenberg et al. 2000). The effect size, *d*, refers, in our case, to the difference in plant performance and population size in the presence or absence of herbivores. The plant performance in the absence of herbivore constituted the control group, while plant performance in the presence of herbivore was the treatment group. In the presence of an herbivore, a positive effect size, *d*, for native or exotic plant implied that an herbivore-related activity was beneficial for the plant. On the other hand, a negative effect size, *d*, meant that the plant performance was negatively impacted by the activity of herbivore.

ANALYSIS OF EXOTIC VERTEBRATE AND INVERTEBRATE HERBIVORE PREFERENCES FOR EXOTIC OR NATIVE PLANTS

In the analysis of exotic herbivore preference for native or exotic plants, again, in our case, the effect size, *d*, for exotic herbivore preference is the difference in amount of tissue eaten or damaged by herbivores between native and exotic plant. Here, the experimental group comprised exotic plant response to herbivory, while the native plant response to herbivory formed the control group. In this case, a positive effect size, *d*, for either vertebrate or invertebrate meant that exotic plant was preferred over the native plant, while a negative effect size, *d*, showed that a native plant was preferred to an exotic plant.

ANALYSIS OF THE EFFECTS OF EXOTIC VERTEBRATE AND INVERTEBRATE HERBIVORES ON PERFORMANCE AND POPULATION SIZES OF EXOTIC PLANTS WITH OR WITHOUT WHICH THEY CO-EVOLVED

The analysis of the effects of exotic vertebrate and invertebrate herbivores on performance and population size of exotic plants with or without which they co-evolved was similar to the first analysis stated above, except that under a categorical fixed model, we classified the exotic herbivore types and plants as having the same geographic origins or not. We used a sub-set of the data employed in the first analysis stated above (i.e., we used only the data on interactions between exotic herbivores and exotic plants, leaving out data on interactions between the herbivores and native plants). Here, a positive effect size, *d*, means that the exotic herbivore (vertebrate or invertebrate) is beneficial to the exotic plant with or without which it co-evolved. On the other hand, a negative effect size, *d*, means that the exotic herbivore is harmful to the exotic plant it co-evolved with or not.

The effect size, *d*, was computed using the formula: $d_i = \frac{\overline{X}^E - \overline{X}c}{S} J$, where $\overline{X}^E$ is the mean response of the experimental group, $\overline{X}^C$ is the mean response of the control group, S is the pooled standard deviation. Mean effect size, Hedges'*d*, was calculated from the individual effect sizes for each study in each of the two data sets using the formula: Hedges'*d* = $\frac{\sum_{i=1}^{n} w_i d_i}{\sum_{i=1}^{n} w_i}$, where d_i is the effect size calculated for the i_{th} study and w_i is the reciprocal of the sampling variance of the i_{th} study. Ninety-five percent confidence limits around the effect size were bootstrapped (1000 iterations) and estimates of the effect size were considered significant only if the resulting confidence intervals did not overlap zero (Gurevitch and Hedges 1999). We tested whether categorical groups (presence or absence of vertebrate or invertebrate herbivores) were homogeneous with respect to effect sizes, *d*, using heterogeneity statistic (Q). We calculated the total heterogeneity (Q_T) for all effects tested across studies on vertebrate and invertebrate herbivores as well as heterogeneity within the herbivore type (Q_W) and between herbivore types (Q_B), and the significance of these statistics was evaluated using a chi-square distribution (Gurevitch and Hedges 1999). A categorical fixed- effects model procedure of meta-analysis was employed with categories being herbivore type (vertebrate or invertebrate) in both analyses.

FAIL-SAFE TESTS

Given that our analyses were based only on published studies except for one manuscript in press and a master's thesis, and studies that show large and significant effects might be more likely to be published than studies that show weak or no effects (i.e. publication bias) (Rosenthal 1979), we calculated Rosenthal's fail-safe numbers for each effect size tested. Fail-safe numbers indicate the number of non-significant, unpublished, or missing studies that would need to be added to the meta-analysis in order to change its results from significant to non-significant (Rosenthal 1979). Results of meta-analysis are considered robust if the computed fail-safe number for each meta-analysis exceeds 5k+10, where k is the number of studies included in a meta-analysis (Rosenthal 1979). A software Meta Win version 2.0 was used in all the analyses (Rosenberg et al. 2000).

RESULTS

EFFECTS OF EXOTIC VERTEBRATE AND INVERTEBRATE HERBIVORES ON PERFORMANCE AND POPULATION SIZES OF EXOTIC AND NATIVE PLANTS

We found that exotic vertebrates and invertebrates have significantly different effects on performance and population sizes of exotic plants (Q_B = 29.20, p = 0.035). The vertebrates have significant positive effects on exotic plants (mean effect size, Hedges'*d*, = 0.2259). On the contrary, invertebrates have significant negative effects on exotic plants (mean effect size, Hedges'*d*, = -0.4499) (Table 1a; Fig.1). Both the exotic vertebrates and invertebrates had similar effects on native plants (Q_B =14.34, p = 0.160), because the bootstrapped confidence intervals of both herbivore mean effects overlap with each other (Fig.1). Overall, invertebrates affect performance and population size of native and exotic plants in the same way (Q_B= 1.02, p = 0.55), while vertebrates affect the native and exotic plants differently (Q_B=36.69, p = 0.022) (Fig.1).

Table 1a: *Mean effect sizes, Hedges´ d, of exotic vertebrate and invertebrate herbivores on performance and population size of exotic plants. Under class, vert. = vertebrate herbivore; inv. = invertebrate herbivore. #Studies = the number of studies for each herbivore type. The effect size is statistically significant only if its bootstrapped confidence interval (Bootstrap CI) does not overlap zero.*

Class	# studies	*d*	df	95 % CI	Bootstrap CI	Bias CI
Vert.	71	0.2259	70	0.1377 to 0.3142	0.0148 to 0.4425	-0.0007 to 0.4279
Inv.	11	-0.4499	10	-0.7105 to -0.1893	-0.6888 to -0.0752	-0.6951 to -0.0865

Table 1b: *Mean effect sizes, Hedges´ d, of exotic vertebrate and invertebrate herbivores on performance and population sizes of native plants. Under class, vert. = vertebrate herbivore; inv. = invertebrate herbivore. #Studies = the number of studies for each herbivore type.The effect size is statistically significant only if its bootstrapped confidence interval (Bootstrap CI) doesnotoverlapzero.*

Class	# studies	*d*	df	95 % CI	Bootstrap CI	Bias CI
Vert.	81	-0.1187	80	-0.1898 to -0.0475	-0.3308 to 0.0584	-0.3466 to 0.0470
Inv.	11	-0.6260	10	-0.9137 to -0.3384	-1.1631 to -0.0530	-1.0672 to 0.0121

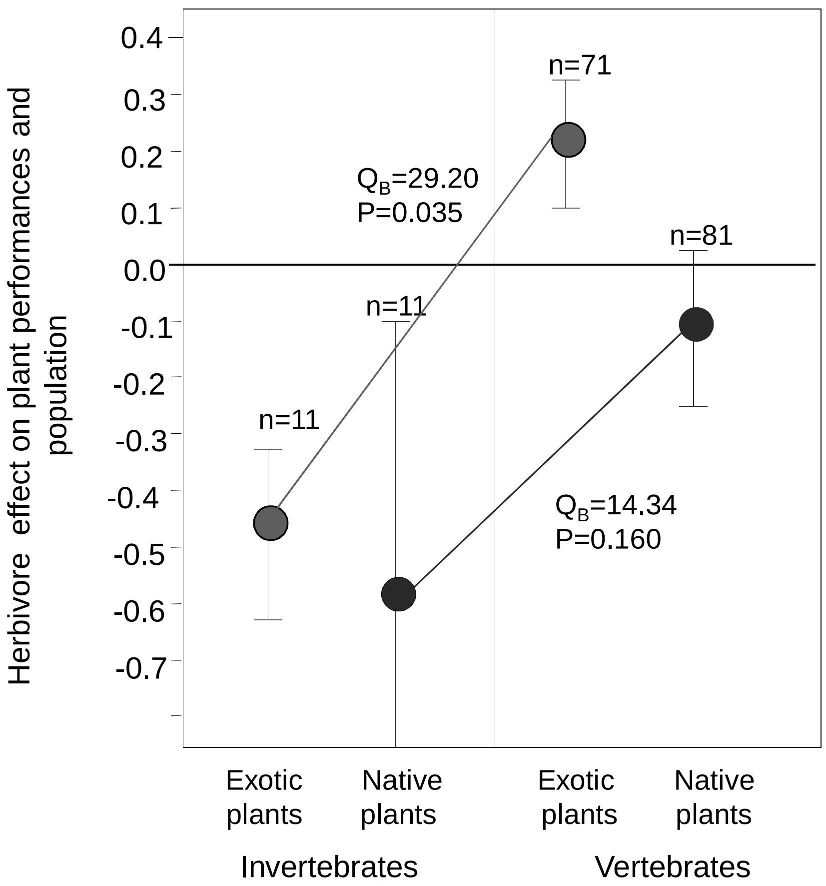

Figure 1: *Mean effects, Hedges'd (represented by the circles), of exotic vertebrate and invertebrate herbivores on population sizes and performance of native and exotic plants. Hedges'd for exotic plants are represented by white circle, and for native plants, it is represented by dark circles. Bars represent bootstrapped confidence intervals of Hedges' d. Numbers on top of the bars are the numbers of studies for each particular case. Values of Hedges'd are statistically significant only if their bootstrapped confidence intervals do not overlap zero. A positive effect size means an exotic herbivore enhanced performance and population size of a plant, while a negative effect size means that the herbivore had overall negative impacts on the plant. Hedges' d values whose bootstrapped confidence intervals overlap with each other are not significantly different from one another (i.e. P>0.05).*

TROPHIC PREFERENCES OF EXOTIC VERTEBRATE AND INVERTEBRATE HERBIVORES FOR EXOTIC OR NATIVE PLANTS

Exotic vertebrate herbivores preferred feeding on native plants to exotic plants (mean effect size, Hedges'*d,* = -0.7069), while exotic invertebrates prefer exotic plants to native ones (mean effect size, Hedges'*d,* = 0.4090) (QB = 49.35, p = 0.021) (Table 2; Fig. 2).

Table 2: *Mean effect sizes, Hedges´ d, of exotic vertebrate and invertebrate herbivore preference for native or exotic plants. Under class, vert. = vertebrate herbivore; inv. = invertebrate herbivore. #Studies = the number of studies for each herbivore type.The effect size is statistically significant only if its bootstrapped confidence interval (Bootstrap CI) does not overlap zero.*

Class	# studies	*d*	df	95 % CI	Bootstrap CI	Bias CI
Vert.	7	-0.7069	6	-1.0633 to -0.3505	-1.086 to -0.053	-1.047 to 0.023
Inv.	64	0.4090	63	0.2824 to 0.5356	0.041 to 0.731	0.066 to 0.750

EFFECTS OF EXOTIC VERTEBRATE AND INVERTEBRATE HERBIVORES ON EXOTIC PLANTS WITH OR WITHOUT WHICH THEY CO-EVOLVED

Exotic vertebrate and invertebrate herbivores differed in their effects on performance and population size of exotic plants that they did not co-evolve with (Q_B= 0.080, p = 0.000). While the exotic vertebrates did not have a significant effect on exotic plants with which they did not co-evolve (mean effect size, Hedges´, *d*, = 0.4551, bootstrapped confidence interval overlaps zero), the exotic invertebrates had a significant negative effect on exotic plants they did not co-evolve with (Hedges´, *d*, = -0.4077, bootstrapped confidence interval does not overlap zero) (Table 3b; Fig.3). However, the exotic vertebrates and invertebrates did not differ in their effects on exotic plants with which they shared evolutionary history (Q_B=0.01389, p = 0.195); both of their effects on the exotic plants were not significantly different from zero (Table 3a; Fig. 3).

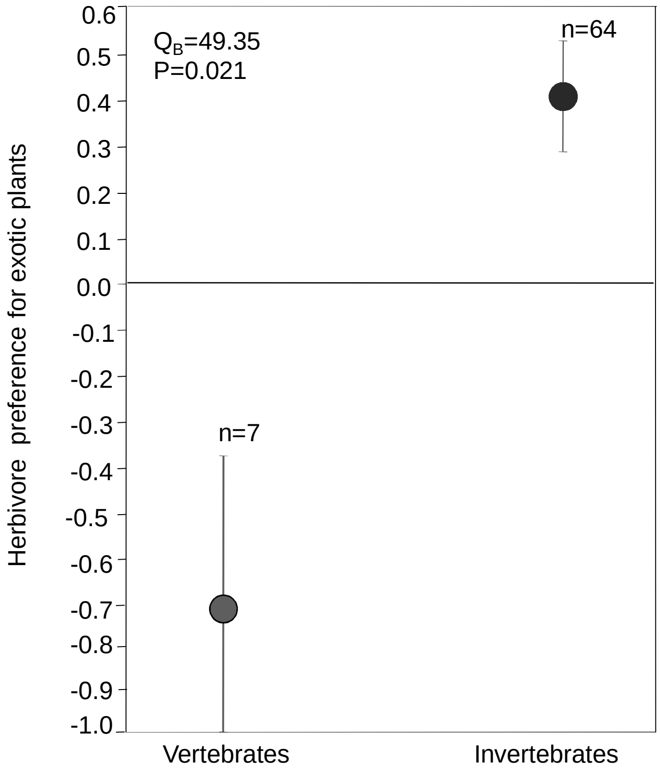

Figure 2: *Mean effects, Hedges'd (represented by the circles), that indicate the trophic preferences of exotic vertebrate and invertebrate herbivores for exotic or native plants. Bars represent bootstrapped confidence intervals of Hedges' d. Numbers on top of the bars are the numbers of studies for each particular case. Values of Hedges'd are statistically significant only if their bootstrapped confidence intervals do not overlap zero. A positive preference means that the herbivores prefer to feed on the exotic plants when they have a choice between exotic and native plants. A negative preference for exotic plants means that the herbivores prefer native plants to exotic plants.*

Table 3a: *Mean effect sizes, Hedges´ d, of exotic vertebrate and invertebrate herbivores on performance and population sizes of exotic plants with which they co-evolved. Under class, vert. = vertebrate herbivore; inv. = invertebrate herbivore. The effect size is statistically significant only if its bootstrapped confidence interval (Bootstrap CI) does not overlap zero.*

Class	# studies	*d*	df	95 % CI	Bootstrap CI	Bias CI
Vert.	17	0.455	16	0.294 to 0.615	-0.029 to 1.001	-0.083 to 0.938
Inv.	6	-0.407	5	-0.810 to -0.005	-0.643 to -0.201	-0.657 to -0.209

Table 3b: *Mean effect sizes, Hedges´ d, of exotic vertebrate and invertebrate herbivores on performance and population sizes of exotic plants with which they did not co-evolve. Under class, vert. = vertebrate herbivore; inv. = invertebrate herbivore. The effect size is statistically significant only if its bootstrapped confidence interval (Bootstrap CI) does not overlap zero.*

Class	# studies	*d*	df	95 % CI	Bootstrap CI	Bias CI
Vert.	54	0.1065	53	-0.003 to 0.215	-0.085 to 0.309	-0.084 to 0.317
Inv.	5	-0.5030	4	-0.991 to -0.015	-1.009 to 0.729	-1.024 to 0.729

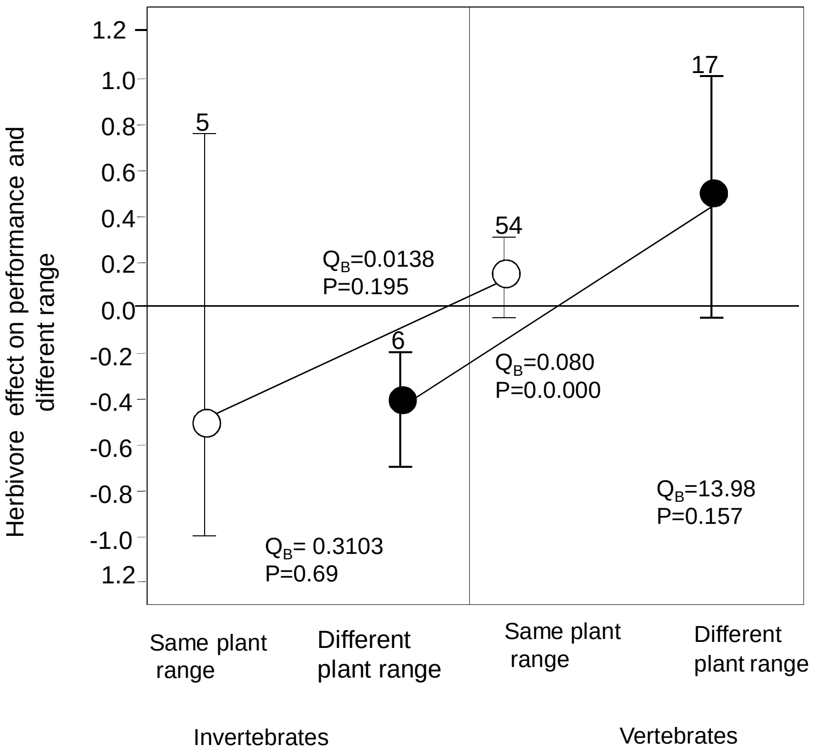

Figure 3: *The mean effects, Hedges'd (represented by the circles), of exotic vertebrate and invertebrate herbivores on population sizes and performance of exotic plants with or without which the herbivores had co-evolved (i.e., same or different plant range, respectively). Numbers on top of the bars are the numbers of studies for each particular case. A value of Hedges'd is statistically significant only if its bootstrapped confidence interval does not overlap zero. A positive value of Hedges'd means an exotic herbivore enhanced performance and population size of an exotic plant, while a negative Hedge'd means that the herbivore had negative impacts on the exotic plant. Hedges'd values whose bootstrapped confidence intervals overlap with each other are not significantly different from one another (i.e., P>0.05)*

In all the analyses above, there were heterogeneities in effect sizes across all studies involving vertebrate and invertebrate herbivores (Q_T), but also within the herbivore type (Q_W) (Table 4).

Table 4: *Heterogeneities of individual effect sizes, d, of exotic vertebrate and invertebrate herbivore on preferences for, and performance and population sizes of native and exotic plants, with and without which they co-evolved.* Q_T *= total heterogeneity of effect sizes across all studies involving both vertebrate and invertebrate herbivores,* Q_W *= heterogeneity of effect sizes within herbivore type (vertebrate or invertebrate). All the heterogeneities were statistically significant (*$p < 0.000$*). Same origin means exotic plant introduced from the same continent as herbivore (i.e. herbivores co-evolved with plants); different origin means exotic plant introduced from a different continent than herbivore (i.e. no co-evolution between herbivore and plants). (A) refers to herbivore effect on exotic plants regardless of whether the herbivores and plants had co-evolved, while (C) takes into account the co-evolution of both herbivores and exotic plants.*

	Native plants		Exotic plants	
Herbivore effect measured	Q_T	Q_W	Q_T	Q_W
(A) Performance and population size:	556.90		459.13	
Vertebrates		474.00		401.35
Invertebrate		68.56		28.58
(B) Trophic preference:			609.60	
Vertebrate				34.00
Invertebrate				526.23
(C) Performance and population size:				
Same origin			401. 34	
Vertebrate				179.79
Invertebrate				19.94
Different origin			28.58	
Vertebrate				207.57
Invertebrate				8.33

FAIL-SAFE TESTS

Rosenthal's fail-safe number for herbivore preferences was 124.6, which is lower than the threshold value of 365 (i.e. 5 * 71 +10) needed for robustness of our results on herbivore preferences for native or exotic plants (Rosenthal 1979). The fail-safe number for herbivore effect on performance and population sizes of native and exotic plants was 74.1, which is also lower than the threshold number of 880 (i.e. 5*174 +10) needed for our results to be robust (Rosenthal 1979).

DISCUSSION

Our results suggests that exotic vertebrate and invertebrate herbivores have disparate effects on plant invasion (Fig.1), and that these effects appear to be independent of whether or not there is a shared area of origin between the exotic herbivores and plants (Fig. 3). The exotic vertebrates had net positive effects on performance and population size of exotic plants, whereas for the native plants, the exotic vertebrates did not have a significant effect (Fig.1). That is to say, while the performance and population sizes of exotic plants were enhanced by the exotic vertebrates, the same attributes of the native plants were not affected by the herbivores. But over time, the exotic vertebrates may, in keeping with the invasional meltdown hypothesis, facilitate or speed up the invasion process by the exotic plants, by consistently enhancing their performance and population sizes over those of the native plants. This can occur when, for example, the population sizes of the native plants remain more or less constant, while those of exotic plants increase through time. The overall effect would be that the relative abundance of exotic plants would increase while that of native plants would decrease over time. In fact, Holmgren et al. (2000) found that grazing by exotic cattle, horses and rabbits in a Chilean matorral resulted in a significant increase in abundance of exotic herbaceous plants, while at the same time, the native herbs decreased in relative abundance. Grazing by exotic cattle and horses increased species richness and ground cover by exotic grasses in a blue oak savanna of southern Sierra Nevada, California (Keeley et al. 2003). Bock et al. (2007) reported that over time, in south eastern Arizona, compared to ungrazed plots, plots grazed by exotic livestock experienced a significant increase in total grass canopy cover by two exotic grasses with a concomitant decline in canopy cover of native grasses. While studying the effects of simulated El Niño Southern Oscillation Event (ENSO), shade, and herbivory on the composition of an herbaceous community in north-central Chile, Manrique et al. (2007) found that exclusion of exotic European rabbits and hares caused an overall increase in density and biomass of native grasses that outcompeted exotic grasses. Best and Arcese (2009) found that exotic Canada geese selectively grazed on exotic grasses while avoiding native forbs within the southern gulf Islands of British Columbia. This selective grazing caused the exotic grasses to overcompensate for the lost biomass, hence proportionally out numbering the native forbs that showed a relative decline in numbers. In contrast to effects of exotic vertebrates, exotic invertebrates suppress performance and population size of both exotic and native plants about equally (Fig. 1).

Through herbivory and soil disturbance, exotic herbivores can influence interactions between native and exotic plant species. Herbivores that promote exotic plants through disturbance might negatively impact native plants without actually consuming them. Alternatively, exotic herbivores that prefer to feed on native plants, hence retarding their performance and population sizes, over exotic plants may favour the exotic plants. In our study, we also looked at trophic preferences of exotic vertebrate and invertebrate herbivores for native or exotic plants and tried to link the results with effects of the same herbivore types on performance and population sizes of plants under field conditions. Whereas the vertebrates preferred to feed on native plants, the introduced invertebrates preferred exotic plants (Fig. 2). Due to their larger sizes relative to invertebrates, vertebrate herbivores generally consume more plant biomass than do the invertebrates (Crawley 1989a). Furthermore, the vertebrates have a tendency to kill plants while invertebrates often only retard plant growth (Parker et al. 2006), although some studies have reported that mollusks kill seedlings of herbaceous and woody plants (Hanley et al. 1996; Joe and Daehler 2008; Hanley and Sykes 2009; Strauss et al. 2009), and even insects can cause deaths of adult trees through rare repeated complete or near- complete defoliation of the trees (Hendrix 1988; Schoonhoven et al. 2005). Hence the preferential feeding of exotic vertebrates on native plants may directly lead to deaths of the native plants, leaving the less preferred exotic plants with more space and growth resources to exploit. The exotic plants may then take advantage of this release and grow fast to occupy the empty space and become invasive. These results suggest that at least some of the effects of exotic vertebrate herbivores on the plant community are due to direct consumption.

However, the negative impacts of exotic invertebrates on both native and exotic plants may not be explained by trophic preferences of the invertebrates. Whereas the invertebrates preferred to feed on exotic plants over the native plants, they affected performance of both groups of plants equally negatively in field experiments (Figs.1 & 2).

The significant negative effects of exotic invertebrates on exotic plants with which they did not share evolutionary history (Fig. 3) support the evolutionary logic that herbivores with no previous co-evolutionary history with host plants will affect the plants more negatively than plants with which they share an evolutionary history. This conclusion should, however, be drawn with caution because of the low power of our test (there were only six studies available to test the evolutionary logic for invertebrates and exotic plants with no similar evolutionary history against five studies involving interactions between exotic invertebrates and plants with a common evolutionary history) (Fig. 3). Our findings on exotic vertebrate effects on exotic plants with or without which they had co-evolved do not, however, support the evolutionary logic. The vertebrates did not have significant effects on performance and population sizes of exotic plants with or without which they were introduced from the same ranges (i.e. continent) (Fig. 3).

As far as we are informed, no meta-analysis has been done to compare preferences for, and impacts on native and exotic plants by vertebrate and invertebrate herbivores in the native ranges of both the herbivores and plants. It is, therefore, difficult to compare our results with previous results on the studies of relative importance of vertebrate and invertebrate herbivores in affecting performance and population sizes of plants within the native ranges of both plants and herbivores. Nevertheless, to get a picture of what results can be obtained from the empirical studies that focus on the relative effects of native vertebrate and invertebrate herbivores on native plant performance within the native ranges, Strauss (1991) found that herbivory by the insects, chrymoselid and cerambycid beetles, was more injurious to ramet growth of a plant, smooth sumac (*Rhus glabra*) than herbivory by a whitetail deer (*Odocoileus virginianus*). Hulme (1994) reported that rodents killed more grass seedlings than molluscs. Hulme (1996a) established that of all three herbivore types, namely, rodents, molluscs, and arthropods; rodents had the greatest effect on plant performance, causing a decline in plant biomass by as much as 50 % and significantly increasing plant mortality.

In summary, our results show that exotic vertebrate herbivores may help initiate or speed up invasion by exotic plants, while exotic invertebrates suppress performance and population sizes of both native and exotic plants.

FUTURE PERSPECTIVE

The ideal meta-analysis of effects of exotic herbivores on performance and population sizes of exotic and native plants would focus on the same attributes of the plants across different studies. However, in our meta-analysis, we included studies that did not all measure the same attributes. While some studies reported any one of the various attributes of plants performance and population size namely, percent cover per unit area of land, survival and plant height, flower and seed production, others reported two or more of them. Our decision to quantify the effects of exotic herbivores using studies that reported different variables was compelled by lack of many papers that report the same variables.

Our computed Rosenthal's fail-safe numbers fell way below the threshold values needed for our calculated overall effect sizes (Hedges'*d*) to be robust. This, therefore, means that our results are just provisional, and that, more studies that report the same attributes of plants attacked by both vertebrate and invertebrate herbivores across different study systems are needed in future in order to authenticate our findings. Moreover, while there were quite a few studies on invertebrates, the numbers of different invertebrate species explored was relatively small. Phylogenetically close herbivores may prefer to feed on related plants (Janz and Nylin1998). Similarly, phylogenetically related plants may suffer the same level of herbivory from a given herbivore (Agrawal and Kotanen 2003). More data are needed on the preferences and impacts of different species of exotic invertebrates in order to more fully understand their effects. Earwigs

(*Forficula auricularia*), for example are very common introduced invertebrate herbivores that have received little attention in this context.

Both vertebrate and invertebrate herbivores may strongly affect plant performance (Crawley 1989a; Palmisano and Fox 1997). Although numerous studies have investigated the effects of vertebrate and invertebrate herbivores on various aspects of plant performance, only very few of such studies have simultaneously examined effects of both types of herbivores on an individual plant (Strauss 1991; Hulme, 1994, 1996a; Palmisano and Fox, 1997; Sessions and Kelly 2001). Studying effects of both vertebrate and invertebrate herbivores, as opposed to only either of them, on performance and population size of individual plants may help identify the more important herbivore guild (i.e. between vertebrate and invertebrate herbivores) that influences plant demography (Hulme 1996a). The data we used in the current meta-analysis all came from studies that looked at only one herbivore guild, i.e., either vertebrate or invertebrate, and not both concurrently, while investigating preferences for, and effects on performance and population sizes of native and exotic plant. The simple reason for this is that there were no data available on factorial experiments that simultaneously investigated exotic vertebrate and invertebrate preferences for, and effects on native and exotic plants. Future studies that aim at understanding how exotic vertebrate and invertebrate herbivores may affect interaction between native and exotic plants should use factorial experimental designs to investigate effects of both vertebrates and invertebrates on the same individual plants. Such studies would give a better understanding of which herbivore type (vertebrate or invertebrate) has more potential to affect demographies of co-occurring native and exotic plants, and hence invasion process of the exotic plants.

In the current paper, we attempted to find out if trophic preferences of exotic vertebrate and invertebrate herbivores for exotic or native plants can aid or hamper invasion by the exotic plants. Various other mechanisms through which herbivores may affect plant invasion have, nevertheless, been reported. These include seed dispersal (Janzen 1984; Malo and Suarez 1995, 1997; Fisher et al. 1996; Campbell and Gibson 2001; Pakeman 2001; Holmgren 2002; Pakeman et al. 2002; Vellend et al 2003; Mouissie 2004; Myers et al. 2004; Manzano and Malo 2006; Loesser et al. 2007; Loydi and Zalba 2008) and soil disturbance (Cushman et al. 2004). A future analysis similar to the one we carried out but including other mechanisms described above would help elucidate the most important mechanisms by which the exotic vertebrate herbivores may influence plant invasion.

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Appendix 1: *Effect sizes, d, of exotic vertebrate and invertebrate herbivores on performance and population sizes of native and exotic plants. Vert = vertebrate herbivores; inv = invertebrate herbivores. A positive effect size means a herbivore enhanced performance or population size of plants while a negative effect size means a herbivore was detrimental to the plant performance or population size.*

Citation	**Site**	**Herbivore**	**Plant type**	**Units**	**Interaction**	**Effect size *d*.**
1	West USA	Horses (vert)	Exotic	Stems/ 50	Dispersal	-0.5039
1	West USA	Horses (vert)	Exotic	Stems/ 50	Dispersal	0.6845
1	West USA	Horses (vert)	Exotic	Stems/ 50	Dispersal	1.3459
1	West USA	Horses (vert)	Native	Stems/ 50	Dispersal	-1.7724
1	West USA	Horses (vert)	Native	Stems/ 50	Dispersal	-0.0854
1	West USA	Horses (vert)	Native	Stems/ 50	Dispersal	-1.3702
1	West USA	Horses (vert)	Native	Stems/ 50	Dispersal	0.4175
2	Argentina	Horses (vert)	Exotic	% cover/	Dispersal	0.7275
3	Australia	Feral pigs &	Exotic	Survival	Disturbanc	1.0467
3	Australia	Feral pigs &	Exotic	Survival	Disturbanc	-0.3413
3	Australia	Feral pigs &	Exotic	Survival	Disturbanc	4.2474
4	Oregon,	Horses (vert)	Exotic	% cover	Browsing	2.3842
4	Oregon,	Cattle (vert)	Exotic	% cover	Browsing	2.8521
4	Oregon,	Horses (vert)	Exotic	% cover	Browsing	1.4461
4	Oregon,	Cattle (vert)	Exotic	% cover	Browsing	1.2811
5	California,	Feral pig (vert)	Native	% cover	Disturbanc	0.3202
5	California,	Feral pig (vert)	Native	% cover	Disturbanc	0.5533
5	California,	Feral pig (vert)	Exotic	% cover	Disturbanc	0.5193
5	California,	Feral pig (vert)	Exotic	% cover	Disturbanc	1.1107
6	Charles	Cattle (vert)	Exotic	% cover	Grazing	0.2285
6	Gunnison,	Cattle (vert)	Exotic	% cover	Grazing	-0.5773
6	Uncompah	Sheep (vert)	Exotic	% cover	Grazing	0.6532
6	Wild horse,	Feral horses (vert)	Exotic	% cover	Grazing	0.1404
7	Oahu,	4 Species of slugs	Native	Leaf area	Herbivory	0.2043
7	Oahu,	4 Species of slugs	Native	Leaf area	Herbivory	3.3424
7	Oahu,	4 Species of slugs	Native	Leaf area	Herbivory	0.0012

Apendix 1 *continued*

7	Oahu, Hawaii	4 Species of	Exotic	Leaf area	Herbivory	0.0139
7	Oahu, Hawaii	4 Species of	Exotic	Leaf area	Herbivory	0.4305
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-1.2587
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-1.1483
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	0.0984
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-0.3368
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-0.9585
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-1.5285
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-1.6242
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-0.1908
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-0.4966
7	Oahu, Hawaii	4 Species of	Exotic	Seedling	Herbivory	-0.2155
8	Arizona, USA	cattle (vert)	Exotic	% canopy	Herbivory	-0.0984
8	Arizona, USA	cattle (vert	Native	% canopy	Herbivory	-1.8440
8	Arizona, USA	cattle (vert	Exotic	% canopy	Herbivory	0.2525
8	Arizona, USA	cattle (vert	Native	% canopy	Herbivory	-1.4122
8	Arizona, USA	cattle (vert	Exotic	% canopy	Herbivory	1.0512
8	Arizona, USA	cattle (vert	Native	% canopy	Herbivory	-1.3718
8	Arizona, USA	cattle (vert	Exotic	% canopy	Herbivory	1.2014
8	Arizona, USA	cattle (vert	Native	% canopy	Herbivory	-0.8986
8	Arizona, USA	cattle (vert	Exotic	% canopy	Herbivory	1.8648
8	Arizona, USA	cattle (vert	Native	% canopy	Herbivory	0.6401
8	Arizona, USA	cattle (vert	Exotic	% canopy	Herbivory	-2.3018
8	Arizona, USA	cattle (vert	Native	% canopy	Herbivory	0.4306
8	Arizona, USA	cattle (vert	Exotic	% canopy	Herbivory	1.3542
8	Arizona, USA	cattle (vert	Native	% canopy	Herbivory	1.8081
8	Arizona, USA	cattle (vert	Native	% canopy	Herbivory	1.9958
9	North Central	Rabbit &	Native	Plants/m^2	Herbivory	1.2462

9	North	Rabbit & hare	Native	Plants/m^2	Herbivory	1.0436
9	North	Rabbit & hare	Native	Plants/m^2	Herbivory	1.3651
9	North	Rabbit & hare	Native	Plants/m^2	Herbivory	1.2758
9	North	Rabbit & hare	Native	Plants/m^2	Herbivory	1.4116
9	North	Rabbit & hare	Native	Plants/m^2	Herbivory	1.1219
9	North	Rabbit & hare	Exotic	Plants/m^2	Herbivory	-0.8588
9	North	Rabbit & hare	Exotic	Plants/m^2	Herbivory	-0.6083
9	North	Rabbit & hare	Exotic	Plants/m^2	Herbivory	-0.3958
9	North	Rabbit & hare	Exotic	Plants/m^2	Herbivory	-0.1129
9	North	Rabbit & hare	Exotic	Plants/m^2	Herbivory	-0.3823
9	North	Rabbit & hare	Exotic	Plants/m^2	Herbivory	0.4441
10	Queensland	cattle (vert)	Exotic	% cover	Herbivory	-0.3910
10	Queensland	cattle (vert)	Exotic	% cover	Herbivory	-2.7049
10	Queensland	cattle (vert)	Native	% cover	Herbivory	0.2921
10	Queensland	cattle (vert)	Native	% cover	Herbivory	-0.4075
10	Queensland	cattle (vert)	Native	% cover	Herbivory	-0.4238
10	Queensland	cattle (vert)	Native	% cover	Herbivory	-1.5053
10	Queensland	cattle (vert)	Native	% cover	Herbivory	-0.4575
10	Queensland	cattle (vert)	Native	% cover	Herbivory	0.5116
11	West	Sheep (vert)	Exotic	% cover	Herbivory	4.3667
11	West	Sheep (vert)	Native	% cover	Herbivory	0.2010
12	Victoria	Sheep (vert)	Native	% cover	Herbivory	-1.7774
12	Victoria	Sheep (vert)	Exotic	% cover	Herbivory	-0.9053
13	Kailua	Cattle & goats	Native	Number of	Herbivory	-1.3472
13	Kailua	Cattle & goats	Exotic	Number of	Herbivory	-0.1591
13	KailuK	Cattle & goats	Native	Number of	Herbivory	-1.6274
13	Kailua	Cattle & goats	Exotic	Number of	Herbivory	-0.8967
13	Kailua	Cattle & fgoats	Exotic	Number of	Herbivory	-0.1208
14	N. Territory	*C. mimosa*	Exotic	Population	Herbivory	-1.6594
15	Colorado	*B. pulicarius*	Exotic	Flower	Herbivory	2.1829
15	Colorado	*B. pulicarius*	Exotic	Fruiting	Herbivory	-0.6660

Appendix 1 *continued*

16	Macquarie, Australia	*Rattus rattus* (vert)	Native	Seedling emergence	Herbivory	-0.3050
16	Macquarie, Australia	*Rattus rattus* (vert)	Native	Seedling emergence	Herbivory	-1.5360
16	Macquarie, Australia	*Rattus rattus* (vert)	Native	Seedling emergence	Herbivory	-1.4455
16	Macquarie,	*Rattus rattus*	Native	Seedling	Herbivory	-1.6299
16	Macquarie,	*Rattus rattus*	Native	Seedling	Herbivory	-1.5486
16	Macquarie,	*Rattus rattus*	Native	Seedling	Herbivory	-1.5645
16	Macquarie,	*Rattus rattus*	Native	Seedling	Herbivory	-0.1919
17	Arizona,	Cattle (vert)	Exotic	Average	Herbivory	-0.5767
17	Arizona,	Cattle (vert)	Exotic	Average	Herbivory	1.0654
17	Arizona,	Cattle (vert)	Exotic	Average	Herbivory	1.6515
17	Arizona,	Cattle (vert)	Exotic	Average	Herbivory	-0.6136
17	Arizona,	Cattle (vert)	Exotic	Average	Herbivory	-0.5502
17	Arizona,	Cattle (vert)	Exotic	Average	Herbivory	-0.5582
18	California,	Cattle (vert)	Native	% cover	Herbivory	-0.2508
18	California,	Cattle (vert)	Native	% cover	Herbivory	0.2617
18	California,	Cattle (vert)	Native	% cover	Herbivory	-0.0134
18	California,	Cattle (vert)	Native	% cover	Herbivory	0.4086
18	California,	Cattle (vert)	Exotic	% cover	Herbivory	0.7032
18	California,	Cattle (vert)	Exotic	% cover	Herbivory	-0.0347
19	California,	Cattle (vert)	Native	% cover	Herbivory	-0.4070
19	California,	Cattle (vert)	Native	% cover	Herbivory	0.2274
19	California,	Cattle (vert)	Native	% cover	Herbivory	-0.0287
19	California,	Cattle (vert)	Native	% cover	Herbivory	0.0518
19	California,	Cattle (vert)	Native	% cover	Herbivory	0.0000
19	California,	Cattle (vert)	Native	% cover	Herbivory	0.6971
20	Newzealand	Hare (vert)	Native	Number of	Herbivory	-0.1420
20	Newzealand	Hare (vert)	Native	Number of	Herbivory	-4.2189
20	Newzealand	Hare (vert)	Native	Number of	Herbivory	-1.5249

Appendix 1 *continued*

20	Newzealand	Hare (vert)	Native	Mean flower	Herbivory	-0.7810
20	Newzealand	Hare (vert)	Native	Mean pod	Herbivory	-0.6711
20	Newzealand	Hare (vert)	Native	Mean pod	Herbivory	-0.2927
20	Newzealand	Hare (vert)	Native	Mean pod	Herbivory	0.4881
21	Mexico	Rabbit (vert)	Native	% cover	Herbivory	-3.0596
21	Mexico	Rabbit (vert)	Native	% cover	Herbivory	-0.5870
21	Mexico	Rabbit (vert)	Native	% cover	Herbivory	-2.8196
21	Mexico	Rabbit (vert)	Native	% cover	Herbivory	3.5706
21	Mexico	Rabbit (vert)	Native	% cover	Herbivory	1.0041
21	Mexico	Rabbit (vert)	Native	% cover	Herbivory	-4.4406
21	Mexico	Rabbit (vert)	Native	% cover	Herbivory	5.0980
6	Bighorn	Cattle (vert)	Native	% cover/m^2	Herbivory	0.3686
6	Bighorn	Cattle (vert)	Native	% cover/m^2	Herbivory	0.0000
6	Bighorn	Cattle (vert)	Exotic	% cover/m^2	Herbivory	-0.1953
6	Charles	Cattle (vert)	Exotic	% cover/m^2	Herbivory	0.0000
6	Charles	Cattle (vert)	Native	% cover/m^2	Herbivory	0.5631
6	Charles	Cattle (vert)	Native	% cover/m^2	Herbivory	-0.1545
6	Charles	Cattle (vert)	Exotic	% cover/m^2	Herbivory	0.5857
6	Gunnison,	Cattle (vert)	Exotic	% cover/m^2	Herbivory	0.1802
6	Gunnison,	Cattle (vert)	Native	% cover/m^2	Herbivory	0.1096
6	Gunnison,	Cattle (vert)	Native	% cover/m^2	Herbivory	0.0860
6	Gunnison,	Cattle (vert)	Exotic	% cover/m^2	Herbivory	0.0630
6	Uncompahgre	Sheep (vert)	Native	% cover/m^2	Herbivory	-0.1000
6	Uncompahgre	Sheep (vert)	Native	% cover/m^2	Herbivory	-0.0509
6	Uncompahgre	Sheep (vert)	Exotic	% cover/m^2	Herbivory	0.0702
6	Wild Horse,	Sheep (vert)	Exotic	% cover/m^2	Herbivory	-0.1735
6	Wild Horse,	Wild horses	Native	% cover/m^2	Herbivory	-0.6997
6	Wild Horse,	Wild horses	Native	% cover/m^2	Herbivory	0.8335
6	Wild Horse,	Wild horses	Exotic	% cover/m^2	Herbivory	-0.5902

Appendix 1 *continued*

6	Bighorn	Cattle (vert)	Native	% cover/m^2	Herbivory	0.4492
6	Bighorn	Cattle (vert)	Native	% cover/	Herbivory	0.4462
6	Bighorn	Cattle (vert)	Exotic	%	Herbivory	1.7143
6	Charles	Cattle (vert)	Exotic	%	Herbivory	-0.1366
6	Charles	Cattle (vert)	Native	%	Herbivory	-0.7085
6	Charles	Cattle (vert)	Native	%	Herbivory	0.3166
6	Charles	Cattle (vert)	Exotic	%	Herbivory	0.1089
6	Gunnison,	Cattle (vert)	Exotic	%	Herbivory	-0.0590
6	Gunnison,	Cattle (vert)	Native	%	Herbivory	0.3517
6	Gunnison,	Cattle (vert)	Native	%	Herbivory	0.2531
6	Gunnison,	Cattle (vert)	Exotic	%	Herbivory	0.1585
17	Arizona,	Cattle (vert)	Native	Canopy	Grazing	-0.3719
17	Arizona,	Cattle (vert)	Native	Canopy	Grazing	-0.5122
22	Patagonia,	Cattle (vert)	Native	Height,	Browsing	-1.0156
23	New South	Sheep (vert)	Exotic	Seed /plant	Browsing	-5.0971
24	California,	Feral pigs (vert)	Exotic	% forb	Disturbance	0.2084
24	California,	Feral pigs (vert)	Native	% forb	Disturbance	0.0000
24	California,	Feral pigs (vert)	Native	% perennial	Disturbance	-1.2666
24	California,	Feral pigs (vert)	Exotic	% annual	Disturbance	0.2383
24	California,	Feral pigs (vert)	Native	% bulb	Disturbance	-0.0968
25	Washington,	Two slug	Exotic	Seedling	Herbivory	-1.0004
25	Washington,	Two slug	Exotic	Adult	Herbivory	-0.0045
26	California,	*H.brunneipennis*	Native	Proportion	Herbivory	-0.9872
26	California,	*H.brunneipennis*	Native	Proportion	Herbivory	-1.8483
24	California,	Feral pigs (vert)	Native	% perennial	Disturbance	-1.2666
24	California,	Feral pigs (vert)	Exotic	% annual	Disturbance	0.2383
24	California,	Feral pigs (vert)	Native	% bulb	Disturbance	-0.0968
25	Washington,	Two slug	Exotic	Seedling	Herbivory	-1.0004
25	Washington,	Two slug	Exotic	Adult	Herbivory	-0.0045
26	California,	*H.brunneipennis*	Native	Proportion	Herbivory	-0.9872
26	California,	*H.brunneipennis*	Native	Proportion	Herbivory	-1.8483

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Appendix 2: *Effect sizes, d, of trophic preferences of exotic vertebrate and invertebrate herbivores for native or exotic plants. A negative effect size means that a native plant was preferred to an exotic plant. Vert. = vertebrate herbivores; inv = invertebrate herbivores.*

Citation	**Site**	**Herbivore**	**Units measured**	**Effect size *d***
2	Seatle, USA	*A. ater* (inv)	Amount eaten	0.7485
3	New York, USA	*A. subfuscu* (inv)	Amount eaten	-0.1685
3	New York, USA	*A. fasciatu* (inv)	Amount eaten	0.3441
3	New York, USA	*D. reticula* (inv)	Amount eaten	0.9051
4	Isla Victoria,	*C. elaphus* (vert)	% browsed	-0.9244
4	Isla Victoria,	*C. elaphus* (vert)	% browsed	-0.8933
5	MedLos	Cattle + horses	Cover decline	-5.7953
5	MedLos	Cattle + horses	Cover decline	-8.3737
5	MedLos	Rabbit (vert)	Biomass loss	-0.4101
6	Nong Lek, Lao	*P. canaliculata*	Weight loss	2.8111
6	Nong Lek, Lao	*P. canaliculata*	Weight loss	3.1514
6	Nong Lek, Lao	*P. canaliculata*	Weight loss	2.6123
6	Nong Lek, Lao	*P. canaliculata*	Biomass decline	-0.4893
6	Nong Lek, Lao	*P. canaliculata*	Biomass decline	0.6870
7	North Dakota,	Goats (vert)	Production	32.8495
8	California, USA	*Otala lactea*	Biomass eaten	-0.6414
8	California, USA	*Otala lactea*	Biomass eaten	1.6906
8	California, USA	*Otala lactea*	Biomass eaten	-0.9161
8	California, USA	*Otala lactea*	Biomass eaten	-1.3973
8	California, USA	*Otala lactea*	Biomass eaten	-1.1987
8	California, USA	*Otala lactea*	Biomass eaten	-0.6117
8	California, USA	*Otala lactea*	Biomass eaten	0.0000
9	California, USA	Slugs/snail (inv)	Damage score	0.1638
9	California, USA	Slugs/snail (inv)	Seedlings eaten	-0.6497
9	California, USA	Slugs/snail (inv)	Seedlings eaten	-0.9580
9	California, USA	Slugs/snail (inv)	Seedlings eaten	1.5824
9	California, USA	Slugs/snail (inv)	Seedlings eaten	0.9584
9	California, USA	Slugs/snail (inv)	Seedlings eaten	0.4985
9	California, USA	Slugs/snail (inv)	Seedlings eaten	1.4284
9	California, USA	Slugs/snail (inv)	Damage score	1.0813
9	California, USA	Slugs/snail (inv)	Damage score	1.7081
10	Rocky Mountain	*R. conicus* (inv)	Flower damage	0.3883
10	Rocky Mountain	*R. conicus* (inv)	Flower damage	0.6270
10	Rocky Mountain	*R. conicus* (inv)	Flower damage	1.8716

Appendix 2 *continued*

Citation	**Site**	**Herbivore**	**Units measured**	**Effect size *d***
11	Florida, USA	*P. insularum*	Biomass eaten	-1.6777
11	Florida, USA	*P. insularum*	Biomass eaten	-2.7254
11	Florida, USA	*P. insularum*	Biomass eaten	-3.6377
11	Florida, USA	*P. insularum*	Biomass eaten	-16.9782
11	Florida, USA	*P. insularum*	Biomass eaten	-10.4219
11	Florida, USA	*P. insularum*	Biomass eaten	-2.5754
11	Florida, USA	*P. insularum*	Biomass eaten	-2.1676
11	Florida, USA	*P. insularum*	Biomass eaten	-6.3105
11	Florida, USA	*P. insularum*	Biomass eaten	-3.1772
11	Florida, USA	*P. insularum*	Biomass eaten	-1.9683
11	Florida, USA	*P. insularum*	Biomass eaten	-2.2304
11	Florida, USA	*P. insularum*	Biomass eaten	-3.0238
11	Florida, USA	*P. insularum*	Biomass eaten	-1.9720
11	Florida, USA	*P. insularum*	Biomass eaten	-2.6294
11	Florida, USA	*P. insularum*	Biomass eaten	-4.3572
11	Florida, USA	*P. insularum*	Biomass eaten	-1.3147
11	Florida, USA	*P. insularum*	Biomass eaten	-1.0517
11	Florida, USA	*P. insularum*	Biomass eaten	-3.8564
11	Florida, USA	*P. insularum*	Biomass eaten	-0.8899
11	Florida, USA	*P. insularum*	Biomass eaten	-0.9992
11	Florida, USA	*P. insularum*	Biomass eaten	-0.2079
11	Florida, USA	*P. insularum*	Biomass eaten	-0.1653
11	Florida, USA	*P. insularum*	Biomass eaten	-0.4207
11	Florida, USA	*P. insularum*	Biomass eaten	0.0000
12	Florida, USA	*P. insularum*	Biomass eaten	-5.5802
12	Florida, USA	*P. insularum*	Biomass eaten	0.3975
12	Florida, USA	*P. insularum*	Biomass eaten	-6.0816
12	Florida, USA	*P. insularum*	Biomass eaten	-0.9940
12	Florida, USA	*P. insularum*	Biomass eaten	-3.1813
12	Florida, USA	*P. insularum*	Biomass eaten	3.8064
12	Florida, USA	*P. insularum*	Biomass eaten	-3.2180

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CHAPTER 7:

GENERAL DISCUSSION

The objective of this thesis was to explore mechanisms that underlie invasion success of plants using *Brassica nigra* as a model. To that end, various strategies were employed including literature review, meta-analysis, molecular genetics, chemical analysis, and field experiments. Below, I give a summary of the findings from those strategies, compare them with other previous studies, and give recommendations for future research.

As reported in **chapter two,** chloroplast DNA (cpDNA) intron (*trnF*-*trnL*) sequences were used to study phylogeographical relationships of 53 invasive (North American) and native (North African, Mediteranean region and Eurasian) populations of *Brassica nigra*. Thirty seven haploptypes were found. Native populations had higher number and diversity of haplotypes than invasive populations. Twenty five haplotypes occurred among the native populations while 15 haplotypes occurred among the invasive populations. Three haplotypes were shared between invasive and native populations. Four haplotypes (H2, H5, H6, and H18) likely underwent multiple introductions from from distinct populations in the native range to invasive ranges of *B. nigra*. The other 34 haplotypes were private, occurring exclusively in invasive or native populations. There was no genetic differentiation between invasive and native range populations based on cpDNA intron *trnF*-*trnL* marker we used. However, there were genetic differentiations among populations within both invasive and native ranges. A mantel test showed a lack of correlation between pairwise genetic distances (F_{ST}) and geographical distances when data were analyzed for native and invasive populations together or separately for the native populations. However, a mantel test for invasive populations separately revealed a significant positive correlation between pairwise genetic distances (F_{ST}) and geographical distances. Occurrence of private haplotypes among the invasive populations suggests that some native range populations remained unsampled. Therefore, a more extensive sampling would identify putative sources of introductions of those populations.

Because multiple introductions of genotypes from distinct locations in the native range may bring close together previously isolated genotypes, hybridization may subsequently take place between those genotypes leading to creations of new, aggressively invading genotypes (Ellstrand and Schierenbeck 2000; Maron et al. 2004; Dlugosch and Parker 2008). Although multiple introductions are thought to have occured in such invasive plants as *Alliaria petiolata* (Durka et al. 2005), *Ambrosia artemisiifolia* (Genton et al. 2005), *Bryonia alba* (Novak and Mack 1995), and *Hirschfeldia incana* (Lee et al. 2004), it remains unknown whether intra-specific hybridization has subsequently occurred between/among multiple-introduced genotypes of such plants. This is because those studies were not designed to examine whether or not hybridization events occurred following multiple introductions. To date, however, the role that intra-specific hybridization between multiple-introduced genotypes from distinct locations could play in biological invasions has received very little attention

(Culley and Hardiman 2009); only a few reports exist on studies that looked into intra-specific hybridizations between multiple-introduced genotypes (Culley and Hardiman 2009). Microsatellite markers revealed that intra-specific hybridization events took place between distinct genotypes of Brazilian pepper tree, *Schinus terebinthifolius*, (Williams et al. 2005) and a Chinese tree, *Pyrus calleryana*, (Culley and Hardiman 2009) that had been multiple-introduced from distinct locations in the native ranges in South America and China, respectively. The two tree species are currently invasive in North America (Williams et al. 2005; Culley and Hardiman 2009). Allozyme markers revealed that invasive genotypes of the grass, *Phalaris arundinacea L*, (which has invaded some parts of North America) are products of hybridization between distinct genotypes of the same species that had been multiple- introduced from disparate populations in Europe (Lavergne and Molofsky (2007). New genotypes that result from intra-specific hybridizations of multiple-introduced distinct genotypes not only alleviate genetic bottlenecks, but also increase genetic diversity and heritable phenotypic variation for ecologically-important traits (Lavergne and Molofsky 2007). The resulting high evolutionary potential of such hybrids may stimulate rappid selection for increased growth, reproduction and spread of invasive plant species (Lavergne and Molofsky 2007). The hybrids may also have much higher potential to adapt to changing climates enabling them to have increasingly detrimental impacts on native communities and ecosystem services in future (Lavergne and Molofsky 2007). It remains unknown whether or not multiple introductions of disntinct genotypes of *B. nigra* from disparate locations in the native range have been followed by intra-specific hybridizations between/among those genotypes. Thefeore, use of such molecular markers as microsatellites could help elucidate any likely hybridizations event(s) that took place following multiple introductions of *B. nigra* from its native to invasive ranges.

In **chapter three**, it was found that invasive populations of *B. nigra* expressed a significantly lower diversity of glucosinolates, a class of defence compounds, than native populations of the same plant. At the same time, the invasive populations of *B. nigra* expressed higher concentrateion of sinigrin than native populations of the same plant (sinigrin accounted for 98 % of all glucosinolate compounds in invasive populations while in native populations, it accounted for 77 %). Nevertheless, invasive and native populations harboured similar diversities of specialist and generalist herbivores in a common garden. It should, however, be noted that the method of herbivore survey we adopted could not have been adequate to detect a difference in herbivore assemblage between invasive and native populations of *B. nigra*. Production of defence compounds is thought to have both ecological and physiological costs (Strauss et al. 2002; Cipollini et al. 2003; Koricheva et al. 2004). Invasive ranges of various plants harbour lower diversities of generalist and specialist herbivores than do native ranges of such plants (Liu and Stiling 2006). Therefore, invasive populations of *B. nigra* possibly were released from intense damage by a high diversity of both generalist and specialist herbivores in the native ranges. Hence *B .nigra* genotypes that express a low diversty of glucosinolates could have had a higher growth and reproductive success than genotypes that expressed high diversity of glucosinolates. Consequently *B. nigra* genotypes expressing lower glucosinolate diversity could have been selected for by the less diverse herbivore assembly present in the invasive ranges. Additionally, the herbivore assemblage dominated by generalists in the invasive ranges could have selected for *B. nigra* genotypes that express only one compound (i.e. sinigrin) in very high concentration because that one compound is effective against a suite of the generalist herbivores that the invasive populations interact with. This could explain why sinigrin accounted for 98% of the total glucosinolates expressed by invasive populations of *B. nigra*. On the other hand, the relatively higher diversity of generalist and specialist herbivores in the native range vs. invasive range of (Liu and Stiling 2006) could have selected for genotypes of *B. nigra* that express a high diversity of glucosinolates. This could exzplain why native populations of *B. nigra* expressed a significantly higher diversity of glucosinolates than invasive populations of the same plant.

In as much as plants would want to optimize their qualitative chemical defence compounds (e.g. glucosinolates) against herbivores, they also need to balance their interactions with mutualists such as pollinators. Thus both herbivores and pollinators can simultaneously impose selective pressures on plant qualitative chemical defence compounds (Adler et al. 2001; Andrews et al. 2007). Various studies have shown that herbivory can reduce (see Kessler and

Halitschke 2009 for a review) or increase (Poveda et al. 2003) pollinator visit to a plant. In the study reported here, we compared diversities and concentrations glucosinolates between invasive and native populations of *B. nigra*, and also the associated herbivore assembalages. However, as *B. nigra* is an outcrossing plant that is pollinated by insects (Conner and Sterling 1995; Westman and Kresovich 1999), and pollinators can be affected by plant defense compounds (Adler et al. 2001), the pollinators too could be affected by the glucosinolates. Thus pollinators could also exert selective pressure on *B. nigra* to not express certain glucosinolate compounds, when such compounds are detrimental to the pollinators. The idea of herbivores and pollinators simultaneously exerting selective pressures on qualitative defense compounds of plants has received very little attention in general (Kessler and Halitschke 2009), and not at all addressed in the field of biological invasions. For this reason, it would be interesting to study the pollinator assemblages alongside those of the herbivores of the same *B. nigra* populations we used and relate them to glucosinolate diversities and concentrations. Furthermore, studies similar to the one reported here but incorporating the pollinator aspect should be applied to other plant taxa as well in the field of biological invasions.

Results presented in **chaper four** indicate that *B. nigra* underwent rapid post-introduction evolutionary changes to grow taller, produce more biomass, and lighter seeds in larger number than native populations. Invasive populations of *B. nigra* also expressed higher concentrations of sinigrin (a defence compound) (see **chapter three** also) but have lower tolerance of herbivore damage. These evolutionary changes in levels of sinigrin, levels of tolerance of herbivory, stature and seed 'packaging' (i.e. more, lighter seeds) are thought to be the results of release from higher intensity of herbivore damage in the native, relative to invasive ranges in accordance with enemy release hypotheis (Keane and Crawley 2002) and shifting defence hypothesis (Doorduin and Vrieling 2011). Thus a putatively lower herbivore pressure that comes mainly from generalist herbivores in the invasive range could have selected for genotypes of *B.nigra* that express higher level of a qualitative defence compound like sinigrin. Since tolerance is never complete, perhaps it is more effective to defend against the low diverse herbivore community in the invasive ranges through resistnce via sinigrin than to tolerate damage from those herbivores. Through an increased production of sinigrin, the invasive populations of *B. nigra* can effectively deter generalist herbivores, while at the same time increase its growth and reproductive out put. This is because production and expression of high concentrations of a qualitative defence compound such as sinigrin is thought not to incur a lot of physiological costs to the plants (Doorduin and Vrieling 2011). Tall plants get their seeds dispersed over wider area than short plants (Muller-Landau et al. 2008). Therefore, by growing taller and producing more and lighter seeds, the invasive populations of *B. nigra* may increase an area over which it spreads its seeds, contributing to its rapid spread in the invasive range. Here, we studied a trade-off between resistance to, and tolerance of herbivore damage by focusing only on leaf feeding herbivores. However, *B. nigra* is known to interact with root herbivores also (Van Dam et al. 2005). Therefore, a study is needed that compares invasive and native populations of *B. nigra* for a trade-off between resistance to, and tolerance of herbivore that takes into account root feeding herbivores. Furthermore, such as study would benefit by also growing the invasive and native populkations of *B. nigra* with hetero-or conspecific competition. This is because *B. nigra* faces both types of competition in both invasive and native ranges.

In **chapter five,** I present results of a field experiment that was undertaken to compare competitive abilities of invasive and native populations of *B. nigra* when protected from herbivore damage or exposed to natural level of herbivory. Many invasive plant species experience both conspecific and heterospecific competition (e.g. Weir et al. 2003, 2006; DeWalt et al. 2004; Lankau 2008; Ni et al. 2010). In the exotic ranges, invasive plants still undergo damage from herbivores, though to a much lower degree than their native range conspecifics (Liu and Stiling 2006). Therefore, herbivoires likely influence competitive interactions between invasive plants and plant native to invaded ranges. To date, however, no reports exist on how herbivores may influence competitive ability of both invasive and native populations of a plant. The invasive and native populations of *B. nigra* were subjected to both conspecific and heterospecific competition. Conspecific competition involved growing invasive populations in pairewise competition with native populations. Heterospecific competition involved growing the invasive and native populations of *B. nigra* in pairwise competitions with two grass species and two legume species. An insecticide and a molluscicide were then used to release half of the plants from herbivore damage. The other half was exposed to natural level of herbivore damage. Data based on absolute mean trait values show that invasive populations of *B. nigra* grew taller than native populations of *B. nigra* when protected from herbivore damage or not regardless of the type of competition. Other traits like seed yield and plant biomass were similar for invasive and native populations regardless of exposure to herbivore damage and type of competition they were subjected to. Competitive abilities of the invasive and native populations were compared using relative interaction intensity index (RII). RII values range from -1 to 1, with negative values indicating competitive interactions and positive values indicating facilitative interactions (Armas et al. 2004). A zero value indicates a net effect of no interaction between plants (Armas et al. 2004). RII data indicate that invasive populations of *B. nigra* benefited from protection from herbivory when grown with heterospecific competitors (legumes and grasses). This is indicated by positive mean RII values for invasive populations under insecticide treatment. The legumes/grasses seemingly facilitated fitness of the invasive populations in the absence of, or under low level of insect damage only but did not do so under natural level of nsect damage. On the contrary, native populations benefited from insect damage because they had mean positive RII values when not protected from insect damage. This indicates that fitness of the native populations was facilitated when interacting with the legumes/grasses under natural level of insect damage. However, when protected from insect damage, fitness of the native populations was not facilitated by the legumes/grasses. These data indicate that the native populations of *B. nigra* were more tolerant of insect damage (i.e. compensated for herbivore damage by increasing fitness) than invasive populations because performances of the native populations were enhanced under natural level of herbivory while those of invasive populations were suppressed. In fact, a study that used the same populations of *B. nigra* as used here found that native populations of *B. nigra* were more tolerant of herbivore damage than invasive populations (Oduor et al. 2011). The negative mean RII values for both invasive and native populations when grown with conspecific neighbours regardless of insecticide treatment indicates that there were only competitive interactions (and no facilitation) pitting the invasive populations of *B. nigra* versus native populations of the same plant species . Overall, RII data indicate that invasive populations of *B. nigra* have evolved to be better competitors only in the absence, or under low level of herbivore damage. If the findings of a meta-anslysis by Liu and Stiling (2006) regarding a lower herbivore damage undergone by invasive plants in the invasive ranges also apply to *B. nigra*, then invasive populations of *B. nigra* may have competitive advantage over plants native to ranges where it is invasive.

Finally in **chapter six** a meta-analysis was carried out to compare effects of exotic vertebrate and invertebrate herbivores on exotic and native plants. Herbivores modify various ecological processes including interactions between native and exotic plants that may affect invasion success by the exotic plants (Parker et al. 2006). Exotic herbivores may facilitate the invasion success of exotic plants in accordance with 'invasional meltdown' hypothesis (Simberloff 2006; Nuñez et al. 2008). A previous meta-analysis by Parker et al. (2006) found that exotic herbivores generally caused an increase in abundance and species richness of exotic plants, while at the same time suppressed native plant abundance and species richness. However, it remained unknown whether different types of exotic herbivores have similar effects on native and exotic plants. Using two distinct data sets, we ran meta-analyses to compare exotic vertebrate and invertebrate herbivore preferences for, and effects on performance and population sizes of native and exotic plants. We found that exotic vertebrate herbivores have positive effects on exotic plant performance and population sizes, and no significant effects on native plants. Exotic invertebrates have significant negative effects on performance and population sizes of both exotic and native plants. Vertebrates prefer to feed on native plants relative to exotic plants, while invertebrates prefer the exotic plants to native plants. Thus the exotic vertebrate herbivores may aid invasiveness of exotic plants, in accordance with the invasional meltdown hypothesis, while exotic invertebrate herbivores probably have no net effect on invasion process of the exotic plants. Invertebrate herbivore preferences for exotic plants support the biotic resistance hypothesis, as the native plants probably resist the invertebrate herbivory. We also tested an evolutionary logic that posits that herbivores with similar evolutionary history as plants will affect the plants less negatively than plants with which they have not co-evolved. Our results indicate that there is no consistent pattern in effects of exotic vertebrate and invertebrate herbivores on exotic plants with or without which they have co-evolved. During the meta-analysis, we found that some areas remained unexplored and, therefore, proposed future research. We found that only very few studies had simultaneously examined effects of both vertebrate and invertebrate herbivores on an individual plant (Strauss 1991; Hulme, 1994, 1996a; Palmisano and Fox, 1997; Sessions and Kelly 2001). Studying effects of both vertebrate and invertebrate herbivores, as opposed to only either of them, on performance and population size of individual plants may help identify the more important herbivore guild (i.e. between vertebrate and invertebrate herbivores) that influences plant demography (Hulme 1996a). Therefore, future studies that aim at understanding how exotic vertebrate and invertebrate herbivores may affect interaction between native and exotic plants should use factorial experimental designs to investigate effects of both vertebrates and invertebrates on the same individual plants. Such studies would give a better understanding of which herbivore type (vertebrate or invertebrate) has more potential to affect demographies of co-occurring native and exotic plants, and hence invasion process of the exotic plants.

In the current paper, we attempted to find out if trophic preferences of exotic vertebrate and invertebrate herbivores for exotic or native plants can aid or hamper invasion by the exotic plants. Various other mechanisms through which herbivores may affect plant invasion have, nevertheless, been reported. These include seed dispersal (Janzen 1984; Malo and Suarez 1995, 1997; Fisher et al. 1996; Campbell and Gibson 2001; Pakeman 2001; Holmgren 2002; Pakeman et al. 2002; Vellend et al 2003; Mouissie 2004; Myers et al. 2004; Manzano and Malo 2006; Loesser et al. 2007; Loydi and Zalba 2008) and soil disturbance (Cushman et al. 2004). A future analysis similar to the one we carried out but including other mechanisms described above would help elucidate the most important mechanisms by which the exotic vertebrate herbivores may influence plant invasion.

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CONCLUSIONES

CONCLUSIONES:

1.- Las poblaciones nativas de *Brassica nigra* presentaron una mayor cantidad y diversidad de haplotipos que las poblaciones invasoras. Solo tres haplotipos se encontraban tanto en poblaciones invasoras como nativas, mientras que 34 haplotipos no eran comunes entre ambos grupos. Cuatro haplotipos, procedentes de poblaciones nativas, fueron introducidos en múltiples ocasiones en América del Norte. No se encontró ninguna diferenciación genética significativa entre poblaciones nativas e invasoras.

2.- Las poblaciones invasores de *B. nigra* tienen una menor diversidad de glucosinolatos, presentado una alta concentración de sinigrin. Tanto las poblaciones invasoras como las nativas fueron visitadas por la misma riqueza y diversidad de herbívoros generalistas y especialistas, a pesar de la correlación entre concentración de sinigrin y el número de especies especialistas que visitaron las plantas.

3.- Las poblaciones invasoras presentaron una alta resistencia y baja tolerancia al daño por herbívoros, en comparación con las poblaciones nativas. Además, las poblaciones invasoras tuvieron una mayor tasa de supervivencia y una mayor producción de semillas en ausencia de herbivoría. En general, con o sin herbivoría, las plantas de poblaciones invasoras alcanzaron una mayor altura, mayor biomasa y semillas con menor peso que las nativas. Estos resultados confirman la idea de la evolución rápida que se produce después de la introducción de una especie invasora. *B. nigra* evolucionó en las zonas introducidas hacia un patrón de mayor resistencia y crecimiento.

4.- Bajo una situación de competencia entre plantas procedentes de poblaciones invasoras y nativas, y con la presencia de depredadores, los individuos de poblaciones invasoras produjeron menos semillas, mientras que los individuos de poblaciones nativas produjeron una mayor cantidad de semillas. Estos resultados indican que los factores: herbivoría y competitividad interactúan entre ellos, siendo mejores competidoras las poblaciones nativas bajo estas condiciones.

5.- Nuestros resultados mostraron una sinergia entre plantas invasoras y herbívoros vertebrados exóticos. Estos herbívoros mostraron una preferencia por especies nativas favoreciendo a las especies invasoras, al ser estas menos dañadas. Sin embargo, los herbívoros invertebrados exóticos afectaron por igual tanto a especies de plantas invasoras como nativas. A pesar de esta sinergia, nuestros resultados no mostraron ningún patrón general de cómo los herbívoros vertebrados o invertebrados exóticos pueden afectar a las plantas invasoras, habiendo co-evolucionado con ellas o no en las mismas zonas.