Biol Invasions (2015) 17:2447–2459
DOI 10.1007/s10530-015-0888-1

ORIGINAL PAPER

Invasion of *Brassica nigra* in North America: distributions and origins of chloroplast DNA haplotypes suggest multiple introductions

Ayub M. O. Oduor · José M. Gómez · Maria B. Herrador · Francisco Perfectti

Received: 12 June 2014 / Accepted: 31 March 2015 / Published online: 10 April 2015

Abstract Deciphering the origin of invasive plant species, 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 applied traditional population genetic analyses to unravel the phylogeographical relationships among invasive (North American) and native (North African, Mediterranean region, and Eurasian) range populations of *Brassica nigra* using chloroplast DNA. We sequenced chloroplast DNA intron (*trnF–trnL*) for 284 individuals representing 36 native and 15 invasive range populations of *B. nigra*. Thirty-two haplotypes were found over the whole data set. A similarity between the invasive range and native range populations in genetic diversity combined with results from analyses of molecular variance and gene genealogies suggest that invasive *B. nigra* populations were introduced from multiple sources in the native range. More generally, this study adds to the growing body of data on the genetic patterns involved in biological invasions that is crucial to our understanding of the evolutionary trajectories of invasive populations.

Keywords Invasive plants · *Brassica nigra* · Rapid post-introduction evolution · Multiple introductions · Population admixture · Invasive and native populations · Introduced range · Native range

Chloroplast DNA (cpDNA) sequences of the region *trnF–trnL* have been published in the GenBank (accession numbers KF947115–KF947398).

Electronic supplementary material The online version of this article (doi:10.1007/s10530-015-0888-1) contains supplementary material, which is available to authorized users.

A. M. O. Oduor · J. M. Gómez · M. B. Herrador
Departamento de Ecología, Universidad de Granada, 18071 Granada, Spain
e-mail: jmgreyes@eeza.csic.es

M. B. Herrador
e-mail: mbelenhe@gmail.com

A. M. O. Oduor (✉)
Ecology, Department of Biology, University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany
e-mail: ayub.oduor@uni-konstanz.de; aoduor02@gmail.com

J. M. Gómez
Estación Experimental de Zonas Áridas (EEZA-CSIC), 04120 Almería, Spain

F. Perfectti
Departamento de Genética, Universidad de Granada, 18071 Granada, Spain
e-mail: fperfect@ugr.es

Introduction

Many plant species have been introduced from their native to exotic ranges due to international trade and travels (Hulme 2009). The small percentage of the introduced plant species that has become invasive alter the structures and functioning of native ecological systems (Mack et al. 2000; Pimentel et al. 2001). Rapid post-introduction evolution of various traits (defensive, growth/reproductive, competitive, and dispersal) is thought to be one mechanism that may underlie invasiveness of many introduced plant species (Lee 2002; Allendorf and Lundquist 2003; Durka et al. 2005; Dlugosch and Parker 2008; Whitney and Gabler 2008). The evolution may occur via various processes, such as adaptive evolution in response to selection imposed by novel biotic and abiotic factors, and/or hybridization among introduced distinct genotypes or among related native or exotic species (Allendorf and Lundquist 2003; Kolbe et al. 2004; Durka et al. 2005; Dlugosch and Parker 2008).

However, species introductions are sampling events that should generate genetic bottlenecks in the introduced populations (Nei et al. 1975; Sakai et al. 2001). Thus, recently introduced populations may have low evolutionary potential due to founder effects (Nei et al. 1975; Sakai et al. 2001). The founder effect may affect annual plant species much more than perennial plant species (Austerlitz et al. 2000). This is because annual plants reproduce faster, and hence may quickly establish large populations based only on a limited number of genotypes (Austerlitz et al. 2000). On the other hand, because perennial plants take long to reproduce, their newly founded populations are likely to grow mostly through the arrival of new migrants representing numerous diverse genotypes found in the original populations (Austerlitz et al. 2000). Thus, one could predict that recently founded populations of annual plants will have a low evolutionary potential due to limited heritable genetic variation. Interestingly, however, recent empirical research has found significant differences between invasive and native populations of short-lived (i.e., annuals and biennials) plants in such traits as growth, defence, and reproductive output, suggesting the possibility of rapid adaptive evolution in those invasive plant species (e.g., Oduor et al. 2011, 2013; Turner et al. 2014). Therefore, the question remains as to how recently founded populations of short-lived invasive plant species may overcome evolutionary constraint associated with founder effects to rapidly evolve in their novel environments (the so-called genetic paradox sensu Allendorf and Lundquist 2003; Roman and Darling 2007).

Multiple-introductions (i.e., introductions of diverse genetic lineages from different source populations in the native range) may enable recently introduced populations to overcome founder effects (Lee 2002; Kolbe et al. 2004; Roman and Darling 2007; Hufbauer and Sforza 2008; Dlugosch and Parker 2008). Through this process, the introduced populations may experience a significant increase in standing levels of quantitative genetic variation that natural selection can act upon (Kolbe et al. 2004; Hufbauer and Sforza 2008; Dlugosch and Parker 2008; Rius and Darling 2014). In addition, recombination between diverse genotypes introduced from distinct source populations can create novel genotypes in the exotic ranges (Ellstrand and Schierenbeck 2000; Durka et al. 2005; Roman and Darling 2007). These novel genotypes may enhance fitness of the introduced populations through hybrid vigour, particularly in the face of novel selection pressures (Ellstrand and Schierenbeck 2000; Durka et al. 2005; Roman and Darling 2007).

An inference of multiple introductions can be made from a combination of traditional population genetic summary statistics that describe the quantity of diversity (e.g., allelic richness and haplotypic diversity), population sub-division (F_{ST}), and spatial arrangement of genetic variation (AMOVA, genotypic clustering) in invasive range and native range populations (Kolbe et al. 2004; Voisin et al. 2005; Genton et al. 2005; Taylor and Keller 2007). Applying these techniques, evidence for multiple introductions can be inferred from studies revealing: (1) genetic diversity as high (or higher) in the invasive range populations as in native range populations, (2) a reduction in geographical genetic structure among the invasive range populations relative to native range populations, (3) a novel co-occurrence in the invasive populations of haplotypes that occur in allopatric populations in the native range (Kolbe et al. 2004; Voisin et al. 2005; Genton et al. 2005; Taylor and Keller 2007; Dlugosch and Parker 2008; Gillis et al. 2009; Meyerson and Cronin 2013).

Studies reviewing genetic patterns of invasive organisms have found high genetic diversity in invasive

populations, thus supporting the idea that many invaders are successful colonizers because their populations were established through repeated colonization events from multiple sources (Bossdorf et al. 2005; Dlugosch and Parker 2008; Uller and Leimu 2011). However, the current paucity of empirical data limits a rigorous quantitative testing of this hypothesis in the light of rapid evolution reported for recently introduced populations of annual invasive plant species. In the current paper, we used an annual invasive plant (*Brassica nigra*) to address the question: Were invasive range populations of *B. nigra* founded by multiple introductions from the native range?

Materials and methods

Study species

Brassica nigra (Brassicaceae) (L.) W. D. J. Koch is a self-incompatible annual herb native to Europe, Asia, and North Africa introduced to North America ca. 200 years ago (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). Presently, *B. nigra* is invasive in certain regions of North America, where it can form thick monospecific stands, although generally in disturbed areas (Lankau and Strauss 2008). Recent studies have found significantly higher resistance to herbivory and reproductive output among invasive range populations of *B. nigra* relative to their native range conspecifics, suggesting rapid post-introduction evolution of the invasive populations of *B. nigra* (Oduor et al. 2011, 2013).

DNA sequencing

Seeds of 15 invasive (North American) and 36 native (European, Asian, and African) range populations of *B. nigra* were field-collected by the authors or their collaborators, obtained from the United States Department of Agriculture (USDA) GRIN germplasm collection, or botanical gardens (see Table 1 and Supplementary material S1 for details). Genomic DNA was extracted from six individual seedlings for each of these 51 populations using GenEluteTM Plant Genomic DNA kit (SIGMA). One non-coding region (*trnF–trnL*) of chloroplast DNA (cpDNA) was PCR-amplified using the universal primer pair Tab F and Tab C (Taberlet et al. 1991), and thereafter sequenced. Sequencing was done through the Sanger procedure by Macrogen Co.

The cpDNA sequence data are particularly useful for testing for multiple introductions because: (1) they are uniparentally-inherited and dispersed via seeds, the propagules through which numerous invasive plant species are spread, (2) their uniparental inheritance eliminates the complicating effects of recombination, and (3) they provide ordered haplotypes for inferring ancestor–descendant relationships, based on predictions from the coalescent theory (Avise 2000; Clement et al. 2000; Freeland 2005; Taylor and Keller 2007). The coalescent theory uses relatedness of haplotypes (i.e., gene genealogies) to infer migration routes of populations (Freeland 2005; Taylor and Keller 2007). Based on the theory, the most common ancestral haplotype from which others have diverged has the following features: a high frequency of occurrence, occupies a central position in the haplotype network, has more connections in the network, and has a wider geographic distribution than any other haplotype in the network (Avise 2000; Clement et al. 2000; Freeland 2005). The theory further states that the most recent haplotypes to diverge from the ancestral haplotypes occupy tip-positions in a haplotype network (Avise 2000; Clement et al. 2000; Freeland 2005).

A total of 284 sequences representing all the 51 invasive and native range populations of *B.nigra* were obtained. Forty-three of these populations were represented by six sequences each (Table 1). Of the eight other populations (all from the native range), two were represented by five sequences each, another two by four sequences each, while four populations were represented by two sequences each (Table 1). The sequences were aligned using ClustalW version 7.0 (Tom Hall, Ibis Therapeutics, Carlsbad, California). The sequences were then trimmed to 694 bp, which included Indels. All the sequences have since been published in the Genbank database (Accession numbers KF947115–KF947398) (see Supplementary material S1). The sequences were then subjected to the analyses described below.

Table 1 Populations of *Brassica nigra* that were used in the present study

Country	Population code	Accession number or collector's name	Latitude	Longitude	Range
Afghanistan	AF1[a]	PI274284	34°0′0″N	69°0′0″E	Native
Afghanistan	AF2[b]	CR 2744	†	†	Native
Germany	AL1[a]	PI 633142	51°25′0″N	12°1′0″E	Native
Germany	AL2[a]	PI 633143	51°49′0″N	11°17′0″E	Native
USA	CA1	R. Lankau	†	†	Invasive
USA	CA3	R. Lankau	†	†	Invasive
USA	CA6	R. Lankau	†	†	Invasive
USA	CA7	R. Lankau	†	†	Invasive
USA	CA8	R. Lankau	†	†	Invasive
USA	CA9	R. Lankau	†	†	Invasive
USA	CA10	R. Lankau	†	†	Invasive
USA	CA11	R. Lankau	†	†	Invasive
Canada	CAN1[a]	PI649154	43°40′0″N	79°25′0″W	Invasive
Canada	CAN2[a]	USDA	†	†	Invasive
Denmark	DEN1[b]	CR 2710	†	†	Native
Denmark	DEN2[b]	CR 2762	†	†	Native
Spain	SP1	Royal Botanic Garden, Spain	†	†	Native
Spain	SP2	Royal Botanic Garden, Spain	†	†	Native
Spain	SP3[¥]	Royal Botanic Garden, Spain	†	†	Native
Spain	SP4[¶]	J. M. Gómez	36°28.847′N	6°0.999′W	Native
Spain	SP5[¶]	J. M. Gómez	36°25.391′N	6°3.770′W	Native
Ethiopia	ET1[a]	PI633149	†	†	Native
Ethiopia	ET2[a]	PI 273642	†	†	Native
France	FR1[a]	Ames 15945	†	†	Native
France	FR2[b]	CR 2113	†	†	Native
Great Britain	GB1[b]	CR 2618	†	†	Native
Greece	GR1[b]	CR 2100	37°20′5″N	22°21′08″E	Native
Greece	GR2[b]	CR 2101	†	†	Native
Greece	GR3[b]	CR 2102	†	†	Native
Greece	GR4[b,‡]	CR 2103	37°58′0″N	23°43′0″E	Native
Greece	USDA9[a,¶]	PI 263866	†	†	Native
USA	IL1	J. Conner	†	†	Invasive
USA	IL3	J. Conner	†	†	Invasive
USA	IL4	J. Conner	†	†	Invasive
India	IND1[b]	CR 2755	†	†	Native
India	IND2[b]	CR 2757	†	†	Native
Italy	[¥]IT1[b]	CR 2727	40°10′0″N	16°31′0″E	Native
USA	NY2	J. Conner	†	†	Invasive
USA	NY3	J. Conner	†	†	Invasive
The Netherlands	NL1	M. Macel	51°53′0″N	5°38′0″E	Native
The Netherlands	NL2	M. Macel	51°38′41″N	5°30′23″E	Native
The Netherlands	NL3[b]	CR 2734	†	†	Native
Pakistan	PAK1[b]	CR 2620	†	†	Native
Poland	POL2[a]	PI 358590	†	†	Native

Table 1 continued

Country	Population code	Accession number or collector's name	Latitude	Longitude	Range
Czech Republic	CHQ1[b]	CR 77	†	†	Native
Turkey	TU2[a,¶]	PI 169066	40°2′47″N	27°58′12″E	Native
Turkey	TU3[a,‡]	PI 592737	39°38′5″N	27°53′6″E	Native
Turkey	USDA11[a]	PI 176881	39°52′0″N	32°52′0″E	Native
Russia	SOV2[b]	CR 2700	†	†	Native
Serbia	YU1[b]	CR 2758	†	†	Native
Serbia	YU2[a]	PI 368378	43°52′0″N	18°25′0″E	Native

Populations marked with a superscript ([a]) were obtained from USDA GRIN germplasm collections while those marked with a superscript ([b]) were obtained from IPK, Gatersleben-Germany. Geographical coordinates of populations marked by † were not provided. However, the populations were spaced at least 30 km away from the nearest population of *B. nigra*

Populations marked by ¥ were represented by five sequences each, those marked by ∓ were represented by four sequences each, while those marked by ¶ were represented by two sequences each. The rest of the populations were represented by six sequences each

For the USA populations, *CA* California, *IL* Illinois, *NY* New York. Serbia is formerly Yugoslavia, while Russia is formerly Soviet Union

Data analysis

Comparing genetic diversity in native and invasive ranges

To examine genetic diversity in the invasive and native ranges of *B. nigra*, we computed estimates of the number of polymorphic sites, the total number of haplotypes, haplotypic diversity (h), and nucleotide diversity (π) using ARLEQUIN v 3.0 (Excoffier et al. 2005) and DnaSP v. 5.10.01 (Librado and Rozas 2009). These diversity indices were computed for each population separately within the invasive and native ranges. Differences between the invasive and native ranges in the mean values of within-population h and π were tested using linear mixed-effects (LME) models, in which the h and π were treated as dependent variables while *B. nigra* range (invasive or native) was treated as a fixed-effects independent variable. Populations were treated as a random-effects independent variable. The LME models were run using two categories of data: (1) the whole set of 36 native and 15 invasive populations of *B. nigra*, (2) a randomly selected subset of 15 invasive and 15 native populations (from a pool of 36) of *B. nigra.* The LME models were run in R v3.0.3 (R Development Core Team 2013).

Comparing genetic structure in the invasive and native ranges

To test whether the invasive range populations have a different geographic genetic structure than the native range populations, we performed hierarchical analyses of molecular variance (AMOVA) (Excoffier et al. 1992). The AMOVA analyses were performed on the global data set to compare genetic structuring between invasive and native ranges, and also hierarchically for the native and invasive ranges separately using: (1) individual populations, and (2) populations pooled by country or state of origin. These hierarchical AMOVA divide the total genetic variance into components due to interindividual differences within a population, interpopulation differences within a country/state, and intercountry/state differences within a range. Genetic differentiation between populations/groups of populations was compared by pairwise F_{ST} measures and tested by AMOVA in ARLEQUIN v 3.0 (Excoffier et al. 2005). Significance of genetic differentiation was tested by 1000 random permutations.

Genealogical relationships among haplotypes

To infer the introduction history of *B. nigra* into North America, we determined genealogical relationships among haplotypes detected among invasive range and native range populations using the statistical parsimony algorithm implemented in ARLEQUIN v 3.0 (Excoffier et al. 2005). A minimum spanning tree depicting genealogical relationships among the haplotypes was constructed using HapStar v0.5.

Testing for demographic expansion in the invasive and native ranges

Changes in demographic history can influence the frequency of alleles, the distribution of mutations, and the coalescent times of gene copies (Zhang et al. 2012). Hence, we inferred the effects of past demographic expansion on the current genetic variation in invasive and native ranges using Tajima's *D* and Fu's F_S neutrality tests (Tajima 1989; Fu 1997). The neutrality tests were conducted for the invasive and native ranges separately using DnaSP v. 5.10.01 (Librado and Rozas 2009). In the tests, examination of deviation from neutrality was based on 1000 coalescent simulations. Non-statistical difference from zero rejects the null hypothesis of neutral evolution. Significant negative values of Tajima's *D* and Fu's F_S indicate an excess of young or rare alleles in the genealogy, which suggest recent population expansion or purifying selection (Tajima 1989; Fu 1997), whereas significant positive values indicate processes such as recent population bottlenecks or balancing selection (Tajima 1989; Fu 1997).

Results

Genetic diversity

Over the whole data set (284 individuals representing 51 populations), 40 polymorphic sites, 23 substitutions, 44 indels, and 32 haplotypes (H1–H32) were identified. The overall mean haplotypic and nucleotide diversities were 0.34 ± 0.12 and 0.00032 ± 0.0006 (mean ± SD), respectively. The haplotypic diversity ranged from 0.00 to 0.7 (mean = 0.31 ± 0.28 SD) for the native range populations and from 0.00 to 0.8 (mean = 0.27 ± 0.3 SD) for the invasive range populations. The nucleotide diversity ranged from 0.00 to 0.0018 (mean = 0.00056 ± 0.0008 SD) for the native range populations and from 0.00 to 0.0043 (mean = 0.001 ± 0.0014 SD) for the invasive range populations. Linear mixed-effects models did not find a significant difference between the invasive (n = 36 populations) and native (15 populations) ranges of *B. nigra* in the mean values of these diversity indices ($P > 0.05$). The same pattern held when the diversity indices were compared using the same number of populations for invasive and native ranges (i.e., n = 15 populations for each range).

Distribution of the 32 haplotypes among invasive and native populations of *B. nigra* is detailed in Table 2 and Fig. 1. Thirteen haplotypes occurred in the invasive range while 22 haplotypes were detected in the native range (Table 2; Fig. 1). Haplotype H2 occurred in all the 51 populations sampled, and was the most abundant (detected in 230 individuals) followed by haplotype H14 (detected in 13 individuals), haplotype H1 (detected in seven individuals), and haplotype H5 (detected in three individuals) (Table 2). Haplotypes H15, H21, and H23 were each detected in two individuals (Table 2). Three haplotypes, H1, H2, and H21, were shared between invasive and native ranges (Table 2; Fig. 1). The rest of the haplotypes were private, occurring exclusively in native or invasive range populations (Table 2; Fig. 1). The occurrence of private haplotypes (H6–H13, H20, H28, and H29) among the invasive-range populations (Table 2; Fig. 1) suggests that some native-range populations that were sources of introductions of those haplotypes remained unsampled or that those haplotypes are modern, derived haplotypes within the invasive range.

Genetic structure

Hierarchical AMOVA of all samples (i.e., the global data set) showed almost no differentiation between the native and invasive ranges (0.06 %) but significant differentiation among populations (13.97 %), whereas the majority of genetic variation resided within populations (85.97 %) (Table 3). The AMOVA performed using individual populations from the invasive and native ranges separately revealed significant genetic differentiation among and within populations of *B. nigra* in the native range (Table 3). In the invasive range, there was significant genetic differentiation only within the population (Table 3). Genetic differentiation among native populations was much higher ($F_{ST} = 0.228$) than that among the invasive populations ($F_{ST} = 0.008$) (Table 3). Nonetheless, invasive range exhibited higher within-population genetic variation (99.18 %) than the native range (77.2 %) (Table 3). When comparing genetic differentiation among groups of populations pooled by

Table 2 Frequency distribution of 32 haplotypes (based on *trnL–trnF* region of cpDNA) detected among invasive and native populations of *Brassica nigra*

Populations/Haplotypes	AF	AL	**CA**	**CAN**	CHQ	DEN	ET	FR	GB	GR	**IL**	IND	IT	NL	**NY**	PAK	POL	SOV	SP	TU	YUG
H1	1	2		1						1	1			1						1	
H2	9	9	42	9	1	12	9	11	6	22	15	10	3	13	10	6	1	6	15	10	11
H3	1																				
H4	1																				
H5		1											1							1	
H6			1																		
H7			1																		
H8			1																		
H9			1																		
H10			1																		
H11			1												1						
H12				1																	
H13				1																	
H14					5												5		3		
H15							1														1
H16							1														
H17							1														
H18								1													
H19										1				1							
H20											1										
H21											1		1								
H22												1									
H23												1		1							
H24													1								
H25														1							
H26														1							
H27														1							
H28															1						
H29															1						
H30																			1		
H31																			1		
H32																			1		

Population codes: *AF* Afghanistan, *AL* Germany, *CA* California, *CAN* Canada, *CHQ* Czech Republic, *DEN* Denmark, *ET* Ethiopia, *FR* France, *GB* Great Britain, *GR* Greece, *IL* Illinois, *IND* India, *IT* Italy, *NL* The Netherlands, *NY* New York, *PAK* Pakistan, *POL* Poland, *SOV* Russia (formerly Soviet Union), *SP* Spain, *TU* Turkey, and *YU* Serbia (formerly Yugoslavia). Populations from the invasive range are marked in bold font

country/state of origin within each range separately, only the native range groups of populations exhibited a significant among-population genetic structure (Table 3). There was, however, no significant genetic structure within groups of populations for either native or invasive range (Table 3).

Genealogical relationships among the haplotypes

A statistical parsimony analysis of the 32 chloroplast haplotypes produced a starburst pattern of haplotype network with a common central haplotype H2. The ancestral haplotype H2 differed from most of the other haplotypes (H1, H3, H4, H5, H6, H11, H14, H15, H16, H17, H18, H19, H20, H22, H23, H24, H26, H27, and H31) by one mutational step (Fig. 1). The rest of the haplotypes differed from the ancestral haplotype H2 by mutational steps ranging from two (e.g., H12) to 10 (H30) (Fig. 1).

Demographic expansion

The hypothesis of recent demographic expansion or purifying selection was supported only for the invasive range populations (Tajima's $D = -2.22$; $P = <0.01$ and Fu's $F_S = -4.47$; $P < 0.02$). For the native range populations, the neutrality tests did not reject a scenario of selective neutrality and population equilibrium (Tajima's $D = -0.89$; $P > 0.1$ and Fu's $F_S = -0.99$; $P > 0.1$).

Discussion

Multiple introductions of *B. nigra* to North America

During biological invasions, the loss of genetic diversity associated with bottlenecks may be offset by multiple introductions from several source populations in the native range (Lee 2002; Kolbe et al. 2004; Roman and Darling 2007; Hufbauer and Sforza 2008; Dlugosch and Parker 2008). The present results suggest that *B. nigra* has not undergone a bottleneck of genetic diversity in its invasive range, likely as a result of multiple introductions from several genetically distinct populations in the native range. The likely multiple introduction events are indicated by: (1) similar levels of nucleotide and haplotypic diversities between the invasive and native ranges, (2) the AMOVA results revealing almost no genetic differentiation between the native and invasive ranges, (3) a novel co-occurrence in the invasive populations of haplotypes that occur in allopatric native range populations (i.e., haplotypes H1 and H2), and (4) divergence of invasive range haplotypes (i.e., haplotypes H8 and H12) from haplotypes that occur in allopatric populations in the native range (i.e., haplotypes H1 and H15). The invasive range populations in Illinois harbour two phylogenetically distant haplotypes (H1 and H21) that are present in allopatric native range populations in Europe and Asia (Table 2; Fig. 1). Haplotype H12 found in a Canadian population was derived directly from haplotype H15, which occurred in allopatric native populations in Ethiopia and Serbia (formerly Yugoslavia); hence the Canadian population could have been introduced from these two native range populations (Table 2; Fig. 1). Similarly, haplotype H8 found in a Californian population was derived directly from haplotype H1, which is found in spatially separated populations in Afghanistan, Germany, Greece, The Netherlands, and Turkey. Hence, the Californian populations were likely introduced from all or any one of these native populations (Table 2; Fig. 1).

The geographical structure of genetic variation in the native range may influence the level of genetic diversity within introduced populations (Novak and Mack 1993; Kolbe et al. 2004; Zardus and Hadfield 2005). For instance, if the native range populations exhibit low among-population genetic structuring, then most of the genetic variation is partitioned within a population, and hence even a single introduction event from a native source population could eliminate or minimize founder effects in the introduced populations (Novak and Mack 1993; Kolbe et al. 2004; Zardus and Hadfield 2005). On the other hand, high genetic differentiation among populations in the native range may mean that only a tiny fraction of the total genetic variation in the native range is partitioned within a population, and hence a single or only few introduction events from such native range populations would lead to founder effects in the introduced populations (Novak and Mack 1993; Kolbe et al. 2004; Zardus and Hadfield 2005; Le Roux et al. 2011). As our AMOVA results show, the native range populations of *B. nigra* had higher among-population genetic variation than the invasive range populations

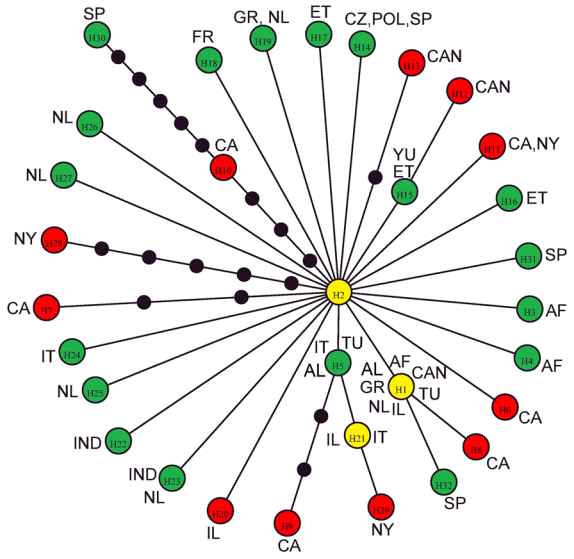

Fig. 1 A minimum spanning tree showing genealogical relationships among 32 haplotypes (H1–H32) detected in invasive and native populations of *Brassica nigra*. Each *line* connecting the ellipses represents a mutational step between haplotypes. There were ten mutational steps between haplotype H2 (ancestral haplotype) and haplotype H30 (the most recent haplotype to diverge). Haplotypes marked in *yellow* were found in both invasive and native ranges, haplotypes marked in *red* occurred exclusively in the invasive range, while haplotypes marked in *green* occurred in the native range only. The ancestral haplotype H2 occurred in all the populations sampled. Population codes: *AF* Afghanistan, *AL* Germany, *CA* California, *CAN* Canada, *CHQ* Czech Republic, *DEN* Denmark, *ET* Ethiopia, *FR* France, *GB* Great Britain, *GR* Greece, *IL* Illinois, *IND* India, *IT* Italy, *NL* The Netherlands, *NY* New York, *PAK* Pakistan, *POL* Poland, *SOV* Russia (formerly Soviet Union), *SP* Spain, *TU* Turkey, and *YU* Serbia (formerly Yugoslavia)

(i.e., 22.79 % vs. 0.82 %, for native and invasive populations respectively) (Table 3). On the other hand, invasive range populations of *B. nigra* exhibited higher within-population genetic variation than the native range populations (99.18 vs. 77.21 %, for invasive and native populations respectively) (Table 3). Thus, it is likely that multiple introductions converted the high level of among-population genetic variation in the native range to high within-population genetic variation in the introduced range. Such a transformation of high among-population genetic variation into a high within-population genetic variation likely due to multiple introductions has previously been reported in invasions by other plant (e.g., Novak and Mack 1993; Genton et al. 2005; Lavergne and Molofsky 2007; Rosenthal et al. 2008; Le Roux et al. 2011) and animal species (e.g., Stepien et al. 2002; Kolbe et al. 2004; Zardus and Hadfield 2005).

The observation of private haplotypes in the invasive populations (Table 2; Fig. 1) suggests that our sampling in the native range does not encompass all sources of the invasive North American populations. An alternative explanation could be an in situ emergence of novel haplotypes following introduction. However, this latter hypothesis appears more unlikely: *B. nigra* was introduced to North America ca. 200 years ago (Bell and Muller 1973; Feeny and Rosenberry 1982; Westman and Kresovich 1999), that is, ca. 200 generations ago (since the plant is annual). The time scale of the current study is much more restricted than in traditional phylogeographic studies, and the evolution of new haplotypes appears unlikely given the slow mutation rates in the *trnL–trnF* intergenic spacer region (0.07270 ± 0.09689 insertions per deletions per locus per Myr) (Smith et al. 2008). This is even more improbable when the private haplotypes diverge by more than one mutational step from other haplotypes within the invasive range, since this would involve multiple mutation events. Thus, future phylogeographic study of *B. nigra* should sample the native range more widely and intensively.

Recent demographic and range expansion of *B. nigra* in North America

Results of the neutrality tests for invasive range populations suggest either purifying selection acting on the invasive populations or recent population demographic expansion of *B. nigra* in North America. This cpDNA intergenic spacer region, as a noncoding locus, is unlikely to be under selection. Thus, the results of neutrality test more likely indicate recent population demographic expansions in the invasive range. As the AMOVA results show a lack of genetic structure among populations in the invasive range (likely due to an on-going gene flow within the invasive range), we suggest that recent population demographic expansion and range expansion of *B. nigra* in North America occurred simultaneously.

Multiple introduction events may precipitate admixture and colonization success

Multiple introductions may lead to introduced populations that are admixtures (i.e., that contain the genetic

Table 3 Hierarchical analysis of molecular variance (AMOVA) (based on *trnL–trnF* region of chloroplast DNA) testing for genetic variation among and within populations of *Brassica nigra* in the invasive and native ranges

Source of variation	*df*	Sum of squares	Variance components	Percentage variation	*P*
(a) Global data set (invasive vs. native range)					
Between ranges	1	0.502	0.00018	0.06	0.255
Among populations	47	22.219	0.03905	13.97	<0.001
Within populations	243	58.412	0.24038	85.97	<0.001
Total	291	81.134	0.27961	0.06	0.255
$F_{ST} = 0.14$					
(b) Individual populations					
Native range					
Among populations	33	16.98	0.055	22.79	<0.001
Within populations	167	31.34	0.188	77.21	<0.001
Total	200	48.32	0.244		
$F_{ST} = 0.228$					
Invasive range					
Among populations	14	5.24	0.0029	0.82	0.210
Within populations	76	27.07	0.359	99.18	<0.001
Total	90	32.31	0.36		
$F_{ST} = 0.008$					
(c) Populations pooled by country or state					
Native range					
Among populations	16	10.61	0.04	16.31	<0.001
Within populations	177	36.75	0.21	83.69	
Total	193	47.36	0.24		
$F_{ST} = 0.163$					
Invasive range					
Among populations	3	1.20	0.0006	0.15	0.5
Within populations	86	31.18	0.36	99.85	
Total	89	32.3	0.36	Total	89
$F_{ST} = 0.0015$					

The AMOVA were performed (a) for the global data set comparing invasive versus native range, and separately for the native and invasive ranges using: (b) individual populations, and (c) populations pooled by country or state of origin

information of several native populations) following secondary contact among previously allopatric native populations (Verhoeven et al. 2011; Rius and Darling 2014). Such admixture may promote colonization success of invasive populations through various processes (Dlugosch and Parker 2008; Verhoeven et al. 2011; Rius and Darling 2014). First, the admixture can increase the level of standing genetic variation within a population that enables the population to respond to natural selection (Kolbe et al. 2008). Second, recombination between genotypes from genetically differentiated populations can increase variation in quantitative traits upon which natural selection will act (Orians 2000; Facon et al. 2005). Finally, admixture can also lead to hybrid vigour (Dobzhansky 1952). A growing number of empirical studies have detected admixture in invasive populations of plants and animals (e.g., Williams et al. 2005; Kang et al. 2007; Kolbe et al. 2007; Taylor and Keller 2007; Rosenthal et al. 2008; Gillis et al. 2009; Chun et al. 2010; Montarry et al. 2010; Keller et al. 2012; Le Roux et al. 2013). The present results also suggest that some invasive populations of *B. nigra* are composed of admixtures of haplotypes from allopatric native range

populations. Although a growing number of studies detect admixture in invasive species, the challenge remains as to whether admixed populations are more successful colonizers than nonadmixed populations (Rius and Darling 2014). Thus, future studies are needed that test whether admixed *B. nigra* populations are more aggressive invaders (e.g., have higher growth and competitive ability) than non-admixed *B. nigra* populations.

Conclusion

Taken together, our results suggest that *B. nigra* in North America has not undergone a bottleneck of diversity, likely as a result of multiple introduction events from the native range. More generally, this study adds to the growing body of data on the genetic patterns and processes involved in biological invasions, which will hopefully lead to an increased understanding of the post-introduction evolution of invasive plant species.

Acknowledgments We thank Yedra García for help with DNA extraction and Sharon Y. Strauss for comments on an earlier version of this manuscript. This study was supported by a Grant from the foundation Banco Bilbao Vizcaya Argentaria (BBVA-BIOCON06/064). We are grateful to North Central Regional Plant Introduction Station (NCRPIS)-USA, IPK, Gatersleben (Germany) and Real Jardín Botánico de Madrid (RJB-CSIC) for providing us with *Brassica nigra* seeds from their germplasm collections. We received additional *B. nigra* seeds from field collections done by Richard A. Lankau, Mirka Marcel,and Jeffrey K. Conner. A.M.O. Oduor received further financial support from a Ph.D. scholarship granted by Agencia Española de Cooperación Internacional para el Desarrollo (MAEC-AECID).

References

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. Conserv Biol 17:24–30

Austerlitz F, Mariette S, Machon N et al (2000) Effects of colonization processes on genetic diversity: differences between annual plants and tree species. Genetics 154:1309–1321

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge, MA

Bell DT, Muller CH (1973) Dominance of California annual grasslands by *Brassica nigra*. Am Midl Nat 90:277–299

Bossdorf O, Auge H, Lafuma L et al (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11. doi:10.1007/s00442-005-0070-z

Chun YJ, Fumanal B, Laitung B, Bretagnolle F (2010) Gene flow and population admixture as the primary post-invasion processes in common ragweed (*Ambrosia artemisiifolia*) populations in France. New Phytol 185:1100–1107. doi:10.1111/j.1469-8137.2009.03129.x

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659. doi:10.1046/j.1365-294X.2000.01020.x

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. doi:10.1111/j.1365-294X.2007.03538.x

Dobzhansky T (1952) Heterosis: a record of researches directed toward explaining and utilizing the vigor of hybrids, Nature and the origin of heterosis. Lowa State College Press, Ames, pp 218–223

Durka W, Bossdorf O, Prati D, Auge H (2005) Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America. Mol Ecol 14:1697–1706. doi:10.1111/j.1365-294X.2005.02521.x

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? Proc Natl Acad Sci U S A 97:7043–7050

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491. doi:10.1007/s00424-009-0730-7

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50. doi:10.1111/j.1755-0998.2010.02847.x

Facon B, Jarne P, Pointier JP, David P (2005) Hybridization and invasiveness in the freshwater snail *Melanoides tuberculata*: hybrid vigour is more important than increase in genetic variance. J Evol Biol 18:524–535. doi:10.1111/j.1420-9101.2005.00887.x

Feeny P, Rosenberry L (1982) Seasonal variation in the glucosinolate content of North American *Brassica nigra* and *Dentaria* species. Biochem Syst Ecol 10:23–32. doi:10.1016/0305-1978(82)90047-3

Freeland JR (2005) Molecular ecology. Wiley, New York

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

Genton BJ, Shykoff JA, Giraud T (2005) High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. Mol Ecol 14:4275–4285. doi:10.1111/j.1365-294X.2005.02750.x

Gillis NK, Walters LJ, Fernandes FC, Hoffman EA (2009) Higher genetic diversity in introduced than in native populations of the mussel mytella charruana: evidence of population admixture at introduction sites. Divers Distrib 15:784–795. doi:10.1111/j.1472-4642.2009.00591.x

Hufbauer RA, Sforza R (2008) Multiple introductions of two invasive *Centaurea taxa* inferred from cpDNA haplotypes. Divers Distrib 14:252–261. doi:10.1111/j.1472-4642.2007.00424.x

Hulme PE (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. J Appl Ecol 46:10–18. doi:10.1111/j.1365-2664.2008.01600.x

Kang M, Buckley YM, Lowe AJ (2007) Testing the role of genetic factors across multiple independent invasions of the shrub Scotch broom (*Cytisus scoparius*). Mol Ecol 16:4662–4673. doi:10.1111/j.1365-294X.2007.03536.x

Keller SR, Gilbert KJ, Fields PD, Taylor DR (2012) Bayesian inference of a complex invasion history revealed by nuclear and chloroplast genetic diversity in the colonizing plant, *Silene latifolia*. Mol Ecol 21:4721–4734. doi:10.1111/j.1365-294X.2012.05751.x

Kolbe JJ, Glor RE, Rodríguez Schettino L et al (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–181. doi:10.1038/nature02807

Kolbe JJ, Glor RE, Schettino LR et al (2007) Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. Conserv Biol 21:1612–1625. doi:10.1111/j.1523-1739.2007.00826.x

Kolbe JJ, Larson A, Losos JB, de Queiroz K (2008) Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. Biol Lett 4:434–437. doi:10.1098/rsbl.2008.0205

Lankau RA, Strauss SY (2008) Community complexity drives patterns of natural selection on a chemical defense of *Brassica nigra*. Am Nat 171:150–161. doi:10.1086/524959

Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc Natl Acad Sci U S A 104:3883–3888. doi:10.1073/pnas.0607324104

Le Roux JJ, Brown GK, Byrne M et al (2011) Phylogeographic consequences of different introduction histories of invasive Australian *Acacia* species and *Paraserianthes lophantha* (Fabaceae) in South Africa. Divers Distrib 17:861–871. doi:10.1111/j.1472-4642.2011.00784.x

Le Roux JJ, Richardson DM, Wilson JRU, Ndlovu J (2013) Human usage in the native range may determine future genetic structure of an invasion: insights from *Acacia pycnantha*. BMC Ecol 13:37. doi:10.1186/1472-6785-13-37

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391. doi:10.1016/S0169-5347(02)02554-5

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452. doi:10.1093/bioinformatics/btp187

Mack R, Simberloff D, Lonsdale MW et al (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Appl 10:689–710

Meyerson LA, Cronin JT (2013) Evidence for multiple introductions of *Phragmites australis* to North America: detection of a new non-native haplotype. Biol Invasions 15:2605–2608. doi:10.1007/s10530-013-0491-2

Montarry J, Andrivon D, Glais I et al (2010) Microsatellite markers reveal two admixed genetic groups and an ongoing displacement within the French population of the invasive plant pathogen *Phytophthora infestans*. Mol Ecol 19:1965–1977. doi:10.1111/j.1365-294X.2010.04619.x

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution (N Y) 29:1–10

Novak SJ, Mack RN (1993) Genetic variation in *Bromus tectorum* introduced populations. Heredity (Edinb) 71:167–176

Oduor AMO, Lankau RA, Strauss SY, Gomez JM (2011) Introduced *Brassica nigra* populations exhibit greater growth and herbivore resistance but less tolerance than native populations in the native range. New Phytol 191:536–544

Oduor AMO, Strauss SY, Garcia Y, et al (2013) Herbivores mediate different competitive and facilitative responses of native and invader populations of *Brassica nigra*. Ecology 94:2288–2298

Orians CM (2000) The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant—Herbivore interactions. Am J Bot 87:1749–1756. doi:10.2307/2656824

Pimentel D, McNair S, Janecka J et al (2001) Economic and environmental threats of alien plant, animal and microbe invasions. Agric Ecosyst Environ 84:1–20

R Development Core Team (2013) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rius M, Darling JA (2014) How important is intraspecific genetic admixture to the success of colonising populations? Trends Ecol Evol 29:233–242. doi:10.1016/j.tree.2014.02.003

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464. doi:10.1016/j.tree.2007.07.002

Rosenthal DM, Ramakrishnan AP, Cruzan MB (2008) Evidence for multiple sources of invasion and intraspecific hybridization in *Brachypodium sylvaticum* (Hudson) Beauv. in North America. Mol Ecol 17:4657–4669. doi:10.1111/j.1365-294X.2008.03844.x

Sakai AK, Allendorf FW, Holt JS et al (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Smith CI, Pellmyr O, Althoff DM et al (2008) Pattern and timing of diversification in *Yucca* (Agavaceae): specialized pollination does not escalate rates of diversification. Proc Biol Sci 275:249–258. doi:10.1098/rspb.2007.1405

Stepien CA, Taylor CD, Dabrowska KA (2002) Genetic variability and phylogeographic patterns of a nonindigenous species invasion: a comparison of exotic versus native zebra and quagga mussel populations. J Evol Biol 15:314–328

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109. doi:10.1007/BF00037152

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Taylor DR, Keller SR (2007) Historical range expansion determines the phylogenetic diversity introduced during contemporary species invasion. Evolution (N Y) 61:334–345. doi:10.1111/j.1558-5646.2007.00037.x

Turner KG, Hufbauer RA, Rieseberg LH (2014) Rapid evolution of an invasive weed. New Phytol 202:309–321. doi:10.1111/nph.12634

Uller T, Leimu R (2011) Founder events predict changes in genetic diversity during human-mediated range expansions. Glob Chang Biol 17:3478–3485. doi:10.1111/j.1365-2486.2011.02509.x

Verhoeven KJF, Macel M, Wolfe LM, Biere A (2011) Population admixture, biological invasions and the balance

between local adaptation and inbreeding depression. Proc R Soc B Biol Sci 278:2–8. doi:10.1098/rspb.2010.1272

Voisin M, Engel CR, Viard F (2005) Differential shuffling of native genetic diversity across introduced regions in a *brown alga*: aquaculture vs. maritime traffic effects. Proc Natl Acad Sci U S A 102:5432–5437. doi:10.1073/pnas.0501754102

Westman AL, Kresovich S (1999) Simple sequence repeat (SSR)—based marker variation in *Brassica nigra* genebank accessions and weed populations. Euphytica 109:85–92

Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, "invasive traits" and recipient communities: challenges for predicting invasive potential. Divers Distrib 14:569–580. doi:10.1111/j.1472-4642.2008.00473.x

Williams DA, Overholt WA, Cuda JP, Hughes CR (2005) Chloroplast and microsatellite DNA diversities reveal the introduction history of Brazilian peppertree (*Schinus terebinthifolius*) in Florida. Mol Ecol 14:3643–3656. doi:10.1111/j.1365-294X.2005.02666.x

Zardus JD, Hadfield MG (2005) Multiple origins and incursions of the Atlantic barnacle *Chthamalus proteus* in the Pacific. Mol Ecol 14:3719–3733. doi:10.1111/j.1365-294X.2005.02701.x

Zhang B, Edwards OR, Kang L, Fuller SJ (2012) Russian wheat aphids (*Diuraphis noxia*) in China: native range expansion or recent introduction? Mol Ecol 21:2130–2144. doi:10.1111/j.1365-294X.2012.05517.x