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Genomic admixture increases fitness during a biological invasion

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Abstract

During biological invasions, multiple introductions can provide opportunities for admixture among genetically distinct lineages. Admixture is predicted to contribute to invasion success by directly increasing fitness through hybrid vigour or by enhancing evolutionary potential within populations. Here, we demonstrate genome-wide admixture during an invasion that substantially boosted fitness in the cosmopolitan weed, *Silene vulgaris*. We identified three divergent demes in the native European range that expanded from glacial refugia and experienced historical admixture in a well-known suture zone. During recent invasion of North America, multiple introductions created additional opportunities for admixture. In common garden experiments, recombinant genotypes from North America experienced a two-fold increase in fitness relative to nonrecombinants, whereas recombinant genotypes from Europe showed no lasting fitness benefits. This contrast implicates hybrid vigour behind the boost in fitness and supports the hypothesis that admixture can lead to fitness increases that may catapult invasion into a new range.

Introduction

The globalization of human commerce has caused numerous species to be intentionally or inadvertently introduced to new locations, where their populations may expand to become widespread and have destructive effects on native biodiversity, ecosystem functioning, and agricultural productivity (Chapin *et al.*, 2000; Sakai *et al.*, 2001). When species invade a new range, their initial colonization and subsequent range expansion often involves the fission and fusion of populations. As a result, invasive species can experience dramatic changes in genetic diversity and population structure (Wares *et al.*, 2005; Dlugosch & Parker, 2008). The impact these genetic changes have on the fitness of introduced populations is of central importance for understanding the forces that promote and retard biological invasions (Lee, 2002; Keller & Taylor, 2008), and more generally, for understanding the evolutionary consequences of range expansions (Excoffier *et al.*, 2009).

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During colonization, founder effects may reduce genetic diversity in newly established populations relative to the source population (Dlugosch & Parker, 2008). The proliferation of invasive species in the face of such bottlenecks is hypothesized to stem from multiple introductions from genetically diverse sources (Frankham, 2005). Admixture occurs when lineages from different sources come into contact and recombine their genomes – an outcome that is an increasingly recognized feature of many contemporary species invasions (e.g. Gaskin & Schaal, 2002; Kolbe *et al.*, 2004; Whitfield *et al.*, 2006) as well as historical invasions following expansion from glacial refugia (Petit *et al.*, 2003). Although many population genetic studies of invasions have reported evidence of multiple introductions, the impact of admixture on the fitness of introduced populations has received little empirical validation (Kolbe *et al.*, 2007; Lavergne & Molofsky, 2007; Facon *et al.*, 2008).

Addressing the fitness consequences of admixture during invasion depends on deciphering a complex interaction of historical, genetic and evolutionary processes. Ideally, we would understand (i) how a history of isolation and migration generated genetic divergence among lineages as well as opportunities for secondary contact, (ii) the magnitude or extent of admixture at the

individual level as a result of recombination among divergent genomes, (iii) the immediate fitness effects of such admixture, and (vi) how the effects of admixture play out over subsequent generations. This requires sampling genotypes from across the native and introduced ranges and pairing observations of both genome-wide diversity and fitness on the same replicate lines.

In this study, we reconstruct the migration history of a widespread colonizing weed, detect multiple episodes of past admixture and estimate how these events have influenced the genetic makeup and fitness of genotypes from the native and introduced ranges. Through a combination of population genetic analyses and common garden field experiments on replicated family lines, we provide direct evidence that admixture during invasion increased fitness through a more than two-fold boost in fruit production over genotypes with less mixed ancestry.

Methods

Study species

The plant genus *Silene* (Caryophyllaceae) has emerged as a model system in ecology and evolutionary biology, including the study of biological invasions (Bernasconi *et al.*, 2009). The herbaceous short-lived perennial, *Silene vulgaris* (Moenche) Garke, exhibits a widespread distribution in its native Eurasia and has become a cosmopolitan weed of human disturbance throughout the Americas and Australia (Randall, 2002; Taylor & Keller, 2007; Keller *et al.*, 2009). In North America, *S. vulgaris* was introduced during the late eighteenth and early nineteenth centuries, occurring as a weed bordering agricultural fields near Boston and Quebec City (Cutler, 1785; Pursh, 1814). Later, it was noted growing out of mounds of dirt, gravel, sand and refuse from ship ballast at the port cities of Philadelphia and New York (Martindale, 1876; Brown, 1878). Thus, every indication is that the introduction was an unintentional by-product of human immigration and commerce. Since its introduction, *S. vulgaris* has expanded its range across most of temperate North America, frequently occurring in networks of patchily distributed populations that undergo local extinction and recolonization (McCauley *et al.*, 2003).

Collections and DNA isolation

We obtained specimens of *Silene vulgaris* from across a broad geographical area, spanning the breadth of the known ranges in Europe and North America. Specimens were either collected as seeds (maternal families from field grown plants) or as leaf tissue dried on silica gel. Seeds were germinated at the University of Virginia greenhouse, and genomic DNA was isolated from either fresh or dried leaf tissue from a single member of each family using Qiagen DNeasy plant mini kits.

Amplified fragment length polymorphisms (AFLPs)

To examine the history of the nuclear genome of *S. vulgaris* during range expansion and invasion, we assayed genome-wide variability for 374 individuals (218 from throughout Europe, 156 from North America) at 267 amplified fragment length polymorphic (AFLP) loci using a standard protocol (Vos *et al.*, 1995). Specifically, 50–100 ng of genomic DNA was digested at room temperature overnight with a pair of restriction enzymes (*Eco*RI and *Mse*I; New England Biolabs) and ligated to double-stranded adapters in a single 10-μL reaction. Preselective and selective PCR amplification was performed in 10-μL reactions according to the ABI Plant Mapping protocol (Applied Biosystems). Selective amplification products were then pooled together (one FAM and TAMRA labelled primer per well) and separated by electrophoresis on an ABI 377 or ABI 3130xl automated sequencer.

Fluorescent peaks were sized against the ROX-500 standard using Genemapper v4.0 software (Applied Biosystems). All samples were normalized to the sum of the sample signal within Genemapper, calculated across the entire project. Peaks > 50 RFU were scored within a size range of 100–400 bp. We ran 30–40 sample duplicates per primer pair on each different electrophoresis platform (377 vs. 3130xl sequencers) to identify the platform-specific size range for segregating loci and define homologous bins across platforms. Automated scoring was manually verified for all samples by the same observer. Peak presence/absence was converted to binary (0/1) coding for further analyses.

Chloroplast DNA (cpDNA) polymorphism

To test for the presence of nonrandom associations between cytoplasmic and nuclear alleles, and whether admixture during invasion had disrupted them, we assayed chloroplast DNA (cpDNA) polymorphism to compare with the AFLP nuclear genotypes. Using published cpDNA sequence data (Ingvarsson & Taylor, 2002; Ingvarsson *et al.*, 2003; Taylor & Keller, 2007), we searched the sequence alignments for polymorphisms in insertions/deletions (indels) or restriction fragment length polymorphisms (RFLPs). Of three major regions in the genealogy (hereafter called clades A, B, and C), two could be positively assigned using indel or RFLP polymorphism, whereas the third was assigned by process of elimination (Fig. S1). Clade A was defined by a 7-bp insertion in an intergenic spacer between *trn*L (UAA) and *trn*F (GAA). Primers were designed to amplify a 123-bp region that flanks this indel polymorphism (forward: 5′ tttaggtcttcaaaaagaggaactc 3′; reverse: 5′ gcaggcagtactccgttgag 3′). PCR was performed in 25-μL reactions, and

8.5 μL was run out on a 3% agarose gel for 80 min. at 105 V. Clade B was defined by a single nucleotide polymorphism (G/T) that created a *Psi*I RFLP within the same *trn*L-*trn*F intergenic spacer. This region was PCR amplified in a 25-μL reaction using universal primers as previously described (Ingvarsson & Taylor, 2002). Following amplification, 8.9 μL of PCR product was combined with 0.1 μL of *Psi*I enzyme and 1 μL of enzyme buffer (New England Biolabs) and digested at 37 °C. The resulting products were visualized on 3% agarose gels. We performed a set of positive controls on our cpDNA haplotyping by analysing 31 samples for the previously mentioned indel/RFLP assays and by direct sequencing their PCR products on an automated sequencer. All 31 samples were congruent in their assignments across methods. A total of 384 samples were typed for their cpDNA clade, encompassing 214 European and 170 North American samples.

Common Garden Field experiment

To determine the potential consequences of admixture for plant fitness, we measured fruit production in common gardens as part of a larger experiment designed to test for genetic differences in quantitative traits between Europe and North America. Two common garden sites in North America were established to assess plant performance under field conditions. The inclusion of more than one garden site is important to control for the influence of genotype-by-environment interactions when testing for genetic differences between ranges (Williams *et al.*, 2008). We located our two garden sites near the latitudinal peripheries of the species' current range in eastern North America to provide an overall contrast in environment. This facilitated testing the genetic effects of principal interest (admixture; range of origin) while controlling for variance in plant fitness arising in different environments. One site 'ON' was located in Ontario, Canada (N 45.8642, W −79.4362) and another 'VA' in Virginia, USA (N 37.8577, W −78.8208). The ON site was located in a farm field no longer under cultivation. A small population of *S. vulgaris* (< 5 plants) grew in the field and was destroyed prior to planting. Natural populations were located ca. 1 km away on the roadside. The VA site was located in a hayfield near the Rockfish River, Nelson County. There were no *S. vulgaris* previously growing in the field, but natural populations existed along the roadsides and adjacent hayfields ca. 1 km away.

Six weeks prior to planting at each site, seeds were surface sown in a completely randomized design into plug trays filled with a standard potting mix (Promix HP). Trays were placed into one of two Percival growth chambers at the University of Virginia set to a diurnal photoperiod of 12 : 12 (light : dark) and a temperature cycle of 21 and 12 °C during light and dark phases, respectively. Trays were checked daily, misted as necessary and rotated within chambers to reduce position effects. Four seedlings per family were randomly chosen for planting into the field (total $N = 2$ sites × 2 continents of origin × 100 families/cont × 4 plants/family = 1600 plants). In addition, a full replicate of seeds were germinated to replace transplant mortality after initial planting.

The common gardens consisted of four rectangular subplots, each containing 200 plants. Plants were spaced 0.5 metres apart within rows, and 0.75 m apart between rows. Seedlings were transplanted into the field during spring 2005. Prior to planting, the existing vegetation was cut back to ground level, and individual holes ca. 15 cm in diameter were created with a post-hole digger. Seedlings were planted directly into the ground and watered in. Seedlings lost to transplant mortality that occurred up to 2-week post-planting were replaced with a replicate seedling from the same family at the same stage of growth. Plants were watered as needed during early summer 2005 at ON to offset drought conditions when plants were young. The existing vegetation was cut back periodically at each site to avoid over-competition.

Weekly censuses were conducted throughout the 2005 growing season and again in 2006. During each census, plants were checked for the number of newly produced mature fruits. Flowers were marked on their calyces with a small dot from a liquid paint pen to avoid recounting in subsequent censuses. Mature fruits were harvested prior to dehiscence to avoid seed contamination at the sites. Total fitness was estimated as the sum of fruit production across years per plant, which integrates both individual survivorship and fecundity.

Statistical analyses

Population structure

To test for native range genetic structure and assign introduced genotypes to their ancestral source regions, we used Bayesian model-based clustering implemented in Structure v2.2 (Pritchard *et al.*, 2000), which accounts for the presence of recessive alleles by integrating over genotypic uncertainty and thus accommodates dominant markers such as AFLPs (Falush *et al.*, 2007). We ran an admixture model with correlated allele frequencies among demes (the *F*-model), which models a scenario of demes simultaneously diverging because of genetic drift from a common ancestral population while allowing for ongoing genetic exchange and recombination between demes (Falush *et al.*, 2003). Batch runs were carried out on a Linux cluster hosted by the Research Computing Lab at the University of Virginia. We conducted a search for the most likely value of K by performing 10 replicate runs at each value of K ranging from 1 to 10, and an additional single run for values of K from 11 to 18. Runs had a burn-in of 200 000 iterations, followed by parameter estimation over an additional 1 000 000 iterations. For $K = 2$ and 3, we observed a bimodal likelihood distribution, with some replicate runs

returning distinctly lower values of Ln(Pr(X|K)), most likely a result of the MCMC chain getting stuck in a region of lower likelihood (Pritchard *et al.*, 2007); thus we followed Pritchard *et al.* (2000) in analysing the mode with the higher Ln(Pr(X|K)). We adopted the ΔK selection criterion, whereby the best model was the one that maximized the second order rate of change in Ln(Pr(X|K)) as a function of K (Evanno *et al.*, 2005).

We estimated gene diversity (average expected heterozygosity) and genetic divergence among demes ($\theta^{(II)}$) using Bayesian estimators for dominant data, implemented in the software HICKORY v1.1 (Holsinger *et al.*, 2002). Individuals were grouped into demes based on the majority-rule of their assignment scores from STRUCTURE. We ran HICKORY with flat priors, with 50 000 burn-in iterations followed by 500 000 sampling iterations. To account for possible inbreeding, we used *f*-free models that estimate gene diversities and θ over the full range of possible values for the inbreeding coefficient.

We tested for nonrandomness in the distribution of genetic diversity in Europe by dividing the sample into three physiographic regions: Eastern Europe, Southern Europe, Western Europe, according to previously defined physiographic boundaries (Taylor & Keller, 2007). These regions reflect physical barriers and known dispersal corridors for plants and animals since the last ice age (Taberlet *et al.*, 1998; Hewitt, 2000). Differences in the frequencies of AFLP demes and cpDNA clades across regions were tested using a χ^2 test of independence. We similarly evaluated the potential for founder effects during invasion to shift the relative frequencies of each deme and clade by analysing the change in frequency between ranges with a χ^2 test of independence. We tested for founder effects subsampling AFLP diversity within demes using two methods: (i) we compared ranges for the average levels of gene diversity within demes, estimated using HICKORY and (ii) we compared ranges for the amount of variation among multilocus genotypes. We approached the latter analysis by first ordinating each multilocus genotype using principal coordinates analysis (R PACKAGE v4.0 by P. Legendre). We then used the first and second PCs to test for a change in intra-demic genotypic variability using Levene's test of homogeneity of variances between ranges, conducted separately by deme. Under a bottleneck hypothesis, the extent of multilocus diversity should drop as rare alleles become lost or reduced in frequency, resulting in a reduction in variance among AFLP genotypes ordinated along the principal coordinates.

Admixture and recombination of genetic variance

To quantify admixture, we tested for the dissolution of allelic associations through recombination by measuring linkage disequilibrium (LD) within the nuclear genome. We estimated a composite index of genome-wide LD across all 267 AFLP loci using the standardized measure of allelic covariance, r_d, in MULTILOCUS v1.2 (Agapow & Burt, 2001). We determined r_d separately for Europe and North America and tested the null hypothesis of linkage equilibrium (no association) within each range using 500 randomizations of the data.

We also examined changes in r_d among pairs of loci where ancestral LD was expected to be strongest, identified as the 50 loci showing the greatest divergence among demes (measured by F_{ST}) following locus-by-locus AMOVA in ARLEQUIN V3.1 (Excoffier *et al.*, 2005). LD among these 50 loci was visualized by producing heatmaps of pairwise r_d. Change in the among-locus covariance structure between Europe and North America was then tested using (i) a Mantel's test on the European and North American r_d matrices in ARLEQUIN, with 10 000 permutations to assess significance, and (ii) hierarchical comparisons of the variance-covariance matrices, using the method of common principal components implemented in the software CPC (Phillips & Arnold, 1999). In hierarchical matrix comparisons, two matrices are compared for their degree of similarity, as measured by their number of shared principal components and associated eigenvalues. Matrix comparisons followed the Flury hierarchy, starting with unrelated structure and continuing through one or more common principal components, up to matrix proportionality and finally total matrix equality. To avoid singularity, one locus had to be dropped from the hierarchical matrix comparison because it was fixed in North America (i.e. had zero variance).

Mating among historically isolated lineages may also disrupt patterns of association between genomes, particularly between cytoplasmic (chloroplast and mitochondrial) and nuclear genomes. Thus, we predicted that admixture occurring since the invasion of North America should produce shifts in the associations between the chloroplast clades and nuclear AFLPs. Cytonuclear associations were tested using a general linear model, with chloroplast clade assignments predicted by range and the first two PCs from the AFLP genotypes.

Admixture-fitness correlation

To determine whether admixture had consequences for plant fitness, we paired our AFLP genotype assignments from STRUCTURE with field estimates of fitness, measured in the common garden experiments. Our goal was to have genetic ancestry information for a member of each of the 200 families planted into the common gardens. However, after losses caused by incomplete germination and mortality, the number of families with both types of data was reduced to 174 (88 European and 86 North American). Family means of total fruit production (summed over 2 years) were calculated for each site to avoid pseudoreplication when paired with the AFLP assignments. Log-transformed family mean fruit production was then used as a proxy for fitness in an analysis of covariance to test for dependency of fitness on the level of admixture present within a family, and how this

relationship varies across ranges. Range was a fixed effect and degree of admixture was the covariate. Site was included as a blocking factor, and treated as a fixed effect. We defined an index of admixture (H_A) using a standardized version of the Shannon–Weaver diversity index,

$$H_A = \frac{-\sum_{i=1}^{n} f_i \log(f_i)}{\log(n)}$$

where f_i is the fractional assignment of an individual to Structure deme i and n is the total number of Structure demes. We used an angular transformation to improve normality of H_A. Analysis of covariance using maximum likelihood estimation was performed by first running the full model specifying all effects. Following a significant range × admixture interaction, we ran models separately by range with all other factors specified as earlier.

Results

Genetic structure in Europe and North America

The evolutionary history of *Silene vulgaris* in its native range resulted in significant structuring of nuclear genomic diversity, with Bayesian clustering favouring a model of three divergent demes (Figs 1 and S2). These demes exhibited significant divergence in AFLP allele frequencies ($\theta = 0.079$; 95% credible interval = 0.052–0.123), as well as a geographical distribution with strong regional structure between Eastern, Western and Southern Europe ($\chi^2 = 165.45$, $P < 0.0001$). The pattern of population structure was highly congruent with the Quaternary history of other temperate European plants and animals (Hewitt, 2000), suggesting demes diverged during historical isolation in southern refugia and subsequently expanded northward across Europe (Fig. 1). Consistent with this, we observed a zone of admixture between demes in the vicinity of the central Alps (Fig. 1 inset), probably the result of secondary contact during post-glacial expansion. There was also some evidence of long-distance mixing of genotypes elsewhere in Europe; for example, Eastern European (red) genotypes in Iberia and Southern European (blue) genotypes in Ireland, perhaps the result of older glacial cycles or more recent human-mediated transport (Fig. 1). In contrast, the majority of genotypes elsewhere in the native range maintained relatively pure ancestry, indicating persistent geographical isolation.

During the invasion of North America, all three ancestral European demes became established, suggesting the invasion process involved multiple introductions from different geographical localities in Europe. Nuclear AFLP variability showed little evidence for a continent-scale bottleneck, with 260 of 267 loci remaining polymorphic in North America, and gene diversity dropping a modest 2% ($H_T = 0.237$; 95% credible interval = 0.214–0.261) compared to Europe ($H_T = 0.241$; 0.217–0.270). However, although the invasion process did not impose a range-wide bottleneck of diversity, the frequencies of North American genotypes with majority membership in each deme were significantly different than those observed in Europe ($\chi^2 = 39.09$, $P < 0.0001$), perhaps reflecting a founder effect caused by biased sampling among demes from different geographical regions of Europe. Introduced genotypes descended from the Eastern European deme were in greatest abundance, and nearly doubled in frequency in North America (35% in EU; 62% in NA). In contrast, the frequency of genotypes descended from Southern Europe was sharply reduced in North America (32% in EU; 8% in NA) (Fig. 1).

Despite evidence for a founder effect shifting deme frequencies, there was a little indication that diversity within demes had been bottlenecked. Gene diversities within demes showed only slight reductions in North America relative to Europe (0.7–5%), and none were significantly different between ranges (Table S1). The amount of genotypic variance along the first two principal coordinates of AFLP loci was also of similar magnitude between ranges, suggesting that North American colonists were a representative sample of the genetic diversity found within each deme in Europe (Table 1). Only the Western European deme showed a significant change in variance between ranges, although the direction was that of higher genotypic variance in North America, contrary to expectations from a bottleneck.

The invasion history in North America left no regional geographical pattern in the distribution of demes, with genotypes from nearby populations frequently assigned to different demes (Fig. 1). Genetic divergence among demes remained significant in North America ($\theta = 0.086$; 95% credible interval = 0.062–0.119). However, there were multiple genotypes that showed evidence of hybrid ancestry, as evidenced by their mixed assignment scores from Structure (Fig. 1 inset). Taken together, the data suggest the invasion process involved a biased yet diverse set of multiple introductions that generally mixed demes at a regional scale and interdemic mating occurred locally where demes have come into recent secondary contact.

The impact of invasion on linkage disequilibrium

When mating occurs between divergent lineages, independent assortment and recombination should erode statistical associations that developed during historical isolation. To test whether this process is evident from the admixture during invasion, we compared genotypes from Europe and North America for levels of linkage disequilibrium (LD) among the 267 AFLP loci. Both continents exhibited significant multilocus LD ($P < 0.002$), but the magnitude of LD in North America ($r_d = 0.0053$) was ~ 40% lower than that in Europe ($r_d = 0.0088$). Among the 50 AFLP loci that contributed most strongly to the

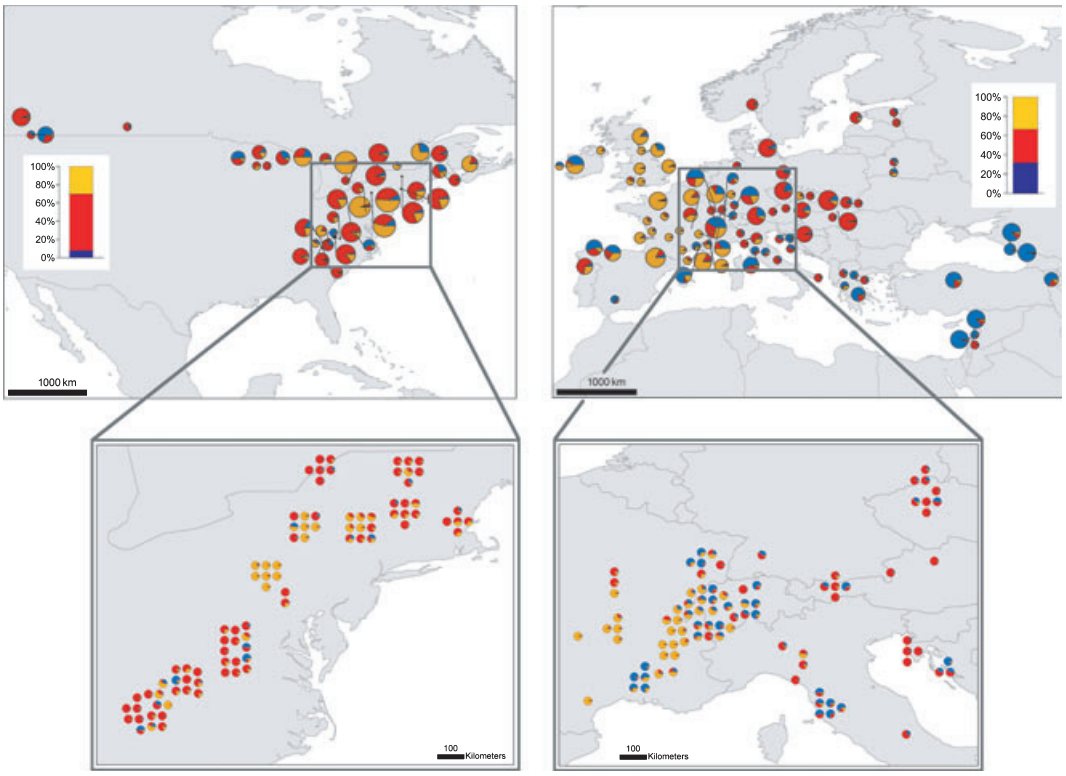

Fig. 1 Structure Bayesian model-based clustering of multilocus amplified fragment length polymorphisms (AFLP) genotypes. In the upper panels, pies represent population averages of deme frequencies, with pie diameter proportional to sample size. Vertical bar plots show the relative frequencies of demes before and after invasion. Insets highlight the areas of admixture on each continent. Within the insets, each circle represents the proportional assignment of a single individual's genome to each deme. Individuals with overlapping sampling locations are offset for clarity.

genetic distinctiveness of demes in Europe, pairwise LD was correlated across ranges (Mantel's $r = 0.349$, $P < 0.0001$; Fig. 2a). However, there was an overall decrease in blocks of loci showing strong pairwise LD in North America and an increase in pairs showing weak to no LD (Fig. 2b, c). Hierarchical comparisons between allele frequency covariance matrices in Europe and North America also supported a change in the structure of LD across ranges (Table 2). Model selection revealed substantial support for shared common principal components across ranges (AIC = 1222), but substantially less support for matrix proportionality (AIC = 1967) or strict matrix equality (AIC = 2022).

Table 1 Levene's test of homogeneity of genotypic variance, defined by the first two principal coordinates of 267 Amplified fragment length polymorphisms (AFLP) loci. Homogeneity of genotypic variance across ranges was tested separately for each deme.

	Deme	Ratio of genotypic variance in NA:EU	d.f. (num, den)	*F*-test	*P*
PC1	Southern	0.78	1, 79	0.33	0.5689
	Eastern	0.91	1, 171	0.23	0.6345
	Western	1.52	1, 118	4.70	0.0322
PC2	Southern	0.62	1, 79	0.76	0.3851
	Eastern	1.25	1, 171	0.66	0.4194
	Western	2.13	1, 118	10.22	0.0018

The effects of admixture were also evident in the associations between nuclear and cytoplasmic genomes within each range. In Europe, there was weak but significant spatial structuring of cpDNA clades among geographical regions ($\chi^2 = 12.27$, $P = 0.0155$), in a pattern similar to the distribution of nuclear demes (Fig. S3). This created significant cytonuclear associations in the native range ($\chi^2 = 32.96$, $P < 0.0001$), with cpDNA clade A most frequently associated with a Western European nuclear deme background and cpDNA clade C most often associated with an Eastern or Southern European nuclear background (Fig. 3). Cytonuclear disequilibria were also present in North America ($\chi^2 = 10.38$, $P = 0.035$), but the associations have evolved since invasion. The dominant trend was a shift of chloroplast clades towards the Eastern European nuclear background, which showed the largest increase in frequency in North America, resulting in a significant

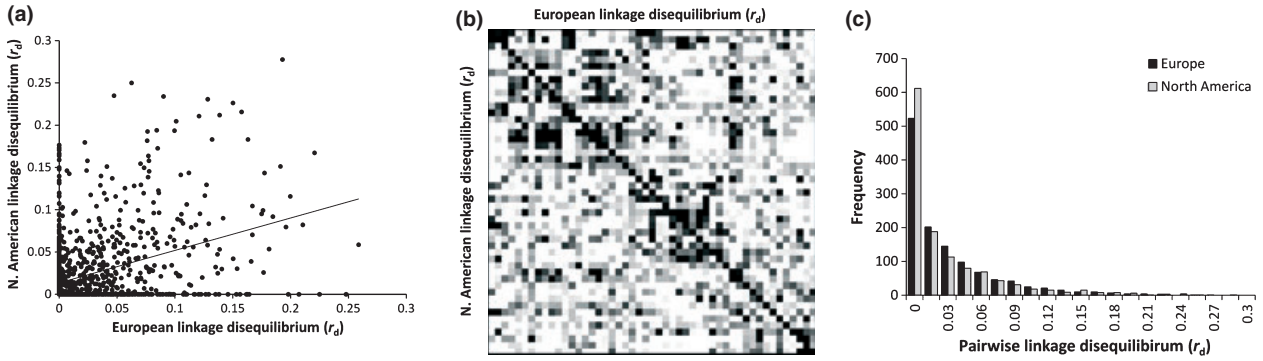

Fig. 2 Partial dissolution of nuclear and cytonuclear associations during invasion of North America. (a) Mantel's test of correlation across ranges in pairwise linkage disequilibrium among the 50 AFLP loci with the largest F_{ST} (b) Heatmap of pairwise linkage disequilibrium (r_d), with darker colours denoting higher values of r_d. (c) Frequency distribution of pairwise r_d among European and North American genotypes.

Table 2 Hierarchical comparison of North American and European variance–covariance matrices. χ^2 values and associated *P* values are from likelihood ratio tests comparing model fit at a given level in the hierarchy to the next highest model. CPC(*n*) indicates a model where *n* principal components are shared between two matrices, whereas 'full CPC' indicates a model where all the principal components are shared but their eigenvalues differ. Akaike information criterion (AIC) favoured a Full CPC model of shared principal components (highlighted in bold) as the simplest model which best explained the data.

Model hierarchy					
Upper	Lower	AIC	χ2	d.f.	*P*
Equality	Proportionality	2022.4	57.80	1	0.0000
Proportionality	Full CPC	1966.6	840.42	48	0.0000
Full CPC	**CPC (4)**	**1222.2**	**949.55**	**990**	**0.8177**
CPC (4)	CPC (3)	2252.6	19.93	45	0.9996
CPC (3)	CPC (2)	2322.7	47.26	46	0.4211
CPC (2)	CPC (1)	2367.5	25.11	47	0.9963
CPC (1)	Unrelated	2436.4	82.36	48	0.0015
Unrelated	2450.0				

change in cytonuclear disequilibria across continents (χ^2 = 6.60, *P* = 0.010).

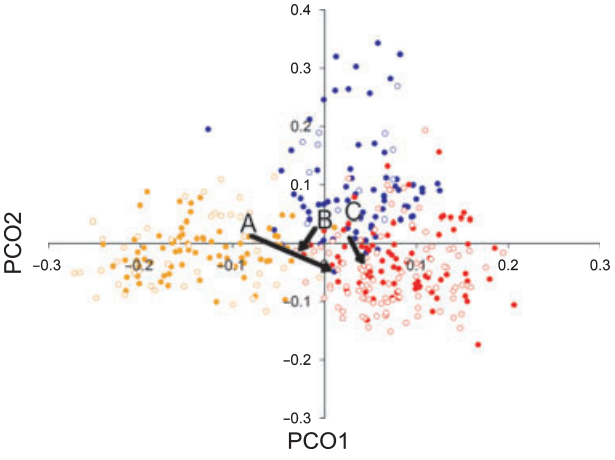

Fig. 3 Change in cytonuclear disequilibria during invasion. Points are individual genotype values from a principal coordinates analysis of all 267 amplified fragment length polymorphisms (AFLP) loci, colour coded according to the three demes identified by Structure (see also Fig. 1). Letters indicate the mean position of European AFLP genotypes associated with each of the three chloroplast clade haplotypes. Arrows show the mean direction of change in AFLP principal coordinate space experienced by each haplotype following invasion of North America.

Fitness consequences of admixture

Based on 2 years of fruit production in common garden experiments, genotypes from Europe and North America were significantly different in the effect that admixture had on fitness (*P* < 0.05; Table 3). When analysed separately by continent of origin, there was a highly significant relationship between admixture and fitness among North American genotypes (β = 1.35, R^2 = 0.0647, *P* < 0.01), but not among European genotypes (β = −0.01, R^2 = 0.0009, *P* > 0.5) (Table 3, Fig. 4a). The magnitude of the fitness increase among admixed North American plants was substantial: families with admixture scores above the median value had more than a two-fold boost in fitness (105%) compared to those with admixture scores below the median (Fig. 4b). European genotypes with admixture above the median showed a more modest 56% increase in fitness, but this difference was not statistically significant (Table 3).

Discussion

Admixture arising from multiple introductions from genetically diverse sources is hypothesized to be a primary driver behind evolution during biological invasions. Here, we have shown that the post-glacial expansion and contemporary invasion of a widespread weed created multiple episodes of admixture between

Table 3 Analysis of covariance for fruit production among *Silene vulgaris* families collected from Europe and North America and grown in two common gardens.

	Source	d.f. (num, den)	*F*-test	*P*
Pooled across continents	Site	1, 314	219.25	< 0.0001
	Continent of origin	1, 314	0.14	0.7112
	Admixture index	1, 314	4.70	0.0308
	Continent × admixture index	1, 314	4.86	0.0282
By continent				
Europe	Site	1, 151	97.85	< 0.0001
	Admixture index	1, 151	0.00	0.9806
North America	Site	1, 162	102.25	< 0.0001
	Admixture index	1, 162	9.01	0.0031

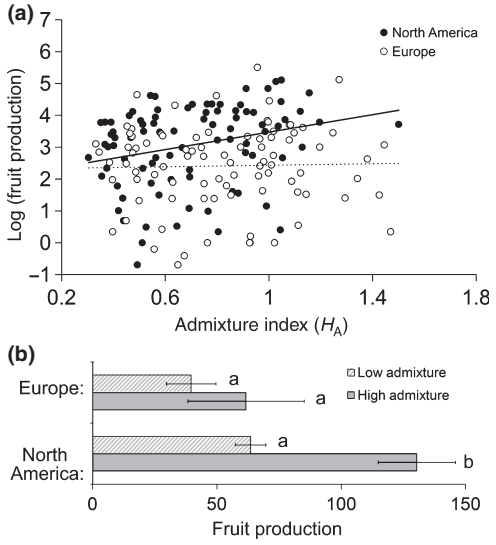

Fig. 4 Relationship between admixture among nuclear amplified fragment length polymorphisms (AFLP) demes and fitness through fruit production. (a) Analysis of covariance, testing heterogeneity among ranges for the relationship between admixture and fitness. Admixture index is plotted with an arcsine-square root transformation. Regression lines show significant (solid) and nonsignificant (dotted) relationships between admixture and log-transformed fruit production among North American and European families, respectively. The partial R^2 values (analysis of residuals after removing the effects of site) are 0.06470 for North America and 0.00086 for Europe. (b) Mean (±) SEM of fruit production, binned into high and low admixture levels relative to the median value per continent, showing the interaction between continent of origin and admixture. In both plots, fitness was estimated as family means of total fruit production among reproductive plants during the 2005–2006 growing seasons averaged over two common garden sites. Bars sharing letters do not differ following Tukey's *post-hoc* comparisons in an analysis of variance.

genetically distinct demes. Comparisons among these episodes revealed that novel occurrences of admixture are capable of generating major increases in plant fitness; over two-fold greater than fitness in the absence of admixture. Our study offers strong support for the hypothesis that the recombination of diversity arising from multiple introductions can substantially boost the fitness of introduced genotypes, confirming a central prediction from evolutionary biology for why some species become weedy and invasive. In the following paragraphs, we discuss these results in detail and offer potential mechanisms behind the fitness effects of admixture that can be tested with future experiments.

Genetic impacts of range expansion and invasion

Population subdivision, founder effects and admixture can exert strong effects on the magnitude and distribution of genetic diversity across a species' range. During biological invasions, these demographic effects may act to reorganize ancestral patterns of diversity that evolved within the species' native range, leaving distinct genetic signatures as colonists disperse to and establish populations within new geographical regions (Excoffier *et al.*, 2009). In this study, we uncovered significant regional-scale genetic structure in the native European range of the colonizing weed, *Silene vulgaris*. This structure consisted of three divergent demes, whose distributions closely correspond to known pathways of post-glacial expansion into Europe from southern refugia (Dumolin-Lapegue *et al.*, 1997; Taberlet *et al.*, 1998; Hewitt, 2000). The presence of *S. vulgaris* individuals with multiple genetic ancestries in Central Europe suggests an historical episode of admixture occurred among demes. This region of admixture in *Silene* corresponds to a well-known suture zone for many other European species of plants and animals, representing secondary contact between divergent lineages that were isolated in glacial refugia in the Iberian, Italian and Balkan peninsulas (Hewitt, 2000; Widmer & Lexer, 2001; Petit *et al.*, 2003). Thus, admixture of *Silene*'s native range probably arose as a consequence of post-glacial expansion, as demes migrated out of southern refugia and met in Central Europe.

During the invasion of North America, genotypes from all three ancestral demes were introduced, and there was no evidence for a bottleneck of diversity within demes. This likely reflects multiple introductions from Europe and is consistent with the high levels of chloroplast genetic diversity in North America observed in previous studies of *S. vulgaris* (McCauley *et al.*, 2003; Taylor & Keller, 2007). While all three demes were introduced, there was evidence for a substantial change in the relative frequencies of demes across ranges. This skew likely reflects a regional-level founder effect caused by differential immigration of genotypes into North America from separate regions in Europe. While the spatial precision with which we can assign colonists is coarse, a proportionally greater fraction of North American genotypes are descended from Eastern Europe and a much smaller fraction from Southern

Europe. The dramatic reduction in the frequency of genotypes from Southern Europe is perhaps initially surprising, given the similarity in latitude between *S. vulgaris* occurrence in North America and the distribution of the Southern deme in Europe. However, major differences exist between the continental climate of North America and the Gulf Stream mediated climate of Europe, affecting how climate scales with latitude and longitude (Menzel *et al.*, 2005). These climate–latitude differences between continents are evidenced by opposing phenotypic clines in *S. vulgaris* life history traits across ranges, indicating that matching latitudes do not correspond to matching selective environments (Keller *et al.*, 2009).

Although a selectively neutral founder effect may offer the simplest explanation for the shift in deme frequencies between ranges, an alternative hypothesis is that deme frequencies evolved in response to interdemic selection during invasion. Given the large number of introduced colonies that fail to establish during an invasion, there would seem to be ample opportunity for selection to act at the population level (Lewontin, 1965). For example, demes may differ in climate-base niche tolerances such that the extent of suitable sites may favour the establishment and spread of some demes over others. Ecological niche models parameterized separately for each *S. vulgaris* deme in Europe suggest that demes do differ in climate-based niche dimensions but are similar in the size of the area predicted to be suitable for invasion into North America (S. R. Keller and D. R. Taylor; unpublished data). Nevertheless, the hypothesis of interdemic selection during colonization and range expansion is intriguing; the differential proliferation of genetically distinct demes may provide excellent testing grounds for empirically addressing the long-held debate regarding the importance of selection above the level of the individual (Wade & Goodnight, 1998).

Genetic evidence for admixture within the genome

Population genetic studies have revealed that mixing of different lineages following multiple introductions is an important feature of many biological invasions (Frankham, 2005; Wares *et al.*, 2005; Roman & Darling, 2007; Dlugosch & Parker, 2008). The reorganization of genetic variance that can arise from admixture among multiple introductions may in turn drive the evolution of invasiveness (Ellstrand & Schierenbeck, 2000; Rieseberg *et al.*, 2007). Most studies that report admixture during invasion do so at the demographic or population level, as evidenced by a reduction in population structure, an increase in overall alleleic diversity or heterozygosity, or the novel co-occurrence of alleles within introduced populations relative to native range populations (Kolbe *et al.*, 2004; Genton *et al.*, 2005; Taylor & Keller, 2007; Gillis *et al.*, 2009). Fewer studies have documented signals of admixture within the genome of invasive genotypes as a result of recombination (Whitfield *et al.*, 2006; Lavergne & Molofsky, 2007).

Our current results in *Silene* show linkage disequilibrium among AFLP loci was reduced by $\sim 40\%$ following invasion of North America. Some of this reduction in LD could have resulted from the shift in deme frequencies accompanying the founder effect, whereas some could have resulted from recombination among demes following admixture. Because founder effects impact the whole genome and thus have similar effects across loci, this should lead to proportional changes in genetic covariance matrices (Lande, 1980). In contrast, recombination should cause departures from proportionality, as loosely linked associations are eroded quickly by independent assortment and recombination, while associations between loci in closer physical proximity take longer to dissipate (Slatkin, 2008). We found that invasion involved both a reorganization of allelic covariance among nuclear loci and a disruption of associations between the nuclear and chloroplast genomes. Collectively, these results point to the process of invasion reshaping the ancestral genetic associations that accumulated within and between genomes over evolutionary time in the native range.

Fitness effects of admixture and recombination

Large increases in fitness may serve to catapult a species' expansion into a new range, even if the initial fitness effects are transient over longer time periods (Ellstrand & Schierenbeck, 2000; Drake, 2006). Admixture among demes may be viewed as a result of 'intraspecific' hybridization and are thus not different in principle from the forces that cause inter-specific plant hybrids to often become invasive. One key difference may be that in interspecific crosses, heterozygosity is often rendered permanent in crosses between species by allopolyploidy, cytological changes or irreversible transitions to asexual reproduction (Ellstrand & Schierenbeck, 2000; Moody & Les, 2002; Rieseberg *et al.*, 2007).

At the intraspecific level, admixture could affect the fitness of introduced populations, and hence the evolution of invasiveness, in three important ways (Ellstrand & Schierenbeck, 2000; Rieseberg *et al.*, 2007). First, admixture may increase genetic variance within populations and thereby increase the evolutionary potential to respond to novel selection pressures (Kolbe *et al.*, 2004; Lavergne & Molofsky, 2007). Second, recombination between demes may segregate unique phenotypes among progeny that may be favoured by selection in the introduced range (Facon *et al.*, 2008). Third, invasive genotypes that are recent hybrids between demes may exhibit hybrid vigour (i.e. heterosis) because of the masking of genetic load (Moody & Les, 2002; Drake, 2006). These mechanisms generate different predictions for how admixture affects fitness in individuals and populations over time. If admixture acts to increase the evolutionary potential of invading populations, then the

individual effects of admixture may not be immediate, but recombination and selection should increase mean population fitness over time. If admixture acts to generate favourable phenotypic novelties, then recombination should be associated with increases in fitness that are maintained over time, with the mean population fitness increasing with the frequency of novel variants. Finally, if admixture principally results in heterosis because of the masking of genetic load, then the fitness benefits arising from dominance are immediate (i.e. maximal in the F1) but will diminish over generations of random mating with the subsequent loss of between-source heterozygosity (Lynch, 1991; Lynch & Walsh, 1998; Drake, 2006). Of course, these genetic mechanisms are not mutually exclusive and could be operating simultaneously.

The separate episodes of admixture during post-glacial expansion in Europe and contemporary invasion in North America allowed a direct comparison between ranges of how admixture affected plant fitness. We found a significant relationship between admixture and fruit production, but only in North America, where admixture was relatively recent, having occurred during the $\sim$ 200 years since initial introduction. In contrast, no association between admixture and fitness was detected in Europe where admixture is likely much older, probably having occurred during post-range expansion following the end of the last ice age. Given this difference in fitness between recent and historical admixture, one hypothesis is that heterosis is the genetic mechanism increasing fitness in *S. vulgaris*, which predicts that fitness is greatest among early hybrid generations as is commonly observed in many crop systems (Lynch, 1991; Lynch & Walsh, 1998). Experimentally, heterosis has been previously shown with controlled crosses among North American populations of *S. vulgaris*, in which hybrid vigour was evident in F1 hybrids, but these crosses were performed without the knowledge of deme membership (Bailey & McCauley, 2006). Other studies have shown that *S. vulgaris* experiences increased selfing and inbreeding depression within isolated local neighbourhoods (Taylor *et al.*, 1999; Emery & McCauley, 2002; Glaettli & Goudet, 2006), suggesting the potential for heterosis upon outcrossing is high. Future experiments that outcross *S. vulgaris* within and between demes from each continent, and that evaluate fitness in F1 and later generation hybrids, are necessary to test heterosis as a mechanism driving fitness increases during *Silene*'s invasion of North America.

An alternative hypothesis is that admixture facilitates a response to selection, either through increased quantitative genetic variance or the production of novel recombinant phenotypes. It is well known that the rate of adaptation is proportional to the additive genetic variance for fitness (Fisher, 1930), and thus we would expect the mean fitness of admixed populations to increase over time relative to recently admixed or nonadmixed populations. If this were the case, recombinant European genotypes should show higher fitness than North American genotypes as a result of responding to selection over a longer period, yet we observed the opposite effect. It is also possible that genotype-by-environment interactions could have prevented European genotypes from attaining their maximum possible fitness. Establishing common gardens on each continent would control for these effects.

An important next step in evaluating admixture's impact during invasion would be to measure how population vital rates or the probabilities of colonization and extinction vary with the degree of recombination within populations. If the effect of admixture is to increase genotypic fitness by raising offspring production, as was demonstrated here, then we would expect rates of population growth to increase, leading to larger and more productive populations that have lower probabilities of extinction, and perhaps increased capacity to establish daughter colonies in an ongoing range expansion (Lewontin, 1965; Wade & Goodnight, 1998; Richards *et al.*, 2003; Drake, 2006). Such feedbacks between evolutionary and ecological dynamics should be expected during invasions, when genetic diversity has ample opportunity to become re-organized and in ways that affect individual-level and mean population fitness (Facon *et al.*, 2006).

Although the fitness effects of admixture are profound, hybrid vigour cannot be solely responsible for the evolution of invasiveness in *S. vulgaris*. After holding the effects of admixture constant, North American plants still had significantly higher mean fruit production (likelihood ratio test of model with and without effects of continent and continent*admixture interaction: $\chi^2 = 28.8$, $P < 0.0001$), suggesting an additional role of adaptation stimulating the invasiveness of *S. vulgaris* in North America. Thus, a major focus for the future should be on parsing out the relative contributions of different evolutionary forces (e.g. admixture, founder effects, natural selection) to the establishment, proliferation and long-term persistence of invasive species.

Conclusions

The reorganization of genetic variance during biological invasion has been hypothesized to create large increases in the fitness of introduced genotypes. We used a combination of population genetic analyses and common garden field experiments to assess the extent of admixture and its effects on fitness in the invasive weed, *Silene vulgaris*. Using nuclear and chloroplast DNA markers, we documented genetic divergence among demes within the native range, and how the post-glacial history of expansion influenced contemporary patterns of genetic structure within and between genomes. During contemporary invasion of North America, admixture and recombination among demes contributed to the dissolution of allelic disequilibria within the introduced range. Finally, we showed that admixed genotypes exhibited dramatic

increases in fitness during recent invasion, but not after historical expansion in the native range, consistent with a mechanism of transient hybrid vigour via heterosis. This work provides some of the best empirical support that admixture during biological invasion can substantially boost the fitness of introduced genotypes, potentially leading to the rapid emergence of invasiveness.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Chloroplast diversity assay.

Figure S2 Structure output from Bayesian clustering of AFLP genotypes.

Figure S3 Chloroplast clade distribution.

Table S1 Bayesian most probable estimates of gene diversity (95% credible interval) for North American and European *Silene vulgaris* genotypes descended from each ancestral deme.

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Table S1. Bayesian most probable estimates of gene diversity (95% credible interval) for North American and European *Silene vulgaris* genotypes descended from each ancestral deme. Estimates are from a Hickory model run for 50,000 burn-in iterations followed by 500,000 sampling iterations.

Deme	Europe	North America
Eastern	0.217 (0.196 – 0.242)	0.215 (0.195 – 0.241)
Southern	0.253 (0.227 – 0.282)	0.239 (0.217 – 0.262)
Western	0.220 (0.202 – 0.243)	0.216 (0.198 – 0.236)
Mean	0.230 (0.209 – 0.255)	0.223 (0.204 – 0.244)

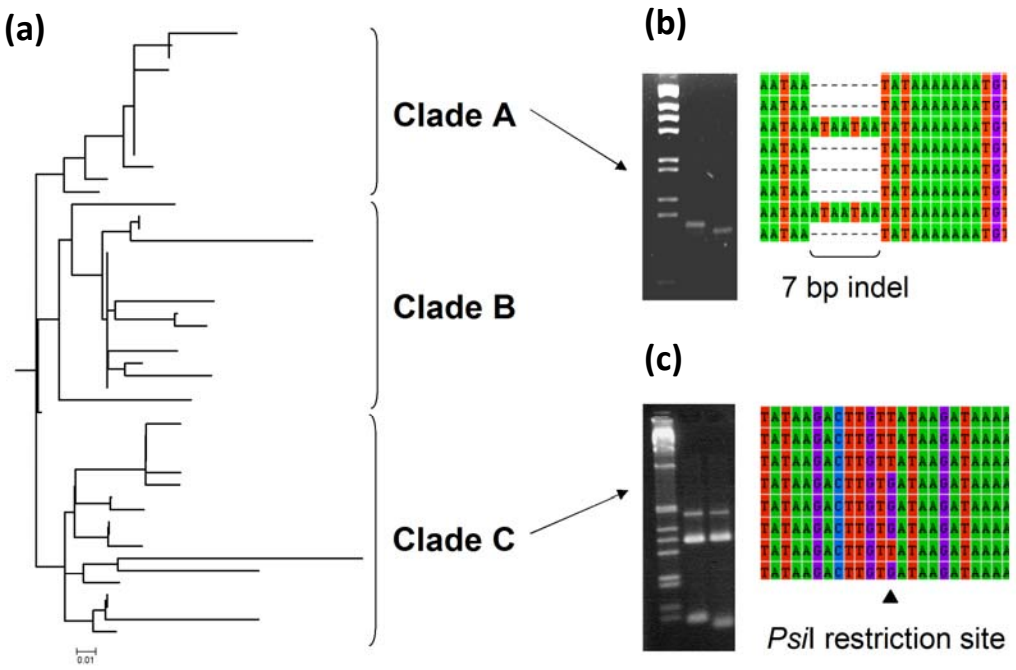

Fig. S1. Chloroplast diversity assay. (a) Neighbor-joining tree of *Silene vulgaris* based on ~1.8 kb of cpDNA, showing three main clades. The tree is rooted with *Silene latifolia* as an outgroup (not shown). (b) and (c) Partial alignments of the *trn*L-*trn*F regions and sample gel results illustrating the assay used to assign cpDNA clade membership of individuals. Clade B was assigned by process of exclusion.

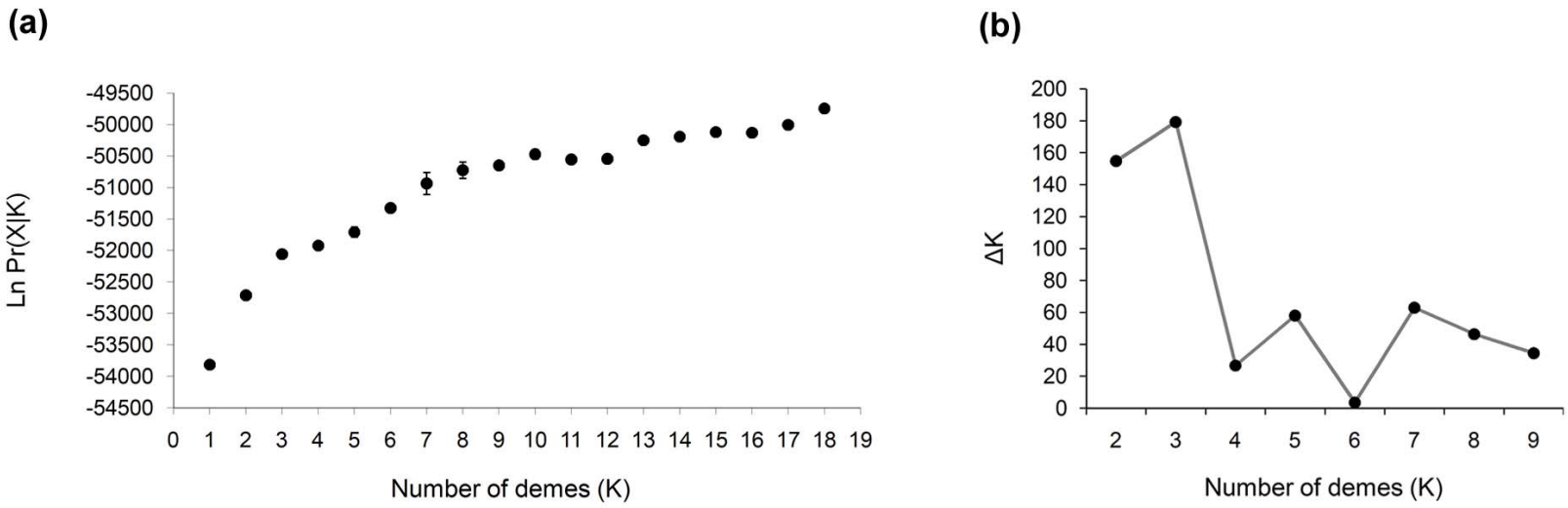

Fig. S2. STRUCTURE output from Bayesian clustering of AFLP genotypes. (a) Ln Pr(X|K) averaged over 10 replicate runs at each value of K = 1-10 or a single replicate at K > 10. Error bars are SEM's over replicates ($K \leq 10$), and are plotted but not visible when the values were very small. (b) ΔK model selection criteria of Evanno et al. (2005), showing support for K = 3 demes.

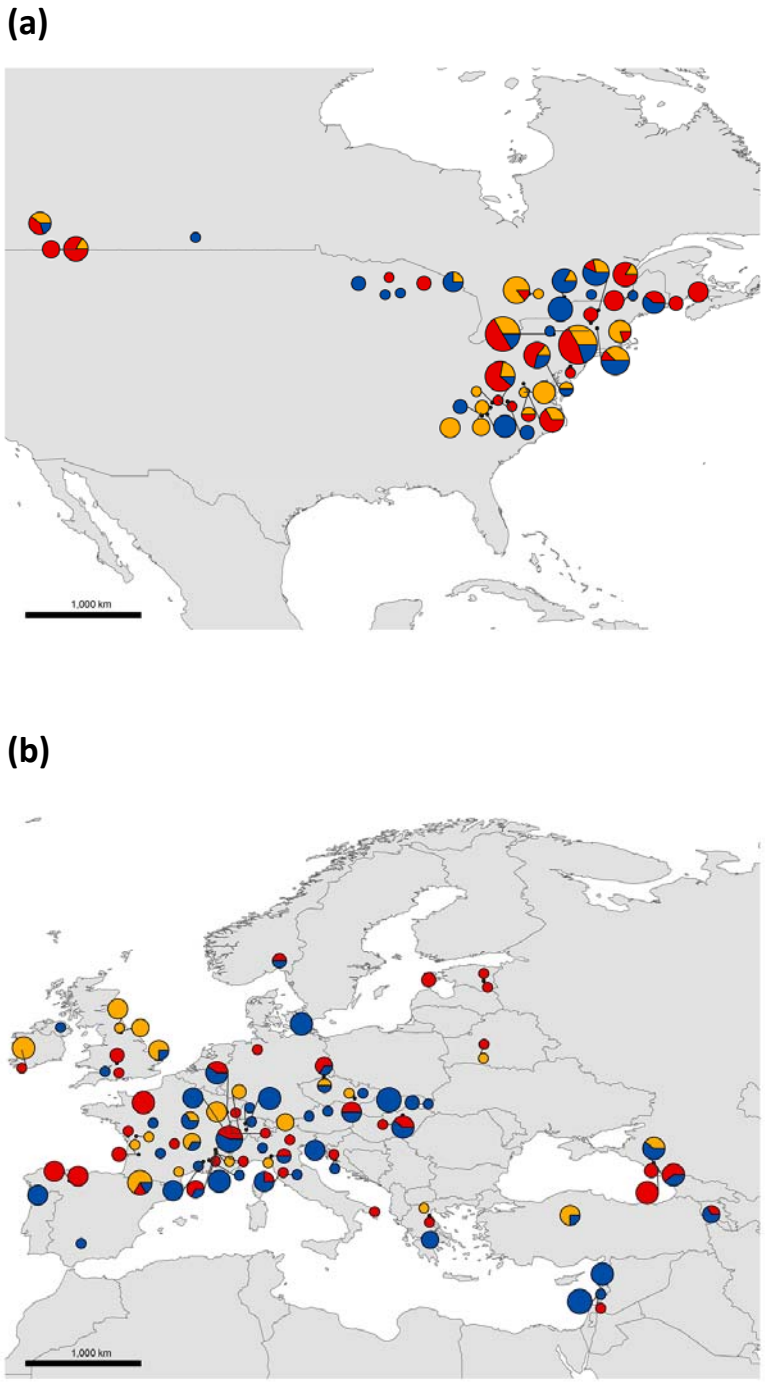

Fig. S3. Chloroplast clade distribution. Maps showing the distribution of chloroplast clades A (orange), B (red), and C (blue) in North America (a) and Europe (b). Each circle represents a collecting site, with size proportional to the number of individuals haplotyped. Overlapping locations have been offset slightly for clarity.