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HISTORICAL RANGE EXPANSION DETERMINES THE PHYLOGENETIC DIVERSITY INTRODUCED DURING CONTEMPORARY SPECIES INVASION

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For a species rapidly expanding its geographic range, such as during biological invasion, most alleles in the introduced range will have their evolutionary origins in the native range. Yet, the way in which historical processes occurring over evolutionary time in the native range contribute to the diversity sampled during contemporary invasion is largely unknown. We used chloroplast DNA (cpDNA) gene genealogies and coalescent methods to study two congeneric plants, *Silene latifolia* and *S. vulgaris*. We examined how phylogenetic diversity was shaped by demographic growth and historical range expansions in the native European range, and how this history affected the diversity sampled during their recent invasion of North America. Genealogies from both species depart from neutrality, likely as a result of demographic expansion in the ancestral range, the timing of which corresponds to shortly after each species originated. However, the species differ in the spatial distribution of cpDNA lineages across the native range. *Silene latifolia* shows a highly significant phylogeographic structure that most likely reflects different avenues of the post-glacial expansion into northern Europe from Mediterranean refugia. By contrast, cpDNA lineages in *S. vulgaris* have been widely scattered across Europe during, or since, the most recent post-glacial expansion. These different evolutionary histories resulted in dramatic differences in how phylogenetic diversity was sampled during invasion of North America. In *S. latifolia*, relatively few, discrete invasion events from a structured native range resulted in a rather severe genetic bottleneck, but also opportunities for admixture among previously isolated lineages. In *S. vulgaris*, lack of genetic structure was accompanied by more representative sampling of phylogenetic diversity during invasion, and reduced potential for admixture. Our results provide clear insights into how historical processes may feed forward to influence the phylogenetic diversity of species invading new geographic ranges.

KEY WORDS: **Chloroplast DNA, coalescent, invasion, mismatch distribution, phylogeography, range expansion, *Silene*.**

The dynamics of a species' geographic range is a central, yet understudied, problem in evolutionary biology. Phylogeography has provided many important insights into geographic range expansions because the pattern of colonization often leaves enduring signatures in the genome (Hewitt 2000; Petit et al. 2003). The evolutionary history of a species prior to expansion plays a central role in deciphering the phylogeography of subsequent range changes because it is precisely this history that determines the distribution of phylogenetic diversity from which colonists are drawn. Chance events during colonization then interact with prior evolutionary history to affect the amount and organization of phylogenetic diversity across a species' range.

Invasive species, defined as those that permanently establish and spread into ecosystems in which they were previously

absent (Kolar and Lodge 2001; Lee 2002), provide interesting natural experiments for investigating how history and chance affect phylogenetic diversity during range expansions. Indeed molecular methods have proven indispensable for estimating the genetic diversity of inocula (Amsellem et al. 2000; Tsutsui et al. 2000; Gaskin et al. 2005), identifying the source of invading propagules (de la Vega et al. 1991; Novak and Mack 2001; Saltonstall 2002), and detecting when lineages that were geographically separate in the native range have come into contact within the area of introduction—so-called admixture (Guinand and Easteal 1996; Gaskin and Schaal 2002; Kolbe et al. 2004).

However, although the genetics of contemporary invasions are often well characterized, the alleles present in invaders usually originate during, and are affected by, processes that occur over evolutionary time in the native range (Davies et al. 2000). For example, many species have experienced periods of rapid demographic increases and range expansions during the warm interglacial periods of the Pleistocene (Hewitt 1996, 2000; Taberlet et al. 1998). We use the term "expansion" in this context instead of "invasion" to denote range changes that took place over evolutionary time without human intervention. These historical expansions can have lasting genetic effects (Tajima 1989; Rogers and Harpending 1992; Ray et al. 2003). It is important to recognize, therefore, that the genetic consequences of modern invasions may be affected as much by historical processes that have occurred in the sources from which invasions are drawn, as they are by contemporary processes that influence the number and diversity of invasive propagules (Travisano et al. 1995; Schaal et al. 2003).

The phylogeographic history of a species in its native range determines the distribution of diversity available for invasion to sample. The number of individuals and sources introduced (i.e., propagule pressure, Kolar and Lodge 2001) then determines whether invasion captures this diversity representatively. For example, low propagule pressure would likely sample within a phylogeographic region, producing low diversity inocula. Higher propagule pressure increases the likelihood of sampling across phylogeographic regions, creating opportunities for admixture in the introduced range. Alternatively, invasive propagules originating from an unstructured native range are more likely to be a representative sample of the diversity present, and will be less sensitive to the magnitude of propagule pressure. Thus, to understand factors affecting genetic diversity during invasion, we need to understand the evolutionary history of the distribution from which it is drawn. Otherwise, it will be unclear *why* genetic diversity becomes bottlenecked (or elevated), or if admixture is occurring, whether it is among lineages with an ancient or a more recent history of isolation. Answers to these questions provide a more detailed view of the invasion process and reveal what implications the sample of diversity has for future evolutionary change.

Gene genealogies are a rich source of information into a species' evolutionary past that could be put to broader use for the study of invasions (Schaal et al. 2003). First, genealogies provide historically ordered alleles, which permit the evolutionary relationships among invasive and non-invasive lineages to be deduced. Second, applying coalescent theory to the distribution of mutations in gene genealogies reveals much about a species' demographic history (Tajima 1989; Hudson 1990; Griffiths and Tavaré 1994; Schaal and Olsen 2000). In fact, some of the most significant insights into the genetics of invasions have been achieved by applying coalescent theory to studies of human migration history. Humans are an invasive species with a recent evolutionary history of colonization, range expansion, and rapid demographic growth (Cann et al. 1987; Rogers 1995; Watson et al. 1997; Thompson et al. 2000; Alonso and Armour 2001). These are many of the same phenomena population geneticists are studying during contemporary invasions by nonhuman species. Yet an evolutionary history of range expansion and population growth has never been explicitly connected to the diversity sampled during invasion.

In this paper, we use gene genealogies to study how evolutionary history affects the genetics of contemporary invasion in two related plant species, *Silene latifolia* and *S. vulgaris*. Both species are weeds of agriculture and disturbed land that have widespread native ranges in Eurasia and have recently invaded North America. Previous studies using morphology and flavonoid genes (Mastenbroek et al. 1983), RAPDs (Vellekoop et al. 1996), and Y-chromosome sequence data (Ironside and Filatov 2005) support a major genetic subdivision between east-central and western Europe in *S. latifolia.* In *S. vulgaris*, population genetic analyses show high levels of cytoplasmic diversity among populations in east–central Europe (Storchova and Olson 2004) and among North American populations (McCauley et al. 2003). We use genealogical data from throughout the native and introduced ranges of both species to show how evolutionary history in the native range has affected phylogenetic diversity introduced during invasion. We show that despite having similar ecologies and histories of demographic expansions in their native ranges, these two species show markedly different patterns of diversity sampled during invasion of North America. We also show that by taking a coalescent-based approach to the problem, we can begin to unravel some of the manifold complexities of species invading new geographic ranges.

Materials and Methods

STUDY SPECIES

Silene latifolia Poiret (*S. alba*) and *Silene vulgaris* (Moench) Garcke are short-lived herbaceous perennials with a history of human association. These species are commonly found in disturbed areas such as roadsides, railroad embankments, cultivated fields, and abandoned lots (Baker 1948; Marsden-Jones and Turrill 1957).

Although both species have similar ecological and life history characteristics, they differ in their breeding system: *S. latifolia* is dioecious (separate male and female individuals), whereas *S. vulgaris* is gynodioecious (populations are a mixture of female and hermaphrodite individuals).

Silene latifolia and *S. vulgaris* have widespread geographic ranges throughout their native Eurasia. Their ranges broadly overlap, extending from the Atlantic coast (Spain, France, United Kingdom, Ireland) across continental Europe and becoming diffuse in Russia. From the south, the distributions extend from North Africa (Morocco to Egypt) and the Middle East northward to Scandinavia (Atlas Florae Europaeae: Jalas and Suominen 1986). Both are believed to have originated in the Middle East or Mediterranean region, and have since colonized most of Europe, possibly with the spread of agriculture (Baker 1948; Marsden-Jones and Turrill 1957; Mastenbroek et al. 1983; Vellekoop et al. 1996; Runyeon and Prentice 1997). Similarly, both species appear to have been introduced to North America during the late eighteenth or early nineteenth centuries (Cutler 1785; Pursh 1814; McNeill 1977), likely as a contaminant of clover seed or in ship ballast (Martindale 1876). Each invasion began along the eastern seaboard and spread rapidly south and west (Antonovics et al. 2003; Keller et al., unpublished ms). *Silene latifolia* and *S. vulgaris* are designated as invasive by the USDA (http://plants.usda.gov), and considered to be agricultural pests in Canada (Agriculture and Agri-Food Canada).

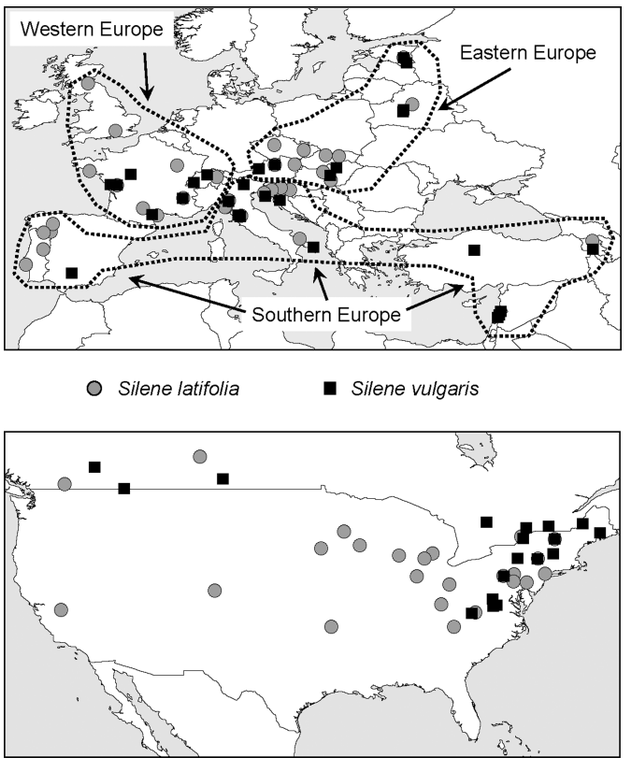

Figure 1. Map of collection sites in North America and Europe. Dotted lines show the phylogeographic groupings tested in AMOVA.

DNA SEQUENCING

Samples were collected as seeds or leaf tissue dried on silica gel from across the geographic ranges of the species in Europe and North America (Fig. 1). Genomic DNA was isolated from one haphazardly chosen plant per site using DNeasy plant miniprep kits (Qiagen, Valencia, CA).

To address the phylogenetics of expansion and invasion, we applied predictions from coalescent theory to gene genealogies based on chloroplast DNA (cpDNA) sequence data. cpDNA sequence data are particularly suited to phylogeographic studies in plants because they (1) provide ordered alleles for inferring ancestor-descendent relationships (Schaal et al. 2003), (2) are essentially asexual, permitting the effects of population history to be inferred from the genealogy without the complicating effects of recombination, (3) are highly polymorphic in these species (McCauley 1994; Ingvärsson et al. 2003; McCauley et al. 2003), and (4) are uniparentally inherited and dispersed through seeds, the same propagules we trace when reconstructing the invasion process. Four cpDNA regions consisting of one intron and three intergenic spacers were PCR amplified and sequenced, following previously reported methods (Ingvärsson and Taylor 2002; Ingvärsson et al. 2003). All sequences for these four cpDNA regions from both species have been deposited in the Genbank database (accession numbers available as supplementary material online). Sequence alignments were made using the default options in CLUSTALW embedded within MEGA 3.1 (Kumar et al. 2004). Alignments were manually adjusted to minimize the number of distinct gaps caused by insertion/deletion events (indels) and nucleotide polymorphisms in the vicinity of the indels. Data from all four regions were concatenated for a total length of about 1800 bp (1876 bp in *S. latifolia* and 1799 bp in *S. vulgaris*), including indels. These regions were sequenced in a total of 62 *S. latifolia* and 50 *S. vulgaris* individuals.

Indels represented a large portion of the variation in these 4 cpDNA regions. Indels located in introns and intergenic spacers of cpDNA are known to possess useful phylogenetic information, and significantly increase the resolution of phylogenies at the intraspecific level for several taxa (Gielly and Taberlet 1994; Hamilton et al. 2003), including *Silene* (Ingvärsson et al. 2003). Length variation in the chloroplast genomes of *S. latifolia* and *S. vulgaris* includes single and di-nucleotide repeat motifs, as well as nonrepetitive indels. Repeat indels evolve at a faster rate than nonrepetitive indels or nucleotides, and are more prone to homoplasy (Ingvärsson et al. 2003). In contrast, nonrepetitive indels are thought to have similar substitution rates as nucleotides, and

nearly identical levels of homoplasy. Therefore, repeat indels were excluded from analysis, whereas binary (0/1) coding was used for nonrepetitive indels and nucleotide substitutions. Indels were coded as a single binary locus, regardless of length (Ingvärsson et al. 2003). When more than one length variant was nested completely within another, this was conservatively regarded as a single locus, with the full-length gap coded as either present or absent.

ANALYTICAL METHODS

Haplotype networks were constructed using 95% statistical parsimony (Templeton et al. 1992) implemented in the software TCS 1.21 (Clement et al. 2000). Phylogeographic structure in the native range was investigated by dividing Europe into three geographical regions (eastern, western, and southern; Fig. 1). The boundaries for these hypothesized genetic subdivisions were defined based on previous studies of *S. latifolia* in Europe, and comparative phylogeographic patterns in Europe among other species of plants and animals. The division between eastern and western Europe was based on evidence from *S. latifolia* that points to a genetic break beginning north of the Austrian Alps and extending northward through Germany to the North Sea (Mastenbroek et al.1983; Vellekoop et al. 1996; Ironside and Filatov 2005). This area also corresponds to a phylogeographic suture zone observed in studies of the post-glacial expansion of other European species (reviewed in Taberlet et al. 1998; Hewitt 2000). The boundary for southern Europe was defined by physiographic features that mark putative glacial refugia along the Mediterranean Sea (Iberia south of the Pyrenees, Italy south of the Alps, and the Balkan Peninsula). Phylogeographic structure was tested using analysis of molecular variance (AMOVA) with the software ARLEQUIN 2.001 (Schneider et al. 2000).

Several haplotypes in each species were recovered only in North America. Given the age of the invasion (ca. 200 years old), these haplotypes almost certainly originated in Europe and thus should be included when estimating the evolutionary history of each species in its native range. However, analyses based on coalescent theory make use of both the relatedness of haplotypes as well as their frequencies in the sample. Nonneutral processes such as rapid demographic growth and selection cause deviations from the neutral model by inflating the frequency of some haplotypes over others. In the current context, if we included all North American samples (with frequencies influenced by the demographics of the recent invasion), our analyses of long-term evolutionary history would likely be biased by the impact of invasion on the frequency distribution of mutations among lineages. To resolve this problem, we included a single sample of each unique North American haplotype in all coalescent simulations. We regard this approach as conservative in the current analyses, as the effect will be to include ancestral diversity known to be present in the genealogy while assigning a low frequency to haplotypes that are not regionally abundant in Europe (as inferred by our sampling).

We tested for the signature of historical demographic expansion in the shapes of the genealogies by calculating Tajima's D and Fu's F_S statistics (Tajima 1989; Fu 1997). Negative values of these statistics indicate an excess of young or rare alleles in the genealogy—evidence of either population expansion or genetic hitchhiking. Calculations were performed in ARLEQUIN and used 1000 simulations to evaluate significance.

To explore the genealogical history of *Silene* in Europe, we performed coalescent simulations in GENETREE 9.0 (Griffiths and Tavaré 1994; http://www.stats.ox.ac.uk/∼griff/software.html). For each species, we used the other species as an outgroup to manually reconstruct the ancestral state at each site. Because GENETREE assumes the data fit the infinite sites model of evolution (each site may mutate only once), sites that violated this assumption were removed prior to analysis.

We used GENETREE to estimate the scaled mutation parameter, θ ($= 2N_e\mu$ for haploids, where N_e is the effective size of the cpDNA population and μ is the mutation rate per sequence per generation). We searched the likelihood surface using 100,000 simulations for each of many values of θ and took the maximum likelihood value as our estimate of θ. To estimate μ, we obtained the silent substitution rate for chloroplasts ($1.1–2.9 \times 10^{-9}$ nucleotide substitutions per site per year; Wolfe et al. 1987). However, this mutation rate does not include indel evolution. We formulated a regression model based on our cpDNA dataset to estimate the rate of indel evolution from the nucleotide evolution observed. To accomplish this, statistical parsimony networks were built for each species using only the nucleotide polymorphisms. We then mapped the indels onto each network and obtained the number of nucleotide and indel changes for each connection between extant haplotypes. Linear regression was used to predict the number of indel changes, given the number of nucleotide changes, and yielded the equation: number indels $= 0.711 + 0.748 \times$ (no. nucleotides). The 95% confidence interval for the slope (0.410–1.09) was significantly greater than zero but not different than one, confirming previous observations that the two types of mutations occur at comparable rates (Ingvärsson et al. 2003). We applied this equation to the range of values for the cpDNA silent substitution rate (K_S) reported by Wolfe et al. (1987) to obtain the combined substitution rate for indels and nucleotides. From the resulting set of corrected estimates, we took the median value of $\mu = 2.8 \times 10^{-9}$ substitutions (nucleotides and indels) per site per year (range = 2.0–5.1). Although uncertainty in μ exists due to a limited fossil record in plants (Wolfe et al. 1987), conclusions from our analyses were not qualitatively different when using the upper or lower range of estimates (data not shown). We adjusted μ to a per-sequence per-generation value by multiplying by the number of bases in the concatenated dataset and by the generation

time (taken to be ~two years, based on life history data from field experiments). Using the relationship $\theta = 2N_e\,\mu$, we then solved for species-wide chloroplast N_e.

Population growth can have a pronounced effect on θ; therefore, we used GENETREE to fit a population growth parameter (β) to the data. We explored the joint likelihood surface of θ and β by running 100,000 replicate simulations for each of many values of θ and β and taking the joint maximum likelihood values as our estimators (e.g., Thompson et al. 2000). We tested if population growth significantly improved the model using a likelihood ratio test (Neter et al. 1996). This test competes two nested models using the statistic $\Delta = -2\log_e(L_1/L_2)$, where L_1 is the likelihood of the model with β and L_2 is the likelihood of the model without β. Δ is approximately X^2 distributed with one degree of freedom.

We then simulated the coalescent for 1,000,000 replicates using the ML estimates of θ and β. From these we obtained the time to the most recent common ancestor (T_{MRCA}) and the ages of individual mutations (in coalescent units, T). For haploids, time is measured in N_eTg years, where g is the generation time.

We explored the distribution of relatedness among haplotypes in North America and Europe by generating the mismatch distribution (the frequency of pairwise differences among individuals). Mismatch distributions are sensitive to demographic events in the evolutionary history of the sample, and thus are useful indicators of past population expansion (Rogers and Harpending 1992; Rogers 1995; Schneider and Excoffier 1999). Specifically, rapid demographic growth leads to a smooth, unimodal mismatch distribution, whose moments can be used to estimate the timing of the expansion, $\tau = 1/2\mu$ generations (Rogers 1995). We generated the mismatch distribution in *Silene* among European haplotypes and fit it to a model of sudden demographic expansion using ARLEQUIN. We also included unique North American haplotypes, as above. We converted our estimate of τ to years using our estimate of μ (obtained above) and multiplying by 2 (number of years per generation).

Mismatch distributions can also reveal recent admixture among divergent lineages. If admixture is occurring, we expect bimodal or multimodal frequency distributions of pairwise differences, attributable to a few differences among haplotypes within clades, and greater differences among haplotypes between clades. We tested for admixture in the *Silene* invasions by calculating the mismatch distribution among all North American haplotypes.

To investigate the cpDNA diversity sampled during invasion, we calculated the total number of haplotypes, private haplotypes, segregating sites, average number of pairwise differences, and gene diversity for North America, Europe, and both continents pooled using ARLEQUIN. We determined whether the diversity sampled during invasion was a representative sample from the genealogy of each species by measuring phylogenetic diversity (PD), equivalent to the sum of the branch lengths represented in invasive haplotypes (Crozier 1997; Purvis et al. 2000). We compared the observed PD to a null expectation by constructing a neighbor-joining tree in PAUP* (Swofford 1998) and calculating the observed PD of invasive haplotypes using the software MESA (http://www.agapow.net/software/mesa/releases/1.9.22). Keeping that tree topology, we then randomly sampled individuals with replacement from the tree (keeping the proportion of "invasive" haplotypes constant) and recalculated PD for each pseudosample. This was repeated 1000 times to generate a null distribution against which the observed value of PD among invasive haplotypes was compared.

Results

HAPLOTYPE NETWORKS AND POPULATION STRUCTURE

From the 62 *Silene latifolia* and the 50 *S. vulgaris* individuals sampled, we identified 38 and 33 cpDNA haplotypes, respectively. Each species had 54 segregating sites that defined the genealogical relationships among the haplotypes (Fig. 2). The networks of both species contained a large number of unique haplotypes, with a few common haplotypes, and possessed high species-wide gene diversity (Table 1).

The spatial distribution of *S. latifolia* haplotypes showed a clear phylogeographic structure in Europe (Fig. 2). Haplotypes from eastern and western Europe formed divergent groups descended from haplotypes currently distributed in southern Europe. AMOVA showed this structuring among European regions is highly significant ($\Phi_{ST} = 0.270$, $P < 0.0001$; Table 2). Invasive haplotypes originated from both eastern and western Europe, suggesting that North America experienced multiple introductions from each of these two phylogeographic regions.

By contrast, *S. vulgaris* showed no clustering of haplotypes with region ($\Phi_{ST} = 0.016$, $P = 0.27$; Table 2). Haplotypes introduced to North America were also widely dispersed in the genealogy, suggesting either multiple introductions or a single introduction from a diverse source (Fig. 2).

EVOLUTIONARY HISTORY AND POST-GLACIAL EXPANSION IN EUROPE

Despite the clear difference in the degree of phylogeographic structure, *S. latifolia* and *S. vulgaris* had remarkably similar demographic histories in Europe. Coalescent simulations (assuming demographic equilibrium) returned values of the scaled mutation parameter, θ, in close agreement for the two species (Table 3). Incorporating population growth (β) into the simulations significantly improved the model fit for both *S. latifolia* ($\Delta = 12.70$, $P = 0.0007$) and *S. vulgaris* ($\Delta = 13.64$, $P = 0.0004$). From these models, the effective size of the cpDNA population for both

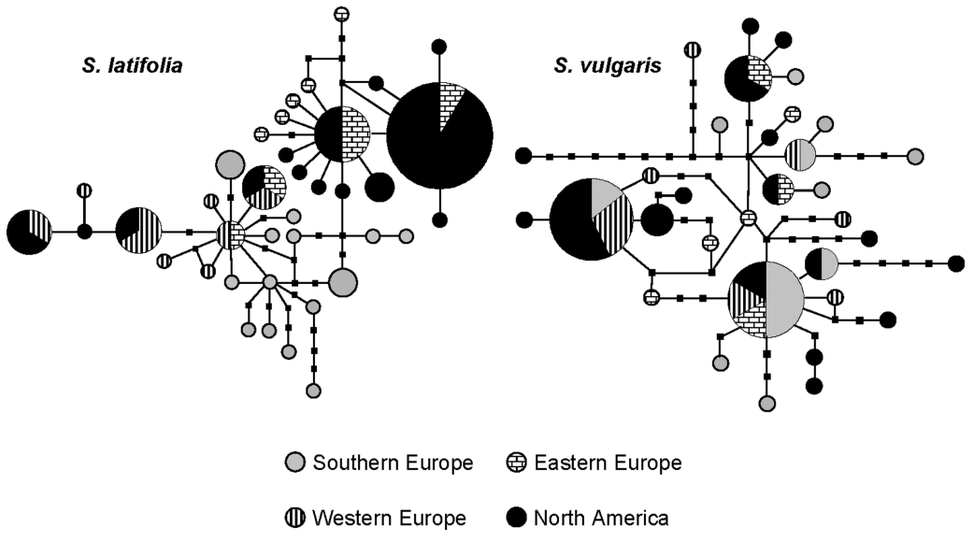

Figure 2. Statistical parsimony haplotype networks. Circles are sampled haplotypes (size proportional to frequency) and small filled squares are inferred haplotypes not recovered in the sample. Each link between haplotypes, regardless of length, represents one mutational step.

species was estimated to be ~2.5 million individuals (Table 3). The maximum likelihood estimate of T_{MRCA} (mean ± SD) was $T = 0.165 \pm 0.013$ for *S. latifolia* and $T = 0.171 \pm 0.019$ for *S. vulgaris*, placing coalescence to the common ancestor of extant haplotypes over 800 kya (thousand years ago) for both species (Table 3).

Both species showed an excess of mutations that were either rare or recently derived, indicated by significantly negative values of Tajima's *D* and Fu's F_S, suggesting either a population expansion or a selective sweep (Table 4). Pairwise differences among European haplotypes showed a unimodal mismatch distribution that provided a good fit to a model of sudden demographic growth (*S. latifolia*: $P = 0.41$; *S. vulgaris*: $P = 0.86$), further supporting a history of population expansion (Fig. 3). The dates of onset for these demographic increases were similar for each species, estimated to be 521 kya for *S. latifolia* and 604 kya for *S. vulgaris* (Table 4).

Several mutations in the *S. latifolia* genealogy defined the phylogeographic structure observed with AMOVA, and hence likely reflect important historical migration events (Fig. 4). The divergence of one group from an ancestral southern European gene pool occurred 725 ± 127 kya. The haplotypes that colonized western Europe represent a subset of this diversity. A second group in southern Europe diverged from the ancestral pool 483 ± 61 kya. This group then gave rise to a subset of haplotypes that originated 330 ± 64 kya that went on to colonize eastern Europe. Thus the origin of the phylogeographic subdivision between eastern and western Europe likely occurred > 400,000 years ago.

GENETICS OF INVASION INTO NORTH AMERICA

Silene latifolia in North America has undergone a substantial bottleneck of diversity, with fewer segregating sites, lower pairwise differences, and lower gene diversity compared to Europe (Table 1). Observed PD of North American haplotypes was only 51% of the diversity expected if the invasion were drawn randomly from the genealogy ($P < 0.001$; Fig. 5). This bottleneck may result from either a founder effect during the colonization of North America by a set of genetically similar individuals, or

Table 1. Estimates of cpDNA genetic diversity in Europe, in North America, and overall for *Silene latifolia* and *S. vulgaris*.

	S. latifolia			*S. vulgaris*		
	Europe	North America	Total	Europe	North America	Total
No. samples	36	26	62	28	22	50
No. haplotypes (# private)	29 (24)	14 (9)	38	21 (16)	17 (12)	33
Segregating sites	48	17	54	34	36	54
Mean pairwise differences (SD)	5.14 (2.55)	3.35 (1.77)	4.90 (2.42)	4.87 (2.45)	6.56 (3.22)	5.64 (2.75)
Gene diversity (SD)	0.990 (0.009)	0.835 (0.070)	0.956 (0.018)	0.963 (0.024)	0.965 (0.028)	0.965 (0.015)

Table 2. Analysis of molecular variance (AMOVA) among eastern, western, and southern European cpDNA haplotypes for *Silene latifolia* and *S. vulgaris*. Geographic regions are defined in Figure 1.

Species	Source of variation	df	Sums of squares	Variance component	Percentage of variation
S. latifolia	Among regions	2	21.397	0.76650	27.03***
	Within regions	32	66.232	2.06975	72.97
	Total	34	87.629		
S. vulgaris	Among regions	2	5.500	0.03809	1.56^{ns}
	Within regions	25	60.214	2.40857	98.44
	Total	27	65.714	2.44666	

***$P < 0.0001$; ns, not significant.

from demographic events that occurred postcolonization that reduced the diversity initially introduced. Although an overall bottleneck in diversity is evident, there has also been an admixture caused by multiple introductions from eastern and western Europe. The signature of admixture is evident in the bimodal mismatch distribution for North America, which shows invasion by haplotypes originating from two highly differentiated sources (Fig. 3).

The situation for *S. vulgaris* was quite different. *Silene vulgaris* in North America had slightly more segregating sites and higher pairwise differences, and equivalent gene diversity compared to Europe (Table 1). North America also contained an amount of PD comparable to random sampling from the genealogy ($P = 0.51$; Fig. 5). The mismatch distribution of North American *S. vulgaris* was unimodal, although the overall shape of the distribution was more ragged than for Europe (Fig. 3). This is consistent with *S. vulgaris* being sampled representatively from an unstructured native range.

Both species had fewer private haplotypes in North America compared to Europe (Table 1). Consistent with other indices of diversity, *S. latifolia* showed a greater proportional reduction in private haplotypes (63%) compared to *S. vulgaris* (25%), although the magnitude of reduction was not statistically significant (Fisher's exact test: $P = 0.28$). Thus, some of the difference between species in PD bottlenecked during invasion may be attributable to the sampling of rare haplotypes, but this alone cannot explain the observed differences in diversity. Rather, the haplotypes of *S. latifolia* introduced to North America originated more recently (on average) than haplotypes that were not introduced (300 kya versus 589 kya; $t = -3.53$, df $= 29$, $P = 0.001$), because invaders mostly come from the younger Eastern European clade (Fig. 4). In contrast, the mean age of *S. vulgaris* haplotypes invading North America was more similar to haplotypes that contributed no colonists (403 kya versus 524 kya; $t = -1.35$, df $= 20$, $P = 0.19$). This difference in haplotype age contributed to the bottlenecking of evolutionary history during invasion by *S. latifolia*, but not by *S. vulgaris*.

Table 3. Maximum likelihood estimates of coalescent parameters for *Silene latifolia* and *S. vulgaris* in Europe.

Species	Model[1]	θ	β	N_e	T_{MRCA}±SD (kya)
S. latifolia	β=0	19	–	0.90×10^6	298±24
	β>0	57	20	2.71×10^6	894±72
S. vulgaris	β=0	18	–	0.89×10^6	304±34
	β>0	48	18	2.38×10^6	811±90

[1]Model refers to maximum likelihood coalescence simulations which included no population growth ($\beta = 0$) or exponential population growth ($\beta > 0$). Likelihood ratio tests indicate inclusion of $\beta > 0$ significantly improves the fit of the model to the data (see Results).

Discussion

The results of this study provide clear insights into how the genetics of invasion by two closely related plant species, *S. latifolia* and *S. vulgaris*, have been influenced by their different histories of postglacial expansion in the native range. Although the two species have comparable genealogical histories, they differ in the spatial distribution of lineages in Europe. This has resulted in dramatic differences in how phylogenetic diversity was sampled during their invasion of North America.

Below, we discuss the phylogenetic history of the two species in their native range. We then interpret how this history interacted with the invasion process to determine the diversity present in the introduced range.

POSTGLACIAL COLONIZATION OF EUROPE

The genealogical results suggest that *S. latifolia* and *S. vulgaris* have similar preglacial histories and post-glacial expansions into Europe, but rather different histories of dispersal since that time. For both species, the T_{MRCA} is similar (~800–900 kya) with similar demographic histories of population expansion (significantly negative D and F_S). The T_{MRCA} for both can be traced to the mid-Pleistocene, whereas the estimated dates of expansion (~500–600

Table 4. Evidence for the occurrence and timing of population expansions of *Silene latifolia* and *S. vulgaris* in Europe.

Species	D	F_S	Sudden demographic expansion model		
			Model fit[1]	τ (95% C.I.)	Onset of expansion (95% C.I.)
S. latifolia	−2.01*	−25.46***	$P = 0.41$	5.469 (3.101–7.017)	521 kya (295–668)
S. vulgaris	−1.89*	−25.21***	$P = 0.86$	6.084 (3.484–8.318)	604 kya (346–826)

[1]Refers to the goodness of fit of a model of sudden demographic expansion to the data. Nonsignificant *P* values indicate a good fit of the presumed model to the data. * $P < 0.01$; *** $P < 0.0001$.

kya) suggest a proliferation of each species shortly after the onset of extreme climate oscillations which characterized the glacial cycles of the last 700 kya (Webb and Bartlein 1992; Hewitt 1996). Initial demographic expansions such as these are likely to overwhelm the signature of subsequent expansions (Rogers 1995). Thus, although *Silene* almost certainly expanded and retreated several times during the glacial cycles of the Pleistocene (Hewitt 1996), the initial expansions would have obscured these subsequent events.

The species, however, are significantly different in how cpDNA lineages are currently distributed in space. The pattern of post-glacial range expansion in *S. latifolia* is particularly clear. Chloroplast DNA lineages in eastern and western Europe are different subsets of the lineages found in southern Europe. This general pattern of post-glacial expansion is similar to that seen in a variety of plant and animal taxa (Hewitt 1996, 2000; Taberlet et al. 1998). The age of mutations that define these lineages suggest that Europe was colonized from genetically distinct refugia, probably located in the Iberian and Balkan Peninsulas. Our results for the chloroplast genome are consistent with the phylogeographic structure present in the *S. latifolia* Y-chromosome (Ironside and Filatov 2005), suggesting similar patterns of seed and pollen flow that occurred at the regional scale.

Our data confirm the existence of phylogeographic structure between eastern and western Europe for *S. latifolia* (Mastenbroek et al. 1983; Vellekoop et al. 1996), but are ambiguous as to the migration events that distributed the haplotypes. It has been hypothesized that with the advent of human agricultural practices, evolution of a weedy lifeform occurred in southern Europe (Baker 1948; Mastenbroek et al. 1983). Weedy genotypes from

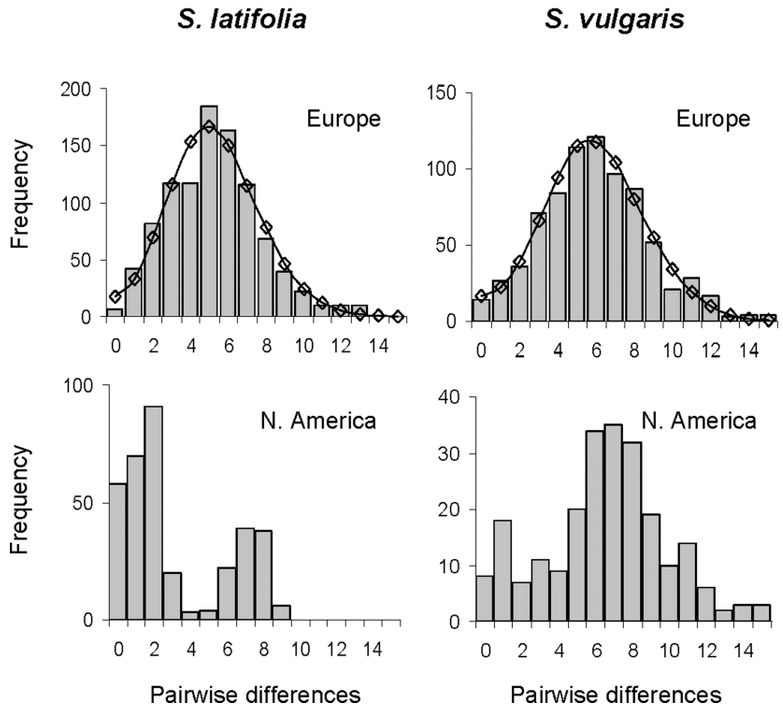

Figure 3. Mismatch distributions among cpDNA haplotypes in Europe and North America. Filled bars are the observed distributions. Solid lines with diamonds are the fitted distributions under the sudden demographic expansion model of Rogers (1995).

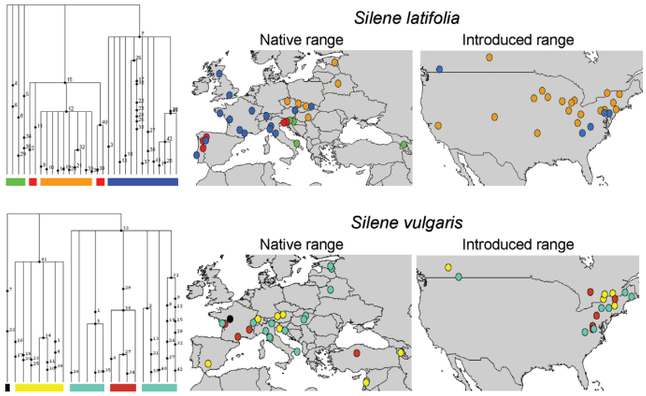

Figure 4. Evolutionary history of *Silene* in Europe and invasion of North America. Gene trees show the distribution of mutations among haplotypes, with mutation age measured along the Y-axis in coalescent units (see Methods). Major groups defined by mutations are color coded and mapped.

divergent refugia are then thought to have migrated northward with the spread of agriculture, resulting in the different "races" currently distributed in eastern and western Europe (Mastenbroek et al. 1983; Vellekoop et al. 1996). Our results clearly point to divergence between eastern and western European lineages as a result of population subdivision that originated > 400 Kya. We cannot rule out that *S. latifolia* remained in these refugia until humans inadvertently dispersed them with the spread of agriculture into northern Europe. However, the fact that *S. latifolia* in southern Europe more often occupies natural habitats such as open woodlands and montane limestone screes (Mastenbroek et al. 1983), suggests that the evolution of weediness may have occurred outside of refugia. These data, along with the evidence that *S. latifolia* experienced rapid demographic expansions well before the spread of agriculture (ca. 6–8 kya), suggest that *S. latifolia* initially colonized Northern Europe in a post-glacial expansion unassisted by humans (sensu Hewitt 2000).

For *S. vulgaris*, the spatial pattern of expansion is less clear, with lineages widely scattered across the continent. The *S. latifolia* data suggest Europe was colonized from several distinct refugia. If *S. vulgaris* colonized Europe from a single refugium that was a melting pot of lineages, then the current differences in genetic structure could be a consequence of differences in the ancestral structure of those refugia. Although we cannot reject this idea, the similar distributions of mutations on the genealogies suggest similar histories for the two species during the course of the Pleistocene.

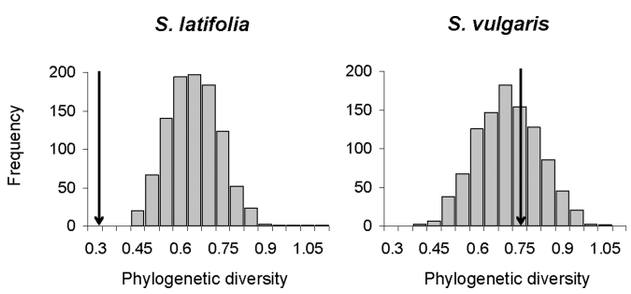

Figure 5. Phylogenetic diversity (PD) sampled during invasion of North America. Filled bars show the null distribution of PD under random sampling. Arrows show the observed PD encompassed by invasive haplotypes.

An alternative interpretation of the data is that differences in phylogeographic structure reflect relatively recent differences in dispersal across Europe. Although both species are short-lived perennials with similar dispersal ecologies, *S. vulgaris* is a self-compatible hermaphrodite whereas *S. latifolia* is dioecious and thus incapable of selfing. This difference in reproductive assurance is thought to be an important determinant of colonizing ability (i.e., Baker's law: Baker 1955; Taylor et al. 1999; Kolar and Lodge 2001). Thus, the current phylogeographic structure of *S. vulgaris* in Europe could reflect a more successful colonizing ability that has subsequently diluted the signature of historical range expansion. *Silene vulgaris* also has cytoplasmic male sterility (or CMS), and differences in population structure of cpDNA lineages could reflect a role of selection on the distribution of the cytoplasmic genomes (Ingvärsson and Taylor 2002; Olson and McCauley 2002; Tsitrone et al. 2003).

CONTEMPORARY INVASION OF NORTH AMERICA

The haplotypes sampled during the invasion of North America by *Silene* represent a subset of the total phylogenetic diversity (PD) present. Both species exhibit similar levels of species-wide polymorphism (Table 1) and experienced a sudden demographic expansion into Europe (Table 4); thus the amount of PD available for invasion was roughly similar. However, the PD actually sampled during invasion differs markedly between the species.

North American haplotypes of *S. latifolia* come from a few local sections of the entire genealogy that correspond to the phylogeographic regions of eastern and western Europe (Fig. 2). The

mismatch distribution of North American *S. latifolia* clearly shows the presence of divergent lineages among the introduced haplotypes (Fig. 3). The result is an admixture of anciently separated (> 400 kya) lineages in the introduced range, an increasingly common feature of biological invasions (e.g., Kolbe et al. 2004). However, even though propagule pressure was high in the sense that sampling occurred across phylogeographic subdivisions, the sampling within each region was sufficiently restricted to result in a strong overall reduction in PD. Furthermore, most (but not all) North American haplotypes were descended from a recently derived eastern Europe clade. As a result, the invasion captured relatively little evolutionary history (Fig. 4), and a clear bottleneck occurred on a continent-wide basis (Fig. 5). This may represent either a founder effect from colonization of North America by a set of genetically similar individuals, or demographic effects that occurred post-colonization that reduced the diversity initially introduced. In either case, a strong bottleneck of PD occurred despite multiple introductions that admixed anciently separated lineages.

It may seem counterintuitive that the invasion process can simultaneously result in bottlenecking and admixture, but the reasons become clear when we consider how diversity is structured in the native range along with what the data reveal about the amount of propagule pressure. The abundance of low pairwise differences in the North American mismatch distribution (the first mode in Fig. 3) demonstrates that each invasion episode sampled a specific subset of the available PD. In other words, a large proportion of the haplotypes introduced differed by few or no mutations, having come from the same phylogeographic region in Europe. The abundance of high pairwise differences (the second mode in Fig. 3) marks haplotypes separated by many more mutations. Because phylogenetically distant haplotypes of *S. latifolia* are spatially separated in Europe, this points to the occurrence of a minimum of two invasion episodes. However, the amount of propagule pressure during invasion was not so great as to make the mismatch distribution continuous, as it is in Europe. Thus multiple introductions, even ones that each involved a genetic bottleneck, produced an admixture of divergent east and west European lineages within North America. The evolutionary consequences of admixture between historically separated, recently bottlenecked populations may be very different compared to admixture among recently separated, non-bottlenecked populations. For example, the degree of heterosis following hybridization between lineages will depend on how inbred the lineages are initially, an outcome that may bear directly on the evolution of invasiveness (Ellstrand and Schierenbeck 2000). This suggests an additional layer of complexity may exist for species experiencing admixture during invasion.

In *S. vulgaris*, the lack of phylogeographic structure in the native range enhanced the probability that a genetically diverse inoculum was obtained, even if the introduced lineages originated from a single geographic region. This lack of structure precluded any novel admixture of divergent native range populations and avoided a genetic bottleneck in North America. These results are consistent with previous findings of high cpDNA diversity in both the native and introduced ranges of *S. vulgaris* (McCauley et al. 2003; Storchova and Olson 2004). Interestingly, North American haplotypes do show a slight overabundance of low pairwise differences in the mismatch distribution, suggesting that some local sampling may have occurred during invasion (Fig. 3). However, because cpDNA haplotypes in *S. vulgaris* are scattered randomly across Europe, there is little more we can conclude definitively about the invasion process. The distribution of pairwise differences observed in North America is consistent with a large number of introductions, a single introduction from a diverse inoculum, and everything in between.

Taken together, our results suggest that the evolutionary history of these species had a profound influence on the phylogenetic diversity captured during their recent invasion. Two species that are otherwise similar in their ecology and genealogical history differ in the current spatial distribution of cpDNA lineages across their native ranges. This has resulted in markedly different population genetic patterns during invasion of North America. These data have implications for the evolution of species invading new geographic ranges if the cpDNA lineages are representative of the nuclear genomes, and hence the traits affecting invasiveness. If this is the case, then for species like *S. vulgaris* with an unstructured native range, invasion may result in less severe bottlenecks of species-wide diversity, fewer opportunities for admixture of previously isolated lineages, and less potential for the redistribution of genetic variance in the introduced range. Conversely, species with a structured native range, such as *S. latifolia*, are more likely to experience severe bottlenecks, have greater opportunities for admixture, and may therefore experience a greater population genetic change during invasion. Because bottlenecks and admixture can have important phenotypic consequences (e.g., inbreeding depression, heterosis, outbreeding depression), these two scenarios present very different implications for the evolution of a species during invasion of a new geographic range.

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