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ORIGINAL ARTICLE

Comparative phylogeography of red maple (*Acer rubrum* L.) and silver maple (*Acer saccharinum* L.): impacts of habitat specialization, hybridization and glacial history

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ABSTRACT

Aim We analysed variation in chloroplast DNA (cpDNA) in red maple (*Acer rubrum* L.) and silver maple (*Acer saccharinum* L.) across a large part of their geographic ranges. *Acer rubrum* is one of the most common and morphologically variable deciduous trees of eastern North America, while its sister species *A. saccharinum* has a more restricted habitat distribution and displays markedly less morphological variation. Our objective was to infer the impact of biogeographic history on cpDNA diversity and phylogeographic structure in both species.

Location Deciduous forests of eastern North America.

Methods We sequenced 1289 to 1645 bp of non-coding cpDNA from *A. rubrum* ($n = 258$) and *A. saccharinum* ($n = 83$). Maximum parsimony networks and spatial analysis of molecular variance (SAMOVA) were used to analyse phylogeographic structure. Rarefaction analyses were used to compare genetic diversity.

Results A total of 40 cpDNA haplotypes were recovered from *A. rubrum* (38 haplotypes) and *A. saccharinum* (7 haplotypes). Five of the seven *A. saccharinum* haplotypes were shared with nearby samples of *A. rubrum*. SAMOVA recovered four phylogeographic groups for *A. rubrum* in: (1) south-eastern USA, (2) the Gulf and south-eastern Coastal Plain, (3) the lower Mississippi River Valley, and (4) the central and northern regions of eastern North America. *Acer saccharinum* had significantly lower haplotype diversity than *A. rubrum*, and novel haplotypes in post-glaciated northern limits of its range were shared with *A. rubrum*.

Main conclusions This is the first study of *A. rubrum* to report a distinct phylogeographic group centred on the lower Mississippi River, and the first to examine data comparatively with *A. saccharinum*. We hypothesized that *A. rubrum* would display stronger phylogeographic structure and greater haplotype diversity than *A. saccharinum* because of its greater geographic range, and ecological and morphological variation. This hypothesis was supported by the cpDNA analysis. The sharing of cpDNA and chloroplast simple sequence repeat (cpSSR) haplotypes in areas of geographic overlap provides evidence of introgression, which led to an increase in haplotype diversity in both species, and to novel phylogeographic structure in *A. rubrum*. We recommend that introgression be considered, along with other potential causes, as an explanation for the phylogeographic structure of cpDNA in plants.

Keywords

Acer, **chloroplast DNA, eastern North America, genetic diversity, haplotype sharing, introgression, maples, SAMOVA.**

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INTRODUCTION

A principal goal of comparative phylogeography is to infer biogeographic history from recurrent patterns in the geographic distribution of genetic variation in co-distributed taxa (Avise, 2000). Comparative phylogeography of trees has been especially useful in reconstructing Quaternary forest distributions, particularly in Europe, where genetic and fossil evidence have been used to infer locations of glacial refugia and individualistic patterns of post-glacial migration (e.g. Petit *et al.*, 2003; Petit & Vendramin, 2007). There have been fewer such studies in eastern North America (reviewed in Soltis *et al.*, 2006). While several phylogeographic studies in eastern North America (e.g. Sewell *et al.*, 1996) agree with the palynological records of species ranges during the Last Glacial Maximum (LGM) (Davis, 1981; Delcourt & Delcourt, 1987), some recent studies have found endemic chloroplast DNA (cpDNA) haplotype variation outside areas of fossil occurrence (McLachlan *et al.*, 2005). These 'cryptic refuges' (i.e. LGM forests with no fossil record) or cryptic populations may have harboured deciduous broadleaf tree species at low population densities (Stewart & Lister, 2002). It is important to know the northern extent of LGM species distributions in order to precisely estimate post-glacial migration rates (Clark, 1998; McLachlan *et al.*, 2005), to gauge the resilience of tree populations to climate change (Petit *et al.*, 2008), and to evaluate expectations about the genetic consequences of range expansions (Excoffier *et al.*, 2009). Phylogeographic evidence can complement fossil-based reconstructions of Quaternary forests, particularly for regions in which fossil records are not available (Cruzan & Templeton, 2000).

Because species vary in traits associated with dispersal and colonization, the study of closely related species may best reveal biogeographic histories by controlling for shared ecological traits. For example, the widespread North American northern red oak (*Quercus rubra*) showed low levels of cpDNA differentiation (G_{ST}) compared with European white oaks (Magni *et al.*, 2005). Because all oaks share traits relating to reproduction and dispersal, the differences in phylogeographic structure were best explained by historical contingencies. In this case, available evidence suggests that northern red oak maintained a single large geographic range in North America during the LGM, whereas European oaks were relegated to the southern peninsulas of Europe where they became highly differentiated.

Studies of closely related species may occasionally show evidence of introgression (via hybridization), which could also affect interpretations of phylogeographic structure (Albach *et al.*, 2006; Maliouchenko *et al.*, 2007). For example, the birches *Betula pubescens* and *Betula pendula* are broadly distributed across Europe and share cpDNA haplotypes in areas of post-glacial expansion, either through hybridization or retention of ancestral polymorphism (Maliouchenko *et al.*, 2007). The European ashes *Fraxinus excelsior* and *Fraxinus angustifolia* also exhibit wide-ranging and shared haplotypes, which suggests a history of hybridization within glacial refugia or during post-glacial recolonization (Heuertz *et al.*, 2006). Novel environments, such as those found in post-glacial landscapes, may promote hybridization and introgression by bringing disparate species into contact and by providing conditions that favour hybrid genotypes (Stebbins, 1974; Excoffier *et al.*, 2009).

We performed a comparative phylogeographic analysis of the sister species red maple (*Acer rubrum* L.) and silver maple (*Acer saccharinum* L.). While red maple and silver maple share some life-history traits, they show divergent patterns of habitat specificity and range-wide morphological variation. Our objectives were: (1) to quantify phylogeographic structure across the range of *A. rubrum* and *A. saccharinum*, (2) to look for evidence of introgression in the form of geographic associations of shared haplotypes, and (3) to compare the phylogeographic structure and haplotype diversity of the two species. Because *A. saccharinum* has greater habitat specialization, a more restricted geographic range and less geographic variation in morphology, we expected it to exhibit less cpDNA haplotype diversity and weaker phylogeographic structure than *A. rubrum*. Because of the relatively long estimated time since divergence (Pliocene) of red and silver maple (Renner *et al.*, 2008), we did not expect to find shared ancestral polymorphism. Our study of red maple builds upon results of a previous phylogeographic study (McLachlan *et al.*, 2005), which documented phylogeographic structure across the North American range and novel haplotypes in regions well beyond the LGM fossil distributions for maples (Jackson *et al.*, 2000).

MATERIALS AND METHODS

Study species

Since their origin in the Cretaceous, maples (genus *Acer*, family Sapindaceae) have speciated extensively in the temperate broadleaf forests of the Northern Hemisphere (Wolfe & Tanai, 1987; Renner *et al.*, 2007) where they comprise the largest tree genus after *Quercus*. *Acer* consists of 124–156 species (van Gelderen *et al.*, 1994; de Jong, 2002), *c.* 80% of which are endemic to Asia. *Acer rubrum* and *A. saccharinum* are the most recently diverged *Acer* sister species in North America (Renner *et al.*, 2007). Based on a fossil calibrated phylogeny, Renner *et al.* (2008) estimate that the two species diverged from a common ancestor in the Pliocene. The two species belong to section *Rubra* (Pax), one of the 16 sections of the genus *Acer*, which also includes the closely related Japanese red maple *Acer pycnanthum* K. Koch (van Gelderen *et al.*, 1994). *Acer pycnanthum* is currently restricted to central Honshu, Japan (Ogata, 1965; Saeki, 2005).

Acer rubrum is the most widely distributed and abundant deciduous tree species in eastern North America (Walters & Yawney, 1990). Its range spans *c.* 24° of latitude, 40° of longitude and from sea level to 1370 m elevation (Sargent, 1922; Walters & Yawney, 1990). It occurs on virtually every type of glaciated and non-glaciated landform within its range,

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from excessively drained to very poorly drained soils, and on sites of both high and very low nutrient availability (Barnes & Wagner, 2004). Rarely found in pure stands, red maple occurs in 56 of the 88 non-tropical forest cover types of the eastern USA (Eyre, 1980). Numerous studies have demonstrated great genetic and phenotypic variation among and within populations, as well as clinal variation in ecological traits such as phenology and growth related to day length, length of growing season, temperature and preference for wet versus dry sites (Perry & Wang, 1960; Townsend *et al.*, 1982; Farmer, 1997; Bauerle *et al.*, 2003). In their description of *A. rubrum* L., Gleason & Cronquist (1991, p. 353) conclude: 'Morphologically, cytologically and ecologically variable, but indivisible'. There are currently three broadly accepted geographic varieties: *A. rubrum* var. *rubrum* Sarg., *A. rubrum* var. *drummondii* Sarg. and *A. rubrum* var. *tridens* Wood.

Acer saccharinum is also widely distributed in eastern North America but does not reach the latitudinal extremes of *A. rubrum* or the extent of its occurrence west of the Mississippi River. It is markedly site differentiated from *A. rubrum*, occurring in full sun along the bank and adjacent terrace (first bottom) of river floodplains that are inundated during the first part of the growing season, and in microsites of very poorly drained swamps and ice-block depressions. In the latter ecosystems, the two species may grow side by side. Despite ecological and cytological barriers to hybridization

Table 1 Comparison of selected natural history characteristics of *Acer rubrum* and *A. saccharinum**†.

	Acer rubrum	*Acer saccharinum*	Reference
I. Geographic range and ecosystem characteristics:			
(1) Geographic range (see also Figs 1 & 2)	Eastern North America; greater north–south latitude range (var. *rubrum*: common in northern region; var. *tridens*: common in Mid and Deep South; var. *drummondii*: dominant in swamps in lower Mississippi River Valley)	Eastern North America excluding mountains, and the south-eastern USA and Gulf Coastal Plains including Florida and parts of the Great Plains. Extending farther west in the central Great Plains	Ellis (1963), Gabriel (1990), Walters & Yawney (1990)
(2) Pre-European settlement primary habitat	Swamps, mesic to wet-mesic sites in upland forests	River floodplains and stream banks	Barnes *et al.* (2004)
(3) 20th-century current habitat	Swamps; diverse, wet-mesic to dry upland forests (var. *drummondii*: deep swamps)	River floodplains and stream banks	Sargent (1922)
II. Leaf morphology:			
(1) Major characteristics (see also Fig. 6)	(var. *rubrum*: 5-lobed. Shallowly dissected, green and glabrous below; var. *tridens*: smaller, narrower, 3-lobed, green, glabrous below; var. *drummondii*: broader, 3–5 lobed, densely woolly pubescent below)	5–7 lobed. Deeply dissected, glaucous silvery-white below	
(2) Variation among individuals	Very high	Low	
III. Reproductive characteristics:			
(1) Chromosome number (x = 13)	(var. *rubrum*: $2n = 78$, 104‡; var. *tridens*: $2n = 78$; var. *drummondii*: $2n = 65$)	$2n = 52$	Ellis (1963), Santamour (1965)
(2) Flowering time in Michigan	Late March–May	Early March–April	
(3) Pollination	By wind	By wind	
(4) Samara length	(var. *rubrum*: 1.9–2.5 cm; var. *tridens*: 1.9–2.5 cm; var. *drummondii*: 3.2–6.4 cm)	4–8 cm	Sargent (1922), Harrar & Harrar (1962), Cooperrider (1995)
(5) Seed weight, seeds per pound	22,860	1780	Olson & Gabriel (1974)

*Where differences among the intra-specific varieties of *A. rubrum* are known, they are shown in brackets. Otherwise, the description in column *Acer rubrum* mainly refers to *A. rubrum* var. *rubrum* because we assume that most studies on *A. rubrum* have been done for var. *rubrum* unless the other varieties were mentioned.

†Only major differences among the taxa for the respective characteristics are presented; for finer-scale details see the text.

‡Santamour (1965) also reported $2n = 91$, 97 and 98 for some individuals.

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(Table 1), natural hybrids have been reported in herbarium collections (*Acer × freemanii* E. Murray) (Ellis, 1963) and controlled crosses have been made to produce horticultural varieties, such as the Freeman maple (Freeman, 1941; Murray, 1969; Santamour, 1993). Ten intra-specific subspecies, varieties and forms (excluding horticultural cultivated varieties) have been recognized (Index Kewensis, Gray Card Index), and are based on minor differences in leaf morphology. No geographic taxa have been recognized. A comparative summary of *A. rubrum* and *A. saccharinum* including information on the intra-specific taxa of *A. rubrum* is presented in Table 1.

Leaf collection

For *A. rubrum*, leaf collections were made of 258 individuals (115 localities) representing its occurrence throughout a large part of its geographic range in eastern North America; of these, 57 were collected from a provenance-test plantation located near Delaware, OH, which was established in 1974 by the United States Department of Agriculture (USDA) Agricultural Research Service. The plantation includes trees from 55 geographic sources, from 27 states and 4 provinces of Canada. In 2005 and 2007, we collected foliage from at least one tree from each seed source. The rest of the foliage collections ($n = 201$) were from mature trees located at 60 natural sites in Florida, South Carolina, Georgia, Louisiana, Texas, Illinois, Michigan (USA) and Ontario (Canada) (see Appendix S1 in Supporting Information). This sampling design included individuals from areas not represented in the seed-source test in Ohio as well as the representative geographic regions and site-specific ecosystems where the three varieties of *A. rubrum* are known to occur.

We collected representative intact shoots from tree populations at 25 of the 60 sites, including three populations each from Florida, Georgia, Louisiana, Texas, Illinois and Ontario, and seven from Michigan (Fig. 1). Leaf samples from 5 to 11 individual trees per population were sequenced, for a total of 164. Voucher foliage specimens of individuals of each population were created to determine morphological differences among varieties. Based upon the physical habitat conditions

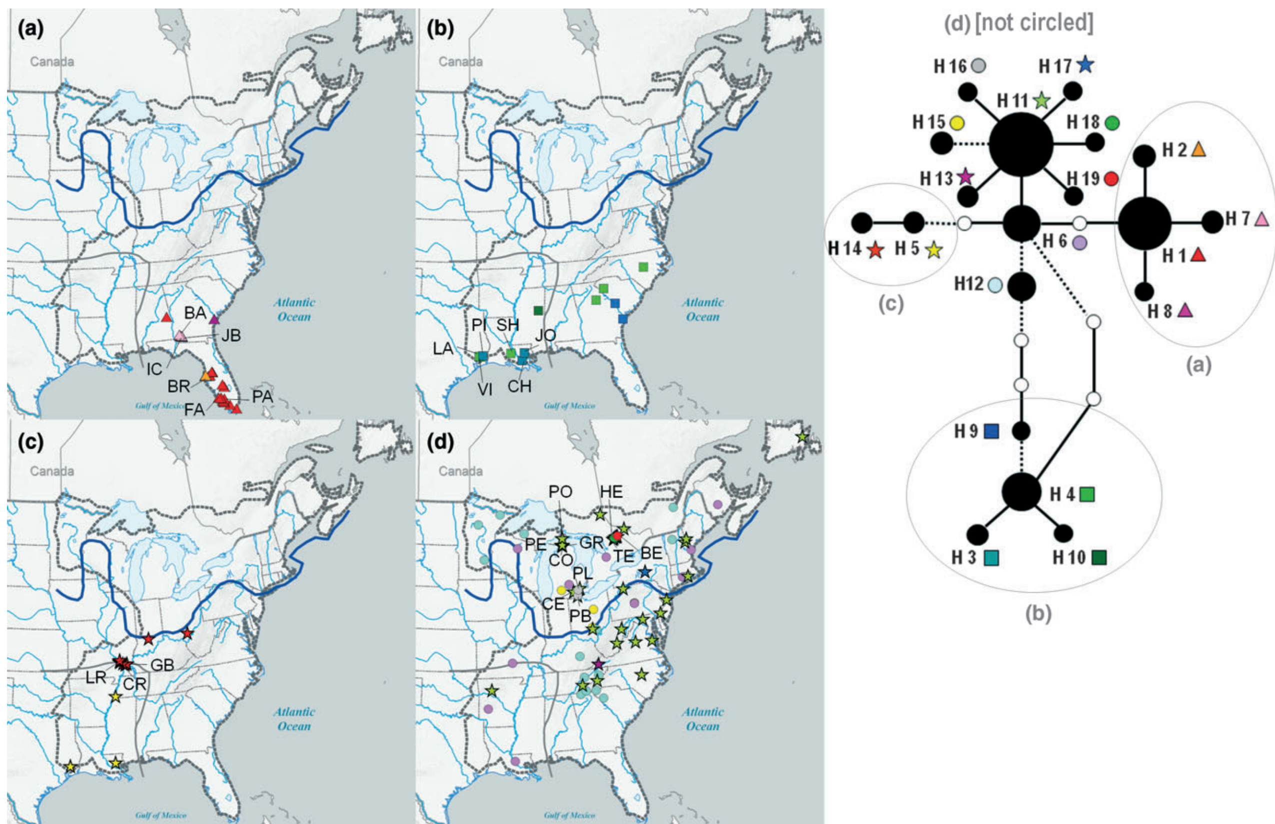

Figure 1 Geographic distribution of 19 non-simple sequence repeat (non-SSR) chloroplast DNA (cpDNA) haplotypes of *Acer rubrum* in eastern North America, and the corresponding parsimony network ($n = 258$). The four phylogeographic groups are indicated in panels (a)–(d). In the network, bold and dashed lines represent nucleotide substitution and indels, respectively; circle size is proportion to haplotype abundance, and unfilled circles represent hypothetical intermediate forms. Haplotypes shared with *A. saccharinum* are shown as stars. Alphabetical symbols on the map are abbreviations of the names of sites where population sampling was conducted (see Appendix S1). Some of the haplotypes are not shown on the map because of spatial overlap with other haplotypes. Grey dashed lines delimit current species range limits. The thick grey line indicates reconstructed geographic range at 16,000 yr BP based on fossil evidence for maples (Delcourt & Delcourt, 1987). The dark blue line illustrates the southern extent of the Laurentide Ice Sheet at the Last Glacial Maximum.

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and morphological examination in the field and laboratory, our working hypothesis was that individuals from Florida and Georgia represent *A. rubrum* var. *tridens*, those from Louisiana, Texas and Illinois represent *A. rubrum* var. *drummondii*, and those from Michigan and Ontario represent *A. rubrum* var. *rubrum*.

For *Acer saccharinum*, leaves of 15 individuals were collected from a silver maple provenance-test plantation located near Carbondale, IL, which was established in 1987 by the Southern Illinois University. This plantation includes trees from 15 seed sources from 13 states and one province of Canada. We visited the plantation in 2009 and collected leaf tissue from one tree from each seed source. In addition, 68 individuals were collected from 26 natural sites in Michigan (eight sites), Ontario (one), New Jersey (three), Massachusetts (one), Illinois (nine), Washington, DC (one), Tennessee (one) and Arkansas (one). One of the Ontario individuals was a leaf specimen in the Algonquin Park Herbarium (specimen no. 5231; collected by Dan Strickland, Algonquin Provincial Park Visitor Centre). At the seven sites, we collected leaf materials for DNA analyses from five to eight individuals of silver maple populations (Fig. 2).

Laboratory procedures

Leaf samples were dried in silica gel at the time of collection. DNA extraction was performed using either Qiagen DNeasy extraction kits (Qiagen Inc., Valencia, CA, USA) or a slightly modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1987). The DNA aliquot from each tree was assigned an identification number (Lab ID), which is linked to information on geographic location, herbarium label information or labels for trees sampled in the plantation (Appendix S1). Three non-coding regions of cpDNA (*trn*L 3′ exon–*trn*F, *atp*B–*rbc*L and *trn*H–*psb*A) were amplified using primers developed by Taberlet *et al.* (1991), Terachi (1993), Sang *et al.* (1997) and Tate & Simpson (2003), respectively. The polymerase chain reaction (PCR) contained 1× Qiagen buffer, 3.75 μM $MgCl_2$, 100 μM of each deoxyribonucleotide triphosphate (dNTP), 2 μM of each primer, 0.25 units of Taq polymerase (Qiagen) and 1.0 μL of undiluted template DNA. The thermal cycle for *trn*L 3′ exon–*trn*F was 95 °C for 15 min, followed by 35 cycles of 95 °C for 1 min, 50 °C for 30 s and 72 °C for 40 s. The thermal cycle for the other regions was the same except for the annealing and extension procedures, which were performed at 50 °C for 30 s and 72 °C for 50 s for *atp*B–*rbc*L and at 57 °C for 30 s and 72 °C for 45 s for *trn*H–*psb*A, respectively. Forward and reverse DNA strands were sequenced using Big Dye Terminator Cycle Sequencing Kit v. 3.1 chemistry (Applied Biosystems Incorporated, ABI) on an ABI 3730 sequencer. DNA chromatograms were edited and aligned using ChromasPro v. 1.34 and ClustalW in mega 4 (Tamura *et al.*, 2007). Indels were coded as single changes, but information on single base-pair-length mutations in chloroplast simple sequence repeats (cpSSRs) was not used for initial identification of haplotypes. Instead, we identified another set of haplotypes distinguished only by cpSSR, i.e. cpSSR haplotypes. Henceforth we refer to non-SSR haplotypes simply as 'cpDNA haplotypes' to distinguish them from the cpSSR haplotypes.

Two loci used by McLachlan *et al.* (2005), *atp*B–*rbc*L (170 bp) and *trn*H–*psb*A (353 bp), could be aligned with our data sets. We therefore combined their cpDNA haplotype data with ours and developed a haplotype map in a higher spatial resolution than to that of our original data. The total number of samples and sampling sites were 387 and 232, respectively. Haplotypes were identified with nucleotide substitutions and indels excluding cpSSR regions.

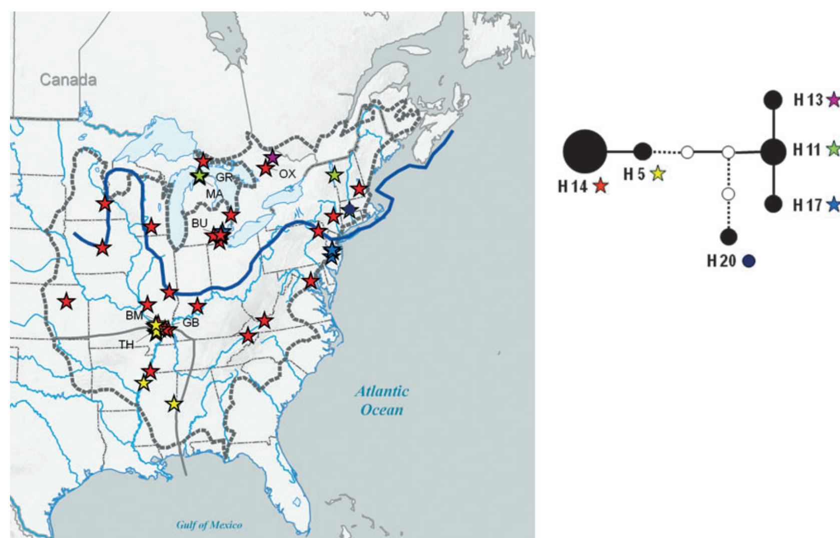

Figure 2 Geographic distribution of the six chloroplast DNA haplotypes recovered from *Acer saccharinum*, and associated haplotype network (n = 83). Haplotypes shared with *A. rubrum* are indicated as stars. See Fig. 1 for additional description of haplotype and map legends.

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Data analyses

To compare the haplotype diversity in *Acer rubrum* and *A. saccharinum*, while accounting for differences in sample sizes, the number of haplotypes after rarefaction to 20 individuals was calculated for each species using the program CONTRIB v. 1.02 (Petit *et al.*, 1998). The spatial boundaries of cpDNA haplotype variation in *A. rubrum* and *A. saccharinum* were examined using spatial analysis of molecular variance (SAMOVA), which maximizes the proportion of genetic variation apportioned among groups through simulated annealing (Dupanloup *et al.*, 2002). SAMOVA was run with 100 replications. We repeated the analyses with different numbers of groups (K) until the F_{CT} value (the proportion of genetic variation among groups) reached a plateau. Haplotype networks were created in TCS v. 1.21 (Clement *et al.*, 2000) using statistical parsimony. In order to illustrate haplotype diversity among putative varieties of *A. rubrum* and between *A. rubrum* and *A. saccharinum*, we created a dendrogram of haplotype clusters based on relative Sørensen distance measures using the software PC-ORD (McCune *et al.*, 2002).

As a further test of phylogeographic structure, we compared N_{ST} and G_{ST} using the program PERMUT (Pons & Petit, 1996). N_{ST} is defined as a measure of genetic differentiation among populations when a measure of the distance between haplotypes is taken into account, whereas G_{ST} is an unordered measure of genetic differentiation that does not incorporate relationships (distances) among haplotypes. PERMUT tests if the observed N_{ST} is larger than the G_{ST} by counting how many permutated G_{ST} values are larger than the observed N_{ST}. A significantly higher N_{ST} value indicates that the relative distribution of phylogenetically related haplotypes contributes to the overall geographical structure of the species, which is one definition of phylogeographic structure (El Mousadik & Petit, 1996). Because our samples came from localities in which only one or a few individuals were sampled, we performed the N_{ST} versus G_{ST} test among groups of populations defined by SAMOVA. Alpha was set at 0.05.

RESULTS

Spatial patterns and diversity of cpDNA sequences

The total aligned length of the three cpDNA regions for *A. rubrum* (n = 258 individuals) and *A. saccharinum* (n = 83) was 1289 to 1625 bp. The combined data set (n = 341) contained 28 polymorphic sites (Table 2). Nucleotide substitutions and indels (i.e. non-SSR polymorphisms) in 21 sites were distributed among 20 haplotypes. Seven polymorphic cpSSR loci were found within the non-coding cpDNA spacer regions in both species, three of which were too long and difficult to score (e.g. repeats greater than 16 bp long). The analysis focused on the remaining four cpSSR loci, for which a total of 14 multi-locus haplotypes were identified.

Nineteen of the 20 cpDNA total haplotypes were found in *A. rubrum*, and these showed phylogeographic structure. Haplotype group 1a (H1, H2, H7 and H8; Fig. 1a) occurred exclusively in the south-eastern USA (i.e. Florida and adjacent Georgia and Alabama). A second group (H3, H4, H9 and H10; Fig. 1b) occurred in the Gulf and south-eastern Coastal Plain (Texas, Louisiana to North Carolina). A third group (H5 and H14; Fig. 1c) was located in east Texas, the lower Mississippi River Valley (i.e. south of the confluence of the Mississippi and Ohio Rivers to the Gulf of Mexico) and in southern Indiana and Ohio. The remaining haplotypes (Fig. 1d) were mostly located in the central and northern regions of eastern North America from South Carolina, Tennessee and Arkansas to Canada. Three haplotypes (H6, H11 and H12) were common and occurred throughout a large part of the species' range, primarily the Appalachian Mountains, the Mid-Atlantic States and New England.

Five of the six cpDNA haplotypes (H5, H11, H13, H14 and H17) in *A. saccharinum* represented a subset of the haplotypes encountered in *A. rubrum* (Fig. 2). H14 was the most abundant shared haplotype (66%), followed by H11 (18%) (Appendix S2). Five of the seven *A. saccharinum* populations demonstrated similar haplotype composition with each of their neighbouring populations of *A. rubrum*. For example, haplotype H14 was abundant in populations of both species in

Table 2 Summary of polymorphisms and number of chloroplast DNA haplotypes identified in *Acer rubrum* and *A. saccharinum*.

	No. of polymorphic sites*†‡				No. of haplotypes§		
Species (sample size)	*trn*L–F	*atp*B–*rbc*L	*trn*H–*psb*A	Total	Non-SSR	SSR	Non-SSR and SSR combined
Acer rubrum (258)	4 (3)	8 (3)	15 (14)	27 (20)	19 (7.44)	14 (5.59)	38 (10.73)
Acer saccharinum (83)	3 (2)	4 (0)	7 (6)	14 (8)	6 (3.32)	4 (2.11)	7 (3.44)
Two taxa combined (341)	4 (3)	8 (3)	16 (15)	28 (21)	20	14	40

SSR, simple sequence repeat.

*Sites with insert and deletions of more than one nucleotide were counted as one polymorphic site.

†Typical aligned length of the three regions: *trn*L–F, 389 bp; *atp*B–*rbc*L, 560 bp; *trn*H–*psb*A, 511 bp.

‡Within parentheses: the number of sites of nucleotide substitutions, and insertions and deletions excluding simple sequence repeats (SSR) (i.e. non-SSR polymorphisms).

§Within parentheses: the number after rarefaction to 20 individuals.

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southern Illinois (see Figs 1 & 2 for geographic locations of the populations). Similarly, H11 was abundant in two populations of *A. saccharinum* from northern Lower Michigan (MA, GR) and in four populations of red maple in the same area (CO, PE, GR, PO).

Geographic structure was also found in the distributions of cpSSR haplotypes. For *A. rubrum*, populations in Florida and adjacent states contained the endemic cpSSR haplotypes Hssr1 and Hssr2 (Fig. 3a). Four cpSSR haplotypes were identified for *A. saccharinum*, all of which were shared with *A. rubrum* (Fig. 3b, Appendix S2). Hssr5 is very common in *A. saccharinum*, and the other haplotypes were located in the north-eastern part of the distribution. The number of cpSSR haplotypes after rarefaction to 20 individuals was lower in *A. saccharinum* (2.11) than in *A. rubrum* (5.59), which corresponds to the numbers of haplotypes identified by non-SSR polymorphisms (3.32 vs. 7.44) (Table 2). When both SSR and non-SSR polymorphisms were combined, 38 and 7 haplotypes were identified in *A. rubrum* and *A. saccharinum*, respectively. Five haplotypes were shared by the two species (Appendix S2). The network of 14 cpSSR haplotypes in *A. rubrum* indicated extensive homoplasy (Fig. 3a).

Spatial analysis of molecular variance (SAMOVA)

SAMOVA identified four phylogeographic groups of *A. rubrum* based on spatial locations and cpDNA haplotypes (SAMOVA groups 1–4 in Fig. 4a). F_{CT} reached a plateau when the number of groups (K) was set at 4 (0.79; Table 3). SAMOVA group 1 was located in the south-eastern USA and characterized by populations with H1, H2, H7 and H8. SAMOVA group 2 was delineated in the north and west of SAMOVA group 1 and corresponded to the region where haplotypes H3, H4, H9 and H10 occur. SAMOVA group 3 in the lower Mississippi River

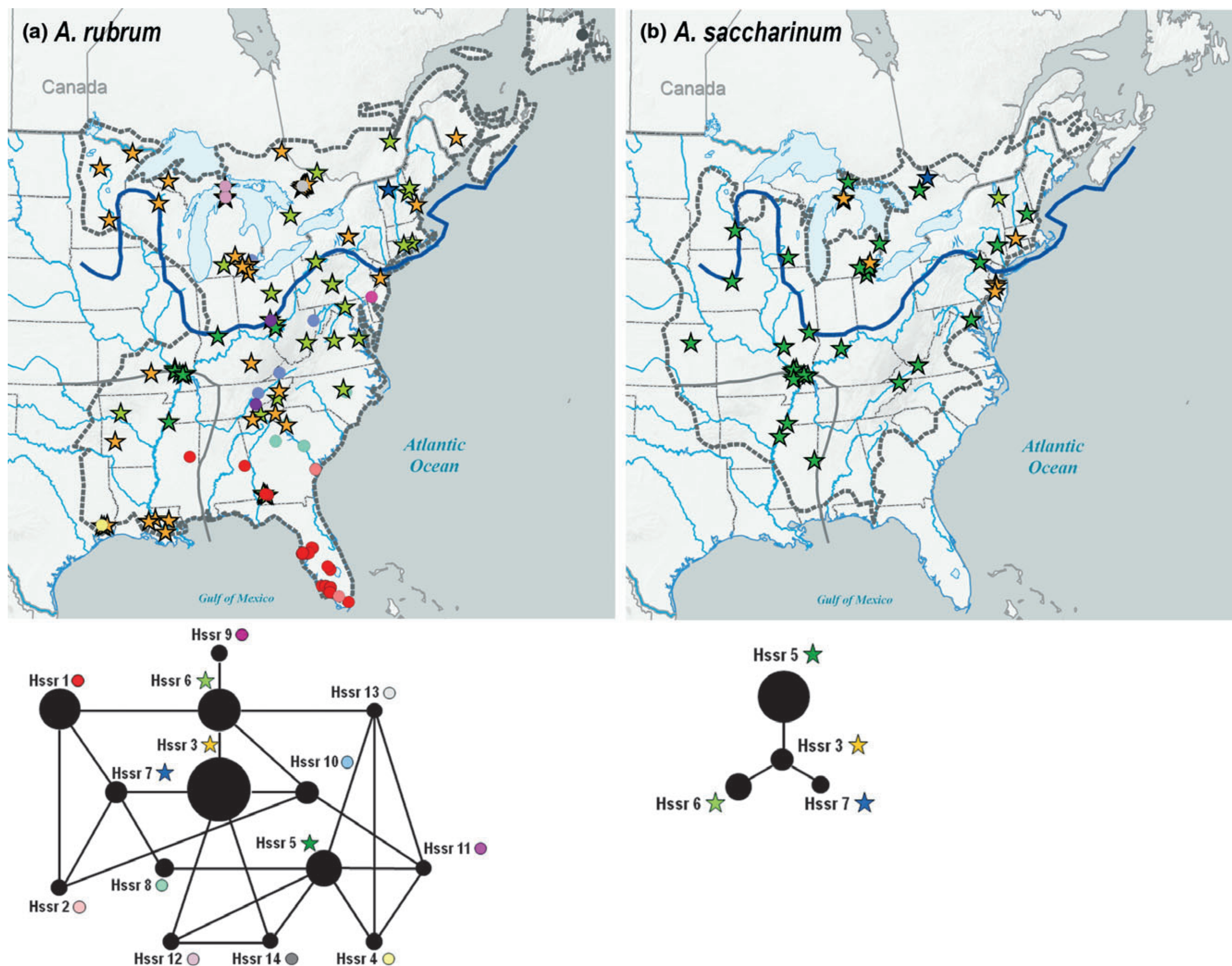

Figure 3 Geographic distribution of (a) 14 simple sequence repeat (SSR) haplotypes of *Acer rubrum*, and (b) four SSR haplotypes of *A. saccharinum*, in eastern North America based on SSR variation of chloroplast DNA (n = 258 for *A. rubrum* and n = 83 for *A. saccharinum*). The SSR haplotypes were numbered from the south to north. Haplotypes shared with *A. saccharinum* are indicated as stars. Some of the SSR haplotypes are not shown on the map due to their geographic overlap with other haplotypes. Other aspects of the haplotype network legend are the same as in Fig. 1.

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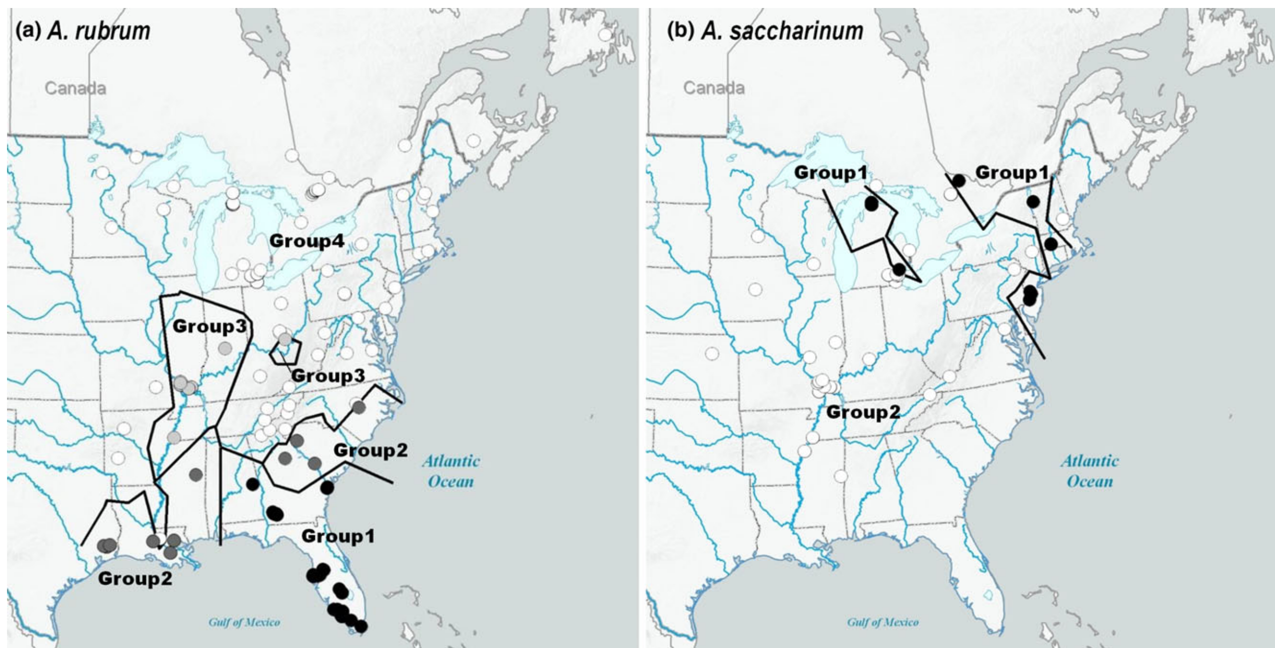

Figure 4 Maps of genetic boundaries for (a) *Acer rubrum* (n = 258) defined by spatial analysis of molecular variance (SAMOVA) at K (number of groups of populations) = 4, and for (b) *A. saccharinum* (n = 83) at K = 2. Boundaries were based on nucleotide substitution and indels excluding simple sequence repeat (SSR) polymorphisms.

Valley and Ohio contained haplotypes H5 and H14. The genetic boundaries identified by SAMOVA correspond well to spatial patterns of the haplotype clades in Fig. 1. For *A. saccharinum*, SAMOVA detected two major phylogeographic groups (F_{CT} = 0.91). SAMOVA group 1 was located in a small area along the north-eastern USA, Ontario and Michigan, while SAMOVA group 2 was distributed in the remaining area (Fig. 4b). Similar phylogeographic patterns were found in cpSSR data of *A. rubrum* and *A. saccharinum* (Appendices S3 & S4).

Table 3 Comparison of fixation indices corresponding to the groups of populations detected by spatial analysis of molecular variance (SAMOVA) in *Acer rubrum* and *A. saccharinum* in eastern North America based on three chloroplast DNA regions: *trn*L 3′ exon–*trn*F, *atp*B–*rbc*L, and *trn*H–*psb*A.

No. of groups (K)	*Acer rubrum*			*Acer saccharinum*		
	F_{SC}	F_{ST}	F_{CT}	F_{SC}	F_{ST}	F_{CT}
2	0.768	0.895	0.546	0.412	0.945	0.906
3	0.677	0.885	0.645	0.228	0.944	0.927
4	0.335	0.861	0.791	−0.118	0.943	0.949
5	0.197	0.856	0.821	−0.142	0.942	0.949

F_{SC}, Proportion of genetic variation between populations within groups.
F_{ST}, Proportion of genetic variation between populations and groups overall.
F_{CT}, Proportion of genetic variation among groups.

N_{ST} versus G_{ST} tests of phylogeographic structure

N_{ST} of *Acer rubrum* (0.801) was significantly higher ($P < 0.001$) than G_{ST} (0.575) based on the four SAMOVA groups. Because PERMUT was not able to test between only two groups recovered for *A. saccharinum* in the SAMOVA, we analysed the SAMOVA groups derived for $K = 3$. N_{ST} (0.758) was significantly higher ($P < 0.001$) than G_{ST} (0.525) using this approach, indicating phylogeographic structure. There was no significant difference between N_{ST} and G_{ST} ($P > 0.1$) with $K = 4$ ($N_{ST} = 0.817$, $G_{ST} = 0.711$) in *A. saccharinum*.

Haplotype data combined with information from a previous study

The sequence data combined with the previous study on *A. rubrum* (McLachlan *et al.*, 2005) contained 23 cpDNA haplotypes (Appendix S2). Seventeen and 16 haplotypes were found in the McLachlan *et al.* data set and the present data set, respectively, and 10 haplotypes were common to both. Spatial patterns of the haplotype occurrence (Fig. 5) were similar to those based on our original data set (Fig. 1). There were distinctive regional haplotype groups in the south-eastern USA (Hc1, Hc7, Hc8) and in the region of the Gulf and south-eastern Coastal Plain (Hc2, Hc3, Hc4, Hc9 and Hc10). Haplotypes Hc5, Hc11, Hc15 and Hc19 were found in *A. saccharinum* of our data set. It was not possible to perform the SAMOVA on this dataset because of the large number of sites.

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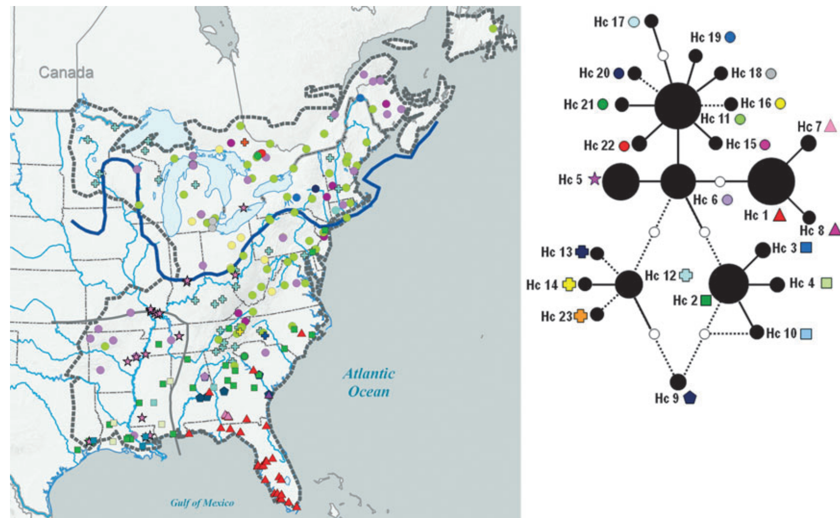

Figure 5 Geographic distribution of 23 haplotypes of *Acer rubrum* in eastern North America, and the associated haplotype network, constructed by combining datasets of chloroplast DNA (cpDNA) sequences from McLachlan *et al.* (2005) and this study (n = 387). Bold and dashed lines in the network represent nucleotide substitutions and indels, respectively, excluding the chloroplast simple sequence repeat (cpSSR) variation. Some of the haplotypes are not shown on the map due to their geographic overlap with other haplotypes. Other aspects of the haplotype network legend are the same as for Fig. 1.

Relationship between intra-specific morphology and molecular variation

Haplotype variation among the 25 populations of *A. rubrum* clustered within the three taxonomic varieties (Fig. 6). In six populations from Florida and Georgia, i.e. representative populations of *A. rubrum* var. *tridens*, H1 was abundant together with H2 and H7. In the six populations from Louisiana and Texas, representing *A. rubrum* var. *drummondii*, haplotypes H3, H4 and H5 were observed. Haplotype H14 was abundant in southern Illinois. In the populations of *A. rubrum* var. *rubrum* from Michigan and Ontario, H6 and H11 were dominant. *Acer saccharinum* populations did not form one cluster but fell into the two clusters comprising *A. rubrum* var. *drummondii* and *A. rubrum* var. *rubrum*. In all cases, the *A. saccharinum* shared haplotypes with samples of *A. rubrum* growing nearby or within the same region.

DISCUSSION

Our analysis recovered four phylogeographic groups for *A. rubrum* in: (1) the south-eastern USA (Florida, Georgia, Alabama), (2) the Gulf and south-eastern Coastal Plain (Texas, Louisiana to South Carolina), (3) the lower Mississippi River Valley, and (4) the southern Appalachians and northern post-glacial regions. This is the first study to encounter a distinct phylogeographic group centred on the lower Mississippi River Valley for *A. rubrum*, and the first to examine data comparatively with *A. saccharinum*. Because the cpDNA haplotypes overlap with geographically co-distributed *A. saccharinum*, we interpret this novel cluster as a probable zone of hybridization. In comparative terms, the level of nucleotide divergence between the geographic samples of *A. rubrum* was of similar or greater magnitude than the divergence between *A. rubrum* and *A. saccharinum*. *Acer saccharinum* had lower haplotype diversity than *A. rubrum* and weaker phylogeographic structure. The pattern in *A. saccharinum* may have resulted from population bottlenecks in the LGM due to ecological and historical factors. The degree of haplotype sharing between the two species underscores the need to consider impacts of introgression in the formation of cpDNA phylogeographic structure.

Phylogeography of *A. rubrum*

In a range-wide cpDNA phylogeographic analysis of *A. rubrum*, McLachlan *et al.* (2005) recovered two overlapping haplotype groups (A and B in their Fig. 3a) located within putative glacial refugia of the southern Appalachian Mountains and south-eastern Coastal Plain (corresponding to our Fig. 1d). This geographic region contained novel and diverse cpDNA haplotypes within 500 km of the terminus of the Laurentide ice sheet. Because LGM palynological cores from this region do not contain maple pollen, the endemic haplotypes were interpreted as evidence of cryptic LGM populations. Our study recovered additional haplotypes, including several that were found only in the formerly glaciated regions and not shared with *A. saccharinum* (e.g. H16, H18, and H19). We do not believe that the evidence is strong enough to suggest that *A. rubrum* occurred even further north during the LGM than proposed by McLachlan *et al.* (2005). These high-latitude haplotypes are uncommon and may occur in unsampled southern locations, or they may be recently derived (e.g. during the Holocene), as suggested by their rarity and by their divergence by single-mutation differences from the widespread haplotype H11 (Fig. 1d).

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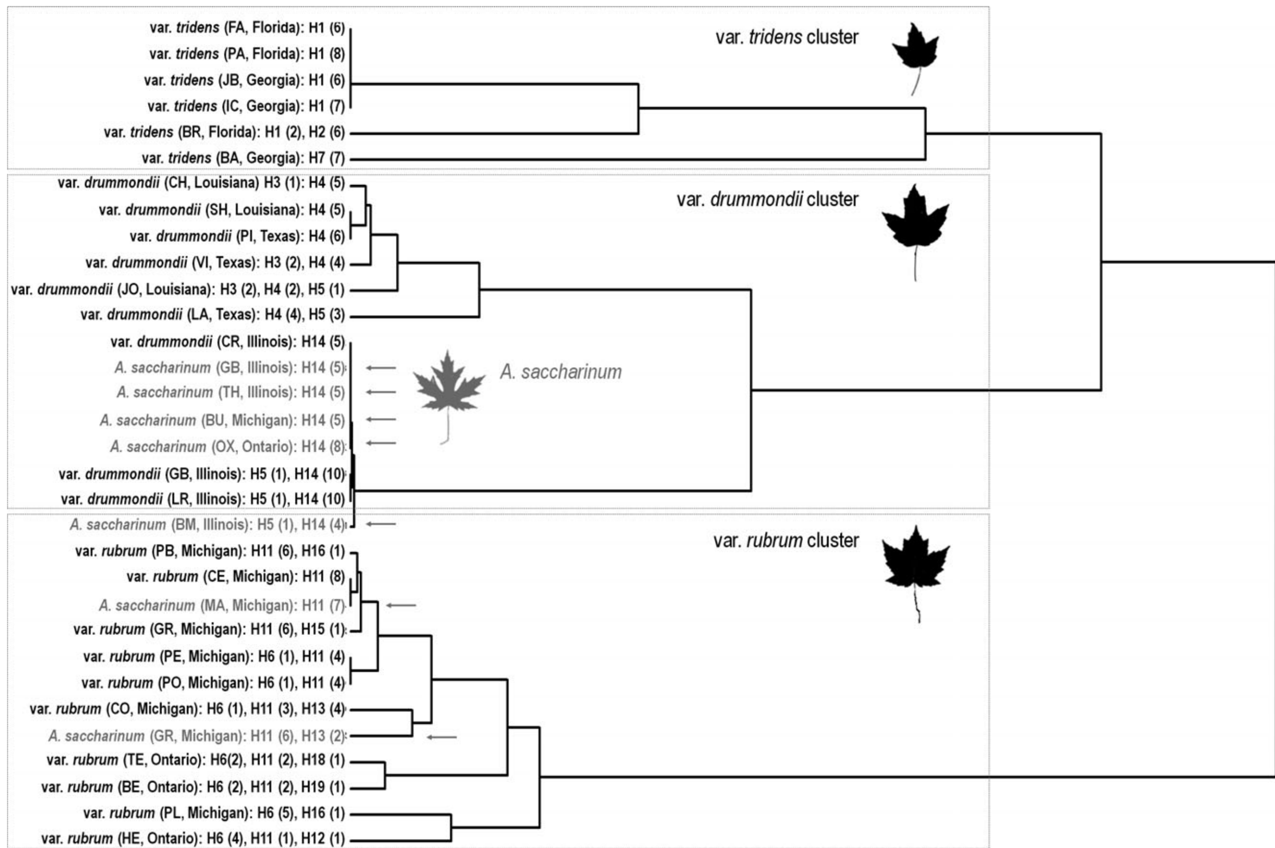

Figure 6 Dendrogram constructed by cluster analysis of the haplotype composition of 25 populations of *Acer rubrum* (var. *tridens*, var. *drummondii* and var. *rubrum*) and seven populations of *A. saccharinum* using relative Sørensen distances. The locality abbreviation is indicated in parentheses after the taxonomic name, and is followed by haplotype identity and number of individuals. See Fig. 2 and Appendix S1 for locations and names of populations, respectively.

McLachlan *et al.* (2005) recovered two additional phylogeographic groups centred in Florida (our Fig. 1a), and in the Gulf and south-eastern Coastal Plain region spanning from Texas and Louisiana to South Carolina, North Carolina, and Georgia (our Fig. 1b). There is no fossil pollen record of maples in peninsular Florida during the LGM (Delcourt & Delcourt, 1987; Jackson *et al.*, 2000), which creates an enigma with respect to the geographic origin of the Floridian haplotypes. The Florida peninsular haplotypes could be derived from low-density cryptic populations that left no fossil record. Alternatively, they could have come from undocumented refugia in the currently submerged Atlantic coastal plain.

Our study recovered a fourth phylogeographic group centred on the lower Mississippi River and Ohio River drainages and extending into southern Ohio (Fig. 1c). The two haplotypes in this group (H5 and H14) were shared with geographically co-distributed *A. saccharinum.* We interpret the geographical overlap of the shared haplotypes as evidence of local introgression, and hypothesize that the most likely parental origin of these haplotypes is *A. saccharinum*, because they are distinctive from other haplotypes in the *A. rubrum* var. *drummondii* cluster (see Fig. 6) and are most abundant and widespread in *A. saccharinum*.

Although *A. rubrum* is a very common tree species in eastern North America, the relationship between intra-specific morphology and molecular variation has not been investigated except for a study on chromosome numbers by Ellis (1963). The present study found a correspondence between the three varieties of *A. rubrum* and cpDNA haplotype groups, which is represented graphically in Fig. 6. Our study provides further evidence of the geographic delimitation and genetic distinctiveness of the three varieties, which justifies their status as separate taxonomic entities.

Contrasts between *A. rubrum* and *A. saccharinum*

There was a clear phylogeographic structure in *A. rubrum* based on the parsimony network (Fig. 1) and SAMOVA (Fig. 4, Table 3). The separately analysed cpSSR data were consistent with cpDNA haplotype data and delimited overlapping geographic groups (Appendices S3 & S4) in *A. rubrum*. Although *A. saccharinum* showed significant phylogeographic structure based on the comparison of N_{ST} and G_{ST}, this structure was weaker than in *A. rubrum*, as indicated by shorter branch lengths in the haplotype network. SAMOVA recognized two groups in *A. saccharinum* compared with the four in *A. rubrum* (Fig. 4), and

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A. saccharinum exhibited significantly lower haplotype diversity in the rarefaction analyses (Table 2).

The lower haplotype diversity and phylogeographic signal in *A. saccharinum* may result from its restricted habitat and geographic distribution. For example, given its relatively narrow riparian habitat preference, it is likely that population bottlenecks have historically been more prevalent at regional scales. At a continental scale, *A. saccharinum* is unable to occupy many parts of the range available to *A. rubrum*, such as the vast coastal plains that would have extended much farther to the east with lowered sea levels of the LGM. The coastal plains do not support *A. saccharinum* because they lack large, meandering rivers with wide alluvial floodplains such as the Mississippi River, with flood water remaining well into the growing season, and they have a prevalence of sands rather than fine-textured alluvial soils. The restricted geographic range of *A. saccharinum*, combined with the relatively narrow distribution of appropriate habitat within its range, would have exacerbated the effects of forest restrictions during the LGM, exposing *A. saccharinum* populations to relatively strong genetic drift, compared with a widespread habitat generalist such as *A. rubrum*.

Introgression and phylogeographic structure in *A. saccharinum*

Of the seven *A. saccharinum* haplotypes recovered in this study, five were shared with proximal *A. rubrum* (Fig. 6). Haplotype sharing might be a result of polymorphism carried from the common ancestor of *A. rubrum* and *A. saccharinum*, or it could result from hybridization and introgression. Because cpDNA evolves relatively slowly, retention of ancestral variation over long time-scales is possible. However, cpSSR polymorphisms of identical lengths are also embedded within the shared cpDNA haplotypes (Fig. 3). Given the rapid stepwise mutation rate of cpSSRs (Provan *et al.*, 2001) it is unlikely that these cpSSR haplotypes would have been maintained through divergence from a common ancestor in the Pliocene. Furthermore, the shared cpDNA haplotypes occur in both species in narrow geographic regions suggestive of hybrid zones. In the lower Mississippi valley, we suggested that the shared haplotypes H14 and H5 were derived from *A. saccharinum*, which finds its favoured habitat in this region. The second region of geographic overlap of haplotype H11 is in the formerly glaciated Vermont and northern Michigan (Fig. 2). Haplotype H11 is widespread in *A. rubrum*, and is nested within other haplotypes associated with *A. rubrum* var. *rubrum* (Fig. 6), suggesting that the haplotype originated in *A. rubrum* and entered *A. saccharinum* through introgression following their post-glacial northern expansion.

Potential reproductive barriers between *A. rubrum* and *A. saccharinum* include asynchronous flowering phenology, cytological variation and difference in habitat preference (Table 1). As Santamour (1993) has pointed out, these are not absolute reproductive barriers, and hybridization and introgression have been reported among even more distantly related species in other diverse tree genera, including oaks (*Quercus*; Belahbib *et al.*, 2001), birches (*Betula*; Palme *et al.*, 2004), poplars (*Populus*; Lexer *et al.*, 2005) and willows (*Salix*; Hardig *et al.*, 2000). Recent studies of introgression between native trees and exotic congeners provide additional evidence of hybridization potential in mulberries (*Morus*; Burgess *et al.*, 2005) and elms (*Ulmus*; Zalapa *et al.*, 2009).

It has long been suggested that hybridization has provided the adaptive genetic variation needed to invade post-glacial habitats (Stebbins, 1972; Excoffier *et al.*, 2009). Introgression may also have an impact on phylogeographic structure and its interpretation. For example, the occurrence of unique haplotypes in northern parts of the *A. rubrum* range has been interpreted as evidence of cryptic northern populations (McLachlan *et al.*, 2005) in regions dominated by tundra and boreal forest trees during the LGM (Jackson *et al.*, 2000). Our study is in general agreement with previous studies of *A. rubrum*. If we had analysed *A. saccharinum* in isolation, however, we might have attributed its restricted northern haplotypes (e.g. H11, H13 and H17 in Fig. 2 and Hssr3, Hssr6 and Hssr7 in Fig. 3b) to the existence of cryptic refugia within the glaciated or boreal habitats of the LGM. Because these northern haplotypes are nearly all shared with *A. rubrum*, a more likely explanation is that they were incorporated in *A. saccharinum* populations following post-glacial expansion and hybridization with *A. rubrum*. This finding suggests that introgression should be considered as an alternative explanation for the origin of phylogeographic groups in other species as well. The role of introgression can be tested by sampling haplotypes from sympatric congeneric species in a comparative phylogeographic context.

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SUPPORTING INFORMATION

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

Appendix S1 Voucher information for sequenced individuals of *Acer rubrum* and *A. saccharinum* and corresponding GenBank accession numbers.
Appendix S2 Haplotype composition of *Acer rubrum* and *A. saccharinum* in eastern North America.
Appendix S3 Comparisons of fixation indices corresponding to the groups of populations detected by SAMOVA in *Acer rubrum* and *A. saccharinum* in eastern North America using chloroplast simple sequence repeat (cpSSR) variation.
Appendix S4 Map of genetic boundaries for *Acer rubrum* (n = 258) defined by SAMOVA at K (number of groups of populations) = 4 based on simple sequence repeat (SSR) polymorphisms.

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BIOSKETCH

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