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Sharing of chloroplast haplotypes among red oak species suggests interspecific gene flow between neighboring populations

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Abstract: The North American red oak species *Quercus rubra*, *Q. ellipsoidalis*, *Q. velutina* and *Q. coccinea* are morphologically similar and showed very low interspecific differentiation at most nuclear genetic markers in our earlier analyses (<10%). However, a few genetic markers showed interspecific differentiation values (up to 84 %) above neutral expectations, a pattern of genomic divergence consistent with models of ecological speciation in the face of gene flow and strong divergent selection. Accordingly, these interfertile species are predicted to maintain differential adaptations to drought while neutral regions of the genome appear to be homogenized by interspecific gene flow. According to this model of maintenance of species integrity by divergent selection with gene flow, we expect a sharing of chloroplast haplotypes between interspecific population pairs. We analyzed maternally inherited chloroplast DNA markers for the first time in interspecific populations of the red oaks (section *Lobatae*) to provide additional evidence for contemporary gene flow between *Q. rubra* and *Q. ellipsoidalis* and between *Q. velutina* and *Q. ellipsoidalis*. Very low interspecific differentiation (G_{ST} = 0.023), but pronounced genetic differentiation among populations from different regions (G_{ST} = 0.277) across species, and sharing of regional chloroplast haplotypes between species in sympatric and neighboring populations provided strong evidence for contemporary interspecific gene flow. The pattern of divergence at chloroplast DNA markers in red oaks suggests interspecific gene flow that resulted in a sharing of chloroplast types while the ecological and morphological distinctness of species was maintained.

Keywords: Quercus rubra, *Q. ellipsoidalis*, *Q. velutina*, chloroplast microsatellites, ecological speciation.

Introduction

Natural hybridization plays an important role in adaptive evolution (Strasburg et al. 2012; Abbott et al. 2013) and the transfer of adaptive genetic variants among species (Arnold 2004; Arnold and Martin 2011). The frequency of hybridization between interfertile species is dependent on pre- and post-zygotic isolation mechanisms, both of which can be affected by the environment (Seehausen et al. 2014). Observational and experimental evidence suggests that selection is a major post-zygotic mechanism in the maintenance of species integrity in hybridizing oaks (Dodd and Afzal-Rafii 2004; Curtu et al. 2007, 2009; de Heredia et al. 2009; Gailing and Curtu 2014; Gailing 2014). Thus, related oak species frequently hybridize in sympatry (Rushton 1993), yet they remain phenotypically and genetically distinct and maintain specific local adaptations for example to drought (Abrams 1990, 1992; Levy et al. 1992; Brendel et al. 2008). At the genome level, interfertile co-occurring European white oak species with different micro-environmental preferences showed a pattern of heterogeneous genomic divergence (Scotti-Saintagne et al. 2004; Goicoechea et al. 2012, 2015) that is predicted as result of strong divergent selection in the face of gene flow (Via and West 2008; Via 2009, 2012).

Genetic marker analyses at genomic and gene-based microsatellite markers indicated interspecific gene flow among *Q. rubra*, *Q. ellipsoidalis* and *Q. velutina,* yet species integrity and different adaptations to drought were maintained (Lind and Gailing 2013; Sullivan et al. 2013; Lind-Riehl et al. 2014; Owusu et al. accepted). As in the European white oaks, these species revealed genomic divergence patterns as predicted by models of divergent selection with gene flow, with most markers showing low interspecific differentiation punctuated by gene loci with differentiation values (F_{ST} values) largely above neutral expectations (outlier loci) (Sullivan et al. 2013; Lind-Riehl et al. 2014). For example, CONSTANS-like 1 was identified as outlier

across multiple population pairs of *Q. rubra* and *Q. ellipsoidalis*, and was nearly fixed on alternative alleles in both species (F_{ST} = 0.55 – 0.84), while the overall neutral interspecific differentiation was below 10% (F_{ST} < 0.10) (Lind-Riehl et al. 2014; Collins et al. 2015). CONSTANS-like 1 is a candidate gene for flowering time (Yano et al. 2000; Alberto et al. 2013) and is involved in growth and development (Herrmann et al. 2010; Hsu et al. 2012).

Additionally, genetic assignment analysis at nuclear DNA markers (genomic and EST-SSRs) in the adult tree, seedling and seed generation provided evidence for gene flow between *Q. rubra* and *Q. ellipsoidalis* as shown by the presence of putative hybrids and introgressive forms that clustered with their parental species (Lind and Gailing 2013; Collins et al. 2015; Owusu et al. accepted). Likewise genetic assignment analyses at genomic and gene-based SSRs revealed evidence for considerable asymmetric gene flow between *Q. ellipsoidalis* and *Q. velutina* (Sullivan 2013; Sullivan et al. submitted).

While we have evidence for interspecific gene flow based on genetic differentiation patterns at genomic and gene-based SSRs (Lind and Gailing 2013; Sullivan 2013; Lind-Riehl et al. 2014; Collins et al. 2015; Owusu et al. accepted) and genome-wide AFLPs (Hipp and Weber 2008), evidence for shared chloroplast haplotypes expected under interspecific hybridization is missing in the red oaks. Chloroplast DNA analyses in more than 2600 white oak populations across Europe revealed a strong phylogeographic pattern, but a nearly complete lack of interspecific differentiation between the two dominant European white oak species *Q. robur* and *Q. petraea* with different soil moisture preferences (Petit et al. 2003b). This lack of differentiation was explained by interspecific gene flow resulting in the invasion of the late-successional species *Q. petraea* into the range of the pioneer species *Q. robur* (Petit et al. 2003b).

In the present study we adapted universal chloroplast microsatellites (cpSSRs) (Weising and Gardner 1999) and cpSSRs originally developed for *Q. robur* (Deguilloux et al. 2003) to assess the distribution of cpDNA haplotypes in the red oaks *Q. rubra*, *Q. ellipsoidalis, Q. velutina* and *Q. coccinea*. Uniparental maternal inheritance and the absence of recombination usually result in very low chloroplast haplotype diversity within populations. However, high differentiation across geographic regions within species can be the result of relatively recent population history, such as postglacial recolonization (Palmé et al. 2003; Petit et al. 2003a; Heuertz et al. 2004; Finkeldey and Gailing 2013). On the other hand, sharing of chloroplast types among closely related species in sympatric or neighboring stands indicated interspecific gene flow, for example for oaks, birches and eucalypts (e.g. Petit et al. 2003b; Palmé et al. 2004; Nevill et al. 2014). In this study we selected sites in which the population pairs of *Q. rubra* - *Q. ellipsoidalis* and of *Q. ellipsoidalis* - *Q. velutina* are sympatric, to test our expectation of shared chloroplast haplotypes. Sharing of regional chloroplast haplotypes between neighboring interspecific population pairs and differentiation among regions independent of the species would strongly implicate recurrent interspecific gene flow. On the other hand, strong differentiation between species would indicate absence of or very limited contemporary interspecific gene flow.

According to the model of maintenance of species integrity by divergent selection with interspecific gene flow we expect a sharing of chloroplast haplotypes among interspecific species pairs.

Materials and methods

Plant materials

Samples were collected from 37 populations including 20 *Q. rubra* populations, 11 *Q. ellipsoidalis* populations, five *Q. velutina* populations and one *Q. coccinea* population (Table 1, Fig. 1, suppl. Fig. 1). *Quercus rubra* / *Q. ellipsoidalis* population pairs (sympatric or neighboring stands) were collected from four geographic regions, and *Q. velutina* / *Q. ellipsoidalis* population pairs (sympatric stands) were sampled from five geographic regions (Table 1, suppl. Fig. 1). The species show largely overlapping distribution ranges in eastern North America (Hipp and Weber 2008). Populations of *Q. rubra*, *Q. ellipsoidalis* and *Q. velutina* can be found in sympatry, but preferentially occur in different micro-environments with regard to water availability (Hipp and Weber 2008; Owusu et al. accepted). Thus, *Querucs ellipsoidalis* is considered as most drought-tolerant species, followed by *Q. coccinea*, *Q. velutina* and *Q. rubra* as the most mesophilic species (Abrams 1990). Analyses at genomic and gene-based microsatellite markers between these population pairs revealed low interspecific differentiation at most nuclear markers, but high differentiation at a few outlier loci suggesting strong divergent selection (Sullivan et al. 2013; Lind-Riehl et al. 2014). Species identity was confirmed based on whole tree silvic and leaf morphological characters (Hipp and Weber 2008; Hipp et al. 2010) for all populations and by genetic assignment analysis at discriminative microsatellite markers for most populations (Hipp and Weber 2008; Lind and Gailing 2013; Lind-Riehl et al. 2014; Owusu et al. accepted). Specifically, morphological species identification was based on leaf, bud and acorn characteristics. For example, end buds are mainly hairless in *Q. rubra*, silky-pubescent in *Q. ellipsoidalis* and *Q. coccinea* and densely canescent in *Q. velutina* (Hipp and Weber 2008). Samples from the sympatric *Q. rubra* / *Q. ellipsoidalis* populations FCF-QR/FCF-QE and ES-

125 QR/ES-QE were identified in the field only based on whole tree silvic characteristics and leaf
126 morphological characters that differentiate between both species (Gailing et al. 2012). For most
127 populations eight genetically identified samples (5 to 8) were randomly selected from each stand
128 for the cpDNA analyses (Table 1). Plant material for four populations (HNF-QR, ONF-QR,
129 ANF-QR and HMNF-QR) was derived from seed-grown seedlings obtained from the J. W.
130 Toumey Nursery (USDA Forest Service, Watersmeet, Michigan). Seeds were collected
131 randomly within populations.

132 **Chloroplast DNA analyses**

133 Total genomic DNA (~ 20 ng) was isolated from fresh or silica gel dried leaf material following
134 the DNeasy96 Plant Kit protocol of Qiagen (Hilden, Germany). A total of ten consensus
135 chloroplast microsatellite primers (*ccmps*) developed for dicotyledonous angiosperms (Weising
136 and Gardner 1999) and three chloroplast microsatellites developed for *Quercus* species (*udt4*,
137 *ukk4*, *ucd4*) (Deguilloux et al. 2003) were tested for amplification and polymorphism on eight
138 samples from eight geographically distant populations including all four species. Since cpDNA
139 alleles are jointly transmitted due to the absence of recombination (Finkeldey and Gailing 2013)
140 corresponding haplotypes can be inferred at different sets of polymorphic markers. Thus, these
141 chloroplast microsatellites have been applied earlier in European white oaks and distinguished
142 several chloroplast haplotypes that had been identified by PCR-RFLP (Gailing et al. 2009; Petit
143 et al. 2002a, b), and there was no indication of size homoplasy for these markers (Gailing et al.
144 2007 a, b; Gailing et al. 2009). Nine markers generated amplification products, three of which
145 (*ccmp2*, *ccmp4* and *udt4*) were found to be polymorphic, and were used to screen 287 samples
146 (5–8 individuals from each population). DNA was diluted (1:20) prior to PCR amplification.
147 PCR reactions were performed in the Applied Biosystems 2720 thermal cycler in a 15 µl reaction

mixture containing 6µl ddH_2O, 3 µl HotFIREPol master mix from Oak Biotechnologies (containing 10 mM Tris–HCL (pH 9.0), 10 mM $MgCl_2$, 2 mM of each dNTP, 0.4U HOTFIREpol Taq DNA polymerase), 2 µl of each forward and reverse primer (5 pmol/µl) and 2 µl of diluted DNA (about 1 ng). The forward primers were labeled with the fluorescent dye 6-FAM. The PCR profile for the three primers was 15 min initial denaturation at 95℃, followed by 35 cycles of 45 sec denaturation at 94℃, 45 sec annealing at 50℃ and 1 min extension at 72℃, with a final extension of 10 min at 72℃. The PCR products were tested on 2 % agarose gels and then diluted for genotyping on an ABI Prism Genetic Analyzer 3730 (Applied Biosystems) at Cornell University. Fragments were scored using GeneMapper v.4.0 (Applied Biosystems).

Data analyses

Polymorphisms in fragment size were identified as different length variants that were combined to define haplotypes (suppl. Fig. 1). Total haplotypic diversity and diversity within populations/species was calculated as H_T and H_S, and as R_T and R_S which take mutational differences among haplotypes into account. Likewise genetic differentiation among populations was computed as G_{ST} and R_{ST} with PermutcpSSR 2.0 (available at http://www.pierroton.inra.fr/genetics/labo/Software/ PermutCpSSR/index.html) as described in Pons and Petit (1995). Since only one population of *Q. coccinea* was analyzed, these samples were excluded for the calculation of G_{ST}/R_{ST} among species. Haplotype networks were created with the program Network 4.6.1.2. (released January 1, 2014, http://www.fluxus-engineering.com/netwinfo.htm) (Bandelt et al. 1995; Bandelt et al. 1999) using the reduced median method. Specifically, the haplotype data were saved as *.ych file and run through star contraction; the contracted data (saved as *.rdf file) were calculated with the reduced median method and results were saved as *.sco file for network calculation.

Results

Out of all cpSSRs tested the three markers *ccmp2*, *ccmp4* and *udt4* showed clear and polymorphic amplification products (suppl. Fig. 2). Combining the observed fragment lengths at these three markers, a total of 23 haplotypes were observed (suppl. Fig. 1), ten of which were present in more than one sample (Table 1). Haplotype 1 (H1) was the most frequent haplotype overall (70.04%) and for each species (Table 1, 2, Fig. 1). The closely related haplotype H2 was rare in most populations, but was dominating in the easternmost *Q. rubra* population ANF-QR. Also the other more frequent haplotypes were shared among at least two species (Table 2, Fig. 1, 2). For example, haplotype H9 was restricted to a single geographic region (OF) and was shared between a neighboring *Q. ellipsoidalis* / *Q. velutina* population pair (Table 1, Fig. 1). As a general pattern, neighboring interspecific population pairs shared haplotypes, strongly suggesting contemporary interspecific gene flow (Fig. 1). As a consequence genetic differentiation among the species *Q. rubra*, *Q. ellipsoidalis* and *Q. velutina* was very low (G_{ST} = 0.023, R_{ST} = 0.016), while much higher genetic differentiation was found among all populations (G_{ST} = 0.277, R_{ST} = 0.329). Haplotypic diversity within populations was comparatively high for each species (Table 3, 4). Within species the highest differentiation among populations was observed for *Q. velutina* (G_{ST} = 0.5771, R_{ST} = 0.5709) as two out of the five populations were fixed for H1 and two other populations showed a high frequency of two other haplotypes with restricted geographic distribution shared with the neighboring *Q. ellipsoidalis* populations (Fig. 1). The genetic differentiation between *Q. rubra* (G_{ST} = 0.206, R_{ST} = 0.253) and *Q. ellipsoidalis* (G_{ST} = 0.240, R_{ST} =0.301) populations was comparatively low. Private haplotypes were present in *Q. rubra*, *Q. ellipsoidalis* and *Q. velutina*, but occurred mostly in single individuals (Table 2). Overall the genetic differentiation between populations at cpDNA markers confirmed earlier observations of

a comparatively weak phylogeographic pattern. Among *Q. rubra* populations, only populations ANF-QR and N-QR did not show H1 as predominant haplotype. Since our sampling was not range-wide for any of the three species and the number of populations for each species was different, variation estimates within and among species are representative for our sampling and might vary in other parts of the species' range.

Discussion

Haplotype diversity

While most samples were collected from adult trees in natural stands subjected to no or limited management, population ES-QR was collected close to a campground, and the high haplotype diversity and occurrence of rare haplotypes in this population suggests that at least some of the trees were planted. For populations HNF-QR, ONF-QR, ANF-QR and HMNF-QR seed-grown seedlings were obtained from a nursery and mixing of reproductive material from different stands within one region was not excluded. However, only one population (ONF-QR) showed a high haplotype diversity which might indicate mixing of reproductive material.

The weak phylogeographic pattern found in the present study confirmed earlier results of PCR-RFLP analyses of cpDNA variation of 66 *Q. rubra* populations collected throughout the species' range (Magni et al. 2005). Similar to our multispecies study, a star-like phylogenetic network was derived with one predominating haplotype occurring in ~ 75 % of the samples (~ 70% in the present study). While our sampling was intensive in the Upper Midwest and focused on interspecific populations pairs, the earlier *Q. rubra* study had a stronger representation of eastern populations. Population differentiation in the latter study (G_{ST} = 0.46) and in the present study was comparatively low (*Q. rubra*, G_{ST} = 0.206; *Q. ellipsoidalis*, G_{ST} = 0.243; *Q. velutina*, G_{ST} =

0.558). Likewise, cpDNA analyses in the northwestern section of the range showed moderate levels of population differentiation (G_{ST} = 0.58) in *Q. rubra* (Birchenko et al. 2009) displaying the highest differentiation values in the southeastern part of the sampling area (Romero-Severson et al. 2003). The overall low differentiation at cpDNA markers was attributed to the relatively large area without major geographical barriers occupied by *Q. rubra* forests during the Last Glacial Maximum (LGM) in North America, with some populations reaching far north close to the glacial margin (Williams 2002; Magni et al. 2005). The existence of one major distribution range during the LGM might have provided only restricted opportunities for northward migration (only a few hundred kilometers) and for long distance founder events (Magni et al. 2005). On the other hand, geographic isolation of refugia and resulting genetic drift might have resulted in new cpDNA lineages and high genetic differentiation among populations from different refugia in Europe (Petit et. 2003a). European white oak populations re-colonized the tundra-like, treeless landscape of central and northern Europe from three discrete glacial refugia, the Iberian and Italian Peninsulas and the Balkans, with distinct chloroplast lineages as reflected in strong cpDNA differentiation among geographic regions (Petit et al. 2002a; Petit et al. 2002b; Petit et al. 2003a). Likewise, the contemporary distribution of cpDNA haplotypes suggested long distance seed dispersal and founder events that resulted in today's strong genetic differentiation at cpDNA markers among populations (Petit et al. 2002a; Petit et al. 2002b; Petit et al. 2003a). In summary, since European white oaks and North American red oaks show similar life history traits, differences in genetic structure at cpDNA markers could have been affected by the isolation among refugia, different opportunities for northward migration and long distance seed dispersal after the Last Glacial Maximum, by interspecific gene flow and by different topologies in Europe and North America (Magni et al. 2005; Petit et al. 2003a).

Sharing of chloroplast haplotypes among species

Similar to interfertile European white oaks (Petit et al. 2003b), no significant differentiation among the red oak species was detectable at cpDNA markers. Low genetic differentiation can be the result of ancestral polymorphism shared among species (Muir and Schlötterer 2005), even in the absence of gene flow, or the outcome of contemporary interspecific gene flow. Our results provide clear evidence for contemporary gene flow between species. Thus, all haplotypes (with a frequency larger than two) were shared among at least two species, and most haplotypes were shared among the three species *Q. rubra*, *Q. ellipsoidalis* and *Q. velutina*. The only population of *Q. coccinea* shared H1 with all other species and the other haplotype, H4, with *Q. rubra*. The sharing of haplotypes (see Fig. 2) among species and differentiation among populations from different geographic regions strongly suggested recurrent interspecific gene flow. Additionally, both frequent and regional haplotypes were shared between species in sympatric and neighboring stands. Thus, regional haplotypes with restricted geographic distribution H9 (region OF) was shared between *Q. ellipsoidalis* / *Q. velutina*, and H7 was shared between *Q. rubra* / *Q. ellipsoidalis* (region NN) and *Q. velutina* / *Q. ellipsoidalis* (region WR). The sharing of haplotypes with restricted geographic distribution between these neighboring stands provides conclusive evidence for interspecific gene flow.

Interspecific gene flow between interfertile red oak species was also reflected in very low interspecific differentiation among the red oak species *Q. rubra*, *Q. ellipsoidalis*, *Q. velutina* and *Q. coccinea* at most nuclear DNA markers (Sullivan et al. 2013) and a pattern of genomic divergence consistent with the maintenance of species integrity in the face of gene flow by divergent selection in neighboring *Q. rubra* / *Q. ellipsoidalis* (Lind-Riehl et al. 2014) and *Q. velutina* / *Q. ellipsoidalis* populations (Sullivan 2013; Sullivan et al. submitted). Genomic

signatures of divergent selection in the red oaks (Sullivan et al. 2013; Lind-Riehl et al. 2014) and the European white oaks (Goicoechea et al. 2012, 2015) between co-occurring interfertile species with different local adaptations suggests that oaks in general are models to study the genomic basis of speciation and maintenance of species integrity with gene flow.

Outlook

The sharing of cpDNA haplotypes was expected according to the model of maintenance of species integrity with gene flow by divergent selection. The availability of genetic linkage maps in *Q. rubra* (unpublished results) and in *Q. robur* (Bodénès et al. 2012; Gailing et al. 2013) and of a genome sequence in *Q. robur* (Plomion et al. 2015) will allow us to compare the genomic distribution of regions under divergent selection across taxonomic sections.

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465 **Table 1.** Sample locations and haplotype frequencies

abbreviation	region	species	N	latitude	longitude	haplotype frequencies									
						1	2	3	4	5	6	7	9	16	19
FCA-QR	FC	*Q. rubra*	8	46°39'09"N	88°30'07"W	7	0	0	0	0	0	0	0	0	0
FCB-QR	FC	*Q. rubra*	8	46°40'28"N	88°31'27"W	7	0	0	0	0	0	0	0	0	0
FCC-QE	FC	*Q. ellipsoidalis*	8	46°39'14"N	88°35'26"W	7	0	1	0	0	0	0	0	0	0
FCE-QE	FC	*Q. ellipsoidalis*	8	46°39'56"N	88°33'20"W	6	0	0	0	2	0	0	0	0	0
FCF-QR	FC	*Q. rubra*	8	46°40'29"N	88°32'06"W	7	0	0	0	0	0	0	0	0	0
FCF-QE	FC	*Q. ellipsoidalis*	8	46°40'29"N	88°32'06"W	6	0	0	0	0	2	0	0	0	0
ES-QR	ES	*Q. rubra*	8	45°04'01"N	86°59'38"W	3	0	0	1	2	1	0	0	0	0
ES-QE	ES	*Q. ellipsoidalis*	8	45°04'01"N	86°59'38"W	6	1	0	0	0	0	0	0	0	0
N-QR	NN	*Q. rubra*	8	45°20'53"N	88°23'17"W	3	0	0	0	0	0	4	0	0	0
N-QE	NN	*Q. ellipsoidalis*	8	45°19'19"N	88°19'53"W	2	0	0	0	0	0	4	1	0	1
C-QR	CN	*Q. rubra*	8	46°42'54" N	91°02'08"W	8	0	0	0	0	0	0	0	0	0
C-QE	CN	*Q. ellipsoidalis*	8	46°44'43"N	91°04'20"W	8	0	0	0	0	0	0	0	0	0
W-QE	WR	*Q. ellipsoidalis*	8	41°04'03"N	87°54'10"W	4	1	0	0	0	0	3	0	0	0
W-QV	WR	*Q. velutina*	8	41°04'03"N	87°54'10"W	0	0	0	0	0	0	7	0	0	0
O-QE	OF	*Q. ellipsoidalis*	8	44°26'59"N	84°12'04"W	2	0	1	0	0	0	0	5	0	0
O-QV	OF	*Q. velutina*	8	44°26'59"N	84°12'04"W	2	0	0	0	0	0	0	5	0	0
R-QE	RE	*Q. ellipsoidalis*	5	44°40'06"N	88°05'28"W	5	0	0	0	0	0	0	0	0	0

R-QV	RE	*Q. velutina*	5	44°40'06"N	88°05'28"W	5	0	0	0	0	0	0	0	0	0
N-QE	NE	*Q. ellipsoidalis*	8	43°02'11"N	85°48'00"W	8	0	0	0	0	0	0	0	0	0
N-QV	NE	*Q. velutina*	8	43°02'11"N	85°48'00"W	8	0	0	0	0	0	0	0	0	0
H-QE	HP	*Q. ellipsoidalis*	8	41°31'35"N	87°26'35"W	3	1	0	0	2	0	0	0	0	0
H-QV	HP	*Q. velutina*	8	41°31'35"N	87°26'35"W	5	1	0	0	1	1	0	0	0	0
HMR-PL	HMR	*Q. rubra*	8	46°53'19"N	87°52'04"W	8	0	0	0	0	0	0	0	0	0
HMR-MI	HMR	*Q. rubra*	8	46°51'21"N	87°51'24"W	7	0	0	0	0	0	0	0	0	0
HMR-LI	HMR	*Q. rubra*	8	46°04'39"N	87°51'18"W	8	0	0	0	0	0	0	0	0	0
HMR-LP	HMR	*Q. rubra*	7	46°51'00"N	87°49'49"W	6	1	0	0	0	0	0	0	0	0
HMR-IH	HMR	*Q. rubra*	8	46°51'13"N	87°04'43"W	4	0	0	0	4	0	0	0	0	0
HNF-QR	HNF	*Q. rubra*	8	46°13'58"N	86°30'36"W	6	2	0	0	0	0	0	0	0	0
ONF-QR	ONF	*Q. rubra*	8	46°13'48"N	88°57'00"W	2	1	1	0	1	0	2	0	1	0
ANF-QR	ANF	*Q. rubra*	8	41°38'23"N	79°06'35"W	2	5	0	0	0	0	0	0	0	0
HMNF-QR	HMNF	*Q. rubra*	8	44°36'37"N	83°04'04'W	8	0	0	0	0	0	0	0	0	0
HL-QR	HL	*Q. rubra*	6	44°07'00"N	84°45'00"W	6	0	0	0	0	0	0	0	0	0
MTU-QR	MTU	*Q. rubra*	8	47°06'02"N	88°32'51"W	7	0	0	0	0	0	0	0	1	0
BR-QR	BR	*Q. rubra*	8	47°27'57"N	87°54'59"W	6	0	0	0	0	0	0	0	0	1
HC-QR	HC	*Q. rubra*	8	35°43'00"N.	88°17'00"W	7	0	0	0	1	0	0	0	0	0
MC-QR	MC	*Q. rubra*	8	34° 45'00”N	88°17'00"N	5	0	1	0	2	0	0	0	0	0
RRT-QC	RRT	*Q. coccinea*	8	37°36'29"N	88°16'17"W	7	0	0	1	0	0	0	0	0	0

Haplotypes H7 and H9 (see Fig. 1) with restricted geographic distribution that are shared among species pairs are highlighted in grey. FC: Ford Forestry Center, MI; ES: Escanaba, MI; NN: Nicolet National Forest, WI; WR: Wolf Road Prairie, IL; RE: Reforestation Road, WI; HP: Hoosier Prairie, IN; NE: Newaygo, MI; CN: Chequamegon National Forest, WI; OF: Oilfields, MI; HMR: Huron Mountain Reserve, MI; HNF: Hiwatha NF, MI; ONF: Ottawa NF, MI; ANF: Allegheny NF, PA; HMNF: Huron Manistee NF,MI; HL: Halfmoon Lake, MI; MTU: Michigan Technological Trails, MI; BR: Brockway Mountain, MI; HC: Tennessee Henderson County, TN; MC: Madison County, AL; RRT: Rim Rock Trail, IL

472 **Table 2.** Haplotype description and frequency

Haplotype	fragment size (bp)			f	f (%)	species			
	ccmp2	*ccmp4*	*udt4*			*Q. rubra*	*Q. ellipsoidalis*	*Q. velutina*	*Q. coccinea*
1	228	116	145	201	70.04	74.91	68.18	57.50	87.50
2	228	116	146	13	4.53	5.71	3.41	2.50	
3	227	116	145	4	1.39	1.25	2.27		
4	227	116	146	2	0.70	0.63			12.50
5	227	115	146	15	5.23	6.30	4.55		
6	226	116	145	4	1.39	0.63	2.27	2.50	
7	226	118	146	20	6.97	3.75	7.95	20.00	
8*	226	118	145	1	0.35	0.63			
9	226	117	146	11	3.83		6.82	12.50	
10*	226	115	146	1	0.35		1.14		
11*	225	116	145	1	0.35			2.50	
12*	229	118	147	1	0.35	0.63			
13*	227	117	145	1	0.35	0.63			
14*	228	120	145	1	0.35	0.63			
15*	229	114	143	1	0.35	0.63			

16	229	116	145	2	0.70	1.25		
17*	229	116	146	1	0.35	0.63		
18*	230	116	145	1	0.35		1.14	
19	234	116	145	2	0.70	0.63	1.14	
20*	224	115	144	1	0.35		1.14	
21*	226	115	143	1	0.35	0.63		
22	228	116	139	1	0.35	0.63		
23*	229	117	146	1	0.35			2.50

473 f: frequency, *: haplotypes observed in a single population (private haplotypes)

474

475 **Table 3.** Genetic variation within populations

Species	population	N	N_a	H_S (seH_S)	Pb[5]-1
Q. rubra	FMF-QR	8	2	0.250±0.180	0.625
	ES-QR	8	5	0.857±0.108	2.750
	HNF-QR	8	2	0.429±0.169	0.893
	ONF-QR	8	6	0.929±0.084	3.286
	ANF-QR	8	3	0.607±0.164	1.518
	HMNF-QR	8	1	0±0	0
	FCA-QR	8	2	0.250±0.180	0.625
	FCB-QR	8	2	0.250±0.180	0.625
	HMR-PL	8	1	0±0	0
	HMR-MI	8	2	0.250±0.180	0.625
	HMR-LI	8	1	0±0	0
	HMR-LP	7	2	0.286±0.196	0.714
	HMR-IH	8	2	0.571±0.094	1
	N-QR	8	3	0.679±0.122	1.607
	C-QR	8	1	0±0	0
	HL-QR	6	1	0±0	0
	MTU-QR	8	2	0.250±0.180	0.625
	BR-QR	8	3	0.464±0.200	1.250
	HC-QR	8	2	0.250±0.180	0.625
	MC-QR	8	3	0.607±0.164	1.518
	mean	7.85	2.3	0.346±0.119	0.8643
Q. ellipsoidalis	FMF-QE	8	2	0.429±0.169	0.893
	ES-QE	8	3	0.464±0.200	1.250

	FCC-QE	8	2	0.250±0.180	0.625
	FCE-QE	8	2	0.429±0.169	0.893
	N-QE	8	4	0.750±0.139	2.143
	C-QE	8	1	0±0	0
	W-QE	8	3	0.679±0.122	1.607
	O-QE	8	3	0.607±0.164	1.518
	R-QE	5	1	0±0	0
	N-QE	8	1	0±0	0
	H-QE	8	5	0.857±0.108	2.750
	mean	7.727	2.455	0.406±0.114	1.062
	W-QV	8	2	0.250±0.180	0.625
	O-QV	8	3	0.607±0.164	1.518
Q. velutina	R-QV	5	1	0±0	0
	N-QV	8	1	0±0	0
	H-QV	8	4	0.643±0.184	1.875
	mean	7.4	2.2	0.300±0.106	0.804
Q. coccinea	RRT-QC	8	3	0.607±0.164	1.518

N: number of samples, N_a: number of haplotypes, H_S: genetic diversity with standard error, Pb[5]-1: haplotypic richness

479 **Table 4.** Genetic diversity of each species and differentiation among populations

species	H_S	H_T	G_{ST}	R_S	R_T	R_{ST}
Q.rubra	0.346	0.436	0.206	2.057	2.415	0.253
Q.ellipsoidalis	0.406	0.536	0.243	2.062	2.518	0.301
Q.velutina	0.300	0.709	0.577	1.804	2.873	0.571
Overall	0.355	0.491	0.277	1.935	2.393	0.329

480 H_S: genetic diversity within populations, H_T: total genetic diversity, R_S: within populations
481 diversity taking mutational differences into account, R_T: total genetic diversity taking
482 mutational differences into account, G_{ST}: genetic differentiation, R_{ST}: genetic differentiation
483 taking mutational differences into account.

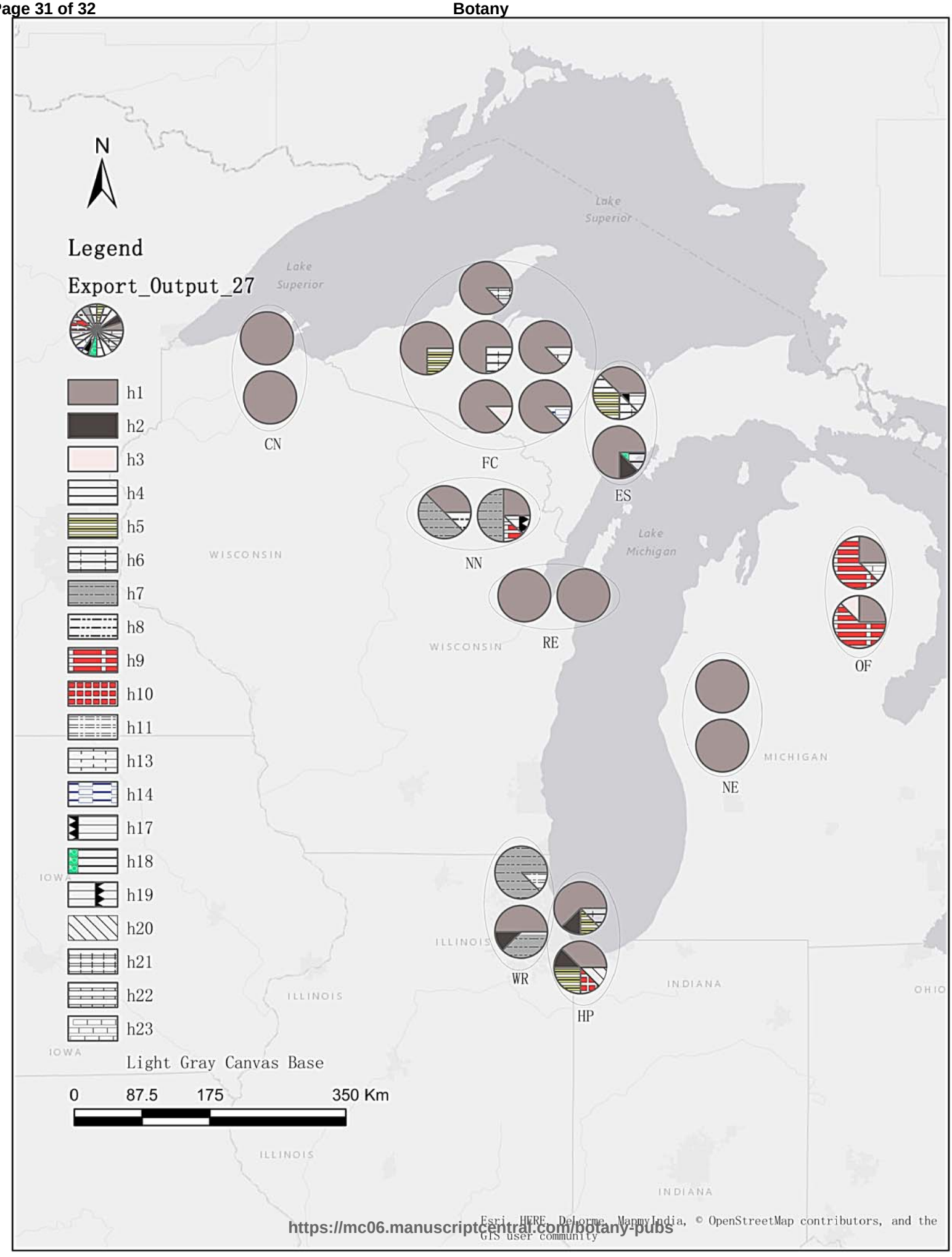
484

485 **Figure Legends**

486 **Fig. 1.** Haplotype distribution shown only for interspecific sympatric stands (see also Table 1).
487 Haplotypes are shared between species in neighboring stands, but differentiated among
488 geographic regions. Region names are included and correspond to names in Table 1.

489 **Fig. 2.** Haplotype networks excluding haplotypes that occur in only a single sample. Sharing of
490 haplotypes among species *Q. rubra* (Qr), *Q. ellipsoidalis* (Qe), *Q. velutina* (Qv) and *Q. coccinea*
491 (Qc) is shown. The size of the circles is proportional to the frequency of haplotypes.

492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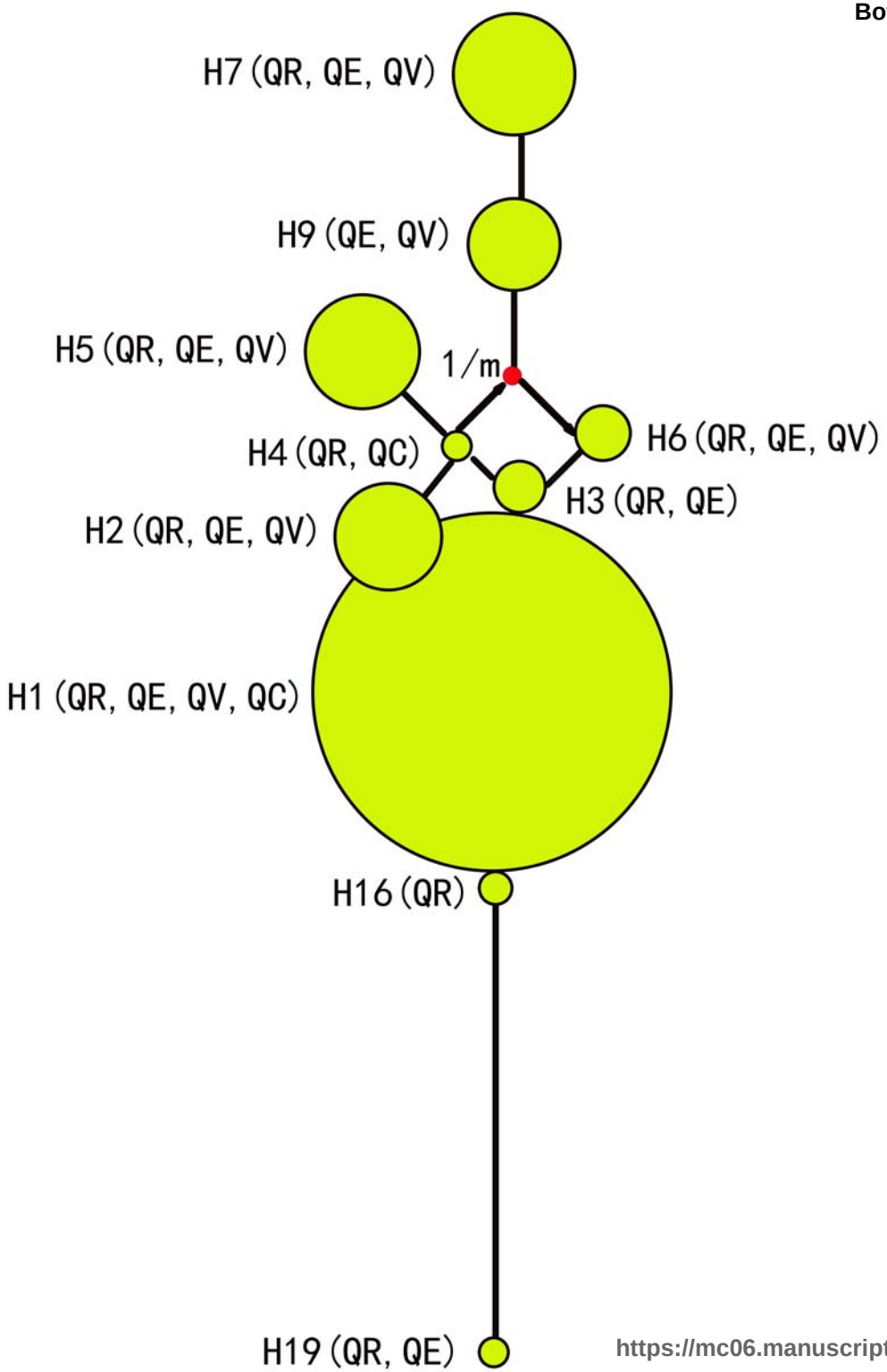
H7 (QR, QE, QV)
H9 (QE, QV)
H5 (QR, QE, QV)
1/m
H6 (QR, QE, QV)
H4 (QR, QC)
H3 (QR, QE)
H2 (QR, QE, QV)
H1 (QR, QE, QV, QC)
H16 (QR)
H19 (QR, QE)