Phylogeographic Analyses of a Boreal-Temperate Ectomycorrhizal Basidiomycete, *Amanita muscaria*, Suggest Forest Refugia in Alaska During the Last Glacial Maximum

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Abstract Beringia, including Alaska and North-Eastern Siberia, has long been a focal point for biogeographical research in a wide range of plant and animal taxa. Whether boreal forest refugia existed in Beringia during the Last Glacial Maximum (LGM) is a major but unanswered question in Quaternary science. We analysed DNA sequence data from *Amanita muscaria* using phylogenetic and coalescent methods to test whether this boreal-temperate ectomycorrhizal fungus was present in Alaska during the LGM. Our data indicates that there are at least two endemic groups showing no detectable migration from or to populations that survived the LGM in Southern refugia. One of these occurs in the boreal forests of Interior Alaska, while the other group is endemic to the maritime rainforests from Southeast Alaska to the Olympic peninsula. These results suggest genetic isolation preceding the LGM and support the hypothesis of glacial forest refugia in Interior Alaska and along the coast of Southeast Alaska and British Columbia.

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1 Introduction

Climatic and geological changes in the Tertiary and Quaternary dramatically influenced the distribution of flora and fauna in North America. In the Tertiary, North America became separated from Eurasia, resulting in vicariant populations of many species of the ancient flora and fauna (Budantsev 1992; Graham 1999). During glacial maxima, plants, fungi and animals were forced to unglaciated refugia, from where they expanded to newly exposed areas in warmer interglacial periods. Reconstruction of these historical events is of paramount importance because they had major influences on past speciation events and are reflected in present day population structures.

Beringia, including Alaska and North-Eastern Siberia, has long been a focal point for biogeographical research in a wide range of plant and animal taxa. This high level of interest arises for two principal reasons. First, due to its diverse landscape and climate and the fact that much of the region remained ice-free during glacial maxima, Beringia served as a refugium for Arctic and subarctic flora and fauna (Adams and Faure 1997; Brubaker et al. 2005; Edwards et al. 2000; Hultén 1968). Second, during much of the Tertiary and the Quaternary periods, Beringia was the major land connection between Asia and North America and provided migration routes to a wide variety of organisms (for example, see Elias et al. 2000; Qian 1999; Swanson 2003). High genetic diversity and evidence for glacial refugia in Beringia have been reported in several, predominantly arctic-alpine animal and plant taxa, such as the Tundra Vole (*Microtus oeconomus*) (Brunhoff et al. 2003), Bighorn Sheep (*Ovis canadensis*) and Dall Sheep (*O. dalli*) (Loehr et al. 2005), Columbian Ground Squirrel (*Spermophilus columbianus*) (MacNeil and Strobeck 1987), Old World Swallowtail (*Papilio machaon*) (Sperling and Harrison 1994), *Amara alpina*, an arctic-alpine ground beetle (Reiss et al. 1999), *Paranoplocephala arctica*, a parasitic cestode of collared lemmings (Wickström et al. 2003), Entire-leaved Avens (*Dryas integrifolia*) (Tremblay and Schoen 1999), Purple Saxifrage (*Saxifraga oppositifolia*) (Abbott and Comes 2003), Arctic Bell-heather (*Cassiope tetragona*) (Eidesen et al. 2007) and the Bog Blueberry (*Vaccinium uliginosum*) (Alsos et al. 2005).

Whether fragments of boreal forest existed in Beringia during the Last Glacial Maximum (LGM) is a major, but, as yet, unanswered question in Quaternary science. Although earlier pollen-based biome reconstructions suggest that all of Beringia was covered by arctic tundra with no conifers (Edwards et al. 2000; Kaufman et al. 2004; Swanson 2003), phylogeographic analyses of DNA sequences and the most recent pollen data support the theory that *Picea glauca* and *P. mariana* existed in small refugia in Alaska (Anderson et al. 2006; Brubaker et al. 2005).

Ectomycorrhizal (ECM) fungi are obligate root-associated mutualistic symbionts of trees in boreal, temperate and tropical forests (Smith and Read 1997; Bas 1969; Härkönen et al. 1994; Simmons et al. 2002) and their past and present populations likely have been shaped by the distribution of their host trees. The likely importance of host trees in the distribution of ECM fungi has been repeatedly noted.

However, despite their ecological importance, very little is known about ECM phylogeography. Furthermore, learning about the phylogeographic history of ECM taxa not only provides information related to the organism in question, but may also help to resolve whether or not their hosts survived the LGM in Beringian refugia. To test the hypothesis of persistent LGM forest refugia in Alaska, we analysed molecular data from *Amanita muscaria*, an ECM fungus native to temperate and boreal forest regions of the Northern Hemisphere.

2 Materials and Methods

Multi-locus DNA sequence data for *A. muscaria* specimens were generated in previous studies (Geml et al. 2006, 2008, and Oda et al. 2004). Geml et al. (2006) reported that there are at least three phylogenetic species clades within the *A. muscaria* species complex that occur in Alaska (referred to as Clades I, II, and III). To our knowledge, Clade I is restricted to North America, while Clades II and III occur in Eurasia and in Alaska. For our tests in this study, 114 ITS rDNA sequences were chosen, which represent the three phylogenetic species. Because the phylogenetic species mentioned are non-interbreeding entities, population-level analyses were conducted separately for each species clade. Multiple sequence alignments were made using Clustal W (Thompson et al. 1997) and subsequently were corrected manually. Identical sequences were collapsed into haplotypes using SNAP Map (Aylor and Carbone 2003) after recoding insertion or deletions (indels) and excluding infinite-sites violations. The analyses presented here assume an infinite sites model, under which a polymorphic site is caused by exactly one mutation and there can be no more than two bases segregating. Base substitutions were categorised as phylogenetically uninformative or informative, and as transitions or transversions. Site compatibility matrices were generated from each haplotype dataset using SNAP Clade and SNAP Matrix (Markwordt et al. 2003) to examine compatibility/incompatibility among all variable sites, with four resultant incompatible sites removed from the data set. This was important as subsequent coalescent analyses assume that all variable sites are fully compatible. Two migration models were used. First, MDIV (Nielsen and Wakeley 2001) was used to determine whether there was any evidence of migration between Alaskan and non-Alaskan populations in each species clade, i.e. to test whether Alaskan populations could have survived the LGM in local forest refugia. For this purpose, specimens were assigned either to the 'Alaskan' or the 'non-Alaskan' group based on their localities. In Clades I and II, the 'non-Alaskan' groups consisted of specimens collected in the contiguous states of the US or in Eurasia, respectively. These represented populations that survived the LGM in Southern refugia. MDIV implements both likelihood and Bayesian methods using Markov chain Monte Carlo (MCMC) coalescent simulations to estimate the migration rate (M), population mean mutation rate (*Theta*), divergence time (T) and the time since the most recent common ancestor (TMRCA). This approach assumes that all populations descended from one panmictic population that may or may not have been followed by migration. For each dataset, the data was simulated assuming an infinite sites model with uniform prior. We used 2,000,000 steps in the chain for estimating the posterior probability distribution and an initial 500,000 steps to ensure that enough genealogies were simulated before approximating the posterior distribution. Second, if MDIV showed evidence of migration, MIGRATE was used to estimate migration rates assuming equilibrium migration rates (symmetrical or asymmetrical) in the history of the populations (Beerli and Felsenstein 2001). We applied the following specifications for the MIGRATE maximum-likelihood analyses: *M* and *Theta* generated from the FST calculation, migration model with variable *Theta* and constant mutation rate. Subsequently, we reconstructed the genealogy with the highest root probability, the ages of mutations and the TMRCA of the sample using coalescent simulations in Genetree v. 9.0 (Griffiths and Tavaré 1994). Ages were measured in coalescent units of 2*N*, where *N* is the population size.

3 Results

Two of the three species clades (Clades I and II) possessed sufficient variation in the ITS to test whether the Alaskan populations were genetically differentiated from non-Alaskan populations. The third, Clade III, was only represented by ten individuals forming three haplotypes with minimal variation. In this species, all Alaskan specimens carried the most frequent haplotype that was shared with Asian specimens as well. The datasets consisted of 653 and 640 characters for Clade I and Clade II, respectively, including gaps.

In Clade I, the ‘North American’ clade, 15 haplotypes were found, of which only one, haplotype D, was found in Alaska (Tables 1 and 2). MDIV showed no evidence of gene flow (M~0) and estimated a divergence time of T~0.9 between the Alaskan and non-Alaskan populations (Fig. 1a). Therefore, in simulations using Genetree, we assumed subdivided population structure and a very low level of migration (close to 0) for the starting backward migration matrix. The coalescent-based genealogy was informative with respect to the resolution of the splitting event between the Alaskan and non-Alaskan populations and inference of the mutational history with respect to variation between and within populations (Fig. 2a).

In Clade II, the ‘Eurasian’ clade, 14 haplotypes were found, six of which occur in Alaska (Tables 1-2). Of these six, five were found only in Alaska (haplotypes C, D, G, H, I), while one (haplotype A) represented the most abundant haplotype in the sample, spanning Eurasia and Alaska. It must be noted, that haplotype D (or subclade II/A in Geml et al. 2006), a lineage endemic to the maritime rainforests of the Pacific Northwest, was considered ‘Alaskan’ as opposed to ‘Eurasian’, despite not being strictly restricted by the political boundaries and occurring as far South as Northwestern Washington state. Apart from this, no other Clade II haplotypes have been found in North America, outside Alaska. Despite the very similar values for Theta (Fig. 1), the population structure in Clade II proved to be very different from

Table 1 Identity of *A. muscaria* haplotypes in our sample. Haplotype letters correspond to those in Table 2 and Fig. 2

Haplotypes	Specimen	Geographic origin
Clade I		
A	45785	Hampshire, Massachusetts, USA
B	45820	Bronx, New York, USA
C	CMP3143	Cochise Co., Arizona, USA
C	RET145-2	Tlaxcala, Mexico
C	CMP0648	Cochise Co., Arizona, USA
C	RET144-10	Tlaxcala, Mexico
D	GAL15330	Fairbanks, Alaska, USA
D	GAL16775	Fairbanks, Alaska, USA
D	GAL15776	Bonanza Creek LTER site, Alaska, USA
D	GAL16654	Fairbanks, Alaska, USA
D	GAL15336	Fairbanks, Alaska, USA
D	GAL17984	Fairbanks, Alaska, USA
D	GAL17982	Fairbanks, Alaska, USA
D	GAL15335	Fairbanks, Alaska, USA
E	RET145-1	Tlaxcala, Mexico
F	CMP1345	Cochise Co., Arizona, USA
G	49100	Cascade, Idaho, USA
H	45840	Lawrence, Massachusetts, USA
I	45843	Hampshire, Massachusetts, USA
J	45060	Amador, California, USA
J	LG382	Santa Cruz Island, California, USA
K	44761	Alpine, California, USA
L	45863	Mendocino, California, USA
L	RET320-1	Fremont Co., Idaho, USA
L	LG882	Santa Cruz Island, California, USA
M	RET271-2	Sussex Co., New Jersey, USA
N	45883	Piscataquis, Massachusetts, USA
N	RET271-3	Somerset Co., New Jersey, USA
O	RET383-3	Newfoundland, Canada
O	RET124-2	Suffolk Co., Massachusetts, USA
O	RET338-9	Clallam Co., Washington, USA
O	RET289-3	Cape May Co., New Jersey, USA
O	RET158-7	Burlington Co., New Jersey, USA
O	RET303-4	Monmouth Co., New Jersey, USA
Clade II		
A	30962	Kitakoma-gun, Yamanashi, Japan
A	30963	Kitakoma-gun, Yamanashi, Japan
A	30976	Kiso-gun, Nagano, Japan
A	30977	Ohno-gun, Gifu, Japan
A	30985	Ohno-gun, Gifu, Japan
A	30964	Gdynia, Poland
A	31445	Surrey, England, UK
A	80048	Surrey, England, UK

(*continued*)

Table 1 (continued)

Haplotypes	Specimen	Geographic origin
A	N10	Magadan district, Magadan region, Russia
A	GAL14284	Denali National Park, Alaska, USA
A	GAL16735	Fairbanks, Alaska, USA
A	GAL5895	Serpentine Hot Springs, N. of Nome, Alaska, USA
A	GAL15453	North Pole, Alaska, USA
A	GAL15461	North Pole, Alaska, USA
A	GAL5505	Denali National Park, Alaska, USA
A	RET036-3	Zürich canton, Switzerland
A	RET309-3	Sogn og Fjordane, Norway
A	RET143-5	Kamchatka, Russia
A	RET141-2	Highlands & Islands Reg., Scotland, UK
A	N3	Ola district, Magadan region, Russia
A	N12	Ola district, Magadan region, Russia
A	N5	Magadan district, Magadan region, Russia
A	N13	Omsukchan district, Magadan region, Russia
A	N6	Magadan district, Magadan region, Russia
A	MP23	Bird Creek Campground, Anchorage, Alaska, USA
A	N15	Tenka district, Magadan region, Russia
A	MP24	Bird Creek Campground, Anchorage, Alaska, USA
A	N16	Anadyr district, Chukot Autonomous Region, Russia
A	N1	Ola district, Magadan region, Russia
A	N11	Ola district, Magadan region, Russia
A	GAL8950	Denali National Park, Alaska, USA
A	GAL5900	Serpentine Hot Springs, N. of Nome, Alaska, USA
A	GAL5946	Quartz Creek, E. of Nome, Alaska, USA
A	GAL16735-2	Fairbanks, Alaska, USA
A	GAL18071	Fairbanks, Alaska, USA
A	GAL16735-3	Fairbanks, Alaska, USA
A	GAL18076	Fairbanks, Alaska, USA
A	GAL15454	North Pole, Alaska, USA
A	GAL18134	Bonanza Creek LTER site, Alaska, USA
A	GAL18136	Bonanza Creek LTER site, Alaska, USA
A	GAL17899	Fairbanks, Alaska, USA
A	GAL18012-6	Fairbanks, Alaska, USA
A	GAL18012-2	Fairbanks, Alaska, USA
A	GAL18012-4	Fairbanks, Alaska, USA
A	GAL18810-2	Homer, Alaska, USA
A	GAL18810-1	Homer, Alaska, USA
A	GAL17691	Fairbanks, Alaska, USA
B	RET107-6	Highlands & Islands Reg., Scotland, UK
C	GAL2005	Bonanza Creek LTER site, Alaska, USA
D	RET264-7	Skamania Co., Washington, USA
D	GAL4247	Glacier Hwy, mile 27, Juneau, Alaska, USA
D	GAL4302	Glacier Hwy, mile 27, Juneau, Alaska, USA
D	RET136-2	Whatcom Co., Washington, USA

(*continued*)

Table 1 (continued)

Haplotypes	Specimen	Geographic origin
D	GAL3643	Glacier Hwy, mile 30, Juneau, Alaska, USA
E	RET149-1	Southern Highlands Prov., Tanzania
E	RET149-2	Southern Highlands Prov., Tanzania
F	N8	Magadan district, Magadan region, Russia
G	GAL18122	Bonanza Creek LTER site, Alaska, USA
H	GAL2814	Dalton Hwy, mile 122, Alaska, USA
I	GAL4810	Denali National Park, Alaska, USA
J	31452	Hampshire, England, UK
J	30965	Gdansk, Poland
J	RET152-6	Baden-Württemberg, Germany
J	RET152-8	Bavaria, Germany
K	30961	Aomori-shi, Aomori, Japan
L	30987	Queenstown, New Zealand
M	N4	Ola district, Magadan region, Russia
N	N14	Tenka district, Magadan region, Russia

that of Clade I discussed above. MDIV showed evidence for intermediate levels of gene flow (M~2.5) between Alaskan and Eurasian populations and estimated no population divergence (T~0) (Fig. 1b). Our analyses using MIGRATE suggested that migration between Alaska and Eurasia has been asymmetrically bidirectional with Alaska receiving more migrants (M_{12}=9,482.98 and M_{21}=33.53; M_{12} referring to the migration from Eurasia to Alaska, and M_{21} *vice versa*). Therefore, in simulations using Genetree, we assumed panmictic population structure. As expected, the coalescent-based genealogy did not show any historical population division (Fig. 2b). However, based on the fact that the specimens from the maritime rainforest ecoregion of the Pacific Northwest of North America formed a well-supported sister group (II/A) to the rest of Clade II in our previous multi-locus phylogenetic analyses (Geml et al. 2006, 2008), we tested in a separate analysis whether or not there was any gene flow between this group inhabiting a special habitat with a restricted distribution and the rest of Clade II, this latter including boreal Alaskan and Eurasian specimens. In this case, MDIV showed no migration (M~0) and estimated a divergence time of T~1.5 between the two populations, i.e. subclade II/A vs. Clade II (Fig. 1c). Nonetheless, the coalescent-based genealogy estimated under an assumption of population subdivision did not differ significantly from the one generated assuming panmixia (Fig. 2c).

4 Discussion

The main goal of this project was to estimate migration between Alaskan and non-Alaskan populations within distinct phylogenetic species in the *A. muscaria* species complex in order to test whether or not these fungi could have survived the

Table 2 Polymorphic sites in haplotypes collapsed after recoding indels and excluding infinite-sites violations from the original dataset for coalescent analyses. Position refers to that in the original alignment, site number is the designation of the given mutation as shown on Fig. 1, site type refers to transition (t), transversion (v), or deletion (-) change with regard to the consensus sequence. Character type indicates either parsimony-informative (i) or non-informative (-) sites. Capital letters refer to haplotype designations on Fig. 2

Phylogenetic species	Clade I	Clade II
Position	1111122223344455	1111111233456
	16660136600132712668	244667233455870506 1
	00134960218778400483	366231200182485654280
Site number	11111111112	111111111122
	12345678901234567890	123456789012345678901
Site Type	ttt-vt-ttttttttttttt	tttttttttvttvvvttvvtt
Character Type	-i-i-ii--------i----	---------i--i--------
Haplotypes:		
A	................C...	....
B	T..2................	G.......CT...........
C	.T...T..............	.G...................
D	.T.................C	..GC.CA...........T.
E	.T...T.C............	....T..T...CC........
F	.T...T...T....G..A..	........C............
G	..A...2........T....	..........T..........
H	...2................	...........A.........
I	....A...............	.............C.A.....
J	......2........T....	..............T......
K	......2.G..G.C......	................A....
L	......2.............	.................A...
M	..........T.G.......	..................A..
N	..................T.	....................A
O	....	

LGM in Alaska. Because non-Alaskan populations most likely survived the LGM in refugia South of the major ice shields, the lack of migration between these and the Alaskan ones would suggest local survival of the latter, implying forest refugia in Alaska.

Overall, we found support for at least two endemic regional populations (haplotype D in Clade I, and haplotype D in Clade II) in different parts of Alaska, both of which exhibited genetic isolation and differentiation from other conspecific populations. Unfortunately, we could not estimate the times of divergence of the clades with certainty due to the lack of fossils and the great variance in nucleotide substitution rates in fungi. Nonetheless, it seems very likely that the major lineages (Clades I-III) separated well before the Pleistocene glacial cycles (Geml et al. 2006). On the other hand, the endemic regional populations likely represent groups that became isolated from the rest of their species in the Pleistocene and survived one or more glacial maxima in local refugia. Our findings support the existence of at least two independent such glacial forest refugia: (1) boreal forest in Interior Alaska; and

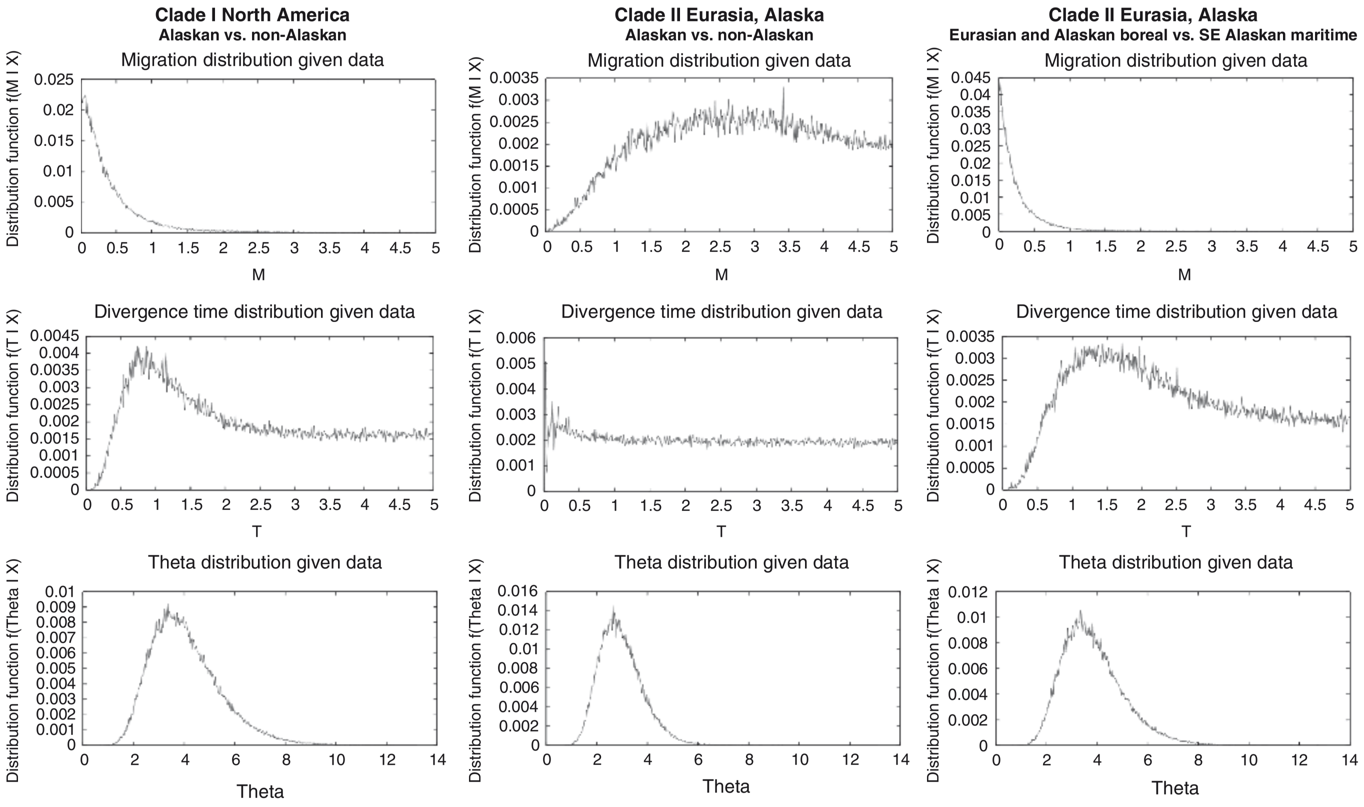

Fig. 1 Posterior probability distributions of migration (*M*), divergence time (*T*) and population mean mutation rate (*Theta*) between population pairs using MDIV. Estimates (axis X) with the highest distribution function (axis Y) values have the greatest probabilities. A and C show examples of zero migration and non-zero divergence time, while graphs in B indicate recent gene flow and no divergence between population pairs

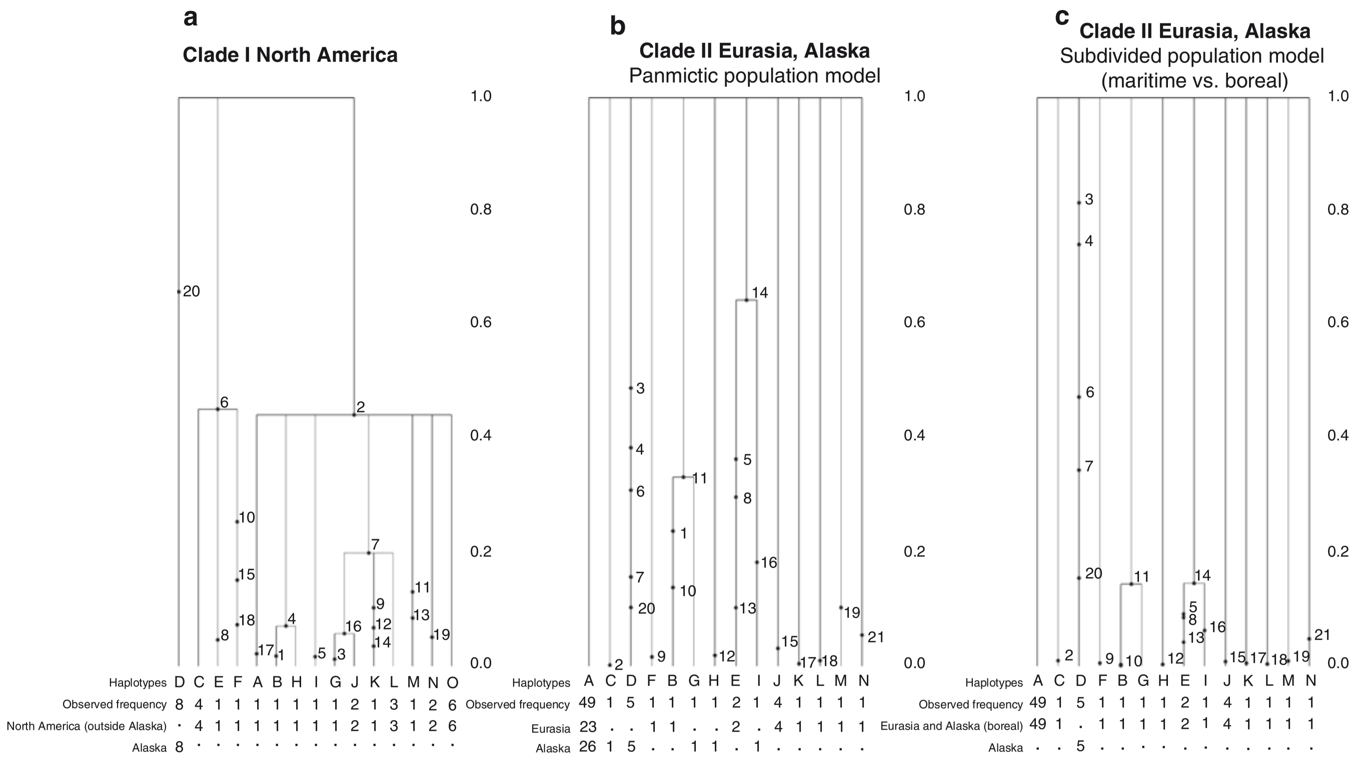

Fig. 2 Coalescent-based genealogies with the highest root probabilities showing the distribution of mutations. The inferred genealogies are based on 2 million simulations of the coalescent. The time scale is in coalescent units of $2N$, where N is the population size. Mutations and bifurcations are time ordered from the top (past) to the bottom (present). Mutation designations correspond to the site numbers in Table 2. The numbers below the tree designate the distinct haplotypes, their observed frequencies in total and in the different geographic regions

(2) maritime rainforest in Southeast Alaska and the Pacific Northwest (Fig. 3). Apart from this, many Alaskan haplotypes in Clade II were shared with specimens from Eurasia. Our results suggesting high migration, most likely both pre- and post-glacial, across the Bering Strait and the similar observed numbers of haplotypes endemic to either Eurasia or Alaska leave open the question whether boreal members of Clade II survived the LGM in Alaska or colonised Alaska from Asia in multiple times in postglacial events.

The possible existence of forest refugia in Interior and Southeast Alaska is also supported by several other independent lines of evidence. For example, although previous palynological biome reconstructions suggest that all of

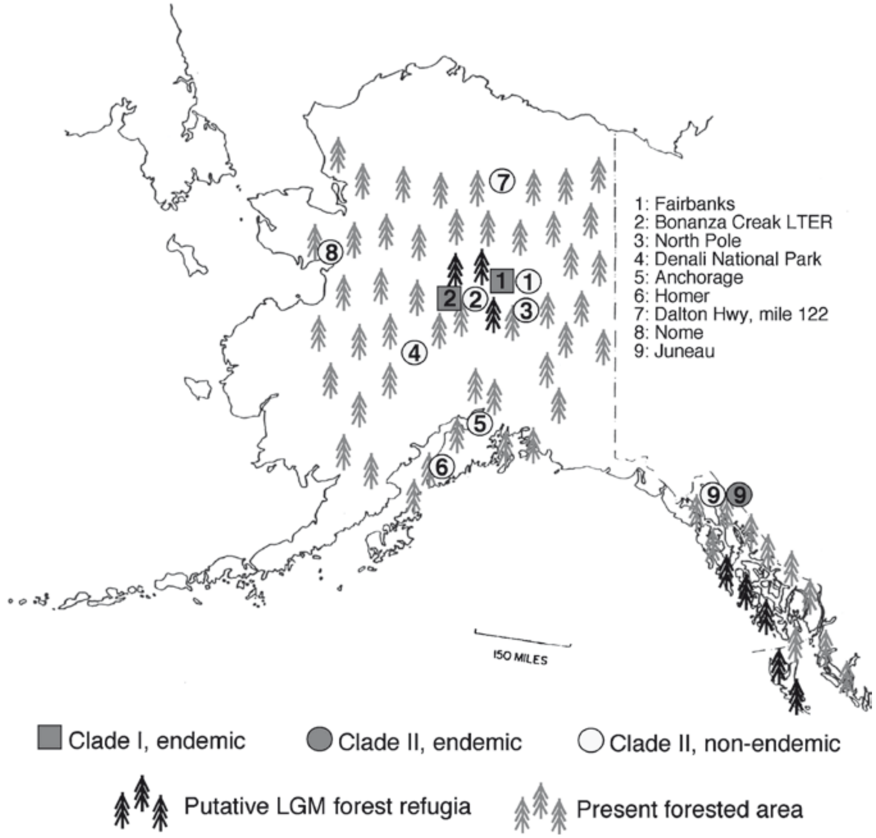

Fig. 3 Outline map of Alaska showing the collecting locations for the 43 Alaskan specimens included in this study. *Dark grey squares* and *circles* indicate sampling localities for endemic haplotypes, while *light grey circles* refer to haplotypes shared between Alaska and Eurasia. Black stylised spruce drawings mark putative forest refugia during the Last Glacial Maximum (LGM) suggested by this and other studies mentioned in the discussion. *Grey* tree figures indicate present forested area, without making a distinction between boreal and maritime forests of Interior and Southeast Alaska, respectively

Beringia was covered by arctic tundra (Edwards et al. 2000; Kaufman et al. 2004; Swanson 2003), recent pollen data by Brubaker et al. (2005) supports the theory that *Picea* forests were present in Eastern Beringia, at least in small fragments restricted to habitats with favourable microclimates. The Northernmost distribution of *Picea*, unlike that of *Betula* and *Populus*, is restricted, by definition, to the boreal region. Therefore, Brubaker's findings indicate the existence of the boreal forest biome in Alaska during the LGM. Similarly, Anderson et al. (2006), surveying forest stands across Northwestern North America, found several chloroplast DNA haplotypes of *Picea glauca* that were unique to Alaska, suggesting local survival. Maroja et al. (2007) analysed mitochondrial DNA sequence data from the spruce beetle (*Dendroctonus rufipennis*) and suggested that the postglacial range expansion of these beetles occurred from three refugia, one of which was in Interior Alaska.

Forest refugia in Southeast Alaska are also supported by genetic studies in several different taxa. For example, this is a region with high mammalian endemism due to its fragmented landscape and complex glacial history. Even animals, as mobile as wolves, have been shown to exhibit a strong signal of independent histories for the coastal and continental populations (Weckworth et al. 2005). Also, phylogenetic studies of Fleming and Cook (2002) on ermine (*Mustela erminea*) showed that there was at least one lineage strictly endemic to Southeast Alaska that likely survived locally during the Wisconsin glaciation. Locations for such glacial refugia in Southeast Alaska have been proposed by Carrarra et al. (2003) based on geological evidence.

In this paper, we demonstrated the use of ECM fungal taxa to indirectly infer glacial vegetation history. Therefore, our results are not only relevant to the mycological community, but to a broader biological community in general, particularly to those with a biogeographic interest.

References

Abbott RJ, Comes HP (2003) Evolution in the Arctic: a phylogeographic analysis of the circumarctic plant, *Saxifraga oppositifolia* (Purple saxifrage). New Phytol 161:211–224

Adams JM, Faure H (1997) Preliminary vegetation maps of the World since the last glacial maximum: An aid to archaeological understanding. J Archeol Sci 24:623–647

Alsos IG, Engelskjøn T, Gielly L, Taberlet P, Brochmann C (2005) Impact of ice ages on circumpolar molecular diversity: insights from an ecological key species. Mol Ecol 14:2739–2753

Anderson LL, Hu FS, Nelson DM, Petit RJ, Paige KN (2006) Ice-age endurance: DNA evidence for a white spruce refugium in Alaska. Proc Natl Acad Sci USA 103:12447–12450

Aylor D, Carbone I (2003) SNAP combine and map. Department of Plant Pathology, North Carolina State University, Raleigh, NC (http://www.cals.ncsu.edu/plantpath/faculty/carbone/home.html).

Bas C (1969) Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. Persoonia 5:285–579

Beerli P, Felsenstein J (2001) Maximum-likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc Natl Acad Sci USA 98:4563–4568

Brubaker LB, Anderson PM, Edwards ME, Lozhkin AV (2005) Beringia as a glacial refugium for boreal trees and shrubs: new perspectives from mapped pollen data. J Biogeogr 32: 833–848

Brunhoff C, Galbreath KE, Fedorov VB, Cook JA, Aarola M (2003) Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. Mol Ecol 12:957–968

Budantsev LY (1992) Early stages of formation and dispersal of the temperate flora in the boreal region. Bot Rev 58:1–48

Carrarra PE, Ager TA, Baichtal JF, VanSistine DP (2003) Map of glacial limits and possible refugia in the Southern Alexander Archipelago, Alaska, during the late Wisconsin Glaciation, Miscellaneous Field Studies Map, MF-2424. US Geological Service, Denver, CO

Edwards ME, Anderson PM, Brubaker LB et al (2000) Pollen-based biomes for Beringia 18, 000, 6000 and 0 14C yr BP. J Biogeogr 27:521–554

Eidesen PB, Carlsen T, Molau U, Brochmann C (2007) Repeatedly out of Beringia: *Cassiope tetragona* embraces the Arctic. J Biogeogr 34:1559–1574

Elias SA, Berman D, Alfimov A (2000) Late Pleistocene beetle faunas of Beringia: where East met West. J Biogeogr 27:1349–1363

Fleming MA, Cook JA (2002) Phylogeography of endemic ermine (*Mustela erminea*) in Southeast Alaska. Mol Ecol 11:795–807

Geml J, Laursen GA, O'Neill K, Nusbaum HC, Taylor DL (2006) Beringian origins and cryptic speciation events in the Fly Agaric (*Amanita muscaria*). Mol Ecol 15:225–239

Geml J, Tulloss RE, Laursen GA, Sazanova NA, Taylor DL (2008) Evidence for strong inter- and intracontinental phylogeographic structure in *Amanita muscaria*, a wind-dispersed ectomycorrhizal basidiomycete. Mol Phylogenet Evol 48:694–701. doi:doi:10.1016/j.ympev.2008.04.029

Graham A (1999) Late cretaceous and cenozoic History of North American Vegetation. Oxford University Press, Oxford

Griffiths RC, Tavaré S (1994) Ancestral inference in population genetics. Stat Sci 9:307–319

Härkönen M, Saarimäki T, Mwasumbi L (1994) Tanzanian mushrooms and their uses 4. Some reddish edible and poisonous Amanita species. Karstenia 34:47–60

Hey J, Wakeley J (1997) A coalescent estimator of the population recombination rate. Genetics 145:833–846

Hultén E (1968) Flora of Alaska and neighboring territories. Stanford University Press, Stanford

Kaufman DS, Ager TA, Anderson NJ et al (2004) Holocene thermal maximum in the Western Arctic (0–180°W). Quat Sci Rev 23:529–560

Loehr J, Worley K, Grapputo A, Carey J, Veitch A, Coltman DW (2005) Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. J Evol Biol 19:419–430

MacNeil D, Strobeck C (1987) Evolutionary relationships among colonies of Columbian ground squirrels as shown by mitochondrial DNA. Evolution 41:873–881

Markwordt J, Doshi R, Carbone I (2003) SNAP clade and matrix. Department of Plant Pathology, North Carolina State University, Raleigh, NC (http://www.cals.ncsu.edu/plantpath/faculty/carbone/home.html).

Maroja LS, Bogdanowicz SM, Wallin KF, Raffa KF, Harrison RG (2007) Phylogeography of spruce beetles (*Dendroctonus rufipennis* Kirby) (Curculionidae: Scolytinae) in North America. Mol Ecol 16:2560–2573

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158:885–896

Oda T, Tanaka C, Tsuda M (2004) Molecular phylogeny and biogeography of the widely distributed *Amanita* species A. muscaria and A. pantherina. Mycol Res 108:885–896

Qian H (1999) Floristic analysis of vascular plant genera of North America North of Mexico: characteristics of phytogeography. J Biogeogr 26:1307–1321

Reiss AR, Ashworth AC, Schwert DP (1999) Molecular genetic evidence for the post-Pleistocene divergence of populations of the arctic-alpine ground beetle *Amara alpina* (Paykull) (Coleoptera: Carabidae). J Biogeogr 26:785–794

Simmons C, Henkel T, Bas C (2002) The genus *Amanita* in the Pakaraima mountains of Guyana. Persoonia 17:563–582

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, Cambridge

Sperling FH, Harrison RG (1994) Mitochondrial DNA variation within and between species of the *Papilio machaon* group of swallowtail butterflies. Evolution 48:408–422

Swanson DK (2003) A comparison of taiga flora in North-Eastern Russia and Alaska/Yukon. J Biogeogr 30:1109–1121

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Tremblay NO, Schoen DJ (1999) Molecular phylogeography of *Dryas integrifolia*: glacial refugia and postglacial recolonization. Mol Ecol 8:1187–1198

Weckworth BV, Talbot S, Sage GK, Person DK, Cook J (2005) A signal for independent coastal and continental histories among North American wolves. Mol Ecol 14:917–931

Wickström LM, Haukisalmi V, Varis S, Hantula J, Fedorov VB, Henttonen H (2003) Phylogeography of the circumpolar *Paranoplocephala arctica* species complex (Cestoda: Anoplocephalidae) parasitizing collared lemmings (*Dicrostomyx spp.*). Mol Ecol 12:3359–3371