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ORIGINAL ARTICLE

Surviving climate changes: high genetic diversity and transoceanic gene flow in two arctic–alpine lichens, *Flavocetraria cucullata* and *F. nivalis* (Parmeliaceae, Ascomycota)

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ABSTRACT

Aim We examined genetic structure and long-distance gene flow in two lichenized ascomycetes, *Flavocetraria cucullata* and *Flavocetraria nivalis*, which are widespread in arctic and alpine tundra.

Location Circumpolar North.

Methods DNA sequences were obtained for 90 specimens (49 for *F. cucullata* and 41 for *F. nivalis*) collected from various locations in Europe, Asia and North America. Sequences of the nuclear internal transcribed spacer (ITS) + 5.8S ribosomal subunit gene region were generated for 89 samples, and supplemented by beta-tubulin (BTUB) and translation elongation factor 1-alpha gene (EF1) sequences for a subset of *F. cucullata* specimens. Phylogenetic, nonparametric permutation methods and coalescent analyses were used to assess population divergence and to estimate the extent and direction of migration among continents.

Results Both *F. cucullata* and *F. nivalis* were monophyletic, supporting their morphology-based delimitation, and had high and moderately high intraspecific genetic diversity, respectively. Clades within each species contained specimens from both North America and Eurasia. We found only weak genetic differentiation among North American and Eurasian populations, and evidence for moderate to high transoceanic gene flow.

Main conclusions Our results suggest that both *F. cucullata* and *F. nivalis* have been able to migrate over large distances in response to climatic fluctuations. The high genetic diversity observed in the Arctic indicates long-term survival at high latitudes, whereas the estimated migration rates and weak geographic population structure suggest a continuing long-distance gene flow between continents that has prevented pronounced genetic differentiation. The mode of long-distance dispersal is unknown, but wind dispersal of conidia and/or ascospores is probably important in the open arctic landscapes. The high genetic diversity and efficient long-distance dispersal capability of *F. cucullata* and *F. nivalis* suggest that these species, and perhaps other arctic lichens as well, will be able to track their potential niche in the changing Arctic.

Keywords
Arctic, biodiversity, climate change, coalescent methods, dispersal, fungi, lichens, migration, persistence, phylogeography.

INTRODUCTION

The arctic tundra is on the brink of significant changes and there are serious concerns among researchers and the public alike over the future of arctic biodiversity. The most serious threats are accelerating climate change and human impact. In addition to the scientific and economic importance of its unique biota, nutrient cycling in the Arctic has major

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consequences for global change. The arctic tundra occupies an area of 8 million km^2 and stores a great portion of the Earth's reactive carbon (Callaghan *et al.*, 2005). The extent of recent climate change is greatest at high latitudes, where further increases in winter and summer temperatures and winter precipitation are predicted. Although plants and animals have been extensively studied (e.g. Reiss *et al.*, 1999; Tremblay & Schoen, 1999; Abbott & Comes, 2003; Brunhoff *et al.*, 2003; Fedorov *et al.*, 2003; Flagstad & Røed, 2003; Wickström *et al.*, 2003; Alsos *et al.*, 2005, 2007; Dalén *et al.*, 2005; Parmesan, 2006; Eidesen *et al.*, 2007; Schönswetter *et al.*, 2007; Marthinsen *et al.*, 2009), the systematics, ecology and phylogeography of fungi in arctic regions remain largely unknown, despite their critical roles in the functioning of these nutrient-poor ecosystems (Callaghan *et al.*, 2004). Among fungi, lichens in particular form an ecologically important and conspicuous part of the arctic vegetation and often display interesting distribution patterns that are rarely found in vascular plants; however, phylogeographical studies on arctic lichens are virtually absent from the literature (Buschbom, 2007; Printzen, 2008).

The ability of fungi to cope with the changing environment is likely to be related to their genetic diversity. According to basic principles of conservation genetics, populations possessing a small amount of genetic diversity are more susceptible to regional extinction during times of stress (e.g. rapid climatic change) than genetically diverse populations (Avise, 2000). Another important aspect is the degree to which they are able to colonize newly exposed, suitable habitats (e.g. following receding glaciers) and to exchange genes with populations inhabiting different geographical regions. The ability to migrate is of particular importance, because climate warming is expected to cause a northward shift in the distribution of many arctic species, and the long-distance dispersal capability of individual species will greatly influence the composition of future arctic communities (Alsos *et al.*, 2007).

Widely distributed species are ideal candidates for studies on the effects of climate changes on biological diversity. For this purpose, we chose *Flavocetraria cucullata* (Bellardi) Kärnefelt & A. Thell and *Flavocetraria nivalis* (L.) Kärnefelt & A. Thell, two widespread arctic–alpine lichens commonly found in the Northern Hemisphere. Both species are present in all five bioclimatic subzones (A–E) of the Arctic *sensu* Walker *et al.* (2005), from shrub tundra to the polar desert (D. A. Walker, University of Alaska Fairbanks, pers. comm.). Both species have also been found in the Southern Hemisphere, albeit only in very few disjunct populations (Bjerke & Elvebakk, 2004). They are characterized by fruticose, yellow-green thalli with: (1) channelled branches and incurved margins (*F. cucullata*, Fig. 1); or (2) ridged and wrinkled surface and only slightly channelled branches (*F. nivalis*, Fig. 2).

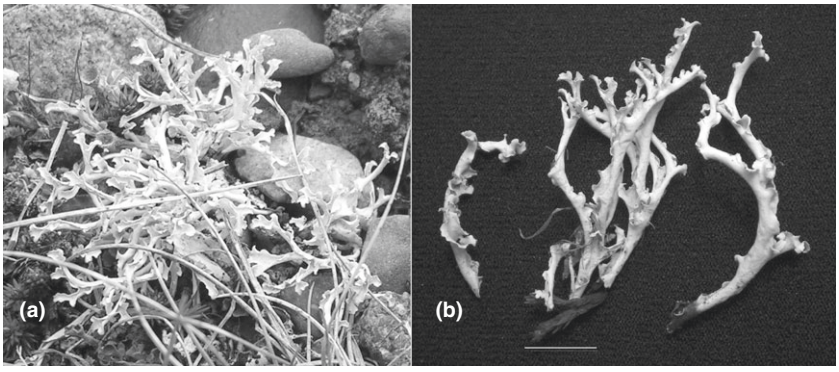

Figure 1 (a) Habitus of *Flavocetraria cucullata* in the field (Toolik Lake, Alaska, USA) and (b) close-up of individual branches against a neutral background, with 1-cm scale bar.

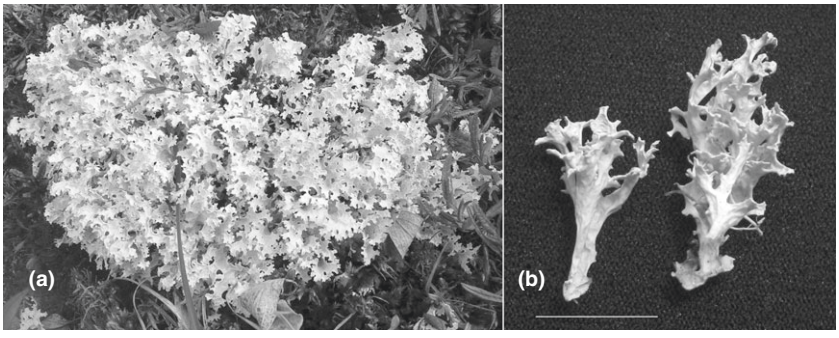

Figure 2 (a) Habitus of *Flavocetraria nivalis* in the field (Atigun River, Alaska, USA) and (b) close-up of individual branches against a neutral background, with 1-cm scale bar.

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Our goals were to assess intraspecific genetic diversity, examine genetic structure and estimate long-distance gene flow in *F. cucullata* and *F. nivalis*. Based on phylogenetic analyses, migration estimates, and genealogies generated using coalescent methods, we infer high and moderate genetic diversity, respectively, and frequent long-distance dispersal in *F. cucullata* and *F. nivalis*. Our results provide the first circumpolar characterization of genetic diversity of an arctic fungus and suggest that at least some arctic lichens have strong potential to adapt to the changing Arctic by tracking their ecological niche and to maintain high genetic diversity through intercontinental dispersal and sexual reproduction.

MATERIALS AND METHODS

Isolates and DNA extraction

Flavocetraria cucullata ($n = 86$) and *F. nivalis* ($n = 94$) specimens were obtained by our own field work, herbarium loans and the collecting efforts of other arctic researchers. DNA was extracted from small samples of dried specimens using the DNeasy® Plant Mini Kit (QIAGEN, Inc., Valencia, CA, USA). Despite our repeated efforts, many herbarium specimens older than 5 years did not yield DNA of a quality suitable for polymerase chain reaction (PCR) and sequencing. We concentrated our efforts on the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA repeat, because this locus has been useful in earlier phylogeographic studies (e.g. Geml *et al.*, 2008; Bergemann *et al.*, 2009) and because its high copy number allowed us to obtain sequence data from specimens with DNA too fragmented for amplification of single-copy genes. Therefore, we generated sequence data from a total number of 90 specimens, namely 49 for *F. cucullata* and 41 for *F. nivalis* (Table 1).

PCR and DNA sequencing

The entire ITS (including ITS1, ITS2 and the 5.8S ribosomal subunit gene region) was amplified in PCR reaction mixtures containing 15.6 μL of Ultrapure Water (Invitrogen, Carlsbad, CA, USA), 2.5 μL of 10 × PCR buffer (0.5 M KCl, 0.1 M Tris HCl pH 8.3, 0.025 M $MgCl_2$), 0.5 μL of 10 mM dNTP mixture, 2.5 mM of each dNTP, 0.25 μL of AmpliTaq® DNA polymerase (Applied Biosystems, Perkin-Elmer, Foster City, CA, USA), 0.25 μL of 50 μM forward primer and reverse primer for the region of interest, and 5 μL of template DNA (100-fold dilution of original DNA extract). PCRs were performed in a 96-well thermocycler (PTC-100 Programmable Thermal Controller; MJ Research, Inc., Waltham, MA, USA) using the following temperature programme for the ITS: 95°C for 2 min, 34 cycles of 95°C for 0.5 min, 56°C for 1 min, 72°C for 2 min, and a final step of 72°C for 10 min, with forward primer ITS1F (Gardes & Bruns, 1993) and reverse primer ITS4 (White *et al.*, 1990).

Because our preliminary analyses revealed deeply divergent ITS phylogroups in *F. cucullata*, we sequenced portions of the beta-tubulin (BTUB) and translation elongation factor 1-alpha (EF1) genes in a subset of the specimens from all ITS clades to test whether the ITS phylogroups represented different phylogenetic species or intraspecific variation. Although there was substantial variation in *F. nivalis* as well, there were no deeply divergent ITS phylogroups warranting multi-locus species delimitation. In *F. cucullata*, the BTUB gene was amplified using primers bt3LM and bt10LM and a temperature programme of 95°C for 2 min, 30 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final step of 72°C for 1 min, following Thell *et al.* (2002). For EF1, a 'touchdown' PCR setting was used with an annealing temperature of 65°C in the first cycle, then successively reduced by 1°C per cycle to 56°C, after which the annealing temperature was maintained at 56°C for the remaining 30–36 cycles, with primers EF1-983F and EF1-1567R (designed by S. Rehner and available from http://www.aftol.org/pdfs/EF1primer.pdf).

Amplification products were electrophoresed in a 1.5% agarose gel and stained with either ethidium bromide or PicoGreen (Invitrogen) for visualization of the bands. PCR products were purified directly using the QIAquick® PCR Purification Kit (QIAGEN). The amplification products were sequenced using the Applied Biosystems (ABI) BigDye 3.1 Terminator kit and an ABI 3100 automated capillary DNA sequencer (Applied Biosystems).

Sequences were deposited in GenBank (*F. nivalis* ITS: GU067685–GU067729; *F. cucullata* ITS: FJ914765–FJ914812, BTUB: FJ914813–FJ914835, EF1: 914836–FJ914860). In addition, all available homologous *Flavocetraria* sequences were downloaded from GenBank and included in the analyses.

Phylogenetic analysis

Sequence data obtained for both strands of each locus were edited and assembled for each isolate using Aligner 1.3.4 (CodonCode Inc., Dedham, MA, USA) or Sequencher 4.5 (GeneCodes, Ann Arbor, MI). Sequence alignments were initiated using ClustalW (Thompson *et al.*, 1997) and subsequently corrected manually. In *F. cucullata*, to test the combinability of DNA sequence data from different loci, the partition homogeneity test (PHT) was performed with 1000 randomized datasets, using heuristic searches with simple addition of sequences and the maximum number of trees saved set to 100. We carried out heuristic searches separately for each locus under the parsimony criterion, using paup* 4b10 (Swofford, 2002), with the maximum number of trees retained set to 100. Gaps were scored as 'missing data'. The bootstrap test (Felsenstein, 1985) was used with 1000 replicates, with 'fast' stepwise-addition. To compare different tree topologies, Shimodaira–Hasegawa tests were used (Shimodaira & Hasegawa, 1999). The High Performance Computing Cluster maintained by the University of Alaska Fairbanks Biotechnology Computing Research Group (http://biotech.inbre.alaska.edu/) was used to run ClustalW. For both species, ITS sequence-type accumulation curves were computed using EstimateS 7.5 (R. K. Colwell, http://purl.oclc.org/estimates).

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Table 1 Geographical origins of *Flavocetraria* specimens sequenced in this study.

Specimen code	Origin
F. cucullata	
FC10	Green Cabin, Banks Island, Northwest Territories, Canada
FC11	Mould Bay, Prince Patrick Island, Northwest Territories, Canada
FC12	Eagle Plains, Richardson Mtns, Yukon Territory, Canada
FC13	Boundary, Interior Alaska, Alaska, USA
FC18	Tangle Lakes, Alaska Range, Alaska, USA
FC19	Isachsen, Ellef Ringnes Island, Nunavut, Canada
FC20	Tangle Lakes, Alaska Range, Alaska, USA
FC21	Tangle Lakes, Alaska Range, Alaska, USA
FC22	Isachsen, Ellef Ringnes Island, Nunavut, Canada
FC23	Tangle Lakes, Alaska Range, Alaska, USA
FC24	Thompson Pass, Chugach Mtns, Alaska, USA
FC25	Franklin Bluffs, Arctic Coastal Plain, Alaska, USA
FC26	Toolik Lake, Brooks Range, Alaska, USA
FC27	Toolik Lake, Brooks Range, Alaska, USA
FC28	Toolik Lake, Brooks Range, Alaska, USA
FC29	Atigun River, Brooks Range, Alaska, USA
FC30	Anmaguligauraq Creek, Brooks Range, Alaska, USA
FC31-1	Franklin Bluffs, Arctic Coastal Plain, Alaska, USA
FC31-2	Franklin Bluffs, Arctic Coastal Plain, Alaska, USA
FC32	Roche Mountanee Creek, Brooks Range, Alaska, USA
FC33	Savage River, Alaska Range, Alaska, USA
FC34	Savage River, Alaska Range, Alaska, USA
FC35	Barrow, Arctic Coastal Plain, Alaska, USA
FC36	Barrow, Arctic Coastal Plain, Alaska, USA
FC38	Ivotuk, Brooks Range, Alaska, USA
FC39	Ivotuk, Brooks Range, Alaska, USA
FC40	Ivotuk, Brooks Range, Alaska, USA
FC41	Atigun Pass, Brooks Range, Alaska, USA
FC42	Finger Mtn, Interior Alaska, Alaska, USA
FC43	Eagle Summit, White Mtns, Interior Alaska, Alaska, USA
FC44	Eagle Summit, White Mtns, Interior Alaska, Alaska, USA
FC45	Wickersham Dome, White Mtns, Interior Alaska, Alaska, USA
FC46	Eagle Summit, White Mtns, Interior Alaska, Alaska, USA
FC105	Dundas, Greenland
FC106	Dundas, Greenland
FC107	Dundas, Greenland
FC108	Great Lyakhovsky Island, New Siberian Islands, Russia
FC110	Labarovaya, Polar Ural, Yamalo-Nenetskiy avtonomnyy okrug, Russia
FC111	Vaskiny Datchy, Yamal Peninsula, Yamalo-Nenetskiy avtonomnyy okrug, Russia
FC112	Nadym, Yamalo-Nenetskiy avtonomnyy okrug, Russia
FC200	Linakhamari, Province of Murmansk, Russia
FC211	Labytnangi, Polar Ural, Yamalo-Nenetskiy avtonomnyy okrug, Russia
FC212	Labytnangi, Polar Ural, Yamalo-Nenetskiy avtonomnyy okrug, Russia
FC213	Great Lyakhovsky Island, New Siberian Islands, Russia
FC214	Great Lyakhovsky Island, New Siberian Islands, Russia
O L142620	Vågå, Oppland, Norway
TROM 510182	Sarfánguaq, Greenland
TROM 194-018-1	Vicinity of La Paz, Province Morillo, Bolivia
TROM 194-018-2	Vicinity of La Paz, Province Morillo, Bolivia
TROM 194-018-3	Vicinity of La Paz, Province Morillo, Bolivia
F. nivalis	
FN6	Wrangell Mtns, Alaska, USA
FN12	Thompson Pass, Chugach Mtns, Alaska, USA
FN13	Atigun River, Brooks Range, Alaska, USA
FN14	Atigun River, Brooks Range, Alaska, USA

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Table 1 Continued

Specimen code	Origin
FN16	Roche Mountanee Creek, Brooks Range, Alaska, USA
FN17	Roche Mountanee Creek, Brooks Range, Alaska, USA
FN18	Savage River, Alaska Range, Alaska, USA
FN19	Savage River, Alaska Range, Alaska, USA
FN20	Savage River, Alaska Range, Alaska, USA
FN21	Barrow, Arctic Coastal Plain, Alaska, USA
FN22	Barrow, Arctic Coastal Plain, Alaska, USA
FN23	Barrow, Arctic Coastal Plain, Alaska, USA
FN24	Atigun Pass, Brooks Range, Alaska, USA
FN25	Eagle Summit, White Mtns, Interior Alaska, Alaska, USA
FN26	Grapefruit Rocks, Yukon-Tanana Uplands, Interior Alaska, Alaska, USA
FN27	Eagle Summit, White Mtns, Interior Alaska, Alaska, USA
FN28	Wickersham Dome, White Mtns, Interior Alaska, Alaska, USA
FN29	Eagle Summit, White Mtns, Interior Alaska, Alaska, USA
FN105	Kettvollen SØ, Os, Hedmark, Norway
FN106	Musaetin, Sel, Oppland, Norway
FN107	Skrikko, Rennebu, Sør-Trøndelag, Norway
FN108	Halvvegesberget, Røyrvik, Nord-Trøndelag, Norway
FN109	Daltinden, Steigen, Nordland, Norway
FN110	Dundas, Greenland
FN111	Dundas, Greenland
FN112	Labarovaya, Polar Ural, Yamalo-Nenetskiy avtonomnyy okrug, Russia
FN113	Vaskiny Datchy, Yamal Peninsula, Yamalo-Nenetskiy avtonomnyy okrug, Russia
FN114	Ny-Ålesund, Svalbard
FN115	Ny-Ålesund, Svalbard
FN116	Ny-Ålesund, Svalbard
FN117	Ny-Ålesund, Svalbard
FN206	Dalnie Zelentsy, Province of Murmansk, Russia
FN207	Puterana Plateau, Mikchanda Mtns, Russia
FN208	Dolgyi Island, Province of Murmansk, Russia
FN209	Laplandsky Reserve, Province of Murmansk, Russia
FN226	Labytnangi, Polar Ural, Yamalo-Nenetskiy avtonomnyy okrug, Russia
TROM I99-004	Parque Nacional Pali-Aike, Tierra del Fuego, Chile
TROM J34-13	Sørelva, Saltdal, Nordland, Norway
TROM J34-14	Sørelva, Saltdal, Nordland, Norway
TROM J22-021	Bjørnoya, Svalbard
TROM J28-04	Tromsdalstud, Troms, Norway

Coalescent analyses

Identical ITS sequences were collapsed into haplotypes using SNAP Map (Aylor *et al.*, 2006) after excluding insertions or deletions (indels) and 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 segregating bases. Base substitutions were categorized as phylogenetically uninformative or informative, and as transitions or transversions. Site-compatibility matrices were generated from each haplotype dataset using SNAP Clade and Matrix (Markwordt *et al.*, 2003; Bowden *et al.*, 2008) to examine compatibility/incompatibility among all variable sites, with any resultant incompatible sites removed from the dataset. Genetic differentiation among geographical populations was analysed using SNAP Map, Seqtomatrix and Permtest (Hudson *et al.*, 1992) implemented in SNAP Workbench (Price & Carbone, 2005). Permtest is a nonparametric permutation method based on Monte Carlo simulations that estimates Hudson's test statistics (K_{ST}, K_S, and K_T) under the null hypothesis of no genetic differentiation. K_{ST} is equal to $1-K_S/K_T$, where K_S is a weighted mean of $K1$ and $K2$ (mean number of differences between sequences in subpopulations 1 and 2, respectively) and K_T represents the mean number of differences between two sequences regardless of the subpopulation to which they belong. The null hypothesis of no genetic differentiation is rejected ($P < 0.05$) when K_S is small and K_{ST} is close to 1. For this test, specimens were assigned to geographical groups based on continents (North America or Eurasia).

Two independent methods were used to determine whether there was any evidence of transoceanic migration between pairs of populations inhabiting different continents. Because we were interested primarily in measuring gene flow across the

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circumpolar Arctic, the few South American specimens sequenced were excluded from these analyses. In *F. nivalis*, two additional analyses were performed on population pairs 'Svalbard' versus 'North America' and 'Svalbard' versus 'Eurasia' to estimate migration between the remote archipelago of Svalbard and the northern continents. Svalbard is the most isolated archipelago in the Arctic and was glaciated (Landvik *et al.*, 1998), making it ideal to test for long-distance colonization and gene flow in arctic organisms. First, we used MDIV (Nielsen & Wakeley, 2001), implemented in SNAP WORKBENCH (Price & Carbone, 2005), employing both likelihood and Bayesian methods using Markov chain Monte Carlo (MCMC) coalescent simulations to estimate the migration (M), where M equals 2× the net effective population size (N_e) multiplied by m (migration rate). Ages were measured in coalescent units of $2N$, where N is the population size. For each dataset, the data were 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. Subsequently, MIGRATE was used to estimate migration rates assuming equilibrium migration rates (symmetrical or asymmetrical) in the history of the populations (Beerli & Felsenstein, 2001). We applied the following specifications for the MIGRATE maximum likelihood analyses: M^* (migration rate m divided by mutation rate μ) and θ ($4N_e\mu$) generated from the F_{ST} calculation, migration model with variable θ, and constant mutation rate. The numbers of immigrants per generation ($4N_em$) were calculated by multiplying θ of the receiving population with the population migration rate M^*. Subsequently, for each species, we reconstructed the genealogy with the highest root probability, the ages of mutations, and the time to the most recent common ancestor (TMRCA) of the sample using coalescent simulations in GENETREE 9.0 (Griffiths & Tavaré, 1994).

RESULTS

Phylogenetic analyses

The entire ITS dataset consisted of 108 sequences and 588 characters, including gaps. Eighty-six characters were variable, of which 66 were parsimony-informative. In the ITS phylogram (Fig. 3), both *F. cucullata* and *F. nivalis* were monophyletic, supporting their morphology-based delimitation. We observed moderately high genetic diversity in *F. nivalis* and very high diversity in *F. cucullata*. The very few South American samples we analysed were genetically very similar to northern individuals in both species, differing by only 1–2 characters (Fig. 3). In *F. cucullata*, we detected four deeply divergent clades, all receiving high bootstrap support. These clades were geographically overlapping, and each of them contained one or more specimens from both North America and Eurasia. The BTUB and EF1 phylograms (see Fig. S1 in Supporting Information), based on 601 and 450 characters, of

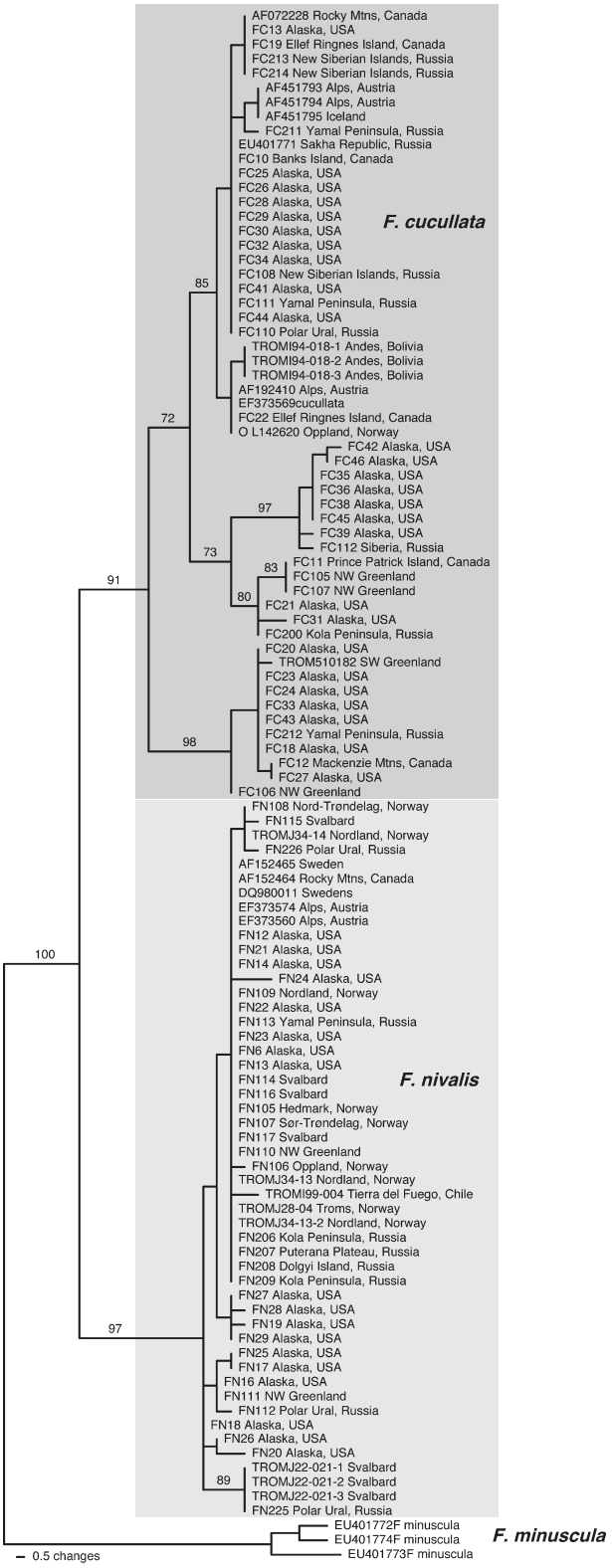

Figure 3 One of 100 equally parsimonious trees (tree length = 114 steps) of *Flavocetraria cucullata* and *F. nivalis* generated from the ITS dataset, with > 70% maximum-parsimony bootstrap values shown above the supported branches. Homologous sequences of *F. minuscula* were used as an outgroup to root the trees.

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Table 2 Polymorphic sites in the internal transcribed spacer (ITS) haplotypes of *Flavocetraria cucullata* and *F. nivalis* collapsed after recoding indels and removing infinite-sites violations from the original ITS dataset for the subsequent coalescent analyses. Haplotype designation, position, site number and designation of the given mutation are as shown in Fig. 5. Position refers to that in the original alignment; site type refers to transition (t), transversion (v) or deletion (−) change with regard to the consensus sequence.

F. cucullata

```
Position                1111111111111112222233444444445
                  4457788999002223344477899011594915799992
                  3624603156120385805807814049324911334679
Site number             1111111111222222222233333333334
                  1234567890123456789012345678901234567890
Site type         t-tttvtvtvtt--vt-ttvttttt-tvtttttvtt-tt-
Character type    -iiii---ii-i---iiiii-iii-ii---iiii-i-ii-
Consensus         G1CCATATCATT11GT1TAGAAATC1AAATTTTTTC1CC1

Haplotypes (frequency)
A (4)             ...TG.G.............................
B (1)             A..........C..........G.......CC.G...TT.
C (2)             .2.TG.....................G.............
D (7)             ..T.....TC.......CGT...C...........T....
E (1)             ..T.....TC..2....CGT...............T....
F (1)             ..T.....TC.......CGT...CT..........T...2
G (2)             ..T.....TC.......CGT...C....G......T....
H (2)             ...............C.................CG...TT.
I (1)             .....A.....C..................C..G...TT.
J (1)             .......G.......C...........C....CG...TT.
K (1)             ..........CC...................C..G...TT.
L (4)             ...........C..........G.......C..G...TT.
M (1)             ...........C..........G.......CC.G...TT.
N (3)             ..............C....G...........CGC..TT.
O (3)             ...TG..............G...2...C..........
P (1)             ...TG.........2.............................
Q (1)             ...TG.........T......G...2..............
R (2)             ...TG...........2...........................
S (10)            ...TG.....................................
T (3)             ...TG.....................G..............
U (1)             ...TG.................................2...
```

F. nivalis

```
Position                                    1112222233445555
                                      3669913600113594811 25
                                      5271928445790134512 21
Site number                                      111111111122
                                      1234567890123456789 01
Site type                             tv-tt-tttvtttttvtt-tt
Character type                          -- ii ----i------- i-
Consensus                             TG1TC2TTTAACAGACAA1CG

Haplotypes (frequency)
A (1)                                 .....1....G..........
B (4)                                 C.2......C.T.......T.
C (1)                                 .T.................T.
D (3)                                 ...C..................
E (1)                                 ...C..........G......
F (1)                                 ....T..............T.
G (1)                                 ....T..C.........G.T.
H (28)                                ..........G..........
I (1)                                 ......C...G..........
J (1)                                 ........C.G..A.A.....
K (3)                                 ....................T.
L (2)                                 ............G.....2T.
M (1)                                 ..........G.....G....
N (1)                                 ..........G.........A
```

which 44 and 11 were parsimony-informative, respectively, also revealed several clades, receiving varying levels of support. Although the number of samples for these protein-coding genes was small, predominantly freshly collected specimens from Alaska and Canada that yielded high-quality DNA, they represented all known ITS phylogroups. All three gene trees were highly incongruent: specimens belonging to divergent clades in one gene genealogy often grouped together in another. For both the BTUB and EF1 datasets, we compared the length of maximum parsimony (MP) trees constructed from the unconstrained dataset with the length of the MP trees under the constraint of monophyly of specimens based on the ITS clades. In all comparisons, the constrained trees were significantly worse (i.e., had significantly more steps) than the unconstrained trees (all $P < 0.05$), again indicating significant incongruence among the gene trees. Similarly, the PHT indicated that there was significant conflict in the phylogenetic signals present at different loci ($P < 0.01$). Pairwise PHT tests carried out in all three possible combinations of gene pairs showed significant incongruence in all cases (all $P < 0.01$).

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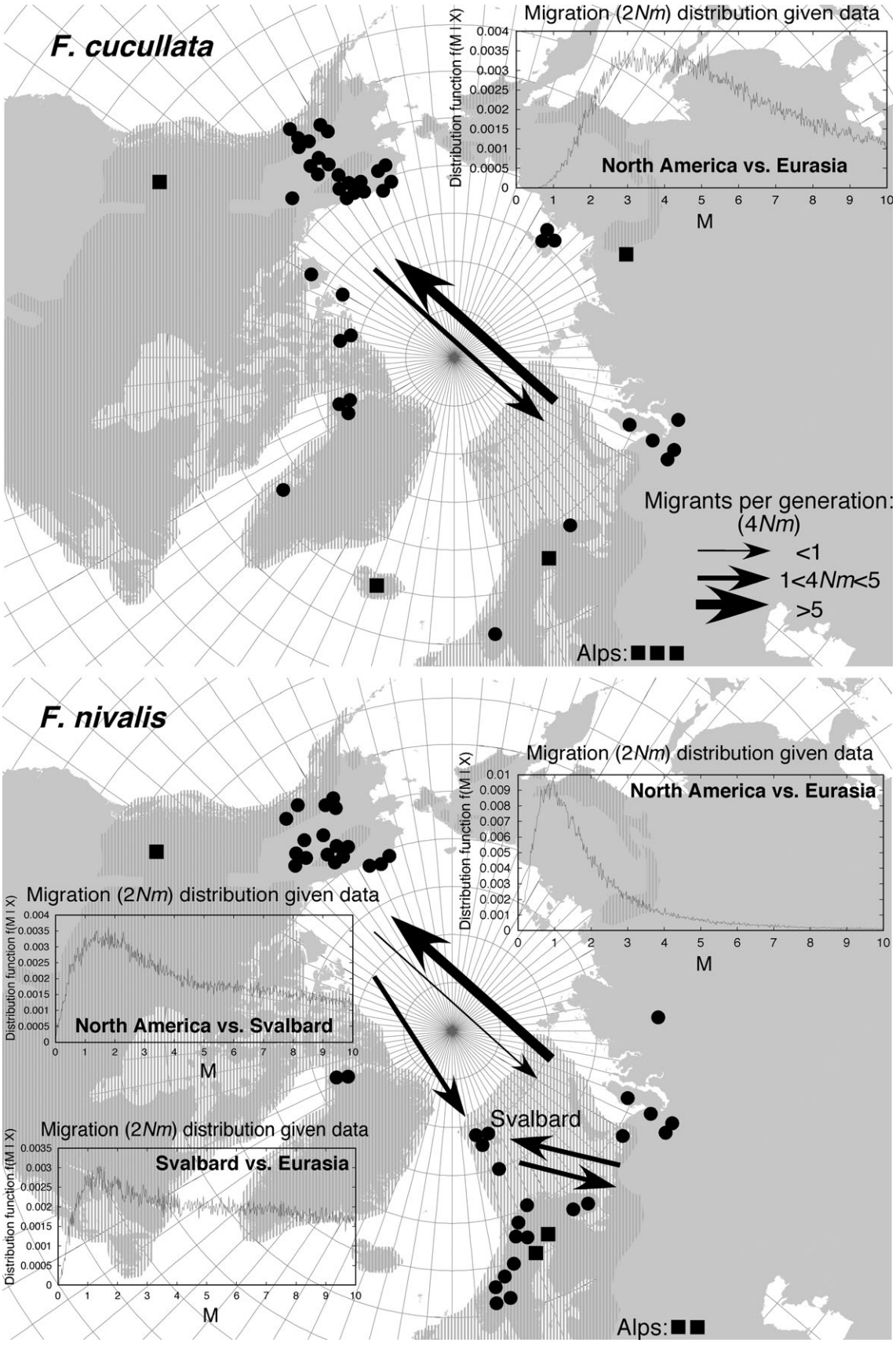

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Hence, there was no evidence for the existence of multiple phylogenetic species in *F. cucullata*, and we treated all *F. cucullata* samples as conspecific for subsequent coalescent analyses.

Coalescent analyses

Estimates of Hudson's test statistics (K_{ST}, K_S and K_T) using a nonparametric permutation method indicated weak, marginally significant, genetic differentiation among North American and Eurasian populations in the two *Flavocetraria* species. The genetic differences within and between continents were similar in both *F. cucullata* and *F. nivalis*, namely $K_S = 7.4109$, $K_T = 7.7436$, and $K_S = 2.1823$, $K_T = 2.2806$, respectively, resulting in $K_{ST} = 0.0430$, $P = 0.022$, and $K_{ST} = 0.0431$, $P = 0.019$, respectively.

In our combined approach, we utilized the complementary strengths of MDIV and MIGRATE to estimate the extent of transoceanic gene flow. For example, MIGRATE was used to estimate the direction of migration, but could not distinguish between shared ancestral polymorphism and recurrent gene flow, whereas MDIV was used to determine if the diversity patterns in North American and Eurasian populations were the result of retention of ancestral polymorphism or recent gene flow. After recoding indels and removing infinite-sites violations (7 sites in *F. cucullata* and 6 sites in *F. nivalis*) from the original ITS datasets, there were 21 and 14 ITS haplotypes in Northern Hemisphere populations of *F. cucullata* and *F. nivalis*, respectively (Table 2). In both species, MDIV showed evidence for moderate to high gene flow between North American and Eurasian populations (Fig. 4) and estimated no population divergence (T not significantly different from 0). MIGRATE detected bidirectional gene flow in most population pairs, although the numbers of migrants per generation were often asymmetrical. For example, in both *F. cucullata* and *F. nivalis*, North America seemed to receive more immigrants from Eurasia than vice versa. In *F. nivalis*, when Svalbard and mainland Eurasia were compared, the numbers of immigrants were similar in both directions, whereas predominantly one-directional (from North America to Svalbard) migration was observed in the other geographical pairing (Fig. 4). In simulations using GENETREE, we assumed a moderately high level of migration ($M = 0.1$). As expected, the coalescent-based genealogies did not show any historical population division and were informative with respect to inferring the mutational history and variation between and within geographical regions (Fig. 5).

DISCUSSION

Climate changes during the Quaternary dramatically influenced the distribution of flora and fauna, particularly at higher latitudes. During glacial maxima, plants, fungi and animals were restricted to unglaciated refugia, from which they recolonized newly exposed areas in warmer interglacial periods. In theory, one can expect to find correlation between genetic diversity and the time a given region has been ice free. Areas colonized recently, in post-glacial times, are expected to have a lower genetic diversity than refugial areas continuously inhabited by the species. The mechanisms explaining these expectations include a limited migration rate and a founder effect, where the colonizing front from the nearest refugium gradually occupies the newly available area, limiting the establishment of genotypes that arrive later from more distant refugia. There are several examples that follow these expectations. For instance, Beringia (including the Yukon Territory, Alaska, Chukotka and north-eastern Siberia) served as a major refugium for arctic flora and fauna during glacial maxima (Hultén, 1968; Adams & Faure, 1997; Edwards *et al.*, 2000; Elias *et al.*, 2000; Abbott & Brochmann, 2003; Brubaker *et al.*, 2005), and high genetic diversity in Beringia and relatively low genetic diversity in formerly glaciated areas have been reported for several animal and plant taxa, such as: the tundra vole, *Microtus oeconomus* (Brunhoff *et al.*, 2003); bighorn sheep, *Ovis canadensis* and Dall sheep, *Ovis dalli* (Loehr *et al.*, 2006); Columbian ground squirrel, *Spermophilus columbianus* (Macneil & Strobeck, 1987); Old World swallowtail, *Papilio machaon* (Sperling & 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); arctic bell-heather, *Cassiope tetragona* (Eidesen *et al.*, 2007); entire-leaved aven, *Dryas integrifolia* (Tremblay & Schoen, 1999); and the purple saxifrage, *Saxifraga oppositifolia* (Abbott & Comes, 2003).

The patterns of genetic diversity described above were not observed in either of the two *Flavocetraria* species. Instead, clades inferred within each species contained specimens from distant geographical regions, and geographical population pairs exhibited moderate to high transoceanic gene flow. This suggests that, in response to climatic fluctuations, both *F. cucullata* and *F. nivalis* have been able to migrate over large distances as a result of an efficient long-distance dispersal capability. In addition, large and diverse populations have served as sources for such migrants, as suggested by the non-asymptotic accumulation curves of intraspecific ITS haplotypes

Figure 4 Geographical locations of *Flavocetraria cucullata* and *F. nivalis* samples and transoceanic migration estimates. Circles refer to newly collected and sequenced samples, whereas squares refer to the geographical origins of sequences from GenBank. Shading indicates areas that were glaciated during the Last Glacial Maximum. Insets show posterior probability distributions of migration ($M = 2N_em$) that were estimated between transoceanic population pairs using Markov chain Monte Carlo coalescent simulations in MDIV. For each dataset, the data were simulated assuming an infinite-sites model, using 2,000,000 steps in the chain, and an initial 500,000 steps to ensure that enough genealogies were simulated before approximating the posterior distribution. Arrow thickness indicates the number of migrants per generation ($4N_em$) in the specified direction in three classes: fewer than 1, 1–5 and more than 5.

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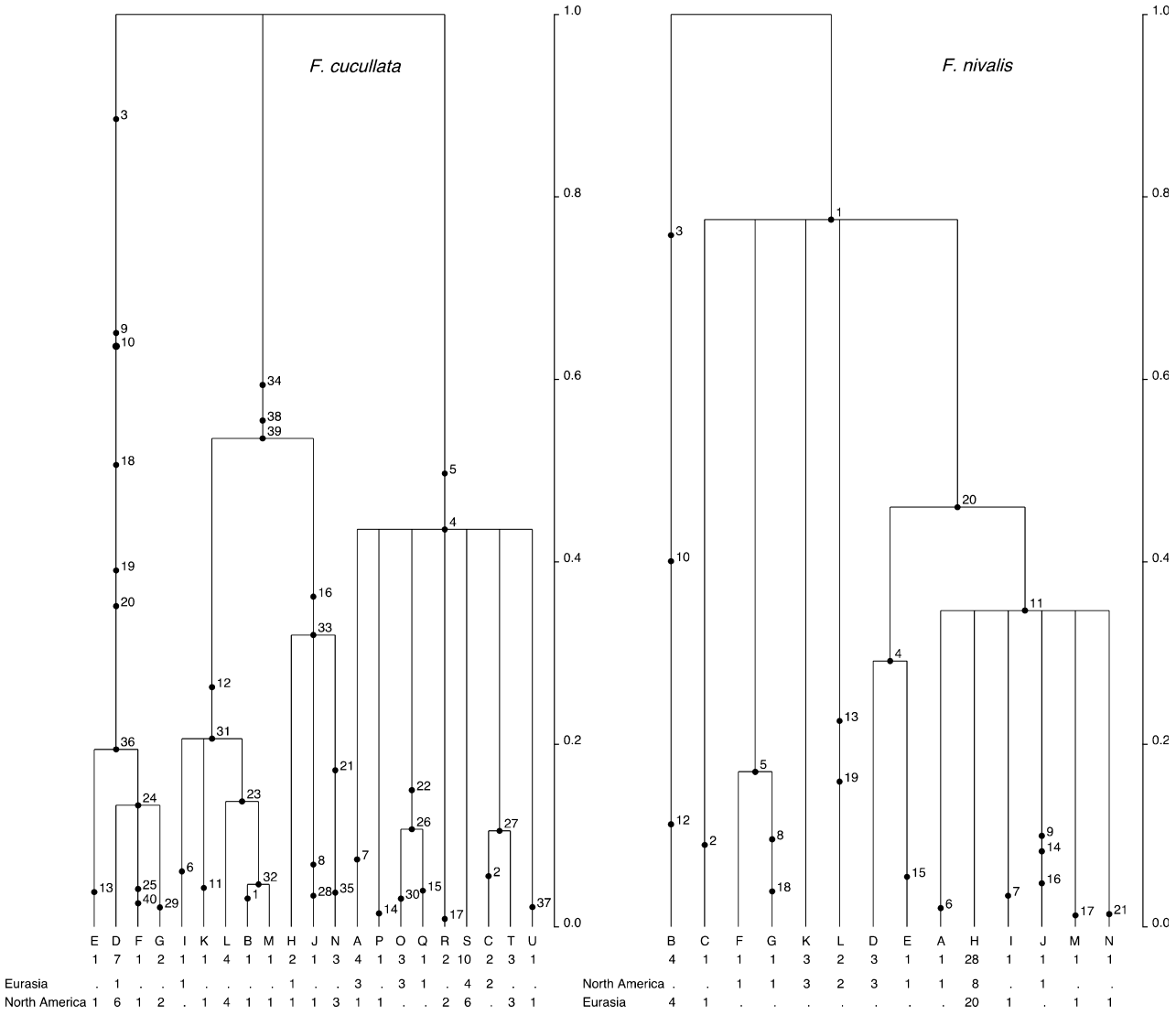

Figure 5 Coalescent-based genealogies of *Flavocetraria cucullata* and *F. nivalis* with the highest root probabilities (likelihood scores: $L = 7.2784 \times 10^{-41}$, SD = 2.3015×10^{-39}; and $L = 2.2766 \times 10^{-23}$, SD = 4.4249×10^{-22}, respectively) showing the distribution of mutations for the internal transcribed spacer (ITS) region. The inferred genealogy is based on 2,000,000 simulations of the coalescent with a Watterson's (1975) estimate of $\theta = 3.8$ (*F. cucullata*) and $\theta = 3.0$ (*F. nivalis*). 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 and their observed frequencies in total and in the different geographical regions.

in our sample (Fig. S2). Similar patterns of circumpolar genetic diversity have been detected in some other arctic organisms, for example in highly mobile animals such as the arctic fox, *Alopex lagopus* (Dalén *et al.*, 2005) and the snowy owl, *Bubo scandiacus* (Marthinsen *et al.*, 2009), as well as in the arctic–alpine lineage of the bog blueberry, *Vaccinium uliginosum* (Alsos *et al.*, 2005).

Many boreal, temperate or tropical fungi show strong intercontinental, sometimes even intracontinental, phylogeographical patterns and limited dispersal, and there is an increasing amount of geographical endemism being discovered (e.g. Taylor *et al.*, 2006 and references therein; Geml *et al.*, 2008; Bergemann *et al.*, 2009). Similarly, most of the very few phylogeographical studies carried out on lichens in the Northern Hemisphere indicate that recent gene flow among continents may be rare or absent and that disjunct distributions have probably existed for a long time and are not the result of recent long-distance dispersal (Högberg *et al.*, 2002; Printzen & Ekman, 2002; Printzen *et al.*, 2003; Palice & Printzen, 2004; Walser, 2004). In the Southern Hemisphere, Lumbsch *et al.* (2008) found some geographical structure in cosmopolitan tropical montane *Thelotrema* species, such as *Thelotrema lepadinum*, although their study was directed towards the phylogenetic reconstruction of the genus *Thelotrema* in Australia using mtSSU markers that showed low amounts of intraspecific variation. In the bipolar *Usnea*

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sphacelata R. Br., the arctic populations sampled were genetically almost uniform, whereas the South American populations were genetically more diverse and shared haplotypes from both hemispheres, indicating a southern origin and recent dispersal to northern high latitudes (Wirtz *et al.*, 2004). In the only population genetic study on arctic lichens published to date, Buschbom (2007) analysed sequence data from amphi-Atlantic populations of the circumpolar *Porpidia flavicunda* (Ach.) Gowan. based on specimens collected in eastern Canada, Greenland and Scandinavia. Buschbom detected low to moderate gene flow, mostly among adjacent regions (e.g. from Greenland to Europe and from Québec and Greenland to Baffin Island), and significant population differentiation between these regions.

Our results are most similar to those obtained for *Porpidia flavicunda*, although in both of the species we investigated the population differentiation was only marginally significant, whereas transoceanic dispersal was more pronounced. The high observed genetic diversity in the Arctic indicates long-term survival at northern high latitudes, whereas the estimated migration rates and the weak geographical population structure suggest continuing long-distance gene flow between continents that has prevented pronounced genetic differentiation. The capability of *Flavocetraria* for long-distance dispersal is also suggested by the fact that the few South American samples we analysed were genetically very similar to northern individuals in both species. Although the mode of dispersal is not known, it is likely that wind dispersal is important, as expected for most lichens. Wind dispersal should be particularly effective in the Arctic as a result of the open landscape, strong winds, and extensive snow and ice cover, as has also been suggested for arctic plants (Alsos *et al.*, 2007). In this regard, the sea ice may be of particular importance for intercontinental dispersal, as it provides a dry surface bridging the continents and archipelagos. Isidia and soredia, vegetative reproductive propagules, are absent in *Flavocetraria* (Thell *et al.*, 2002), whereas apothecia, sexual reproductive structures producing ascospores, are known, albeit rarely observed (Brodo *et al.*, 2001). On the other hand, conidia, one-celled asexual spores, are produced abundantly in black pycnidia at the edges of the erect thallus lobes (Brodo *et al.*, 2001), and thus they seem to be the primary agents of dispersal. Nevertheless, the incongruence we found among gene trees in *F. cucullata* suggests that inter-locus recombination by means of non-selfing sexual reproduction may be frequent enough to maintain high genetic diversity. Thus, ascospores may play some role in the dispersal of *Flavocetraria*, particularly because ascospores are generally considered more resistant to adverse environmental conditions than conidia (Seymour *et al.*, 2005). Besides wind, other possible means of dispersal include spores and conidia being carried by migratory animals, driftwood or drifting sea ice. Currently, we can only hypothesize that the asymmetrical migration rates between Eurasia and North America may be the result of differences in the extent of ice sheets in these continents during glacial maxima. As most of the North American Arctic was glaciated (except for eastern Beringia), the population sizes of *F. cucullata* and *F. nivalis* may have been greater in Eurasia than in North America, possibly resulting in more emigration events from Eurasia during and following glacial maxima. Similar asymmetrical migration patterns have been observed in a number of high-latitude organisms as summarized by Waltari *et al.* (2007). However, additional sampling is needed to elucidate this phenomenon further.

The implications of our results may not be restricted to *Flavocetraria*, but may have wider relevance for studies on the biodiversity, ecology and conservation of arctic fungi in general. The reconstruction of phylogeographical patterns of arctic organisms is of paramount importance, because knowledge of both past migrational history and present-day genetic diversity is essential for improving our predictions of how arctic species and communities will respond to global change. The high genetic diversity and the efficient long-distance dispersal capability of *F. cucullata* and *F. nivalis* suggest that these species, and perhaps other arctic lichens as well, will probably be able to track their potential niche in the changing Arctic.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Figure S1 Phylograms of *Flavocetraria cucullata* based on the BTUB and EF1 datasets.

Figure S2 Accumulation curves of internal transcribed spacer sequence types in *Flavocetraria cucullata* and *F. nivalis*.

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BIOSKETCH

József Geml is interested in the biodiversity, phylogeography and evolutionary ecology of arctic and boreal fungi and their historical and recent responses to changes in the landscape and the climate.

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