A multilocus evaluation of ermine (Mustela erminea) across the Holarctic, testing hypotheses of Pleistocene diversification in response to climate change

4 authors:

Natalie Dawson
University of Montana
13 PUBLICATIONS **217** CITATIONS

SEE PROFILE

Andrew G Hope
Kansas State University
62 PUBLICATIONS **981** CITATIONS

SEE PROFILE

Sandra L. Talbot
United States Geological Survey
199 PUBLICATIONS **3,159** CITATIONS

SEE PROFILE

Joseph A Cook
University of New Mexico
463 PUBLICATIONS **7,022** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Suitability of habitat for black-footed ferret reintroduction at El Malpais National Conservation Area View project

Factors influencing shifting winter distributions of Arctic Geese View project

ORIGINAL ARTICLE

A multilocus evaluation of ermine (*Mustela erminea*) across the Holarctic, testing hypotheses of Pleistocene diversification in response to climate change

Natalie G. Dawson[1,2], Andrew G. Hope[3]*, Sandra L. Talbot[3] and Joseph A. Cook[1]

[1]*Department of Biology and Museum of Southwestern Biology, University of New Mexico, MSC03 2020, Albuquerque, NM 87131, USA,* [2]*College of Forestry and Conservation, University of Montana, Missoula, MT 59812, USA,* [3]*U.S. Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, AK 99508, USA*

ABSTRACT

Aim We examined data for ermine (*Mustela erminea*) to test two sets of diversification hypotheses concerning the number and location of late Pleistocene refugia, the timing and mode of diversification, and the evolutionary influence of insularization.

Location Temperate and sub-Arctic Northern Hemisphere.

Methods We used up to two mitochondrial and four nuclear loci from 237 specimens for statistical phylogeographical and demographic analyses. Coalescent species-tree estimation used a Bayesian approach for clade divergence based on external mutation rate calibrations. Approximate Bayesian methods were used to assess population size, timing of divergence and gene flow.

Results Limited structure coupled with evidence of population growth across broad regions, including previously ice-covered areas, indicated expansion from multiple centres of differentiation, but high endemism along the North Pacific coast (NPC). A bifurcating model of diversification with recent growth spanning three glacial cycles best explained the empirical data.

Main conclusions A newly identified clade in North America indicated a fourth refugial area for ermine. The shallow coalescence of all extant ermine reflects a recent history of diversification overlying a deeper fossil record. Postglacial colonization has led to potential contact zones for multiple lineages in north-western North America. A model of diversification of ermine accompanied by recent gene flow was marginally less well supported than a model of divergence of major clades in response to the most recent glacial cycles.

Keywords
Approximate Bayesian computation, Beringia, coastal refugium, gene flow, island endemism, Mustelidae, North Pacific coast, phylogeography, species tree estimation.

*Correspondence: Andrew G. Hope, US Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, AK 99508, USA. E-mail: ahope@usgs.gov

INTRODUCTION

Cyclical climate change through the Pleistocene (2.6 Ma–12 ka) has been a catalyst for rapid speciation (Brochmann & Brysting, 2008; McCulloch *et al.*, 2010). At higher latitudes, continental ice sheets periodically forced dramatic distributional shifts (Hewitt, 1996), including episodes of range expansion followed by isolation and subsequent divergence. These repeated events underlie the generalized process of

diversification associated with climate cycles (Hope *et al.*, 2012), although responses varied widely. Generally, the ranges of tundra-associated species expanded during glacial advances, while those of forest-associated species contracted (Fedorov *et al.*, 2008; Hope *et al.*, 2013). Diversification has been complicated by migration, secondary contact and associated gene flow, coupled with isolation in multiple refugia (Abbott *et al.*, 2013). In Europe and North America, a model of refugial isolation followed by latitudinal shifts has been

the foundation for understanding the evolutionary consequences of ice ages (Hewitt, 1996; Arbogast & Kenagy, 2001; Petit *et al.*, 2003). Phylogeographical investigations have also tied diversification to repeated longitudinal shifts, including intercontinental movement through Eurasia and across the Bering Isthmus (Cook *et al.*, 2005; Waltari *et al.*, 2007; Hoberg *et al.*, 2012).

We examined these biogeographical concepts by assessing the diversification of the ermine (also called the stoat; *Mustela erminea* Linnaeus, 1758), one of the most widely ranging terrestrial mammals (Reid & Helgen, 2008). Ermine occur through boreal Europe, Asia, North America and Greenland (Fig. 1), on adjacent land-bridge archipelagos (e.g. the British Isles, Japan, Alaska's Alexander Archipelago), and in southern forest remnants that constitute montane sky islands. The earliest ermine fossils in Eurasia and North America date to the late Pliocene (*c.* 3 Ma; Kurtén, 1968) and the middle Pleistocene (*c.* 0.7 Ma; Kurtén & Anderson, 1980), respectively.

Despite a broad distribution, previous molecular assessments have uncovered only shallow regional phylogeographical structure. Fleming & Cook (2002) discovered three proximal but distinct mtDNA lineages: one distributed widely across the Palaearctic and north-western North America (hereafter, the Holarctic clade); a second widespread in North America (the continental clade); and a third endemic to a few islands along the North Pacific coast (NPC; the island clade) coinciding with a hypothesized coastal refugium. Martínková *et al.* (2007) detected another coastal refugium in Ireland dated to the Last Glacial Maximum (LGM; *c.* 21 ka). Ermine may also have occupied High Arctic refugia, where prey including collared lemming (Fedorov & Stenseth, 2002) and ptarmigan (Holder *et al.*, 1999) persisted. Northern and coastal refugia indicate that ermine can persist in periglacial areas despite their typical association with boreal forest (Fleming & Cook, 2002; Martínková *et al.*, 2007).
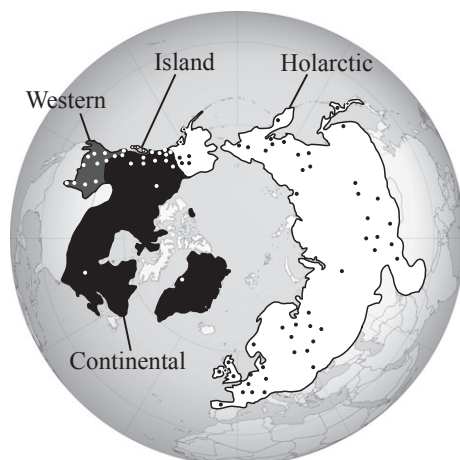

Figure 1 Map of the study area illustrating sampling localities and the range-wide distribution for four major clades of ermine (*Mustela erminea*) as detected in the current study.

Refugial locations and recolonization processes may be inferred from molecular signatures. Other species that share similar distributions with ermine in North America exhibit distinct West Coast lineages, reflecting isolation in coastal refugia (Demboski *et al.*, 1999; Conroy & Cook, 2000) or isolation west of the Rocky Mountains and/or Cascade Range (e.g. Demboski & Cook, 2001; Chavez & Kenagy, 2010). In addition, species consisting of distinct regional lineages across the northern continents indicate multiple refugial areas south of major continental ice sheets during glacial phases (Runck & Cook, 2005; Himes & Kenagy, 2010; Hope *et al.*, 2012). Repeated phylogeographical signatures of other boreal taxa (Arbogast & Kenagy, 2001) allow us to test competing hypotheses concerning the location of Pleistocene refugia for ermine in North America and associated source(s) for recolonization (H_1), and changes in population size, timing and sequence of divergence, and gene flow among contemporary lineages (H_2). We also link previous regional studies of ermine by means of expanded nuclear and range-wide geographical sampling, which are critical for understanding diversification across the Holarctic and for reassessing the conservation implications of coastal endemism along the NPC.

Hypotheses

H_{1a} – North American ermine occupied three glacial refugia, or H_{1b} – more than three refugia during the late Pleistocene. Three distinct clades were identified in north-western North America, and the island clade is likely to have persisted in a NPC refugium (Fleming & Cook, 2002). Fossil ermine dated to the Wisconsinan glacial (*c.* 130 to 12 ka) are known from Alaska and the Yukon Territory (i.e. eastern Beringian refugium) and throughout the conterminous United States (Kurtén & Anderson, 1980; Harington, 2011).

H_{2a} – Cladogenesis of extant ermine lineages began early in their evolutionary history, or H_{2b} – lineages only recently diverged. Considering early- to mid-Pleistocene fossils, ermine may have diverged over deep time-scales. However, if the current distribution is a consequence of recent expansion from a single source followed by allopatric divergence over one or two glacial cycles, clades should exhibit shallow structure. Similarly, gene flow following secondary contact may limit diversification (Abbott *et al.*, 2013). Finally, fluctuations in population size can influence coalescence times. We performed coalescent analyses of population size change and species tree estimation using multiple loci and a mutation rate based on documented vicariance events that are appropriate for investigating recent diversification within ermine. We then used empirical data to form and test multiple models of diversification from simulated data using approximate Bayesian computation (ABC). ABC tests provide a rigorous assessment of population size change, timing of diversification and the extent of gene flow in a mammal species that has been influenced by regional environments across the Holarctic.

MATERIALS AND METHODS

Sampling and sequencing

We analysed DNA sequences from 237 specimens of *M. erminea* (see Appendix S1 in Supporting Information). Our final analyses were based on 541 bp of the cytochrome *b* gene (cyt *b*, n_{tot} = 160), 272 bp of the control region (CR, n_{tot} = 210) and four independent nuclear loci: 261 bp of the serotonin receptor gene (*HTR1B* intron; *n* = 39), 480 bp of the agouti signalling protein gene (*ASIP* intron + exon; *n* = 46), 458 bp of the feline sarcoma proto-oncogene (*FES* intron + exon; *n* = 54), and 520 bp of the growth hormone receptor gene (*GHR* intron + exon; *n* = 35). A single individual of *Mustela frenata* was the outgroup (Appendix S1).

Clade demographics

Genetic diversity and demography were analysed for all clades collectively across loci, and for separate clades with mitochondrial data only. Summary statistics calculated in DnaSP 5 (Librado & Rozas, 2009) included genetic diversity indices, and Tajima's *D* (Tajima, 1989) and Fu's F_S (Fu, 1997) tests for demographic expansion. Population size changes through time were calculated from the coalescent extended Bayesian skyline plot (EBSP) with tree prior for multiple loci (Heled & Drummond, 2008).

Estimation of mutation rate

Few fossils of *M. erminea* (*sensu stricto*) exist and fossil dates are broad (Paleobiology Database; http://www.paleodb.org/). Koepfli *et al.* (2008) suggested a time to the most recent common ancestor (TMRCA) for *M. erminea* and other mustelids of *c.* 3.5 Ma but with a confidence interval *c.* 2 Myr wide. We estimated a mutation rate for cyt *b* using isolation dates (bathymetric and isotopic data; Yoshikawa *et al.*, 2007) for an extant insular lineage of *Mustela nivalis* on Honshu, Japan. We assumed that the closely related ermine shares a similar mutation rate (Kumar & Subramanian, 2002).

Methods for estimating mutation rate were adapted from Hope *et al.* (2010). Briefly, we retrieved GenBank cyt *b* sequences of *M. nivalis* from Honshu, Hokkaido, Russian Primorye and South Korea. Honshu is isolated by the Tsushima and Tsugaru straits, both *c.* 130 m in depth, allowing only limited connections through the Pleistocene. At 0.33 Ma [marine isotope stage (MIS)-10], 0.14 Ma (MIS-6; Yoshikawa *et al.*, 2007) and the LGM (0.021 Ma; MIS-2), sea levels dropped, creating land-bridge connections or narrow frozen straits. A rate greater than 20% Myr^{-1} for the cyt *b* gene would be necessary to accommodate an LGM connection, so this possibility was excluded. In beast 1.6.1 (Drummond & Rambaut, 2007), we grouped samples from Honshu separately and estimated clock rate with a tree root fixed to either 0.33 Ma or 0.14 Ma. These two calibrations resulted in rates for the cyt *b* gene of 2.1% Myr^{-1} and 5.1% Myr^{-1}

(equivalent to 4.2% and 10.2% divergence rates). Although fossils were not incorporated into mutation rate estimation, we used fossils to refine rate estimates a posteriori. Between the two point mutation rates, we considered 2.1% Myr^{-1} to be the most realistic because the coalescence time of ermine and the closely related *M. frenata* that used this rate and based on multiple loci coincided closely with the earliest ermine fossils, and because fossil dates generally underestimate the first occurrence.

Gene trees, species trees and divergence dating

Markov chain Monte Carlo (MCMC) searches of tree space were performed in MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). The convergence of MCMC runs (with burn-ins) was assessed in tracer 1.4 (Rambaut & Drummond, 2007), with burn-ins adjusted for convergence among analyses. Phylograms were visualized in FigTree 1.2.2 (Rambaut, 2009). Haplotype networks for all loci were constructed in Network 4.610 (Bandelt *et al.*, 1999). We used *beast (Heled & Drummond, 2010) to co-estimate all genealogies embedded within a species tree. We also repeated *beast analyses using only mitochondrial loci and using only nuclear loci.

ABC model simulations

We developed 12 models of diversification with accompanying parameters and command lines (see Fig. S1 in Appendix S2) to test hierarchical models of population size, timing and levels of gene flow associated with cladogenesis. For each model, one million simulated datasets were produced in diyabc 1.0.4.46beta (Cornuet *et al.*, 2010) for two mtDNA loci and four autosomal nuclear loci. All parameters were assigned a uniform prior (interval of ± 0.5 × parameter value) except for the rate of gene flow (*ra*, for which the interval remained at default values). All models considered four clades: Holarctic, island, continental and western. The Holarctic and island clades formed a lineage that was sister to the continental and western clades together. Mutation rates for all models were based on empirical values estimated from species tree analyses assuming a cyt *b* rate of 2.1% Myr^{-1}. Transition/transversion ratios (*K*) and the proportion of invariant sites (P_{inv}) were set to mean values for autosomal and mitochondrial loci from empirical data.

The first four models (N_e models; Fig. S1a–d) tested scenarios of population size change through time. All shared a topology consistent with the species tree reconstruction, and divergence times remained constant. All clades underwent a 'bottleneck' following initial divergence, and then their populations grew to reflect population size changes calculated from EBSPs. The population sizes for N_e model 1 followed values obtained from individual clade EBSPs; the population sizes for N_e models 2 to 4 reflected values obtained from an EBSP for all clades combined to represent size changes for the entire species. For N_e model 2, population size was assigned relative to the population sizes of clades, in the ratio

of sizes provided from individual EBSPs, and resulting in a constant total population size through the tree. For N_e model 3, population size was assigned equally among clades and total population was constant through the tree. For N_e model 4, population sizes were constant along each branch, meaning a fourfold increase in total population through the tree from root to tips (i.e. expansion through time). Following simulations, the N_e model most similar to the empirical dataset provided the population sizes that were used in subsequent models.

The second four models (temporal models; Fig. S1e–h) tested different timings of cladogenesis. Temporal model 1 reflected a recent (Pleistocene/Holocene; 21–7 ka) fragmentation of all clades. Temporal model 2 reflected a recent divergence of the Holarctic and island clades and continental and western clades, respectively, but deep divergence of both combined lineages. For temporal model 3, the divergences of extant clades were dated to the onset of the Wisconsinan glacial (*c.* 100 ka); for temporal model 4, the divergences of extant clades were dated to the onset of the Illinoian glacial (*c.* 200 ka). Divergence times from the temporal model most similar to the empirical dataset were assigned to all subsequent models.

The final four models (gene flow models; Fig. S1i–l) tested scenarios of gene flow. Gene flow model 1 was identical to the best temporal model (no gene flow). Model 2 assigned gene flow recently (Pleistocene/Holocene transition) between closely related clades. For model 3, gene flow was assigned both recently and coincident with the Sangamon interglacial (*c.* 130–100 ka). The final model assigned gene flow coincident with the Holocene (21–7 ka) and the two most recent interglacials (*c.* 100 ka; *c.* 200 ka).

Statistical evaluation of simulated data from each set of models included pre-evaluation of model–prior combinations to detect models unable to explain the empirical dataset and the computation of posterior probabilities of models including both direct and logistic regression methods with default values. For additional details of methods, see Appendix S2.

RESULTS

Clade demographics

Mitochondrial genealogies identified four clades generally coincident with geography (Fig. 1). Low genetic diversity characterized all loci (Table 1). Significant geographical expansion (and growth) was evident for the continental and Holarctic clades, with only minimal expansion in the western and island clades (Table 1). Among the nuclear loci, only *ASIP* indicated significant expansion. Timing of population size change from EBSPs indicated growth beginning prior to 50 ka for the Holarctic clade (Fig. 2a) and at *c.* 25 ka for the continental clade (Fig. 2b).

Gene trees, species trees and divergence dating

The relationships among the four major clades were generally consistent across mitochondrial loci, while nuclear genealogies were largely unresolved (Fig. S2 in Appendix S2), although analysis of all loci from species tree reconstruction lent strong support for the four clades, with a sister relationship between the continental and western clades and between the Holarctic and island clades (Fig. 3). Both clade pairs (continental + western; Holarctic + island) were reciprocally monophyletic. Haplotype networks generally reflected the low divergence and low resolution among clades for nuclear loci (Fig. S3 in Appendix S2). Haplotypes within clades were geographically widespread, although some regional affiliations included Europe and Central Asia within the Holarctic clade.

TMRCA estimates for the main clades varied depending on the loci used (Table 2; Fig. 3): estimates were shallower with all six loci and deeper when using only mitochondrial loci.

Table 1 Range-wide demographic statistics for 237 samples of ermine (*Mustela erminea*). Loci include partial mtDNA control region (CR) and cytochrome *b* (cyt *b*) and partial introns from nuclear 5-HT1B receptor gene: serotonin receptor (*HTR1B*), agouti signalling protein (*ASIP*), feline sarcoma proto-oncogene (*FES*) and growth hormone receptor (*GHR*). Statistics include sample size (*n*), sequence length (*l*), number of haplotypes (*h*), haplotype diversity (*Hd*), nucleotide diversity (π), Tajima's *D* and Fu's F_S. Significance of *D* and F_S is indicated as follows: *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

| Gene – group	*n*	*l* (bp)	*h*	*Hd*	π	*D*	F_S
CR – all	210	271	42	0.906	0.0117	−1.696**	−30.143***
CR – continental	52	263	14	0.710	0.0047	−1.945**	−9.931***
CR – Holarctic	129	264	28	0.875	0.0074	−1.978**	−21.830***
CR – island	14	264	3	0.560	0.0029	0.558	0.292
CR – western	15	262	6	0.790	0.0056	−0.198	−1.709
cyt *b* – all	160	541	46	0.914	0.0174	−0.523	−9.959*
cyt *b* – continental	31	541	8	0.499	0.0018	−2.053***	−3.964**
cyt *b* – Holarctic	114	541	33	0.873	0.0045	−1.990**	−26.784***
cyt *b* – island	4	541	2	0.500	0.0009	−0.612	0.172
cyt *b* – western	11	541	3	0.345	0.0010	−1.600*	−0.537
HTR1B	39	259	4	0.538	0.0023	−0.325	−0.571
ASIP	46	480	13	0.872	0.0036	−0.477	−6.127***
FES	54	449	4	0.535	0.0013	−0.265	−0.463
GHR	35	520	6	0.645	0.0016	−0.794	−1.956
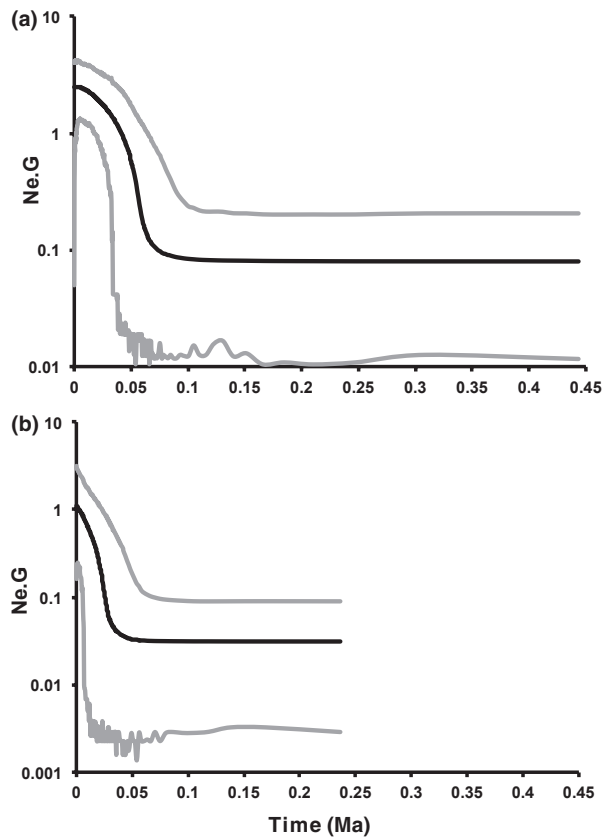
Figure 2 Extended Bayesian skyline plots using six independent loci for (a) the Holarctic clade and (b) the continental clade of ermine (*Mustela erminea*). Plots indicate population growth from past (right) to present (left) including median population size through time (black line) and 95% highest probability distribution (grey interval). Log-transformed *y*-axes represent population size as a function of effective size (N_e) and generation time (*G*).
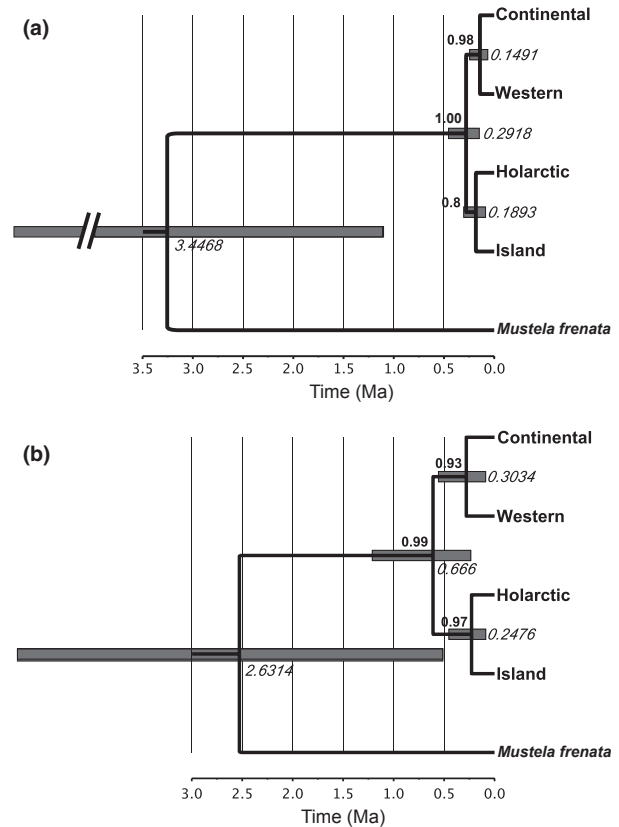

Figure 3 Species trees using (a) six loci or (b) two mitochondrial loci for ermine (*Mustela erminea*). Analyses, performed in *BEAST, provide posterior probability nodal support (bold, left of node) and coalescence times (millions of years ago, Ma; italics, right of node). Shaded bars indicate 95% confidence intervals for coalescence estimates, consistent with values in Table 2. Topologies are ultrametric and proportional to evolutionary time (bottom), which extends from present (right) to past (left).

Conversely, tree roots were older when estimated using six loci than when based only on mtDNA (Fig. 3). This observation was also true for a species tree based only on nuclear loci (Fig. S4 in Appendix S2). Using all loci, the TMRCA for each clade pair coincided with the Illinoian–Saalian glacial (*c.* 150–190 ka; Gibbard & van Kolfschoten, 2004) and the TMRCA of all clades coincided with the pre-Illinoian (*c.* 300 ka; Table 2, Fig. 3). Using only mitochondrial loci increased the divergence estimates to *c.* 300 ka or *c.* 250 ka for pairs of clades and *c.* 660 ka for the whole species (Table 2, Fig. 3). The TMRCA of *M. erminea* and *M. frenata* was estimated at 3.4 Ma or 2.6 Ma (based on six or two loci, respectively; Table 2, Fig. 3).

ABC model simulations

The N_e model most closely resembling the empirical data divided the population size from the combined ermine dataset along branches in proportions reflecting the population sizes that were calculated for individual clades (N_e model 2; Fig. S1). Both direct and logistic regression methods supported N_e

Table 2 Time to most recent common ancestor (TMRCA) estimates (mean and 95% confidence intervals in million years ago, Ma) for ermine (*Mustela erminea*) from species tree reconstructions based on 237 specimens and six loci: cytochrome *b* (cyt *b*); control region (CR); partial 5-HT1B receptor gene; agouti signalling protein; feline sarcoma proto-oncogene; and growth hormone receptor. Reconstructions based on two loci used cyt *b* and CR. TMRCA estimates correspond to those in Fig. 3.

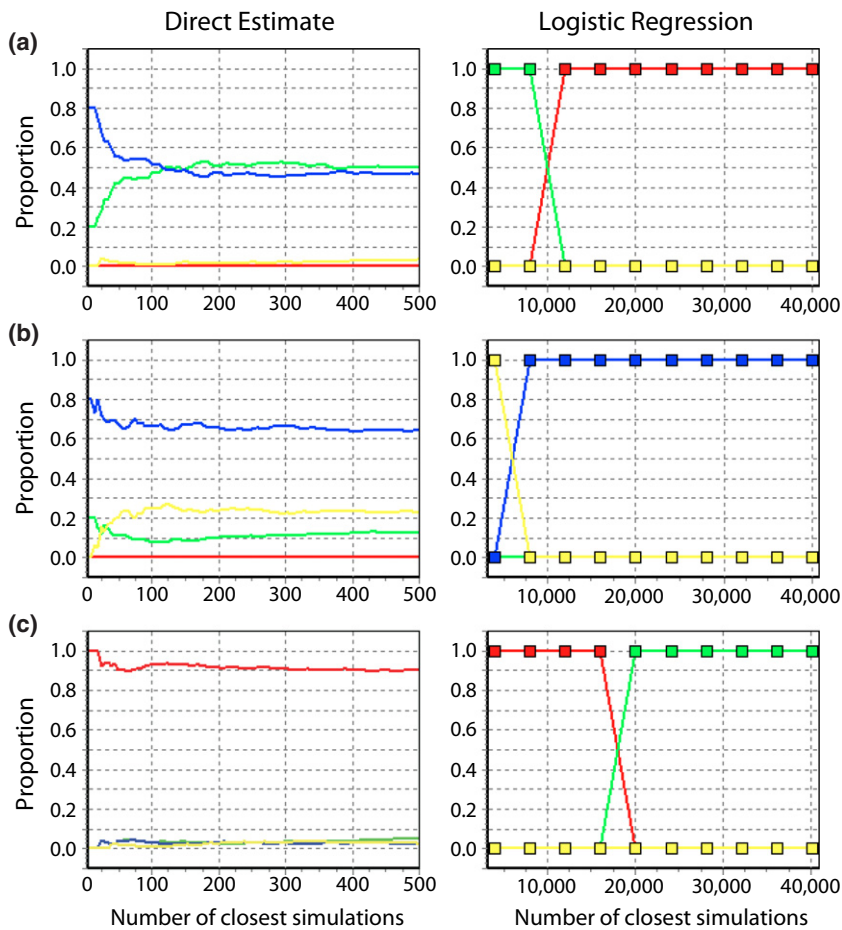| Number of loci	Group	TMRCA (Ma)	95% CI (TMRCA; Ma)
6	1: Continental + western	0.1491	0.0639–0.2442
	2: Holarctic + island	0.1893	0.0863–0.3038
	3: 1 + 2	0.2918	0.1489–0.4552
	4: Tree root	3.4468	1.1169–5.9687
2	1: Continental + western	0.3034	0.0878–0.4527
	2: Holarctic + island	0.2476	0.0868–0.4527
	3: 1 + 2	0.6660	0.2348–1.2117
	4: Tree root	2.6314	0.5125–4.7269

Figure 4 Approximate Bayesian computation model comparisons considering results of direct (left) and logistic regression (right) analyses for (a) N_e models, (b) temporal models and (c) gene flow models of diversification for ermine (*Mustela erminea*). Models are colour-coded within each set: red, model 1; green, model 2; blue, model 3; yellow, model 4. Direct analyses considered the proportion of times that a model was represented in the n closest datasets to empirical data from the 500 closest simulations. Logistic regressions considered the n closest datasets to empirical data reported as 10 point-estimates (regression intercept) with the proportion of the model represented as the dependent variable, and differences between the observed and simulated dataset summary statistics as the independent variable. Models correspond to Fig. S2 in Appendix S2 and data values are reported in Table S1 of Appendix S2.

model 2 (Fig. 4; Table S1 in Appendix S2), although the distributions of simulated data for N_e models 2 and 3 were closely overlapping (Fig. S5 in Appendix S2). Using the population sizes from N_e model 2, simulations indicated that temporal model 3 (divergences dated to the onset of the Wisconsinan glacial) best coincided with empirical data (see Figs 4 & S5 and Table S1). With divergence times set to temporal model 3, gene flow model 1 (no gene flow) most closely resembled the empirical data, although gene flow model 2 (recent gene flow) was a close second, and all four gene flow models produced closely overlapping distributions (Fig. S5).

DISCUSSION

H₁: Location of Pleistocene refugia and sources for post-glacial recolonization

We documented a new clade of ermine in western North America, consistent with the persistence of the species in four refugia in North America during the Wisconsinan glacial. The western and continental clades indicated two southern refugia for ermine in North America that parallel the primary east/west division of several other mammals (Arbogast & Kenagy, 2001) and the Holarctic and island clades reflect Beringian and NPC refugia. Ermine persisted through

several glacial cycles in North America, with the earliest fossils, dating to *c.* 0.7 Ma, coming from Arkansas and Kansas (Kurtén & Anderson, 1980). Fossils from the last interglacial (Sangamon; 130 ka) have been found in the Yukon Territory and Utah, while fossils from the Wisconsinan glacial and the Holocene have been recovered from eastern Beringia (Yukon), the central Rockies (Idaho and Colorado), Texas and Arkansas (Kurtén & Anderson, 1980; Harington, 2011).

A lack of evidence of Holocene population expansion for the western clade, coupled with few common haplotypes, is consistent with contraction into remnant habitat in western North America since the LGM (Fig. 1). Holocene demographic contraction was also suggested for European populations (Martínková *et al.*, 2007). In contrast, the broad distribution and signature of expansion evident for the continental clade were consistent with Holocene colonization of deglaciated regions in Canada and Alaska. There was no evidence to support a High Arctic refugium for ermine during the LGM, although ermine may have occurred in the High Arctic earlier in the Wisconsinan. The lack of significant genetic structure in the continental clade indicated a single geographical source. We suggest that continental ermine spread north from a southern refugium (consistent with fossil evidence) located east of the Rocky Mountains.

Northward movement was likely to have been rapid and coincident with the expansion of boreal vegetation following the LGM (Jaramillo-Correa *et al.*, 2004; Runck & Cook, 2005; de Lafontaine *et al.*, 2010; Hope *et al.*, 2012). Further north, significant expansion also characterized the Holarctic clade (Table 1), reflecting the rapid spread of ermine across Eurasia. The geographical distribution of haplotypes through Eurasia indicated multiple potential sources in Ireland, continental Europe and Central Asia. Fossil evidence from Beringia during the Wisconsinan (Kurtén & Anderson, 1980; Harington, 2011) may represent populations that were subsequently extirpated. Finally, the island clade was restricted to the Prince of Wales (POW) and nearby Haida Gwaii archipelagos (Fleming & Cook, 2002). Low genetic diversity is likely to indicate a small refugial population with a coalescent history that may only span the most recent glacial period.

H₂: Timing and sequence of evolution among ermine lineages

This multilocus coalescent approach revealed a recent history of diversification among extant ermine. TMRCA was no earlier than *c.* 660 ka and possibly as recent as *c.* 122 ka (Table 2, Fig. 3), a surprising finding given that there are European fossils dated to *c.* 3 Ma (Kurtén, 1968). The knowledge that divergence times calculated from mitochondrial loci are often overestimated compared with nuclear loci lends further support to recent diversification (Smith & Klicka, 2013). Even the deeper mitochondrial divergence estimates revealed that the current geographical structure resulted from a recent pulse of diversification, possibly as a consequence of stronger and longer glacial phases in the late Pleistocene (Hope *et al.*, 2013). Cladogenesis at 0.66 Ma (Table 2, Fig. 3b) may coincide with the first intercontinental colonization based on the earliest North American fossils (*c.* 0.7 Ma; Kurtén & Anderson, 1980). Genetic analysis of fossils from the Conrad Fissure in Arkansas might clarify the number and timing of intercontinental colonizations.

Our analyses supported three intercontinental colonization events for ermine. Given the fossil record, ermine are likely to have entered North America through Beringia in the mid-to-late Pleistocene and diverged around 300 ka. A second invasion from Eurasia occurred as late as the penultimate glacial (Illionian; *c.* 130–200 ka), probably along a southern coastal route through western Beringia (Hope *et al.*, 2013), with subsequent isolation along the NPC during the Wisconsinan glacial. In a similar time-frame, southern populations are likely to have diverged along an east/west axis, possibly across the Rocky Mountains. Finally, during the most recent glacial leading up to the Holocene, ermine representing the Holarctic clade colonized eastwards through Beringia. As the Wisconsinan ice sheets receded, the continental clade expanded northwards and attained proximity to the Holarctic and island clades (Fig. 1).

Although there are potential pitfalls of using multiple discordant gene trees to infer clade relationships (Degnan & Rosenburg, 2006), species trees using all loci (Fig. 3) or only nuclear loci (Fig. S4 in Appendix S2) resulted in the same topology and demonstrated that adding loci narrows the confidence intervals around node ages and reduces potential homoplasy near the tree root (Fig. 3). Despite well-supported species trees, stochastic lineage sorting is inherently difficult to distinguish from gene flow (Reid *et al.*, 2012). Multiple mitochondrial haplotypes were widely distributed within both Holarctic and continental clades, indicating population expansion and within-clade connectivity. However, some nuclear haplotypes were shared across clades and individuals sharing alleles were often located near major contact zones (Figs S2 & S3 in Appendix S2), particularly in eastern Beringia between the Holarctic and island clades and in western North America between the continental and western clades (Fig. 1). This may reflect recent colonization, the retention of nuclear alleles, the maintenance of gene flow or some combination of these. Gene flow both within and among major regions may therefore be an important feature of ermine biogeography. The periodicity of glacial cycling, particularly during the early to middle Pleistocene, may have been too brief to initiate persistent differentiation. With additional loci and emerging genomic methods available to assess the degree and timing of hybridization, ermine constitute an excellent group for exploring the dynamics between divergence and intermittent gene flow (Miller *et al.*, 2012; Twyford & Ennos, 2012).

The timing of divergence and potential gene flow is also more rigorously evaluated by statistical simulation of realistic evolutionary scenarios (Beaumont, 2010). Phylogeographical analyses such as ABC set the stage for rigorous statistical tests of hypotheses regarding high-latitude diversification processes (Beaumont, 2010). Preliminary genealogies and species trees provided a null topology for the timing and sequence of differentiation events, while demographic statistics provided estimates of population changes through time. Hierarchical model simulations then provided controls for sets of variables. The best-supported N_e model used population sizes for clades that reflected their relative empirical genetic diversity. The most strongly supported topology from ABC analyses was consistent with cladogenesis resulting from the onset of glacial phases and corresponding regional isolation. This model (no gene flow) exhibited minimally higher support than a model incorporating recent gene flow between the most closely related populations, so the possibility of contemporary hybridization deserves further investigation.

A closer look at islands of the NPC

Refugia coincident with coastal land-bridge archipelagos may support populations that have experienced prolonged isolation due to rising sea levels that maintained separation following glacial phases. The distributions of two distinct and geographically restricted ermine phylogroups are consis-

tent with independent coastal refugia – an Irish refugium (Martínková *et al.*, 2007) and an NPC refugium in North America (Fleming & Cook, 2002; Dawson, 2008). Both constitute land-bridge archipelagos, although the Irish lineage maintained relatively high diversity and is minimally divergent from 'mainland' ermine of the Holarctic clade (isolation dating to *c.* 23 kyr), whereas ermine of the island clade of the NPC exhibited low genetic diversity with population isolation persisting until at least the Last Glacial (prior to 100 ka; Table 2, Fig. 3). The relative timing of divergence and observed levels of genetic diversity have implications for the conservation of distinct lineages and for understanding the influence of coastal refugia on regional patterns of diversity and community assembly. Small populations that have experienced extended isolation in insular refugia – such as those shown by endemic genetic signatures in ermine along the NPC – may represent significant components of a species' genetic legacy, and may be important for considering future persistence.

During the Last Glacial (*c.* 120–11 ka), coastal communities of the NPC were shifted onto the exposed continental shelf, isolated from other mainland areas by the Cordilleran ice sheet and are likely to have been further fragmented by major tidewater glaciers (see Fig. 3 in Carrara *et al.*, 2007; Cook & MacDonald, 2013). Molecular studies have identified cold-tolerant species that exhibit distinct island lineages along the NPC (e.g. Talbot & Shields, 1996; Demboski & Cook, 2001; Topp & Winker, 2008), although few of these endemic lineages are as divergent as in ermine (Soltis *et al.*, 1997; Weckworth *et al.*, 2005; Cook *et al.*, 2006; Dawson *et al.*, 2007; Shafer *et al.*, 2011). Molecular studies on salmon (*Oncorhynchus keta*; Kondzela *et al.*, 1994), spruce grouse (*Falcipennis canadensis*; Barry & Tallmon, 2010) and numerous plants (e.g. Soltis *et al.*, 1997), and a fossil record from caves representing a continuous sequence of radiocarbon ages from 40 to 10 ka (Heaton & Grady, 2003), provide further evidence that POW was a refugium for relatively diverse communities during the Last Glacial, although the community composition changed as the climate warmed. The flora during the Last Glacial consisted of graminoid tundra with willows (*Salix* spp.; ≥ 30 ka) suggesting a cold, arid climate (Brubaker *et al.*, 2005; Zazula *et al.*, 2005). This community was replaced by birch shrub and sedge–moss tundra (15–13 ka) indicating moister summers and warmer winters. The earliest trees arrived *c.* 12 ka (*Picea*) and 11 ka (*Pinus*), based on dated stumps and pollen found near Haida Gwaii (Fedje & Josenhans, 2000). Species such as ermine that persisted in the NPC despite marked community turnover are thus characterized by broad ecological tolerances, although these species may have experienced significant reductions in genetic variability.

Ermine of the island clade are endemic to only a few islands of the POW (MacDonald & Cook, 2009) and Haida Gwaii (Heusser, 1989) archipelagos, but representatives of both the continental and Holarctic clades of ermine have recently colonized a few islands of the NPC. The close prox-imity of multiple lineages presents strong conservation implications for the maintenance of diversity and for evolutionary dynamics including competition, hybridization and the exchange of parasites (Koehler *et al.*, 2007). Further documentation of these three clades throughout the NPC is needed. The close genetic associations of island-clade ermine on Haida Gwaii and POW islands suggest a Last Glacial connection between these islands. Steller's jay (Topp & Winker, 2008) and several lichens and fungi (Geiser *et al.*, 1998) conceivably share similar signatures.

The divergences between pairs of ermine lineages was assessed with the K/θ ratio (Birky, 2013) with values of 5.5 (based on cyt *b*) for island and continental clades and 0.8 for island and Holarctic clades. Values greater than or equal to 4 may represent species-level differentiation under the evolutionary genetic species concept (Birky, 2013). Although we do not yet recommend taxonomic revision, these values, coupled with multilocus analyses, provide strong evidence for multiple evolutionarily significant units (Moritz, 2002) along the NPC. Understanding the vulnerability of archipelago populations is essential, particularly considering that high endemism coupled with environmental variability increases the risk of extinction or extirpation (Weir & Schluter, 2007).

CONCLUSIONS

Multiple species, as exemplified by ermine, attained vast distributions across the northern continents in relatively short time-frames through the late Pleistocene, with intercontinental movement through Beringia being an important consequence of expansion. Common phylogeographical structure over numerous ecological components of Arctic diversity presents the possibility of parallel differentiation among related taxa (Dawson, 2012), but the realization of both common and idiosyncratic responses also provides key insights. Because several mustelids are widely distributed across the Holarctic, assumptions of parallel evolution with niche partitioning or interdependence should be tested. For carnivores such as ermine, we may also predict codifferentiation between specialized predators and their prey, but whereas many small prey species have developed multiple distinct phylogroups across the Holarctic attributable to Pleistocene climate cycles (Hewitt, 1996), predators such as ermine seem to maintain a higher degree of continent-wide connectivity through glacial cycles, highlighting the value of comparative investigation.

Ermine were influenced by localized isolation in coastal and periglacial areas or broad regional differentiation due to barriers such as continental ice sheets. However, reference to these as 'refugia' during glacial periods is potentially misleading for a species that can seemingly thrive and maintain a broad distribution during glacial advances (Bennett & Provan, 2008). Rather, coastal refugia along the NPC or in Ireland (Martínková *et al.*, 2007) are indicative of confined isolates that may or may not have experienced a significant

loss of genetic diversity through potential bottlenecks. True interglacial refugia may be represented by isolated patches of temperate habitat at the southern extent of the boreal biome in the form of sky islands, and yet the genetic evidence still suggests the long-term maintenance of close associations among these terrestrial islands.

The diversification of ermine through the last three glacial cycles parallels the late-Pleistocene evolutionary history of numerous temperate taxa, although the extent of interaction between contemporary lineages along zones of contact remains to be explored. North-western North America, as shown by an increasing number of genetic assessments, was a dynamic evolutionary arena with multiple transition zones between lineages, biomes and regional environments.

ACKNOWLEDGEMENTS

REFERENCES

Abbott, R., Albach, D., Ansell, S. *et al.* (2013) Hybridization and speciation. *Journal of Evolutionary Biology*, **26**, 229–246.

Arbogast, B.S. & Kenagy, G.J. (2001) Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography*, **28**, 819–825.

Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Barry, P.D. & Tallmon, D.A. (2010) Genetic differentiation of a subspecies of spruce grouse (*Falcipennis canadensis*) in an endemism hotspot. *The Auk*, **127**, 617–625.

Beaumont, M.A. (2010) Approximate Bayesian computation in evolution and ecology. *Annual Review of Ecology, Evolution, and Systematics*, **41**, 379–406.

Bennett, K.D. & Provan, J. (2008) What do we mean by 'refugia'? *Quaternary Science Reviews*, **27**, 2449–2455.

Birky, C.W. (2013) Species detection and identification in sexual organisms using population genetic theory and DNA sequences. *PLoS ONE*, **8**, e52544.

Brochmann, C. & Brysting, A.K. (2008) The Arctic – an evolutionary freezer? *Plant Ecology and Diversity*, **1**, 181–195.

Brubaker, L.B., Anderson, P.M., Edwards, N.E. & Lozhkin, A.V. (2005) Beringia as a glacial refugium for boreal trees and shrubs: new perspectives from mapped pollen data. *Journal of Biogeography*, **32**, 833–848.

Carrara, P.E., Ager, T.A. & Baichtal, J.F. (2007) Possible refugia in the Alexander Archipelago of southeastern Alaska during the late Wisconsin glaciation. *Canadian Journal of Earth Sciences*, **44**, 220–244.

Chavez, A.S. & Kenagy, G.J. (2010) Historical biogeography of western heather voles (*Phenacomys intermedius*) in montane systems of the Pacific Northwest. *Journal of Mammalogy*, **91**, 874–885.

Conroy, C.J. & Cook, J.A. (2000) Molecular systematics of a Holarctic rodent (*Microtus*: Muridae). *Journal of Mammalogy*, **81**, 344–359.

Cook, J.A. & MacDonald, S.O. (2013) Island life: coming to grips with the insular nature of Southeast Alaska and adjoining British Columbia. *North Pacific temperate rainforests: ecology and conservation* (ed. by G. Orians and J. Schoen), pp. 19–42. University of Washington Press, Seattle, WA.

Cook, J.A., Hoberg, E.P., Koehler, A., Henttonen, H., Wickström, L., Haukisalmi, V., Galbreath, K., Chernyavski, F., Dokuchaev, N., Lahzuhtkin, A., MacDonald, S.O., Hope, A., Waltari, E., Runck, A., Veitch, A., Popko, R., Jenkins, E., Kutz, S. & Eckerlin, R. (2005) Beringia: intercontinental exchange and diversification of high latitude mammals and their parasites during the Pliocene and Quaternary. *Mammal Study*, **30**, S33–S44.

Cook, J.A., Dawson, N.G. & MacDonald, S.O. (2006) Conservation of highly fragmented systems: the north temperate Alexander Archipelago. *Biological Conservation*, **133**, 1–15.

Cornuet, J.-M., Ravigné, V. & Estoup, A. (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). *BMC Bioinformatics*, **11**, 401.

Dawson, N.G. (2008) *Vista Norteña: tracking historical diversification and contemporary structure in high latitude mesocarnivores*. PhD Thesis, University of New Mexico, Albuquerque, NM.

Dawson, M.N. (2012) Parallel phylogeographic structure in ecologically similar sympatric sister taxa. *Molecular Ecology*, **21**, 987–1004.

Dawson, N.G., MacDonald, S.O. & Cook, J.A. (2007) Endemic mammals of the Alexander Archipelago. *Wildlife management on the Tongass National Forest* (ed. by J. Schoen). Audubon Special Publication, New York.

Degnan, J.H. & Rosenburg, N.A. (2006) Discordance of species trees with their most likely gene trees. *PLoS Genetics*, **2**, e68.

Demboski, J.R. & Cook, J.A. (2001) Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America. *Molecular Ecology*, **10**, 1227–1240.

Demboski, J.R., Stone, K.D. & Cook, J.A. (1999) Further perspectives on the Haida Gwaii glacial refugium. *Evolution*, **53**, 2008–2012.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Fedje, D.W. & Josenhans, H. (2000) Drowned forests and archaeology on the continental shelf of British Columbia, Canada. *Geology*, **28**, 99–102.

Fedorov, V.B. & Stenseth, N.C. (2002) Multiple glacial refugia in the North American Arctic: inference from the phylogeography of the collared lemming (*Dicrostonyx groenlandicus*). *Proceedings of the Royal Society B: Biological Sciences*, **269**, 2071–2077.

Fedorov, V.B., Goropashnaya, A.V., Boeskorov, G.G. & Cook, J.A. (2008) Comparative phylogeography and demographic history of the wood lemming (*Myopus schisticolor*): implications for late Quaternary history of the taiga species in Eurasia. *Molecular Ecology*, **17**, 598–610.

Fleming, M.A. & Cook, J.A. (2002) Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. *Molecular Ecology*, **11**, 795–808.

Fu, Y-X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Geiser, L.H., Dillman, K.L., Derr, C. C. & Stensvold, M.C. (1998) Lichens and allied fungi of southeast Alaska. *Lichenographia Thomsoniana: North American lichenology in honor of John W. Thomson* (ed. by M.G. Glenn, R.C. Harris, R. Dirig and M.S. Cole), pp. 1–24. Mycotaxon, Ithaca, NY.

Gibbard, P. & van Kolfschoten, T. (2004) The Pleistocene and Holocene epochs. *A geologic time scale* (ed. by F.M. Gradstein, J.G. Ogg and A.G. Smith), pp. 441–452. Cambridge University Press, Cambridge, UK.

Harington, C.R. (2011) Pleistocene vertebrates of the Yukon Territory. *Quaternary Science Reviews*, **30**, 2341–2354.

Heaton, T.H. & Grady, F. (2003) The Late Wisconsin vertebrate history of Prince of Wales Island, Southeast Alaska. *Ice Age cave faunas of North America* (ed. by B.W. Schubert, J.I. Mead and R.W. Graham), pp. 17–53. Indiana University Press, Bloomington, IN.

Heled, J. & Drummond, A.J. (2008) Bayesian inference of population size history from multiple loci. *BMC Evolutionary Biology*, **8**, 289.

Heled, J. & Drummond, A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.

Heusser, C.J. (1989) North Pacific coastal refugia: the Queen Charlotte Islands in perspective. *The outer shores* (ed. by G.G.E. Scudder and N. Gessler), pp. 91–106. Queen Charlotte Islands Museum, Skidgate, BC.

Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Himes, C.M. & Kenagy, G.J. (2010) Influence of montane isolation and refugia on population structure of *Sorex palustris* in western North America. *Journal of Mammalogy*, **91**, 1000–1010.

Hoberg, E.P., Galbreath, K.E., Cook, J.A., Kutz, S.J. & Polley, L. (2012) Northern host–parasite assemblages: history and biogeography on the borderlands of episodic climate and environmental transition. *Advances in Parasitology*, **79**, 1–97.

Holder, K., Montgomerie, R. & Friesen, V.L. (1999) A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). *Evolution*, **53**, 1936–1950.

Hope, A.G., Waltari, E., Dokuchaev, N., Abramov, S., Dupal, T., Tsvetkova, A., MacDonald, S.O., Henttonen, H. & Cook, J.A. (2010) High-latitude diversification within Eurasian least shrews and Alaska tiny shrews (Soricidae). *Journal of Mammalogy*, **91**, 1041–057.

Hope, A.G., Speer, K.A., Demboski, J.R., Talbot, S.L. & Cook, J.A. (2012) A climate for speciation: rapid spatial diversification within the *Sorex cinereus* complex of shrews. *Molecular Phylogenetics and Evolution*, **64**, 671–684.

Hope, A.G., Takebayashi, N., Galbreath, K.E., Talbot, S.L. & Cook, J.A. (2013) Temporal, spatial and ecological dynamics of speciation among amphi-Beringian small mammals. *Journal of Biogeography*, **40**, 415–429.

Jaramillo-Correa, J.P., Beaulieu, J., Bousquet, J. (2004) Variation in mitochondrial DNA reveals multiple distant glacial refugia in black spruce (*Picea mariana*), a transcontinental North American conifer. *Molecular Ecology*, **13**, 2735–2747.

Koehler, A.V.A., Hoberg, E.P., Dokuchaev, N.E. & Cook, J.A. (2007) Geographic and host range of the nematode *Soboliphyme baturini* across Beringia. *Journal of Parasitology*, **93**, 1070–1083.

Koepfli, K.-P., Deere, K.A., Slater, G.J., Begg, C., Begg, K., Grassman, L., Lucherini, M., Veron, G. & Wayne, R.K. (2008) Multigene phylogeny of the Mustelidae: resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. *BMC Biology*, **6**, 10.

Kondzela, C.M., Guthrie, C.M., Hawkins, S.L., Russell, C.D., Helle, J.H. & Gharrett, A.J. (1994) Genetic relationships among chum salmon populations in southeast Alaska and

northern British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 50–64.

Kumar, S. & Subramanian, S. (2002) Mutation rates in mammalian genomes. *Proceedings of the National Academy of Sciences USA*, **99**, 803–808.

Kurtén, B. (1968) *Pleistocene mammals of Europe*. Weidenfield and Nicholson, London.

Kurtén, B. & Anderson, E. (1980) *Pleistocene mammals of North America*. Columbia University, New York.

de Lafontaine, G., Turgeon, J. & Payette, S. (2010) Phylogeography of white spruce (*Picea glauca*) in eastern North America reveals contrasting ecological trajectories. *Journal of Biogeography*, **37**, 741–751.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

MacDonald, S.O. & Cook, J.A. (2009) *Recent mammals of Alaska*. University of Alaska Press, Fairbanks, AK.

Martínková, N., MacDonald, R.A. & Searle, J.B. (2007) Stoats (*Mustela erminea*) provide evidence of natural overland colonization of Ireland. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 1387–1393.

McCulloch, G.A., Wallis, G.P. & Waters, J.M. (2010) Onset of glaciation drove simultaneous vicariant isolation of alpine insects in New Zealand. *Evolution*, **64**, 2033–2043.

Miller, W., Schuster, S.C., Welch, A.J. *et al.* (2012) Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. *Proceedings of the National Academy of Sciences USA*, **109**, E2382–E2390.

Moritz, C. (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology*, **51**, 238–254.

Petit, R.J., Aguinagalde, I., de Beaulieu, J.-L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palmé, A., Martín, J.P., Rendell, S. & Vendramin, G.G. (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300**, 1563–1565.

Rambaut, A. (2009) *FigTree v1.2.2*. University of Edinburgh. Available at: http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.4*. University of Edinburgh. Available at: http://beast.bio.ed.ac.uk/Tracer.

Reid, F. & Helgen, K. (2008) *Mustela erminea. IUCN Red List of threatened species*. Version 2013.1. IUCN, Gland, Switzerland. Available at: http://www.iucnredlist.org/details/29674/0.

Reid, N., Demboski, J.R. & Sullivan, J. (2012) Phylogeny estimation of the radiation of western North American chipmunks (*Tamias*) in the face of introgression using reproductive protein genes. *Systematic Biology*, **61**, 44–62.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Runck, A.M. & Cook, J.A. (2005) Postglacial expansion of the southern red-backed vole (*Clethrionomys gapperi*) in North America. *Molecular Ecology*, **14**, 1445–1456.

Shafer, A.B.A., Côté, S.D. & Coltman, D.W. (2011) Hot spots of genetic diversity descended from multiple Pleistocene refugia in an alpine ungulate. *Evolution*, **65**, 125–138.

Smith, B.T. & Klicka, J. (2013) Examining the role of effective population size on mitochondrial and multilocus divergence time discordance in a songbird. *PLoS ONE*, **8**, e55161.

Soltis, D.E., Gitzendanner, M.A., Strenge, D.R. & Soltis, P.E. (1997) Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution*, **206**, 353–373.

Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.

Talbot, S.L. & Shields, G.F. (1996) Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Molecular Phylogenetics and Evolution*, **5**, 477–494.

Topp, C.M. & Winker, K. (2008) Genetic patterns of differentiation among five landbird species from the Queen Charlotte Islands, British Columbia. *The Auk*, **125**, 461–472.

Twyford, A.D. & Ennos, R.A. (2012) Next-generation hybridization and introgression. *Heredity*, **108**, 179–189.

Waltari, E.C., Hoberg, E.P., Lessa, E.P. & Cook, J.A. (2007) Eastward Ho: phylogeographic perspectives on colonization across the Beringian nexus. *Journal of Biogeography*, **34**, 561–574.

Weckworth, B.V., Talbot, S.L., Sage, G.K., Person, D.K. & Cook, J.A. (2005) A signal for independent coastal and continental histories for North American wolves. *Molecular Ecology*, **14**, 917–931.

Weir, J.T. & Schluter, D. (2007) The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science*, **315**, 1574–1576.

Yoshikawa, S., Kawamura, Y. & Taruno, H. (2007) Land bridge formation and proboscidean immigration into the Japanese Islands during the Quaternary. *Journal of Geosciences (Osaka City University)*, **50**, 1–6.

Zazula, G.D., Froese, D.G., Westgate, J.A., La Farge, C. & Mathewes, R.W. (2005) Paleoecology of Beringian "pack-rat" middens from central Yukon Territory, Canada. *Quaternary Research*, **63**, 189–198.

SUPPORTING INFORMATION

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

Appendix S1 Specimens examined, sample localities, GenBank accession numbers.

Appendix S2 Supplementary methods, results (including supplementary tables and figures) and discussion.

BIOSKETCH

This research is part of **Natalie G. Dawson**'s doctoral dissertation. She studies Nearctic mustelids as part of her research and education programme at the University of Montana. She is associate director of the Wilderness Institute within the College of Forestry and Conservation at the University of Montana (UM) and currently directs and teaches the Wilderness and Civilization Program, an experiential education programme at UM.

Author contributions: N.G.D., S.L.T and J.A.C. conceived the ideas; N.G.D. and A.G.H. collected the data; N.G.D. and A.G.H. analysed the data; S.L.T. and J.A.C. secured financial support and provided laboratory facilities; N.G.D. and A.G.H. led the writing; and all authors contributed to writing.

Editor: Luiz Rocha