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DIVERSITY AND DEMOGRAPHY IN BERINGIA: MULTILOCUS TESTS OF PALEODISTRIBUTION MODELS REVEAL THE COMPLEX HISTORY OF ARCTIC GROUND SQUIRRELS

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To assess effects of historical climate change on northern species, we quantified the population history of the arctic ground squirrel (*Spermophilus parryii*), an arctic-adapted rodent that evolved in Beringia and was strongly influenced by climatic oscillations of the Quaternary. Competing hypotheses for the species' population history were derived from patterns of mitochondrial (mtDNA) structure and a bioclimatic envelope model (BEM). Hypotheses invoked (1) sequential isolation of regional populations beginning with the Arctic, (2) deep isolation only across central Alaska, and (3) widespread panmixia, and were tested using coalescent methods applied to eight nuclear (nDNA) loci. The data rejected strict interpretations of all three hypotheses, but perspectives underlying each encompassed aspects of the species' history. Concordance between mtDNA and nDNA geographic structure revealed three semi-independently evolving phylogroups, whereas signatures of gene flow at nDNA loci were consistent with a historical contact between certain populations as inferred by the BEM. Demographic growth was inferred for all regions despite expectations of postglacial habitat contraction for parts of Beringia. Our results highlight the complementary perspectives on species' histories that multiple lines of evidence provide, and underscore the utility of multilocus data for resolving complex population histories relevant to understanding effects of climate change.

KEY WORDS: **Climate change, ecological niche model, phylodemography, *Spermophilus parryii*, statistical phylogeography.**

Contemporary climate change is influencing species distributions, with important consequences for patterns of genetic diversity and species' long-term evolutionary potential (Myers and Knoll 2001; Parmesan 2006). Understanding how species responded to climatic variability in the past is fundamental for anticipating the effects of future warming (Edwards et al. 2005). Repeated episodes of global cooling and warming that marked transitions between glacial and interglacial periods of the Quaternary (the last 2.6 million years [Ma]; Gibbard et al. 2010) provide a framework for quantifying the genetic consequences of climate change.

Elucidating this historical record has proven fruitful in studies of temperate species, resulting in well-supported models of range fluctuation and refugial hypotheses (Hewitt 1996, 2004b).

Past population dynamics of arctic species are not as well understood as they are for temperate biotas. This is remarkable given that the Arctic has been a center for climate change studies (Henry and Molau 1997; ICSU 2004) and the region is particularly sensitive to climatic influences (Sala et al. 2000; IPCC 2007). Arctic organisms exhibited variable and often idiosyncratic responses to glacial–interglacial cycles. Populations of cold-adapted species may have expanded with climate cooling and retracted with warming (Stewart and Lister 2001), but this model represents an over-simplification that by itself does not fully predict the genetic consequences of climate change (e.g., Galbreath et al. 2009). Similarly, phylogeographic studies on northern species reveal genetic signatures of varied population histories, including demographic expansion (Galbreath and Cook 2004), contraction (Fedorov 1999), and stability (Fedorov et al. 1999); loss of diversity through time (Prost et al. 2010); deep (Brunhoff et al. 2003) and shallow (Holder et al. 2000) isolation; and persistence in cryptic (Loehr et al. 2006) and high arctic (Fedorov and Stenseth 2002) refugia. Concordance is most evident in shared patterns of endemism within major refugia (e.g., Beringia; Hewitt 2004b).

Beringia, the area spanning eastern Siberia and northwestern North America (Hultén 1937), provides a natural laboratory for assessing the effects of climate change on northern species. In its role as a high-latitude refugium that maintained considerable species diversity through multiple glaciations, the region contributed to the evolution of endemic northern lineages (Hewitt 2004a). As the crossroads that mediated contact between the largest landmasses of the Northern Hemisphere, it influenced species through episodic opening and closing of dispersal routes (Sher 1999). Glacial-age dispersal across the Bering Land Bridge predominantly flowed from west to east (Waltari et al. 2007b), and played a critical role in shaping Holarctic biotas (Sher 1984).

Beringia's complex history is reflected in the diverse population dynamics of the species that occupy it. Molecular phylogeographic results have begun to resolve the broad outlines of these multifaceted northern histories (Hewitt 2004a), but the majority of these studies are based solely on single-locus mitochondrial (mtDNA) datasets. Stochastic variation in gene genealogies and nonneutral evolution can result in single-locus inferences that do not match the true population history (Ballard and Whitlock 2004), and maternally inherited mtDNA offers direct insight only into the history of females, which can differ from the broader population history of the species. Biparentally inherited nuclear (nDNA) markers may therefore be more useful for resolving species-wide population histories.

Conclusions drawn from mtDNA-based phylogeographic studies provide hypotheses that can be tested against independent nDNA datasets using statistical phylogeographic methods (Knowles 2004). Alternative hypotheses that explicitly address population responses to climate change can be derived from bioclimatic envelope models (BEM; also commonly referred to as ecological niche models), which offer insights into population structure that are entirely independent of the molecular perspective (Carstens and Richards 2007; Waltari et al. 2007a). A BEM is a numerical model that relates geographic occurrences for a species to climatological data to characterize the bioclimatic space that the species occupies (Elith and Leathwick 2009). With appropriate climatological datasets, BEMs can be projected onto different climatic scenarios to infer species' distributions in different time periods. Interpretations of population history derived from BEM predictions can then be tested using molecular data (Richards et al. 2007). In this study, we use a BEM in combination with the results of a mtDNA-based phylogeographic study and the paleontological record to identify competing hypotheses regarding the history of differentiation and demography in the arctic ground squirrel (*Spermophilus parryii*), a northern rodent with an evolutionary history deeply rooted in Beringia (Eddingsaas et al. 2004). We test the hypotheses using a suite of unlinked nDNA markers.

STUDY ORGANISM AND HYPOTHESES

The distribution of *S. parryii* is centered on Beringia (Fig. 1), making the species an excellent target for quantifying the effects of climate change on arctic species. The species is supremely well adapted to arctic environments, capable of surviving in hibernation with body temperatures below freezing (Buck and Barnes 2000), and staying in hibernacula for up to 8 months annually (Buck and Barnes 1999; Sheriff et al. 2010). Furthermore, their middens, which may contain seed caches, whole plants, and insects, have been preserved in permafrost for millennia and provide an exquisite look into Beringian paleoecology (Zazula et al. 2003, 2007), as packrat middens have done for past environments of the southwestern United States (Thompson and Anderson 2000).

A phylogeographic analysis of *S. parryii* based on mtDNA sequences revealed nonoverlapping West, Southwest, Central, and North clades in Alaska that may date to the Middle Pleistocene (Eddingsaas et al. 2004). Hereafter we refer to these major regional phylogroups as the Beringia, Southwest, Southeast, and Arctic groups, respectively, reflecting the new understanding of geographic structure established in the current study. The geographic pattern of mtDNA differentiation is broadly corroborated by morphological (Hall 1981), allozymic (Nadler and Hoffmann 1977), and host–parasite (Holland 1963) data.

We test three competing hypotheses regarding the history of population differentiation in *S. parryii* (Fig. 2). The Ancient Arctic Isolation (AAI) hypothesis is derived from the mtDNA

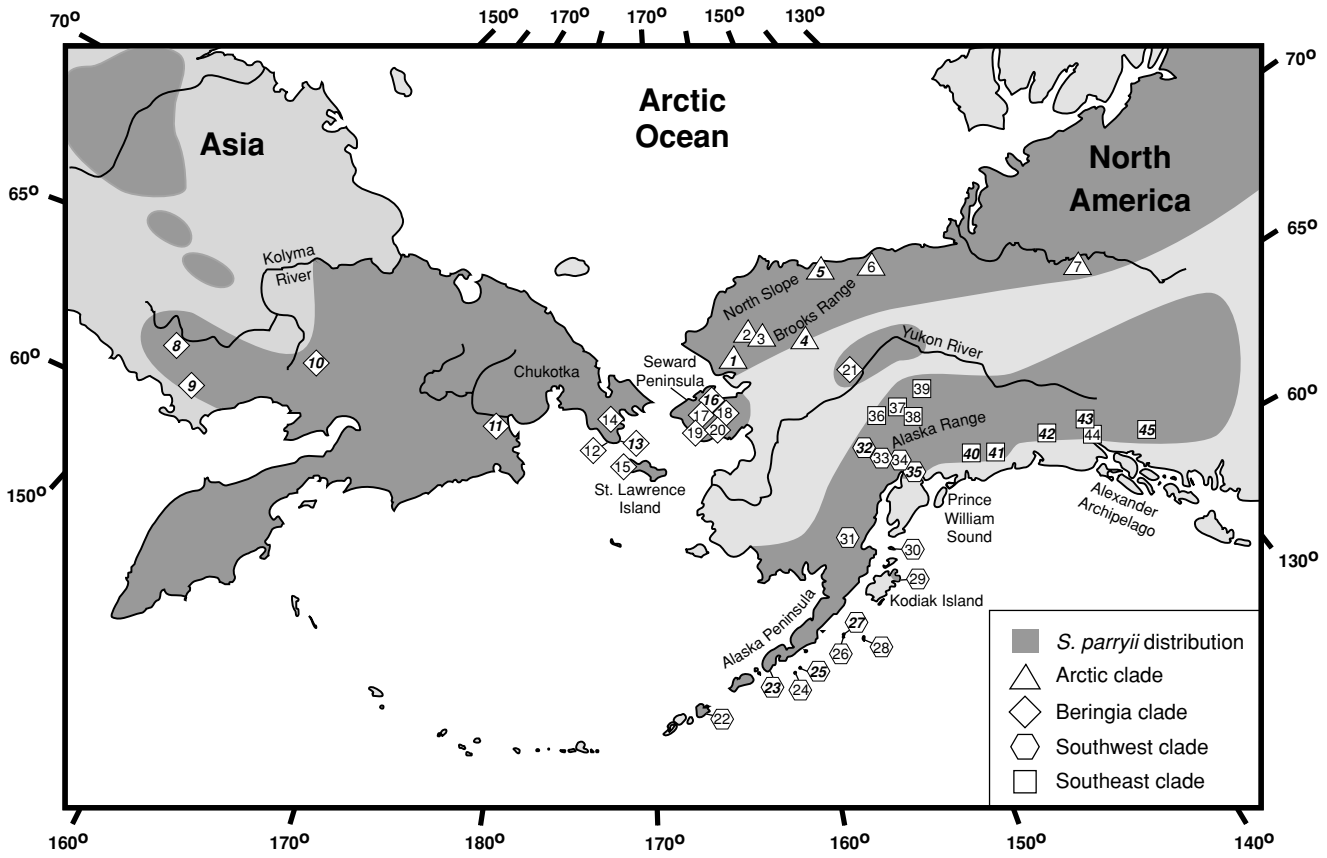

Figure 1. Beringian distribution of arctic ground squirrels (*Spermophilus parryii*) and sampling localities. Locality numbers cross-reference with Table S1. Symbols behind numbers indicate major mtDNA lineages identified with each locality.

phylogeny and predicts a population history that matches the mtDNA tree with deep population subdivision and essentially no gene flow among regions. As alternatives, we develop two hypotheses based on BEM predictions under current, last glacial maximum (LGM; ∼21 thousand years ago [ka]), and last interglacial sensu stricto (LIG; ∼130 – 116 ka) climate (see Results). The north-south split (NSS) hypothesis predicts deep divergence (low gene flow) between populations generally distributed north (Seward Peninsula, Brooks Range, North Slope) and south (Alaska Range, Alaska Peninsula) of the Yukon River, but little structure (high gene flow) within those regions. The Widespread Panmixia (WPX) hypothesis predicts a shallow coalescence for all *S. parryii*, lack of phylogeographic structure, and high gene flow among regional populations.

We also use the paleoenvironmental record and BEM to derive hypotheses for the history of climate-driven demographic fluctuation. Two mtDNA lineages, Southwest and Southeast, occupy parts of Beringia that were heavily glaciated during the LGM (Dyke et al. 2003), so postglacial population expansion is predicted for both regions. Expectations for the Beringia and Arctic populations are less clear-cut. During glacial periods, expansion of steppe-tundra ("mammoth steppe"; Guthrie 1982) in Beringia might have resulted in growth of ground squirrel populations (Zazula et al. 2007), which would predict postglacial population decline for the northern lineages. In contrast, the BEM indicates that the distribution of optimal conditions across nonglaciated Beringia could have remained stable or grown since the LGM (see Results), possibly causing population expansion.

Methods

BIOCLIMATIC ENVELOPE MODELING

We developed a BEM under current (1950–2000) climate conditions and projected it onto simulated climate data for the LGM and LIG using MAXENT 3.3.1 (Phillips et al. 2004, 2006). The BEM and projections were based on 19 environmental parameters commonly applied in bioclimatic modeling (e.g., mean annual temperature). Because MAXENT is relatively robust to autocorrelation between variables, we chose to include all 19 parameters. To test for model over-fitting (Beaumont et al. 2005) we constructed a BEM using only the two highest-contributing parameters, and it was not qualitatively different from the full model (Figure S1). We acquired current and past bioclimatic data from the WorldClim database (http://worldclim.org/) at a resolution of

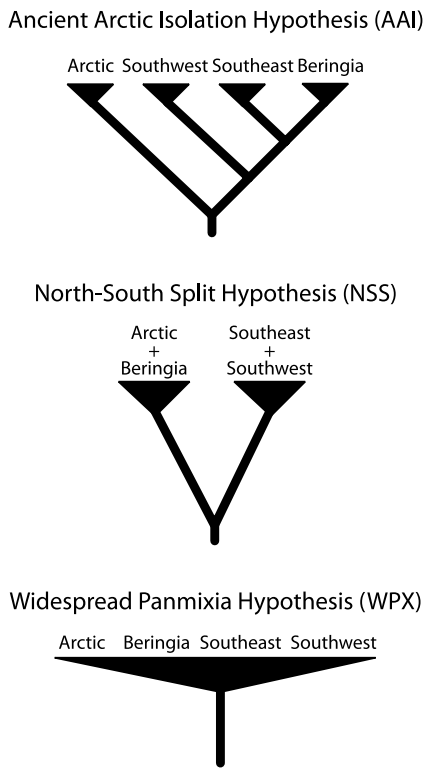

Figure 2. Phylogenetic predictions derived from alternative hypotheses for arctic ground squirrel (*Spermophilus parryii*) population structure.

2.5 min and applied a 145°E to 130°W and 50°–75°N mask. Climate data for the LGM were derived from simulations run using the Community Climate System Model version 3 (CCSM3) (Collins et al. 2006) and Model for Interdisciplinary Research on Climate version 3.2 (MIROC) (Hasumi and Emori 2004), originally available from the Paleoclimate Modeling Intercomparison Project Phase 2 (http://pmip2.lsce.ipsl.fr/) (Waltari et al. 2007a). We used the CCSM3 and MIROC datasets to generate separate LGM predictions and then produced a composite result showing areas where predictions agreed between models. LIG climate data were derived from simulations under the CCSM3 general-circulation model (Otto-Bliesner et al. 2006).

We used the Mammal Networked Information System (http://manisnet.org/) to query 38 museum databases for georeferenced species occurrence records. We also georeferenced a map of sampling localities in Siberia (Chernyavski 1984) to increase the density of records in western Beringia, resulting in 408 total locality records separated by $>0.01°$. To account for geographic variation in sampling effort, we used the target background sampling approach to select pseudo-absence records for the model (Phillips et al. 2009). In this method, background points are drawn from locality records for co-distributed species that have been sampled using methods similar to those used for the target species. We used the same data sources as described above to accumulate locality records for other Beringian small mammals (*Lemmus sibiricus*, *Lemmus trimucronatus*, *Mustela erminea*, *Mustela nivalis*, *Microtus oeconomus*, *Myodes rutilus*, *Sorex cinereus*, *Sorex tundrensis*, *Tamias minimus*, *Tamias sibiricus*), yielding 2120 background points. We trained models on 75% of the locality records, retaining 25% for model testing. We performed 10-fold cross-validation and calculated binomial probabilities for 11 common thresholds to test the hypothesis that the BEM predicts occurrence for test data no better than does a random prediction model.

MOLECULAR DATA COLLECTION

To better resolve the distribution of major mtDNA lineages among Beringian populations of arctic ground squirrels, we expanded on the dataset of Eddingsaas et al. (2004) and Cook et al. (2010), which included data from 96 vouchered *S. parryii* specimens representing 25 localities distributed across Alaska and northwest Canada (Table S1). For the current study, we acquired tissue samples (liver, heart, kidney) from an additional 104 specimens representing a total of 45 localities, including 20 localities not examined previously. *Spermophilus columbianus* ($N = 1$) and *S. richardsonii* ($N = 3$) served as outgroups for phylogenetic analyses (Harrison et al. 2003). Voucher and tissue samples for all specimens are archived at either the University of Alaska Museum of the North or the Museum of Southwestern Biology (Table S1).

We isolated genomic DNA from tissue samples using either Qiagen DNeasy tissue extraction kits or a salt precipitation method modified from Miller et al. (1988). We PCR amplified the mtDNA cytochrome *b* gene (cyt-*b*) in two overlapping fragments. Details of DNA extraction and PCR protocols are described elsewhere (Eddingsaas et al. 2004). PCR products were sequenced in both directions on an ABI 3100 genetic analyzer (Applied Biosystems Inc., Foster City, CA) using ABI PRISM® BigDye™ sequencing chemistry.

To obtain an independent data source for testing phylogeographic and demographic hypotheses, we designed eight anonymous locus primer sets (ALPS; Table 1) using a modification of the method described by Jennings and Edwards (2005). See Appendix S1 for the full protocol. Our goal was to maximize the number of loci rather than number of individuals (Felsenstein 2006), so for ALPS sequencing we selected a subsample of individuals that represented the Arctic ($N = 3$), Beringia ($N = 8$), Southeast ($N = 5$), and Southwest ($N = 5$) regions, as well as *S. richardsonii* ($N = 3$) and *S. columbianus* ($N = 1$). Nuclear loci were PCR amplified as described elsewhere (Galbreath et al. 2009) with a 64°C annealing temperature and sequenced in both directions.

Direct sequencing of heterozygous ALPS loci resulted in electropherograms with distinctly overlapping peaks at variable sites. Allelic phase of heterozygous loci was easily determined in cases where alleles differed at a single position, or if an

Table 1. Primer sequences for anonymous locus primer sets designed for the arctic ground squirrel (*Spermophilus parryii*).

Primer name	Primer sequence (5′ to 3′)
SppaALP2F	GTTCTTCCTCCTGTGCATCC
SppaALP2R	TGGTAGAACACTTGCCTTGC
SppaALP3F	AACACCATGCATCACTTCTCC
SppaALP3R	AGGTTGGTGGACTCTGATGG
SppaALP4F	ATTTGGACTTGGAGCAAAGC
SppaALP4R	GGGGCATTAAATGACAGAGG
SppaALP6F	ATGGTGAGAAAATGCCAAGG
SppaALP6R	AATCCCATCCTATCCCAACC
SppaALP7F	AAACCACATTGGATGATGTCC
SppaALP7R	CATGCAGGAAATTTGTCATCC
SppaALP9F	TTGGTCCTTTCTTCTGTTTGG
SppaALP9R	TGGAGCCACTTATCCTGAGC
SppaALP10F	AGGGGGTATTGTGGATGTGC
SppaALP10R	AATTGTGGCAGGCTAAATGG
SppaALP12F	GGGGATAAAACCACAACAGC
SppaALP12R	CCTGGACCGCATATACTTGG

indel caused an offset that permitted allelic sequences to be traced separately across the electropherogram. For sequences that differed at multiple sites and lacked indels, we either cloned and sequenced PCR products, or inferred allelic sequences using the program PHASE version 2.1 (Stephens et al. 2001; Stephens and Scheet 2005) as implemented in DnaSP version 5 (Librado and Rozas 2009). PHASE was used to resolve allelic sequences for five individuals from one locus (ALP12). The full ALP12 dataset was analyzed simultaneously. PHASE was run 10 times and the results of the run that yielded the best goodness-of-fit to an approximate coalescent model were kept. Independent runs from different starting seeds produced equivalent results, with high probabilities of nonsingleton base calls (> 0.9). Base calls for singleton sites that could not be unambiguously recovered (probability < 0.7) were coded as missing data. Final datasets included both alleles for each sequenced individual whether homozygous or heterozygous.

All sequence datasets were aligned using ClustalW as implemented in MEGA version 4 (Tamura et al. 2007) and alignments were checked by eye. We excluded indels and sites of ambiguous alignment from further analyses. To test for recombination we applied the RDP (Martin and Rybicki 2000), MAXCHI and CHIMAERA (Posada and Crandall 2001), and GENECOV (Padidam et al. 1999) algorithms implemented in the software RDP3 (Martin et al. 2005). We used DNASP to calculate haplotype diversity (h) and nucleotide diversity (π) for the *S. parryii* datasets, and tested for departures from neutrality by comparing Tajima's D (Tajima 1989) and Fu and Li's F^* and D^* (Fu and Li 1993) statistics to 1000 coalescent simulations of a large, neutrally evolving population of constant size. For the coding cyt-*b* locus, we tested for selection using McDonald–Kreitman (MK) tests (McDonald and Kreitman 1991), comparing ratios of fixed and polymorphic synonymous and nonsynonymous substitutions within and between *S. parryii* and outgroups. To assess relative signatures of selection across all loci we employed the Hudson, Kreitman, Aguadé (HKA) test (Hudson et al. 1987) using HKA (Hey 2005). Appropriate inheritance scalars were applied for the mtDNA and nDNA loci (0.25 and 1.0, respectively).

ESTIMATING PHYLOGENIES, GENE FLOW, AND DEMOGRAPHIC HISTORY

As an initial test of phylogeographic concordance across loci, we produced unrooted minimum spanning networks using TCS 1.21 (Clement et al. 2000) (excluding outgroups), and performed maximum likelihood (ML) phylogenetic analyses (including outgroups). To select appropriate models of nucleotide substitution for each locus, we used an ML phylogeny generated under the GTR + I + G substitution model in GARLI version 0.96 (two replicates per run) (Zwickl 2006) as a starting tree for testing alternative nested substitution models via DT-Modsel (Minin et al. 2003). Final GARLI analyses based on the selected models optimized ML tree topologies and model parameter values. Resulting models (Table 2) were used in all subsequent analyses.

We conducted additional ML and Bayesian analyses on a mtDNA dataset of only unique haplotypes. First, we used GARLI to determine the best tree from 10 replicate runs, and assessed nodal support based on 200 bootstrap replicates (five tree searches per bootstrap). Bayesian analyses were conducted using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). We performed six runs from different random number seeds with five chains and 10 million generations, taking samples every 1000 generations. Stationarity and convergence were assessed by ensuring that standard deviations of split frequencies between runs approached zero, visualizing split probabilities in AWTY (Nylander et al. 2008), and comparing summarized tree topologies from separate runs. We discarded 1 million generations from each run as burn-in, combining the remaining trees to produce the final topology.

We employed the coalescent-based *BEAST (Heled and Drummond 2010) method of phylogeny estimation implemented in BEAST 1.5.4 (Drummond and Rambaut 2007), which permits multilocus estimates of species trees and divergence times. We isolated the mtDNA and nDNA datasets in separate analyses to minimize circularity with respect to our test of the AAI hypothesis, which was derived from a phylogeographic analysis of mtDNA. Also, mtDNA and nDNA may have evolved under different population histories (see Results), which would confound attempts to reconstruct a single history from them. We inferred relationships between the two outgroup species and four regional populations, assigning individuals to regions based on their geographic origin and mtDNA identity. We applied

Table 2. Summary statistics for loci sequenced from the arctic ground squirrel (*Spermophilus parryii*). Values include the total number of sequences (*N*), sequence length in base pairs (bp), number of unique alleles or haplotypes (N_h), haplotype diversity (*h*), percent nucleotide diversity (π), Tajima's *D*, Fu and Li's *D** and *F**, and the model of nucleotide substitution selected by Dt-Modsel for each locus.

	N	bp	N_h	*h*	π (%)	*D*[a]	*D**[a]	*F**[a]	Model
ALP2	38	926	9	0.778	0.500	−0.213	−1.393	−1.183	HKY+G
ALP3	38	953	8	0.593	0.122	−1.447	−0.495	−0.933	K80
ALP4	40	940	9	0.653	0.113	−1.324	−0.728	−1.074	K80+G
ALP6	40	935	8	0.800	0.145	−0.661	−0.976	−1.028	F81
ALP7	39	953	12	0.764	0.379	0.513	−0.178	0.051	HKY+I
ALP9	34	944	11	0.813	0.324	−0.362	−0.332	−0.401	HKY+G
ALP10	36	969	5	0.711	0.101	−0.055	−0.008	−0.026	HKY
ALP12	38	865	8	0.734	0.386	−1.000	−0.376	−0.689	HKY+G
Cyt-*b* – total	200	1140	59	0.976	2.522	0.513	1.129	0.999	HKY+G
Cyt-*b* by lineage:									
Arctic	31	1140	8	0.791	0.184	−0.513	0.862	0.512	HKY+G[b]
Beringia	54	1140	19	0.919	0.746	0.343	0.930	0.855	HKY+G[b]
Southeast	43	1140	12	0.900	0.370	−0.800	0.207	0.163	HKY+G[b]
Southwest	72	1140	20	0.931	0.713	−0.128	0.371	0.215	HKY+G[b]

[a]None of the *D*, *D**, or *F** statistics indicate significant departures from neutrality.

[b]Separate models were not selected for individual mitochondrial lineages.

the Yule tree prior and in the multilocus analysis allowed rates to vary among loci, but fixed the molecular clock for each locus based on the results of likelihood ratio tests of clock-like evolution (Felsenstein 1988) performed using PAUP* version 4b10 (Swofford 2000). Analyses were run for 100 (mtDNA) and 500 (nDNA) million generations, with 10% of each run discarded as burn-in. We assessed stationarity by examining parameter trend plots and effective sample size (ESS) values (all >200) using TRACER 1.5 (Rambaut and Drummond 2007). Analyses were repeated three times from different random seeds to confirm that parameter estimates converged on similar values.

The *BEAST method infers relationships based on predefined sets of populations, but does not evaluate patterns of structure. We used the program STRUCTURE 2.3.3 (Pritchard et al. 2000) to obtain an independent assessment of population structure based on the multilocus nDNA dataset for comparison to the distribution of mtDNA lineages. We applied the admixture model of ancestry and both correlated (Falush et al. 2003) and uncorrelated allele frequencies models, running separate analyses for all values of *K* (no. of population clusters) between 1 and 8. Runs proceeded for 100 thousand generations following a burn-in of equal length, and were repeated at least twice to confirm that results were reproducible. Bar plots showing coefficients of cluster membership estimated for each individual were visualized using DISTRUCT 1.1 (Rosenberg 2004).

The history of demographic fluctuation and gene flow among the four major phylogeographic regions was quantified simultaneously using the Bayesian formulation of LAMARC 2.1.3 (Kuhner 2006). For nDNA analyses, we applied locus-specific models of nucleotide evolution, retained the default priors for θ and migration (*M*), and adjusted the growth (*g*) prior to a uniform distribution spanning −1000 to 2000. We used estimates of relative mutation rates among loci taken from the results of the *BEAST analysis. Final analyses were run for 50 million generations following 10 million generations of burn-in with sampling every 5000 generations. Additional independent runs of 5 and 10 million generations (1 million burn-in) confirmed convergence on similar results. We assessed parameter trend plots, ESS values (all >600), and the shape of posterior distributions using TRACER to confirm stationarity. We conducted a comparable LAMARC analysis on the mtDNA data, running three separate analyses of 10 million generations (1 million burn-in) with sampling every 1000 generations. We compared demographic results from LAMARC to pairwise mismatch distributions computed for each mtDNA lineage using DnaSP. A smooth, unimodal mismatch distribution may indicate recent population expansion (Slatkin and Hudson 1991; Rogers and Harpending 1992).

Results

BIOCLIMATIC ENVELOPE MODELING

The BEM for arctic ground squirrels had good predictive power for species occurrence under current conditions. The mean area under the receiver operating characteristic curve (AUC) for the test data from the 10 cross-validation runs was 0.74, indicating that the BEM performed better than a random model (AUC = 0.5). A small standard deviation (0.014) for the AUC showed that model performance was robust to variation in the selection of

datasets for training. Test data were predicted significantly better under the BEM than under a random model based on 11 species occurrence thresholds tested in MAXENT ($P < 0.001$) indicating that model performance is independent of threshold.

We present two thresholds for probability of species occurrence (logistic output) from projections of the BEM on current, LGM, and LIG climatic conditions (Fig. 3). The first is a low threshold of 0.17 that encompassed 99% of occurrence records. A more restrictive threshold of 0.5 provides an indication of regions of highest occurrence probability and has a tighter fit to the known species distribution under current conditions. The prediction for the LIG and LGM indicates that in eastern Beringia, northern (Seward Peninsula, Brooks Range, North Slope) and southern (Alaska Range and Alaska Peninsula) regions were separated by a band of unfavorable conditions. Possible isolation across central Alaska provides the basis for the NSS hypothesis. Under current conditions the NSS pattern is only apparent at the high prediction threshold (Fig. 3). Under the lower threshold, favorable conditions are predicted across much of Alaska and Siberia, implying a lack of population structure that could result in widespread gene flow (WPX hypothesis). A composite map that summarizes minimum prediction probabilities compared across all time periods suggests that populations of the North Slope may have been relatively stable through time. In contrast, squirrel populations across the subarctic may have experienced shifting environmental conditions, due to both climate change and glacial expansion.

PHYLOGEOGRAPHY AND DEMOGRAPHY

We collected 106 new cyt-*b* (GenBank # JF314404-JF314509) and 343 nDNA (among 8 loci; GenBank # JF330430-JF330772)

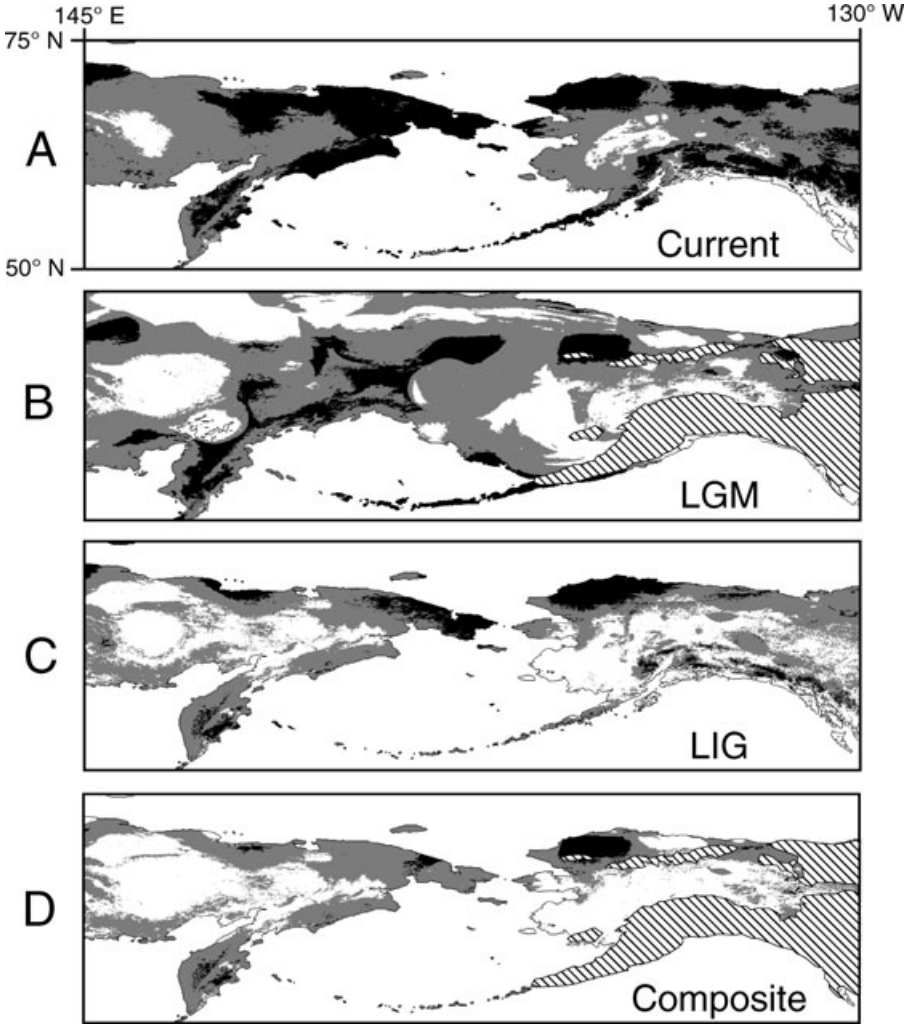

Figure 3. Bioclimatic envelope models for arctic ground squirrels (*Spermophilus parryii*) under current conditions (A), the last glacial maximum (LGM; B), and the last interglacial (LIG; C). Two thresholds for probability of occurrence are shown as different colors: 0.17 (gray) and 0.5 (black). The composite map (D) shows areas of agreement among the three time periods at the two thresholds, indicating areas where moderate and good conditions may have persisted under a range of global climate scenarios. The extent of North American LGM glaciation (Cordilleran ice sheet; Dyke et al. 2003) is indicated by hatching lines in panels B and D.

sequences for this study. None of the loci yielded signatures of recombination. Anonymous loci were not as diverse as cyt-*b*, with mean nucleotide diversity lower by an order of magnitude (Table 2). Tests of clock-like evolution failed to reject a strict molecular clock for any locus ($P > 0.05$). Tajima's D and Fu and Li's F^* and D^* statistics were not significant for any locus ($P > 0.05$), but the MK test on cyt-*b* identified a higher ratio of nonsynonymous to synonymous mutations polymorphic within *S. parryii* (38:101) than fixed between *S. parryii* and *S. columbianus* (3:48; $P = 0.0012$). Tests comparing *S. parryii* as a whole and individual lineages separately to *S. richardsonii* were not significant ($P > 0.15$), suggesting that selection has had a relatively weak influence on shallow genetic patterns. The HKA test did not reject neutrality for the ALPS loci ($P > 0.07$), but inclusion of cyt-*b* produced a significant result ($P < 0.006$). We interpret these results to indicate that the mtDNA and nDNA loci have distinct evolutionary histories stemming from different selective and demographic regimes (Ballard and Whitlock 2004), justifying our decision to conduct analyses of the mtDNA and nDNA datasets separately.

The complete dataset of 199 *S. parryii* cyt-*b* sequences obtained for this study yielded a total of 59 unique haplotypes, but did not reveal deep phylogeographic structure or phylogenetic relationships beyond that which was described previously (Eddingsaas et al. 2004). Addition of new sampling localities clarified the distribution of the four major mtDNA lineages. Our new data show that the Beringia clade, previously known only from the Seward Peninsula, spans Siberia and central Alaska (Fig. 1). The distribution of mtDNA haplotypes was nonrandom, with few shared by more than one population; shared haplotypes always occurred in adjacent populations (Fig. 4). In contrast, nDNA loci revealed less-obvious geographic structure, with many individual alleles shared by several populations (Figure S2).

Both phylogenetic analyses of mtDNA placed *S. richardsonii* as sister to the Arctic clade (Figs. 4 and 5). Paraphyly for arctic ground squirrels was also observed in two nDNA loci (Figure S3), but the multilocus nDNA analysis strongly supported overall monophyly for *S. parryii* (Fig. 5). Relationships among regional subgroups of ground squirrels inferred by the mtDNA and nDNA datasets were not concordant (Figs. 4 and 5), as shown by the fact that the tree topology obtained from one dataset did not occur in posterior samples of trees obtained from the other. The nDNA analysis did not place the Arctic group in the basal position, nor did it indicate that the deepest split subdivided northern (Beringia and Arctic) from southern (Southwest and Southeast) populations.

Divergence time estimates for mtDNA and nDNA differed, particularly with respect to the origin of *S. richardsonii* relative to regional groups within *S. parryii*. Credible intervals of mtDNA-based time estimates for these nodes overlap broadly (Fig. 5) whereas the nDNA analysis placed intraspecific divergence events significantly more recent than splits leading to the outgroups. Based on the 1.52% Ma^{-1} substitution rate for cyt-*b* calculated by Eddingsaas et al. (2004), mtDNA divergence times within the *S. parryii*/*S. richardsonii* group range from 0.2 to 1.7 Ma (including 95% credible intervals) and the basal split in the tree occurred 1.6 to 3.7 Ma. Without an externally calibrated molecular clock for nDNA, we can obtain rough divergence times by assuming simultaneous divergence for mtDNA and nDNA during the event that led to *S. columbianus*. Anchoring the base of the trees in this way yields intraspecific split times for *S. parryii* ranging from 0.016 to 0.374 Ma. However, large error bars on the basal nodes suggest considerable stochastic error associated with this assumption (i.e., coalescence times for mtDNA and nDNA genealogies may not be identical). Accommodating this by matching the highest credible divergence time estimate from the mtDNA analysis to the lowest estimate from the nDNA analysis and vice versa, we calculated a wide range of possible intraspecific divergence times spanning 0.005 to 1.157 Ma.

Analysis of nDNA population structure showed that *S. parryii* does not represent a single panmictic population. Values of the log probability of the data peaked at $K = 3$ and $K = 4$ under the independent allele frequencies and correlated allele frequencies models, respectively. Plots of cluster assignments for individuals show considerable concordance between clusters and mtDNA phylogroups (Fig. 6), with an important exception. In the three-cluster assignment, individuals representing the Beringia and Southwest groups were assigned to the same cluster, consistent with the close relationship retrieved in the *BEAST analysis. The four-cluster assignment showed the same general pattern but also indicated that populations from western Beringia formed a separate cluster from those further east.

Demographic analyses using LAMARC revealed nDNA signatures of population growth for all four regional groups, although the result for the Beringia group was not statistically significant (Fig. 7A). Likewise, the mtDNA analysis showed positively trending, but nonsignificant, growth estimates. Mismatch distributions yielded largely consistent signatures of demographic change. The Southeast, Arctic, and mainland Southwest distributions were relatively unimodal (Fig. 7B) as expected under a history of expansion. Island Southwest populations were more structured. Only the Beringia lineage failed to show an unambiguous signature of expansion.

The LAMARC analysis revealed mtDNA gene flow estimates skewed toward zero, whereas nDNA results recorded nonzero gene flow among all regional groups (Fig. 8). The multilocus analysis yielded a particularly strong signal of gene flow between the Beringia and Southwest groups. Weaker signals between other regions may reflect ancestral polymorphism rather than recent or ongoing gene flow. Distinguishing between ancestral polymorphism and gene flow is a persistent problem in population genetics, especially in expanding populations.

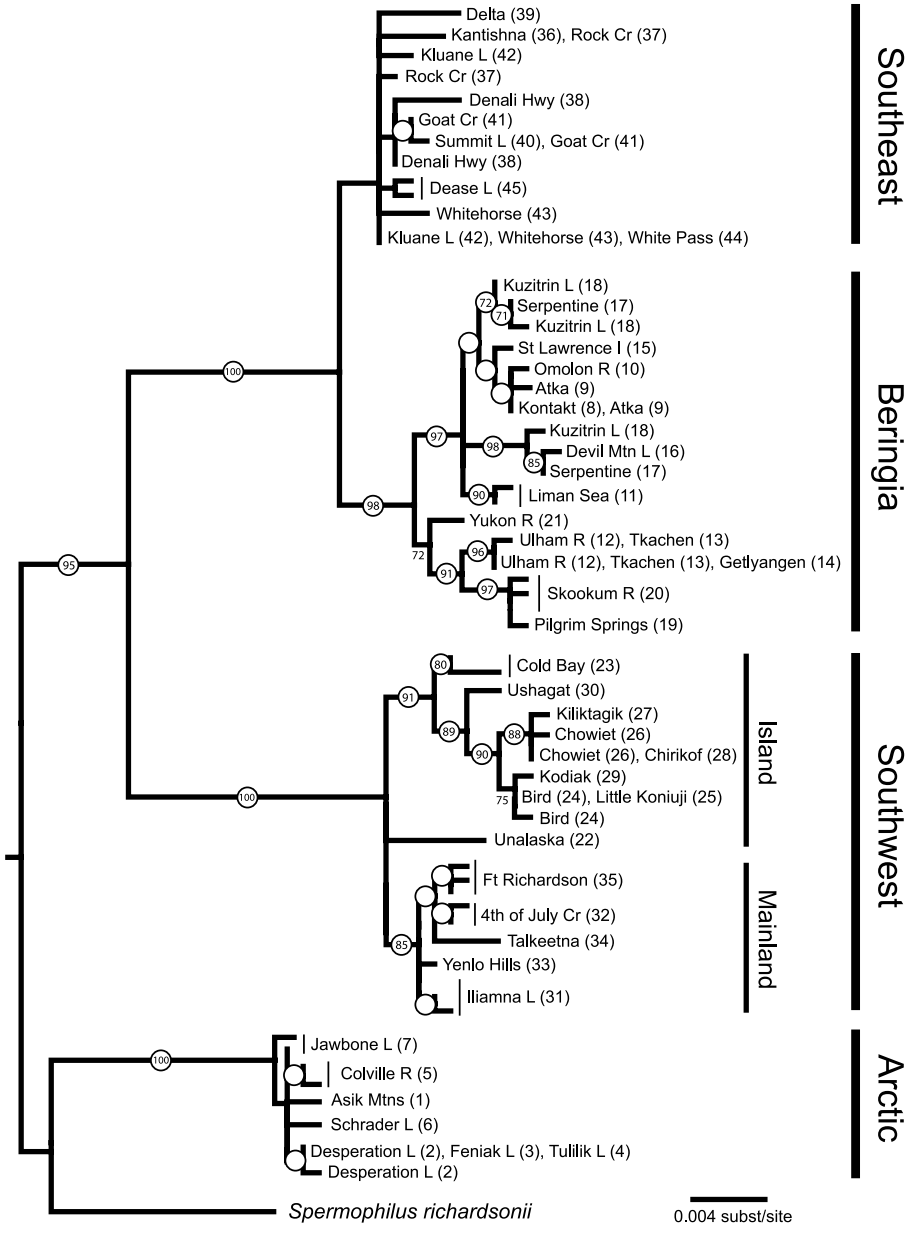

Figure 4. Maximum likelihood phylogeny of arctic ground squirrel (*Spermophilus parryii*) cytochrome *b* haplotypes. Haplotypes are labeled by locality names and locality numbers (in parentheses) that cross-reference with Figure 1 and Table S1. Circles on branches indicate Bayesian posterior probabilities ≥ 0.95. Numbers in circles or on branches are maximum likelihood bootstrap percentages ≥ 70. The outgroup, *Spermophilus columbianus*, has been trimmed for clarity.

Discussion

POPULATION HISTORY OF ARCTIC GROUND SQUIRRELS: BEM VERSUS mtDNA

The hypotheses that motivated this study represent competing models for understanding population-level consequences of historical climate change. The AAI hypothesis predicts long-term regional isolation despite episodic climatic oscillations, whereas the NSS and WPX models predict that fluctuations in climate permitted occasional episodes of gene flow between at least some regional groups. Viewed in another way, these hypotheses represent a test of whether patterns of population structure in *S. parryii* are predicted more accurately by mtDNA phylogeography (AAI hypothesis) or by a BEM (NSS or WPX hypotheses). Our data suggest that neither fully predicts the population history of arctic ground squirrels, yet both perspectives capture important aspects of that history.

Patterns of nDNA structure reject strict interpretations of the three main hypotheses. The population tree resolved by multilocus nDNA analysis failed to conform to phylogenetic predictions of either the AAI or NSS hypotheses, indicating that isolation

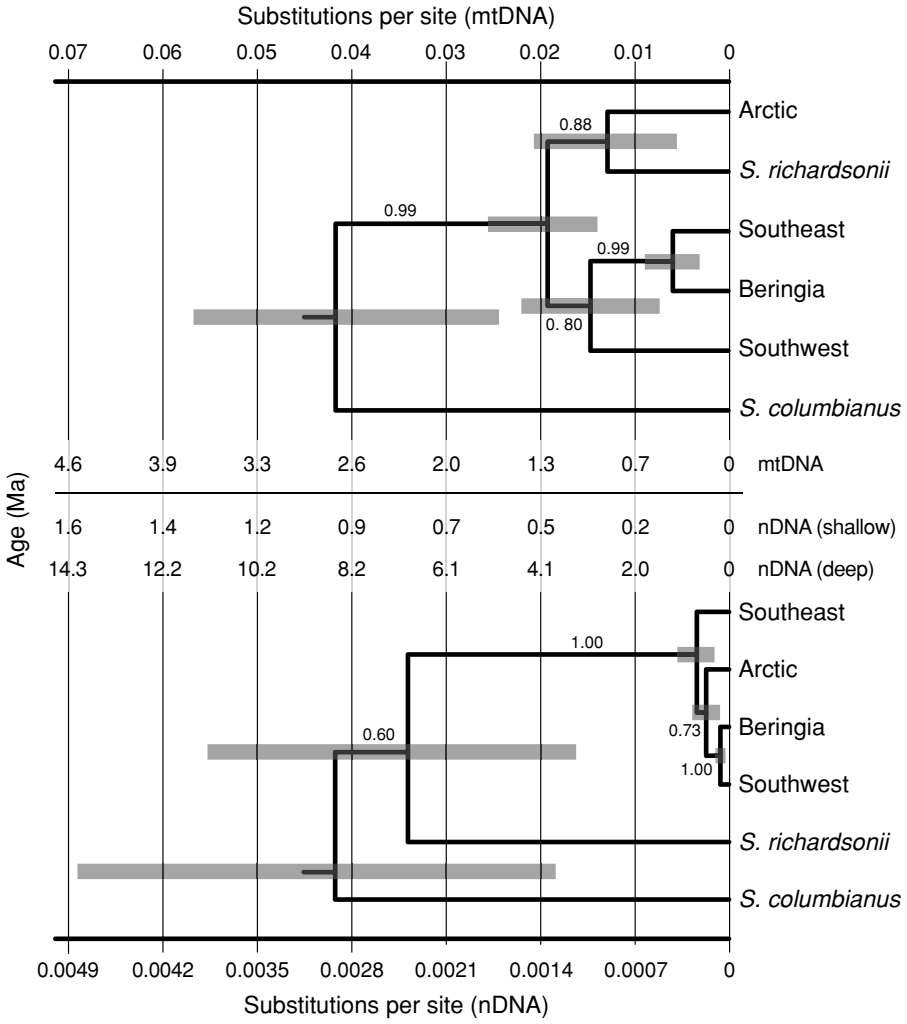

Figure 5. **Results of multispecies coalescent analysis of phylogeny and divergence time for arctic ground squirrels (*Spermophilus parryii*) using *BEAST. The top tree is derived from mitochondrial DNA (mtDNA) alone and the bottom tree is based on eight independent anonymous nuclear (nDNA) loci. Numbers on branches indicate Bayesian posterior probabilities for specific nodes. Scales listed on top and bottom axes indicate divergence times as a function of substitutions per site for mtDNA and nDNA analyses, respectively. Nodes are positioned along the axes to reflect the mean divergence estimate. Gray bars on nodes indicate the 95% credible interval around each estimate. Rows of numbers between the two trees indicate approximate ages in millions of years (Ma) for listed nucleotide divergences. Note that increments are not exactly equal due to a rounding error. The top row gives ages for the mtDNA tree based on a mutation rate of 1.52% Ma^{-1} (Eddingsaas et al. 2004), and for the nDNA tree based on the assumption that mtDNA and nDNA diverged simultaneously at the split leading to *S. columbianus* (as shown). The second and third rows provide age estimates for the nDNA tree calculated respectively by matching the lowest (95%) credible nDNA divergence time for *S. columbianus* to the highest (95%) credible estimate based on mtDNA (shallowest estimates), and vice versa (deepest estimates).**

among regional groups did not track the sequence of population splitting suggested by the mtDNA phylogeny or the BEM under the high prediction threshold. Furthermore, signatures of gene flow between at least two regions (Beringia and Southwest; Fig. 8) reject the possibility of deep isolation among all regions or between northern and southern populations. Although past gene flow occurred, it was not so great as to produce panmixia across the species' range. Nonzero divergence time estimates (Fig. 5), regional nDNA structure (Fig. 6), and equivocal gene flow estimates between some regions (Fig. 8) reject the WPX hypothesis. Thus, at least three regions (Arctic, Southeast, and Beringia/Southwest) are evolving along semi-independent trajectories.

The BEM fared better as a predictor for historical demography than as an indicator of phylogeny. Evidence for demographic growth across nonglaciated Beringia (Fig. 7) is consistent with BEM predictions, but contradicts expectations derived from the post-LGM ecological record. During glacial periods, aridification and expansion of steppe-tundra could have permitted larger arctic

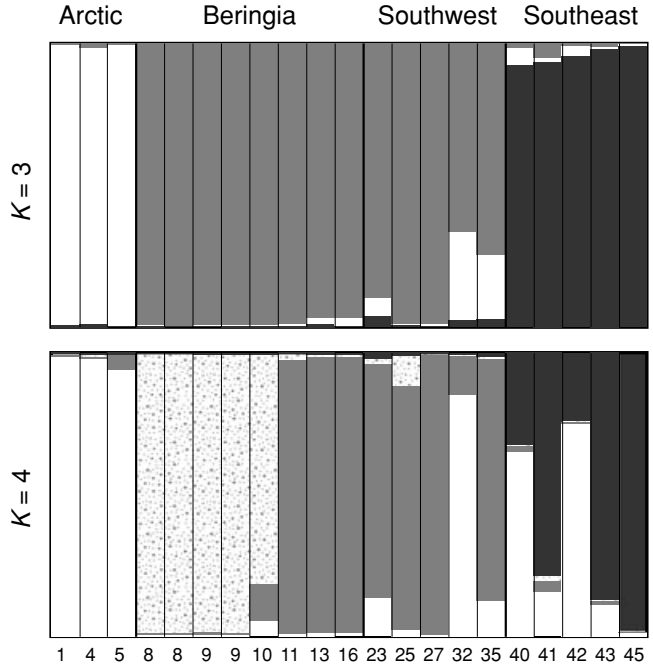

Figure 6. Results of assignments of individual arctic ground squirrels (*Spermophilus parryii*) to population clusters. Bar plots are shown for the best estimates of *K* (number of population clusters) retrieved from analyses based on the independent allele frequencies (top) and correlated allele frequencies (bottom) models. Different fill colors/patterns denote separate population clusters. Each vertical bar provides a proportional representation of the estimated cluster membership for a single individual. Sampling locality numbers for individuals are listed along the bottom axis. Heavy vertical black lines separate individuals into the four major mitochondrial phylogroups.

ground squirrel populations than at present (Zazula et al. 2007), and the fossil record shows that squirrels occurred in parts of central Alaska and Canada's Yukon Territory during the Wisconsinan glacial (10–75 ka) where they are currently absent (Harington 2003). In these areas and in eastern Siberia, suboptimal habitats (e.g., boreal forest) have largely replaced Beringian steppe-tundra since the LGM (Khotinskiy 1984; Zazula et al. 2007).

In general, the molecular data indicate that demographic expansion played an important role in the history of each *S. parryii* lineage, regardless of LGM glacial history or postglacial environmental changes. The strongest indication that postglacial habitat loss diminished populations is in the Beringia group, which spans the region of potentially greatest population decline according to the fossil record (Zazula et al. 2007). However, the timing of expansion is essentially unknown, and there is no guarantee that it specifically represents the period described by the BEM and paleoecological reconstructions. The relatively slow rate of evolution at nDNA loci may result in a lag that allows only deep or prolonged events to be recorded; for example, population expansion associated with the transition between the LIG and LGM could have produced a general signal that was not overwritten by more recent events (Grichuk 1984; Muhs et al. 2001).

THE CHALLENGE OF INTEGRATING BEMS WITH PHYLOGEOGRAPHIC DATA

Combining bioclimatic modeling with phylogeographic perspectives has potential to improve our understanding of processes that underlie patterns of genetic variation (Richards et al. 2007; Waltari et al. 2007a; Kozak et al. 2008). However, several assumptions must be met for meaningful comparisons between BEMs and genetic structure to be made (Elith and Leathwick 2009; Nogués-Bravo 2009), including (1) niche stability for the species over the time period of interest, (2) equilibrium between environmental conditions and the species' distribution, and (3) environmental parameters used in the model represent the most important factors determining the species' distribution. None of these assumptions can be robustly tested with available data, but the last may be of particular concern. We modeled the distribution of *S. parryii* using bioclimatic data in part because of the availability of complementary datasets for the LIG, LGM, and current time periods, but microspatial variation in characteristics such as substrate type, permafrost depth, slope, elevation, or timing of snow melt or snow cover may be more important in determining the distribution of this burrowing rodent (Bee and Hall 1956; Gillis et al. 2005; Sheriff et al. 2010).

Uncertainty regarding the relative importance of factors that determine species' distributions highlights the need for deep knowledge of both species' natural histories and environmental characteristics of the landscapes that they occupy, but such knowledge is rarely available. Even widely used bioclimatic datasets may not accurately reflect conditions for the time periods that they putatively represent. Climate data for the arctic and subarctic, including Alaska and Siberia, are especially prone to error due to low density of weather stations in the region. High-resolution datasets for modern climate are produced by filling gaps by interpolation (Hijmans et al. 2005). Error is compounded for past climate reconstructions, which are derived from computer simulations based on climate models with their own associated errors. These models generally provide global climate parameters at relatively coarse spatial resolutions, which must be statistically downscaled (another form of interpolation and source of error) to increase resolution.

Even if all assumptions are met and a BEM accurately predicts a species' distribution, certain temporal issues must be considered when interpreting the results in light of genetic structure. First, did the observed genetic patterns arise over the same period of time as that which was modeled using the BEM? In this study, BEM predictions date to 130 ka, whereas intraspecific divergence time estimates range broadly across the last 2 Ma. Thus, the BEM may have failed to fully predict the history of

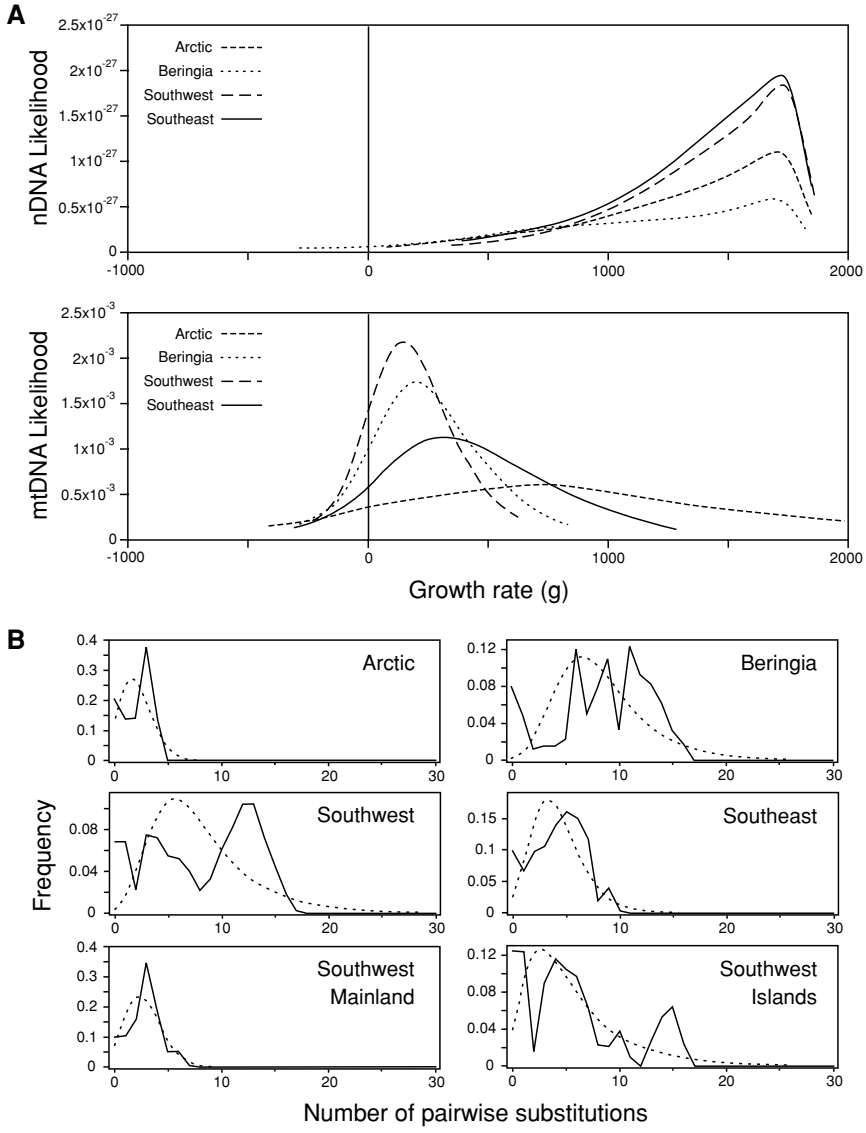

Figure 7. Results of demographic analyses for regional populations of arctic ground squirrels (*Spermophilus parryii*). Probability density curves for growth rate calculated using LAMARC are shown for the nuclear (nDNA) and mitochondrial (mtDNA) DNA datasets (A; top and bottom, respectively), and are truncated to include only 95% credible intervals. In the mismatch distributions (B), solid lines represent the frequency of pairwise nucleotide differences between sequences and dashed lines represent the expected distribution under a model of sudden population expansion (Rogers and Harpending 1992).

population differentiation because it targeted the wrong (i.e., too recent) time period. The BEM's comparative success in predicting demographic trends may be because genetic signatures of demography were necessarily influenced (if not fully overwritten) by events over the time period that it covered. Finally, the coarse temporal resolution of our BEM projections, which provided a snapshot of population structure for only three distinct time periods, could have led to incorrect historical inferences. From the standpoint of population structure, the BEM might have missed a critical paleoenvironmental event. Large gaps between time slices make it difficult to infer population continuity between regions of predicted occurrence from one time period to the next.

Despite the challenges inherent in combining BEMs with genetic perspectives on species' histories, we concur with others (Richards et al. 2007; Kozak et al. 2008) who advocate the approach as a useful tool for phylogeographic studies. In the present study, the use of a BEM to develop testable hypotheses strengthened the hypothetico-deductive framework and provided a focus for interpreting patterns of genetic variation. Further, it highlighted gaps in our understanding of environmental factors that underlie the population history of *S. parryii*. As existing environmental datasets are refined and new ones become available for additional variables, climate models, and time periods, estimates of species' histories derived from distributional modeling should

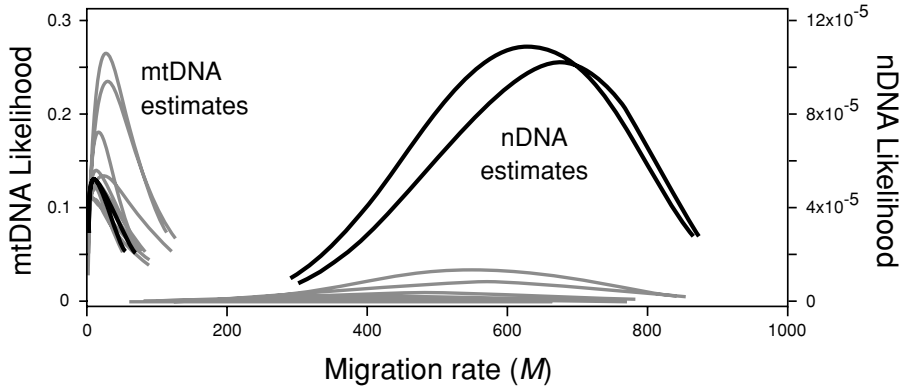

Figure 8. Migration rate estimates between regional populations of arctic ground squirrels (*Spermophilus parryii*). Black curves represent estimates of migration between the Beringia and Southwest regions. Gray curves represent rates between other regions. Probability density curves for migration rate are truncated to include only 95% credible intervals. Left and right y-axis scale bars denote mitochondrial (mtDNA) and nuclear (nDNA) likelihood values, respectively.

improve, with implications for better integration with molecular perspectives.

MULTI-LOCUS PHYLOGEOGRAPHY: mtDNA VERSUS nDNA

There is a growing consensus that multilocus perspectives are necessary to accurately and robustly reconstruct population histories (Edwards and Bensch 2009). A corollary to this is the recognition that mtDNA, which has served as the workhorse molecule of faunal phylogeography for decades, provides only partial resolution of population histories. Criticisms of mtDNA phylogeography often focus on evidence that certain fundamental assumptions of mtDNA evolution (e.g., effective neutrality, lack of recombination, maternal inheritance) can be broken (Ballard and Whitlock 2004). Even if all assumptions are met, stochastic sorting will often cause a single locus to yield genealogical relationships that do not reflect the population history of the species. Stochastic variation may explain why the mtDNA phylogeny suggests a paraphyletic relationship between *S. parryii* and *S. richardsonii* whereas the tree derived from multiple nDNA loci strongly supports monophyly of arctic ground squirrels (Fig. 5).

Incongruence between mtDNA and nDNA phylogeographic signatures could also be exacerbated because these loci track somewhat different population histories due to sex-biased dispersal (Ballard and Whitlock 2004). Female *S. parryii* exhibit strong natal philopatry (Byrom and Krebs 1999), which would limit mtDNA admixture while male dispersers transport nDNA alleles among populations. Males enter hibernation later and emerge earlier than females (Rausch 1953; Sheriff et al. 2010), creating more opportunities for male dispersal. Sex-biased dispersal might explain contrasting signals of gene flow retrieved from mtDNA and nDNA loci, specifically between Southwest and Beringia populations. Although the fourfold difference in N_e between the genomes could contribute to these differences, nonoverlapping estimates for the two datasets (Fig. 8) suggest that even after accounting for coalescent variation that accommodates differences in N_e, rates of mtDNA gene flow among regions could not have matched the high levels detected for nDNA loci.

Significant joint mtDNA/nDNA HKA tests might also be a consequence of different population histories for male and female squirrels. The HKA tests identified an excess of polymorphic sites in cyt-*b* relative to the ALPS loci. The pattern could be explained by a complex scenario of balancing selection at the mtDNA locus, but this seems unlikely given that purifying selection, which reduces variation, is generally more prevalent in mtDNA evolution (Ballard and Whitlock 2004). Instead, population structure at the mtDNA locus caused by low female dispersal rates could cause retention of higher levels of intraspecific polymorphism than expected relative to nDNA. The geographically structured distribution of mtDNA haplotypes, implying local mtDNA differentiation, is consistent with this interpretation. Results of the MK test indicate that selection against new variants, evident in comparisons between *S. columbianus* and *S. parryii*, is operating only weakly if at all at intraspecific scales.

The various factors that can cause patterns of mtDNA structure to not reflect the species-wide population history weigh against its use as the sole source of phylogeographic inference. However, patterns of mtDNA variation may offer useful insight as long as they are interpreted appropriately. Indeed, if female population structure is specifically of interest, maternally inherited mtDNA may be the only tool available, with the caveat that it represents a single locus. More generally, however, we argue that wherever mtDNA and nDNA are congruent one might infer an especially strong signal of population history. For example, with the exception of the relationship between the Beringia and Southwest populations, major geographic patterns of mtDNA and nDNA structure are strikingly concordant for *S. parryii*. Further, both sets of markers provide some evidence for substructure

across western Beringia, and generally consistent demographic results strengthen confidence that ground squirrel populations have expanded.

ORIGINS OF DIVERSITY

Arctic ground squirrels probably evolved in Beringia (Hoffmann 1981), with various lines of evidence pointing to a North American ancestor. For example, the earliest fossil evidence for the species comes from Canada's Yukon Territory and dates to ~740 ka (Zazula et al. 2010). Known fossils from Siberia date only to ~33 ka (Gubin et al. 2001). Furthermore, a mtDNA phylogeny placed *S. parryii* within a clade that consists mostly of North American species (Harrison et al. 2003; Herron et al. 2004), and this result is supported by karyotypic data (Liapunova and Vorontsov 1970).

Geographic distributions of *S. parryii* and its closest relatives (*S. richardsonii* and *S. elegans*) suggest that glacial-age expansion of continental ice sheets initiated speciation, which occurred >0.3 Ma according to our conservative timing estimates. Westward dispersal across the Bering Land Bridge probably occurred more recently given higher levels of genetic diversity in Alaska than in Siberia (Fig. 4; Figures S2 and S3). The shallow fossil record in Siberia is consistent with a Late Wisconsinan arrival, but evidence for nDNA structure subdividing Central Asian and Beringian populations across western Beringia hints at an earlier colonization. The split is roughly concordant with phylogeographic breaks in other species that have been attributed to glacial barriers (Hewitt 2004b). If glacial-age isolation (e.g., during the LGM) caused the structure, then colonization across the land bridge presumably occurred before the Late Wisconsinan. Episodes of climate-mediated dispersal across Beringia with subsequent diversification may have determined patterns of Holarctic diversity in numerous taxa (Hoberg and Brooks 2008).

The presence of three major lineages within Beringia (Beringia/Southwest, Arctic, Southeast) sets *S. parryii* apart from most northern species, which generally exhibit limited phylogeographic structure within the refugium. Various periglacial refugia outside of Beringia probably contributed to population differentiation in some species (Fedorov and Stenseth 2002; Galbreath and Cook 2004), but a lack of evidence for past or present arctic ground squirrel populations in these peripheral zones indicates that they were not responsible for the observed structure. This suggests a role for subrefugial isolation within Beringia in promoting and organizing diversity.

Traditional perspectives on Beringian endemism focused on glacial-age isolation between populations inside and outside the refugium, and downplayed the potential for substructure within Beringia. This follows from the mammoth steppe concept, which emphasizes widespread environmental homogeneity across the Holarctic during the LGM (Guthrie 1982, 2001). However, the Beringian landscape has always included environmental and topographic heterogeneity that may have been biogeographically significant from the perspective of organisms with narrow niche preferences and low dispersal capacities (Hoffmann 1981; Elias and Crocker 2008). For example, during the LGM central Beringia was wetter than neighboring areas and consisted of a mosaic of tundra ecosystems (Elias et al. 1996; Anderson et al. 2004), potentially limiting transberingian dispersal by species that favored drier environments (Guthrie 2001; Elias and Crocker 2008). This could explain why *S. parryii*, which requires dry soils for its burrows, may have colonized Siberia only recently despite its relatively deep history in Alaska.

Distributions for the three arctic ground squirrel lineages suggest a history of differentiation caused by a combination of glacial, paleoecological, and physiographic barriers, rather than climatic factors directly modeled by the BEM. The origin of the Arctic lineage is most easily understood as a consequence of isolation of populations north of the Brooks Range, which was heavily glaciated during glacial maxima. A similar history of isolation may explain differentiation in diverse taxa (e.g., Fedorov and Stenseth 2002; Abbott and Comes 2003; Wickström et al. 2003). The pattern is mirrored in distinct arctic and subarctic faunas (MacDonald and Cook 2009), and indicates that within Beringia, the Brooks Range and its associated glaciers probably formed an important biogeographic barrier during the Pleistocene.

To understand the cause of differentiation between the two more southerly distributed lineages it will be necessary to determine where the lineages evolved, and this will require more thorough multilocus sampling at the population level. An Alaskan center of origin for both lineages might indicate isolation driven by expansion of the Cordilleran ice sheet across the Alaska Range. Alternatively, the Southwest/Beringia lineage could have evolved in western Beringia and subsequently expanded eastward into Alaska. The BEM predicts better conditions for arctic ground squirrels in western and central Beringia than adjacent to the ice sheet in southern Alaska during the LGM (Fig. 3), although this is not a robust result. Dispersers from different isolates would have come into contact in central Alaska during periods of range expansion, but displacement of steppe-tundra by boreal forest during the LIG (Muhs et al. 2001) and Holocene (Ager 1983) probably helped maintain isolation. Populations that represent points of contact between neighboring lineages during periods of range expansion may be among the first to disappear during range retraction, erasing any record of introgression (Galbreath et al. 2009).

CONCLUSIONS

In this study, we reconstructed the population history of *S. parryii* to better understand how past climatic oscillations affected northern species, and to evaluate the alternative historical perspectives

offered by mtDNA phylogeography and paleodistributional modeling. Our data revealed that mtDNA apparently overestimated the amount of population structure and failed to accurately resolve the relationship between *S. parryii* and *S. richardsonii*, although certain patterns of congruence with nDNA provide support for specific historical inferences. Predictions of population structure derived from the BEM were generally not supported, probably due both to failure to meet certain key assumptions of the method and to challenges associated with interpreting paleodistributional predictions of limited temporal resolution.

Distributions of the major arctic ground squirrel lineages implicate glacial and ecological barriers, combined with climate-driven range fluctuation, in structuring regional populations within Beringia. This is consistent with a growing body of literature identifying Pleistocene glacial/interglacial cycles as a major determinant of structure in northern biotas, although it is one of the first instances of such structure evolving within subarctic Beringia (Hewitt 2004b). Climate change and associated paleoecological impacts probably also underlie a general signal of population growth, but lack of resolution regarding the timing of demographic events impedes full understanding of the historical influences that control population size through time. As the field of nDNA phylogeography progresses, improving understanding of the temporal resolving power of nDNA loci should be a priority. For example, how many loci are necessary to infer histories of a given depth and duration? Are multilocus approaches limited by rates of evolution at nDNA loci?

The history of *S. parryii* has now been examined from different perspectives, including mtDNA and nDNA sequences, microsatellites, allozymic and karyotypic data, morphology, life-history, parasitology, fossil record, paleodistributional models, and paleoecology. Each is important and informative, but none independently resolves the full history of the species. This is evident in contrasting genetic signatures retained by nDNA and mtDNA that may in part reflect different life histories of males and females. To date, most phylogeographic studies of northern organisms have focused on variation within cytoplasmic genomes. Our findings show how these single-locus perspectives can provide important corroboration of historical inferences derived from other sources, but on their own have potential to mislead, especially because of stochasticity in the coalescent and failure to fully resolve sex-biased population processes (e.g., male-dominated gene flow). Our analysis underscores the importance of independent lines of evidence for resolving population histories, and represents a step toward improved integration of phylogeography with other fields that focus on understanding past and future environments.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Bioclimatic envelope models for arctic ground squirrels (*Spermophilus parryii*) under current conditions.

Figure S2. Minimum spanning networks recovered from (A) mtDNA lineages and (B) anonymous locus (ALP) datasets for arctic ground squirrels (*Spermophilus parryii*).

Figure S3. Maximum likelihood trees recovered from individual anonymous locus datasets for arctic ground squirrels (*Spermophilus parryii*).

Table S1. Locality identification number, population name, subspecies designation, major mitochondrial clade, approximate latitude and longitude, museum catalog number (UAM—University of Alaska Museum, MSB—Museum of Southwestern Biology), frozen tissue number (AF—University of Alaska Museum, IF—Idaho State University, NK—Museum of Southwestern Biology), genetic loci that were sequenced (cyt-b = cytochrome b; ALPS = anonymous nuclear loci), and GenBank numbers for cyt-*b* sequences for *Spermophilus parryii*, *S. richardsonii*, and *S. columbianus* specimens used in this study.

Appendix S1. Protocol for identifying anonymous nuclear DNA loci.

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