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# Fire and ice: genetic structure of the Uinta ground squirrel (*Spermophilus armatus*) across the Yellowstone hotspot

MARCEL VAN TUINEN,§* KIM O'KEEFE,§† UMA RAMAKRISHNAN‡ and ELIZABETH A. HADLY†
*Department of Biology and Marine Biology, University of North Carolina at Wilmington, Wilmington, NC 28403, USA, †371 Serra Mall, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020, USA, ‡National Centre for Biological Sciences, GKVK Campus, Bellary Road, Bangalore 560065, India

**Abstract**

**The range of the Uinta ground squirrel, *Spermophilus armatus*, is centred over one of the most tectonically active regions today, the Yellowstone hotspot. We document the role of Quaternary tectonic and climatic history on the genetic structure of this species by screening museum and extant individuals throughout its range. Phylogeographic, divergence time, and demographic analyses of partial mitochondrial cytochrome *b* and control region DNA sequences yield insight into the cadence of evolution across three spatiotemporal scales: (i) a relatively deep intraspecific divergence of *S. armatus* into three lineages coincident with the last major volcanic eruption in the region and maintained by the Snake River Plain; (ii) demographic expansion in two lineages corresponding to the time of last deglaciation of the region; and (iii) a recent (< 50 years) local extinction of the third lineage coincident with climatic change and conversion of habitat for agricultural purposes in eastern Idaho. Beyond these inferences, our study highlights the unique value of museum material to phylogeography, and shows that small mammal recolonization of previously glaciated montane 'islands' differs from northward postglacial expansion observed in areas previously covered by continental ice sheets. Montane 'islands' may harbour high genetic diversity because of admixture and recurrent expansion/extinction.**

*Keywords*: glaciation, ground squirrels, Lava Creek Caldera, phylogeography, postglacial recolonization, Snake River Plain, Yellowstone hotspot

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## Introduction

A frequent observation in phylogeographic studies is that the modern distribution of genetic diversity remains associated with the geographic landscape in which species evolved, thus giving credence to vicariance as an important mechanism of species and population subdivision (Avise *et al*. 1987; Riddle 1996). Palaeoclimatic, orogenic or tectonic change is often correlated with species or subspecies divergence through the inference of fossil-calibrated molecular clocks (van Tuinen *et al*. 2004). A prime example of palaeoclimatic change stimulating isolation and divergence of populations involves the waxing and waning of continental ice sheets throughout the Pleistocene (Avise *et al*. 1987; Brunsfield *et al*. 2001; Lessa *et al*. 2003; Hewitt 2004).

Small boreal mammals in particular have provided a good test of the importance of glacial/interglacial cycling to shallow genetic history with recent investigations indicating a central role of dispersal in montane mammal phylogeography (Brant & Orti 2003; Demboski & Sullivan 2003; Eddingsaas *et al*. 2004; Hewitt 2004; Runck & Cook 2005; Waltari & Cook 2005). In high-latitude species, postglacial recovery generally has been rapid after population expansion from individuals surviving in southern refugia (Hewitt 2004). Because mutational buildup is too slow to fix novel genetic diversity subsequent to the Pleistocene, standing genetic diversity in previously glaciated areas is often low.

In contrast, genetic diversity may be much higher in areas previously covered with isolated ice sheets not connected to the continental ice sheets, such as montane

§These authors contributed equally to this manuscript.
Correspondence: Kim O'Keefe, Fax: 650-725-822; E-mail: kokeefe@stanford.edu

glaciers or 'islands', but this concept has little empirical support (Knowles 2000). Understanding the process of recolonization of these isolated glaciated areas is of interest to us for several reasons. First, these areas may be repositories of genetic diversity because of the shorter times between glacial cycles and more frequent, multidirectional recolonization events (Hadly *et al*. 2004). Second, in the face of global warming, they are thought to be vulnerable to population extinctions as cool climate species are forced off these montane islands and are replaced by lower-altitude species (Knowles 2000; Masta 2000; DeChaine & Martin 2005). Montane glaciers are not uncommon, particularly in western North America, where many are found throughout the Rocky Mountains as far south as Mexico (Halfter 1987).

Members of the squirrel family Sciuridae are an informative mammalian system for ascertaining the role that climate plays in diversification among species (Lamb *et al*. 1997; Mercer & Roth 2003). For several reasons, ground squirrels of the genus *Spermophilus* provide for valuable phylogeographic study of mammals. Several species occur today in intermontane regions that have been profoundly influenced by extreme, nonseasonal environmental perturbations such as repeated montane glaciation cycles and volcanism during the Quaternary. In addition, previous work on the phylogenetic relations among North American *Spermophilus* found that closely related species are geographically proximate, suggesting that most species evolved in or near their current ranges (Harrison *et al*. 2003). Thus, geographic barriers that have contributed to allopatric speciation in the genus are likely to be found within the present distribution of the group. In addition, although they have restricted ranges relative to other small herbivores, spermophilines are among the most locally abundant mammals in the north-central Rocky Mountains (Yensen & Sherman 2003). To date, phylogeographic investigation among ground squirrels has been limited to two North American species, the arctic (*Spermophilus parryi*) and antelope ground squirrels (*Ammospermophilus leucurus*). These studies describe two opposing genetic scenarios: (i) a northward postglacial expansion of antelope ground squirrels from southern relict but more genetically diverse populations (Whorley *et al*. 2004), and (ii) maintenance of high genetic diversity through glacial vicariance combined with a Beringian refugium in the arctic ground squirrels (Eddingsaas *et al*. 2004). However, neither study involved species recolonization of isolated glaciers.

Among the North American *Spermophilus* species, the Uinta ground squirrel (*Spermophilus armatus*) is a montane species with a relatively small species range (Fig. 1B) centered over one of the most tectonically active regions in the world today, the Yellowstone hotspot (Pierce & Morgan 1992). The species thus provides an important test for the relative influence of dispersal among montane basins vs. the vicariance caused by separation between basins during glaciation and/or volcanism. The Yellowstone hotspot is so named because it currently resides in Yellowstone National Park and is causing the topographic uplift of the Beartooth Plateau. The uplift increased the altitude of this region enough that it is thought to have contributed to the formation of montane ice caps, as much as a mile thick (Smith & Siegel 2000), that formed during the last glaciation and covered the region as recently as 12 000 BP (Pierce 1979). Thus, the animals present in Yellowstone National Park today have only recently recolonized the region since the last glaciation (Pinedale glaciation 40 000–12 000 BP).

The Yellowstone hotspot has had a long history of influence on the region in which *S. armatus* evolved. Over the last 17 million years, the continent has migrated over the hotspot in a southwestern course resulting in a swath of extreme tectonic instability from the southeast of Oregon, through Idaho and into the Greater Yellowstone ecosystem (GYE). As the hotspot 'migrated', it caused topographic uplift. Repeated caldera eruptions, layers of heavy basalt lava flows, and the gradual cooling and shrinking of the earth's crust that took place after the hotspot moved on resulted in the subsequent topographic collapse that formed the massive snake river plain (SRP) (Smith & Siegel 2000). The SRP, a 500-mile long, 50-mile wide swath of basalt plain (Pierce & Morgan 1992) (see Fig. 1B), has been shown to be a geographic barrier for some fish species (Johnson 2002) and has been hypothesized to restrict movement of *S. armatus* (Davis 1939) and other squirrels in the area (Zegers 1984; Demboski & Sullivan 2003; Yensen & Sherman 2003).

Here, we investigate the recolonization of the previously glaciated parts of the GYE and the influence of tectonic activity in shaping contemporary patterns of genetic diversity of *S. armatus.* We hypothesize that genetic diversity in the montane island situated on the Yellowstone Plateau will be high relative to what would be expected from a unidirectional post-Pleistocene expansion from other portions of the species range and that the SRP has served as a barrier to gene flow. To study these questions, we required a comprehensive sampling of the entire species range. Comprehensive sampling of modern populations across the entire range is challenging because most populations are found in montane regions and have extremely short active seasons (3–4 months). Thus, we have utilized a combination of mostly historic and limited modern specimens from numerous mammal collections. Here, we present results from a phylogeographic study of *S. armatus* through genetic analysis of cytochrome *b* (cyt *b*) and control region (D-loop) sequences obtained from museum, tissue and raptor pellet samples that represent the entire species' distribution. We report the presence of three intraspecific genetic lineages structured by the dynamic tectonic and climatic history of this region, and the historic loss of one

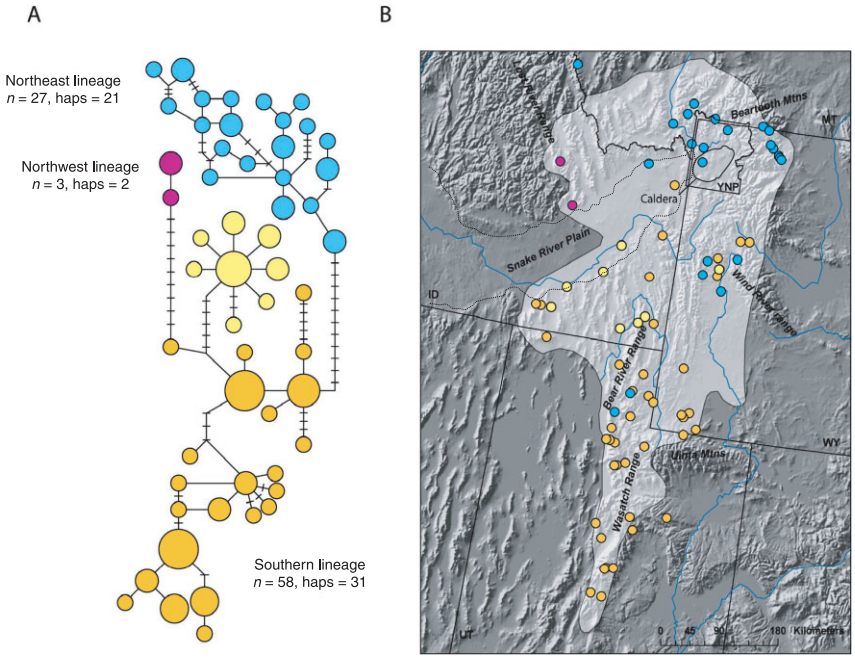


**Fig. 1** (A) Unrooted haplotype network of absolute distances between mitochondrial DNA haplotypes of control region (D-loop). Each circle represents a unique haplotype, its size proportional to the haplotype frequency. Colours represent the three genetic lineages found within the species range from analysis of all sequences in TCS: northwest lineage (purple), northeast lineage (blue), and southern lineage (yellow and gold). The yellow within the southern lineage represents a 'star' phylogeny indicating recent expansion. Haplotypes from this star phylogeny are colour-coded to show the location of the recent expansion within the geographic range. Lines connecting each haplotype represent a single nucleotide substitution, and the hatch marks along those branches represent additional substitutions. Haplotypes with more than one branch connecting them to other haplotypes represent alternative pathways of equal likelihood. (B) Distribution Map of *Spermophilus armatus*. Gray shaded relief is species range derived from Hall & Kelson (1959), georeferenced in ArcGIS and shown with top of the map orientated as due North. State lines and boundaries of the Snake River Plain are shown, representative geographic ranges are labelled and the position of the Lava Creek Caldera in Yellowstone National Park (YNP) that erupted approximately 640 000 years ago is drawn in black. The Yellowstone Plateau referred to in the text overlaps well with the YNP park boundaries. Sampling localities are based on georeferenced collecting localities and are shown by coloured circles. The colours represent the three genetic lineages found within the species' range: northwest lineage (purple), northeast lineage (blue), and southern lineage (yellow and gold). The yellow colour represents an area of recent population expansion and corresponds with the star phylogeny portion of the haplotype network also in yellow. Note that two localities showed genetic identification to *S. armatus* outside of its accepted species range. This phenomenon is likely real, particularly in Utah.

of these lineages. Divergence times estimated for the faster marker (D-loop) yield dates consistent with the timing of tectonic and climatic events across the Yellowstone region. These results reveal the value of museum specimens for phylogeographic study over historic time and provide unique insight into the direct influence of Quaternary environmental change linked to the evolution of mammals of the intermontane American west.

## Materials and methods

### *Taxon sampling*

Individuals were sampled from throughout the current species range from a variety of sources: tissues from field-trapped individuals ($n = 7$); dental material found in raptor pellets from Yellowstone National Park ($n = 5$), and museum skins from several North American museums ($n = 78$). Sequences obtained from the sister species, *Spermophilus beldingi* (Harrison *et al*. 2003), serve as an outgroup ($n = 2$). Specimen number and sampling localities are found in Table S1, Supplementary material. Our total sampling equals 92 individuals from 81 localities, including nine localities originally used in determination of the species' marginal records and three museum specimens collected in 1936, 1940, and 1987 from outside those established margins (Hall & Kelson 1959). Two of the 92 specimens were identified as *Spermophilus elegans* and therefore not used in our analyses. Our total geographic coverage spans 39 counties, and is distributed across the major mountain ranges (Uinta, Wasatch, Wyoming, Wind River, Teton, Absaroka, Lost River), plateaus (Beartooth, Yellowstone, Wasatch), and other suitable habitat within the current distribution of *Spermophilus armatus*. It also

includes historic individuals from the Lost River Range in the northwestern part of the species range, in localities presently devoid of *S. armatus*. Initial sampling regime included limited sampling across the southern part of the range; hence, our expanded sampling included mostly individuals from the southern end of the species range, allowing us to test whether expansion into the northern montane islands could have been from the south alone.

### *Genetic sampling*

In accordance with parallel work in our laboratory on rodents from the GYE, we used a genetic marker (mitochondrial cytochrome *b*) identified previously as phylogeographically informative for *Spermophilus* (Eddingsaas *et al*. 2004), *Ammospermophilus* (Whorley *et al*. 2004), and other squirrel taxa (Arbogast *et al*. 2001; Demboski & Sullivan 2003). We also screened a faster marker (mitochondrial D-loop), which yielded additional resolution for shallow branches. Our initial data set included cytochrome *b* (381 bp) and D-loop (262–263 bp) sequences for 34 individuals. To better test the pattern and directionality of postglacial recolonization, we expanded our sampling to comprehensively cover localities within the southern portion of the species range, focusing this time on the faster evolving D-loop gene. Our final data set includes D-loop sequences for 90 individuals spanning 262–263 bp, seven additional shorter (152 bp) D-loop sequences from individuals showing lower amplification success as well as the 34 cytochrome *b* sequences (381 bp) for a representative subset. Phylogenetic analyses were performed on the cyt *b* and D-loop subset data sets ($n = 34$) separately (see below, Figs 2 and 3, respectively) as well as the two mtDNA segments combined (see below, Fig. 4). A final phylogenetic analysis (see below, Fig. 5) and all of the demographic analyses for *S. armatus* are based on the 'expanded' D-loop data set ($n = 90$; 262 or 263 bp). We refer to Table S1 for the GenBank Accession numbers and Table 1 for primer sequences.

### *Laboratory methods*

DNA extraction, amplification and contamination control of modern and historic specimens followed the published protocol for ancient DNA by Hadly *et al*. (2004). Sequencing of polymerase chain reaction (PCR) products was conducted by Cogenics formerly Genaissance Pharmaceutical, New Haven, Connecticut. We treated museum skins and pellets as specimens like 'ancient DNA' with low-copy mtDNA and high probability for contamination. Additional processing for museum skins included the removal of hair before extraction, which we found to reduce the number of PCR inhibitors. Authentication of mitochondrial sequences included: (i) use of multiple contamination controls, (ii) spatial separation of modern and historic DNA extraction and amplification in separate buildings by different personnel, (iii) sequencing of both forward and reverse directions for multiple overlapping fragments, (iv) repeated extraction and amplification for historic individuals showing unique haplotypes, (v) use of two linked markers for a representative taxon subset to confirm topological consistency, and (vi) cloning (TOPO TA) from PCR products of two representatives from each of the two most divergent lineages.

**Table 1** Primer pairs for cytochrome *b* and D-loop fragments. Fragments for each gene are overlapping

| Primer name | Primer sequence (5′–3′) | Gene |
|---|---|---|
| Sarm1 | AAACCCCCTAAGCACCCCACCTC | Cytochrome *b* |
| Sarm2 | TAGAATTCAGAATATGCATTGAC | Cytochrome *b* |
| Sarm3 | CCATCTATCTAAACAACGAAGCA | Cytochrome *b* |
| Sarm4 | TTTTCGATTAGGCTGACGGTTGG | Cytochrome *b* |
| Sarm10 | ACTATCAAAGATATCCTTGGAGTCC | Cytochrome *b* |
| Sarm11 | CACACCTCCCAGTTTATTAGGG | Cytochrome *b* |
| SarmD10 | ACTCCTATGTAAATCGTGCATT | D-loop |
| SarmD11 | TCCACGGTCATGYTGACGGGTRG | D-loop |
| SarmD12 | CGTYCATAATACTAACATAGTAC | D-loop |
| SarmD13 | GAGACCAAATTTGGTAGGGGATAGTC | D-loop |

### *Phylogenetic analyses*

Phylogenetic analyses were run in PAUP 4.0 beta (Swofford 2003) using the NJ and ML algorithm and with model parameters established from the Akaike information criterion in MODELTEST version 3 (Posada & Crandall 1998). We performed an analysis using unweighted MP with indels specified as a fifth state. To establish the phylogeographic information content and consistency of the observed indels, we compared the resulting MP tree to the unweighted MP, ML/NJ trees in which indels were excluded from analyses. Nodal support was determined by NJ bootstrapping (BS) and ML quartet puzzling with 1000 iterations. Because multiple MP trees were found for every data set, we determined nodal consistency in the MP analysis by estimating the majority rule consensus values with a 50% cutoff and setting the Maxtrees option to 500. Consistency between different methods (NJ, ML and MP) was determined through comparison of nodal support values in the presented mtDNA gene trees (Figs 2–5).

### *Genetic structure*

We used TCS (Clement 2000), which does not assume a bifurcating pattern of genetic divergence, to identify unique haplotypes and to generate an unrooted minimum-spanning network with a 95% plausibility of each linkage between haplotypes. We performed a series of analyses of molecular variance (AMOVA) in ARLEQUIN 3.1 (Excoffier &

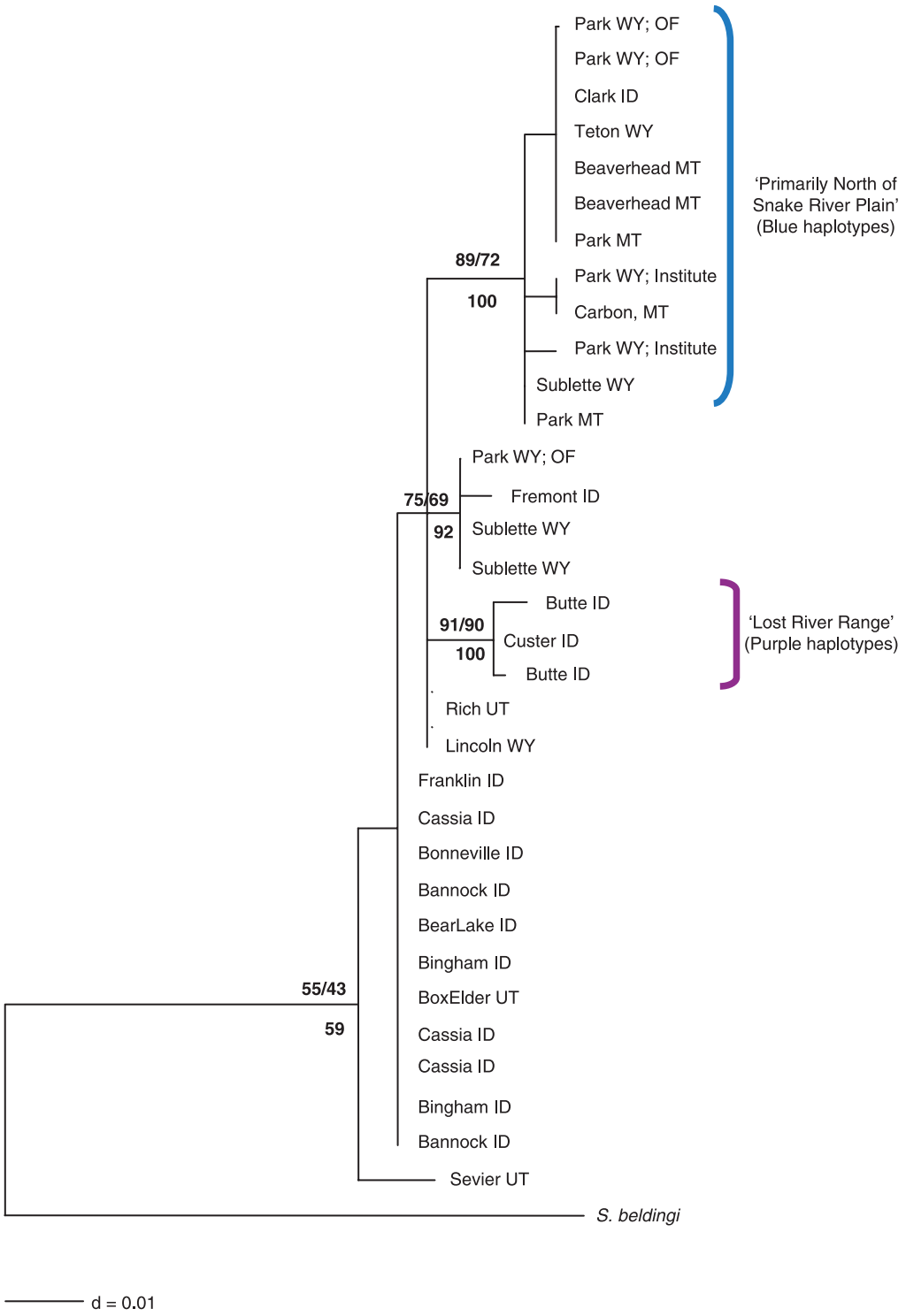


**Fig. 2** mtDNA gene tree of modern and historic individuals of *Spermophilus armatus* based on cytochrome *b* ($n$ = 34, bp = 381). Depicted is the maximum-likelihood tree using the HKY model ($T$ ratio = 22.6) as specified by MODELTEST. Numbers shown above nodes are bootstrap values using neighbour-joining and 1000 iterations (left), and bootstrap values using maximum likelihood in combination with quartet-puzzling and 500 iterations (right). Numbers below nodes identify the frequency of branching support from equally likely maximum-parsimony trees.

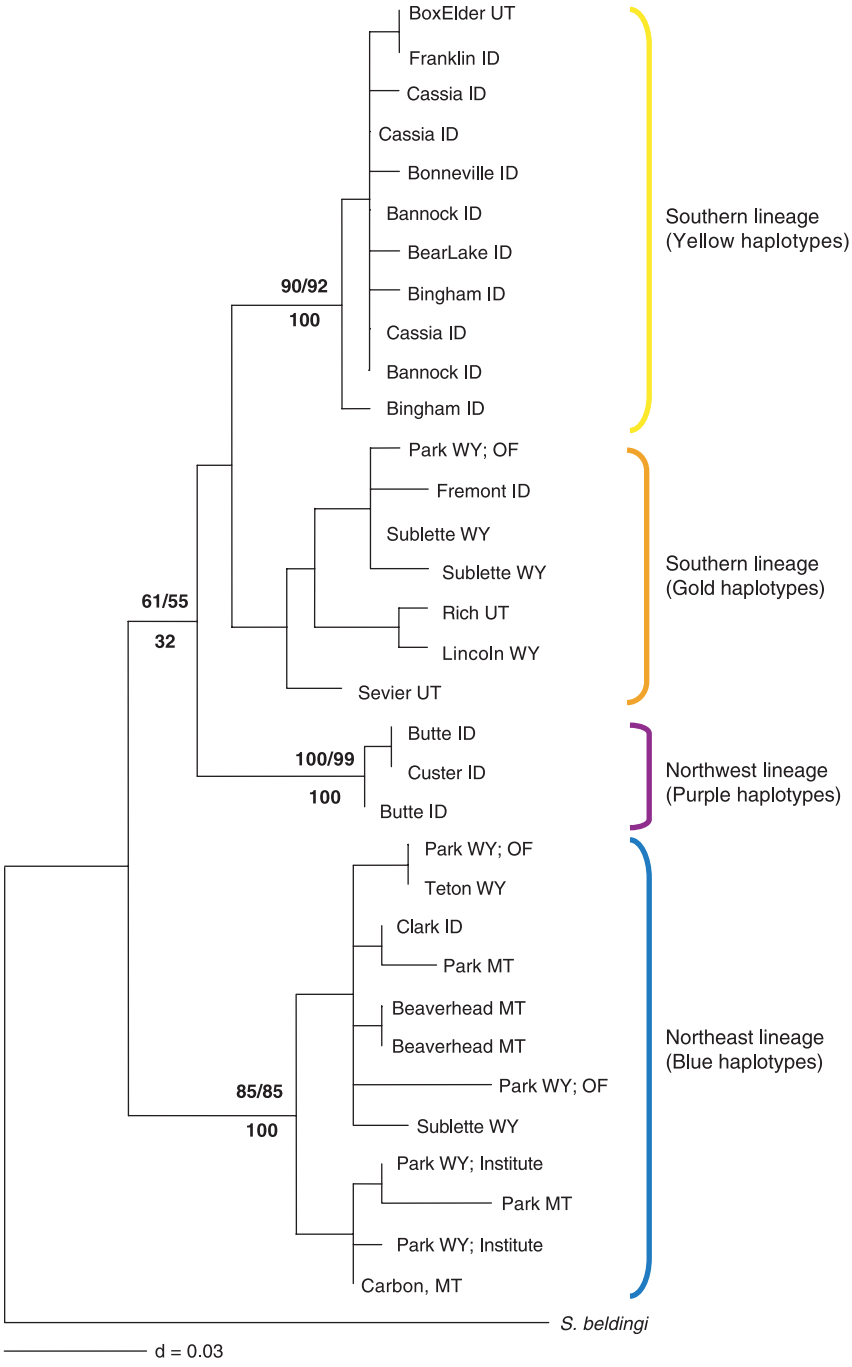


**Fig. 3** mtDNA gene tree from modern and historic individuals of *Spermophilus armatus* based on D-loop ($n = 34$, bp = 262–263) sequences. maximum-likelihood (ML) tree is shown here using the HKY + *G* model (*T* ratio = 63.21, alpha = 0.08) as specified by MODELTEST. Numbers shown at nodes are bootstrap values using NJ and ML (above nodes), and MP consensus values.

Schneider 2005) to determine how much of the observed genetic structure could be explained by distinct geographic divisions. Standard estimates of genetic distance were calculated in ARLEQUIN 3.1 using the best model of evolution for our data set as specified by MODELTEST.

### *Demographic history*

We tested for past changes in population size using several methods. We used Fu's neutrality test (Fu 1997) in ARLEQUIN 3.1, which is a particularly powerful test for detecting sudden and recent expansions in populations with an excess of rare alleles. We also examined the distribution of pairwise differences (mismatch distributions) for each group to look for evidence of past expansion (Rogers & Harpending 1992). In general, populations undergoing recent and sudden expansion exhibit a Poisson-shaped mismatch distribution while populations in equilibrium tend to have ragged distributions (Slatkin & Hudson 1991).

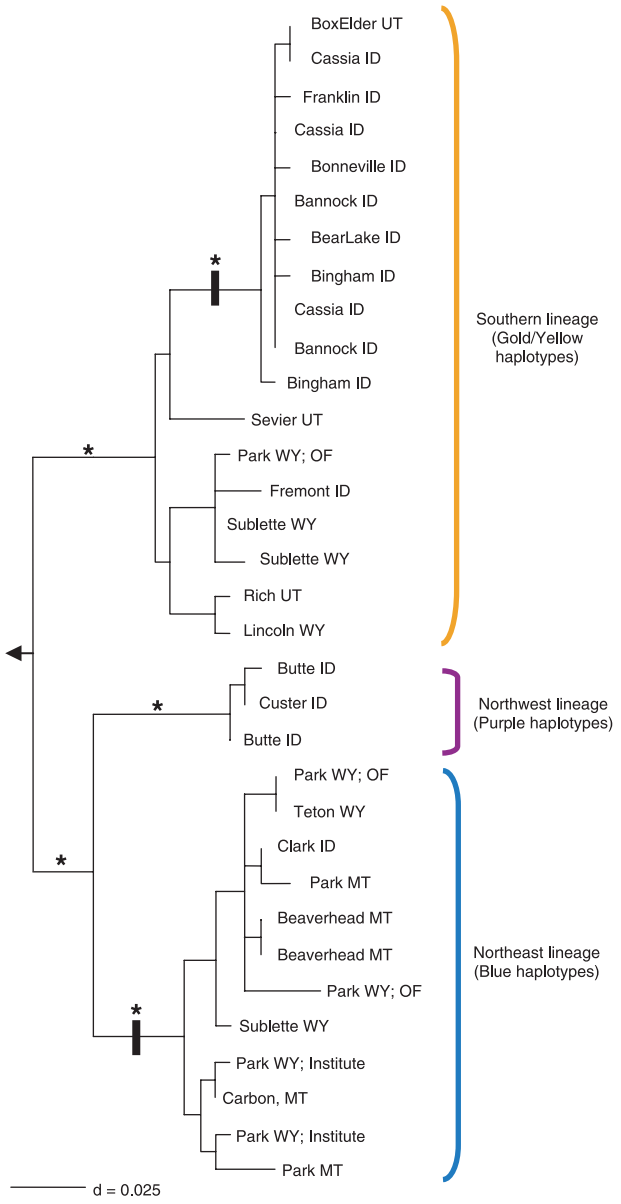


**Fig. 4** mtDNA tree from modern and historic individuals of *Spermophilus armatus* based on a combination of cytochrome *b* and D-loop sequences ($n = 34$, bp = 624). Depicted is a maximum-likelihood tree using a TiM + $G$ + $I$ model and parameters Rmat = 1, 20.2, 0, 0, 36.3, alpha = 0.5151, $I = 0.5824$ as specified by MODELTEST. Asterisks denote nodes with significant ($P = 0.95$) support. Nodes with hatch-bars have additional indel support (see text for discussion).

### *Divergence times*

Approximate sublineage ages were estimated from the faster marker (D-loop) because of its increased resolution for the shallow history of *S. armatus* and our interest in dating population expansions and recent admixture. The fossil record of *S. armatus* in the Pleistocene is too discontinuous to be of use for internal calibration (Eshelman & Sonnemann 2000), which prevented direct calibration of the D-loop molecular rate. We therefore estimated the observed rate of the D-loop data set relative to the cyt *b* rate of molecular evolution and converted this to an absolute rate based on a published cyt *b* rate for *Spermophilus* (1.52% per million years, Eddingsaas *et al.* 2004). This rate was originally calibrated from fossil and geologic evidence suggesting a divergence time of 5.16 million years ago (Ma) between *S. beecheyi* and *S. parryi*. Likelihood models were run with and without a molecular clock constraint using the likelihood ratio test under a chi-square probability distribution to assess whether the data were significantly clock-like. We used MEGA 3.0 to estimate sublineage divergence times from 'net' distances after correction for within-lineage polymorphism. We approximated the MODELTEST-preferred model of TrN + $G$ + $I$ using a TrN + $G$ model with application of a stringent correction for rate heterogeneity across sites ($G = 0.19$) estimated from the Akaike information criterion-averaged alpha ($G$), [not alpha (IG)] output.

## Results

### *Overall amplification success and resulting phylogeographic structure*

DNA was readily amplified from skins when hair is removed and amplification is limited to small length (100–150 bp) fragments. Our protocols resulted in a 92% amplification rate based on 85 skins attempted, five primer pairs (Table 1), and two mitochondrial markers spanning ~650 bp. Genetic analyses reveal that the genetic diversity throughout the entire modern range of *Spermophilus armatus* is relatively low and can be traced to three lineages. The most widespread and abundant lineage is present throughout the south and southwest of the modern distribution (southern lineage, 'SL') and is found exclusively south or east of the SRP (Fig. 1B, yellow and gold: the SRP on this map runs in a north–northeast direction along the Yellowstone hotspot). A second lineage is predominantly found north of the SRP in the northeast of the current distribution (northeast lineage, 'NEL') (Fig. 1B, blue). A third lineage is very rare (represented by only three individuals out of the 88 *S. armatus* that we sampled) and only found historically north of the SRP in the northwest of the species range (northwest lineage 'NWL') (Fig. 1B, purple).

### *Phylogenetic analyses*

*Cyt b.* Our observed substitution pattern for cytochrome *b* (cyt *b*) is consistent with the average mitochondrial pattern among mammals (Irwin *et al.* 1989), with abundance of substitutions at third codon positions, low

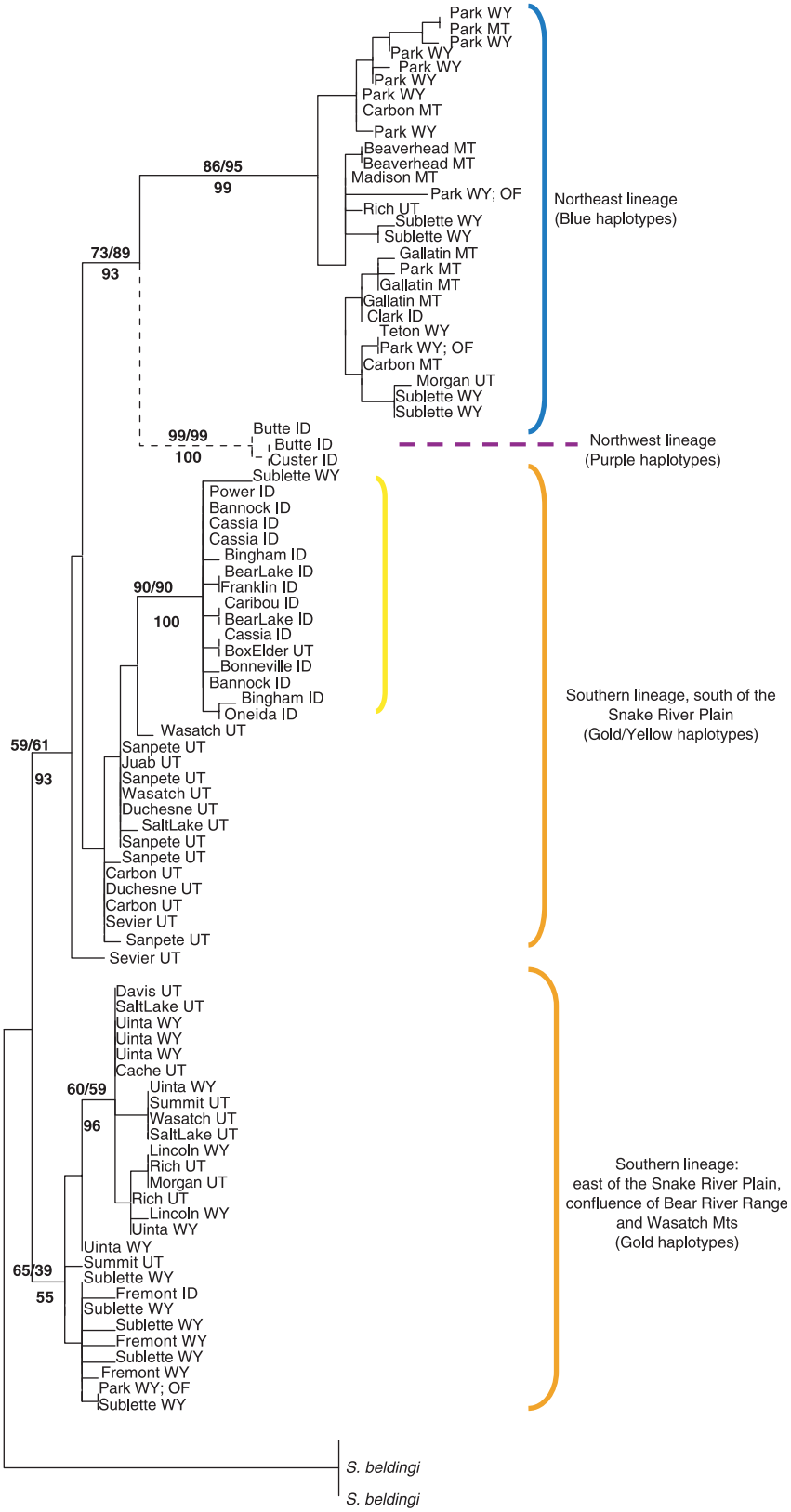


**Fig. 5** mtDNA gene tree of D-loop sequences from modern and historic individuals of *Spermophilus armatus* sampled throughout the species range ($n = 88$, bp = 263). Depicted is the maximum-likelihood tree using a TN + *G* (alpha = 0.19) model specified by MODELTEST. Numbers shown above nodes are: bootstrap values using neighbour-joining and 1000 iterations (left), and bootstrap values using maximum likelihood in combination with quartet-puzzling and 500 iterations (right). Numbers below nodes identify the frequency of branching support from equally likely maximum-parsimony trees. Support < 50% is not shown. Dotted line depicts a genetically distinct lineage only found in historic individuals.

GC content across all sites and especially at third codon positions. All observed substitutions in cyt *b* were transitional, suggestive of a recent species history. Phylogenetic results show tentative geographic clustering among *S. armatus*, low genetic diversity (HKY model, *T* ratio = 22: 2.3% overall pairwise divergence = 2.3%; mean pairwise divergence = 0.95%; see Johns & Avise 1998 for comparison to other species) and limited bootstrap support (Fig. 2). Intermediate support is found for a north and south of the SRP division (75% BS). Three historic individuals north of the SRP and at the western edge of the species range in the Lost River Range form their own grouping (91% BS). A second lineage found primarily north of the Snake River has strong support (89% BS), and includes individuals from northeast Idaho, Montana, northern Wyoming, and the Wind River Mountains. Cytochrome *b* variation was too low (< 1%) to detect structure in the southern lineage (Fig. 2).

*D-loop.* Use of the faster-evolving D-loop mitochondrial marker for these individuals added considerable resolution (Fig. 3). The HKY model again best described this data set, but with an additional correction for rate heterogeneity among sites (*T* ratio = 63.21, *G* = 0.08). The unrooted D-loop phylogeny and that of the cytochrome *b* for the same individuals lack conflict, although the two genes differ in the placement of the root. In species with shallow lineage coalescence, this difference is not unexpected because of a long branch leading from the in-group to the sister-group (Demboski & Sullivan 2003). The D-loop data support the grouping of a distinct northeast lineage (85% BS), and a southern lineage subgrouping in Idaho (90% BS), both of which have support from several unique substitutions and a 1-bp deletion or insertion (Fig. 3).

*Cyt b and D-loop.* As expected from the observed congruence, analysis of the combined fragments (under model TiM + *G* + *I* and parameters Rmat = 1, 20.2, 0, 0, 36.3, *G* = 0.5151, *I* = 0.5824) increases support for both lineages to 96–100% and again supports a distinct lineage in the northwest of the species range near the Lost River Range (100% BS), as well as a clustering of these Lost River individuals within an expanded northeast lineage grouping (73% BS) (Fig. 4). The combined data still provides little information on *S. armatus* from Utah, which is likely due to the limited sampling from the southern range relative to the diversity found there. Thus, we expanded our sampling to include individuals from the entire range, focusing our sequencing efforts on the faster evolving D-loop which is better suited for shallow phylogeographic analysis of the Uinta ground squirrel.

*Expanded D-loop.* The expanded D-loop phylogeny is consistent with that based on cytochrome *b*, but with added resolution from both variable sites and informative indels. The expanded D-loop data set again shows a 100% consistency index for the two indels. Furthermore, it confirms the Snake River Plain as a phylogeographic break, and indicates two areas of admixture (east of the Snake River Plain and Teton Mountains in the Wind River Range, and at the confluence of the Bear River Range and Wasatch Mountains) (Fig. 5).

### *Genetic structure and diversity*

Significant $F_{ST}$ values were found between samples taken from north of the SRP and those existing south of the SRP (D-loop: $F_{ST} = 0.454$, $P < 0.001$). Variation between these two groups ($\Phi_{SC}$) explained more than 45% of the variation across the entire species range. Grouping individuals by the four clades represented in Fig. 5 (northeast lineage, northwest lineage, southern lineage south of the SRP and southern lineage east of the SRP also resulted in a significant $F_{ST}$ value (D-loop: $F_{ST} = 0.482$, $P < 0.001$), increasing the $\Phi_{SC}$ from 45% to 48%. The unrooted haplotype cladogram generated by TCS with a 0.95 probability however, depicts only three haplogroups (Fig. 1A) that correspond with the three best-supported lineages depicted in the mtDNA gene tree in Fig. 5 (northeast, northwest, southern).

The southern lineage (SL) is the most widespread and abundant lineage across the range of the species and is present throughout the south and southwest of the modern distribution. It is the only lineage represented immediately south of the Snake River Plain and west of the Teton and Salt River Mountains (Fig. 1B, yellow and gold). The southern lineage also contains the greatest amount of genetic diversity both in terms of the number of unique haplotypes (31) as well as the mean pairwise distance (6.83 ± 3.26). The northeast lineage (NEL) is found predominantly to the north and northeast part of the current range with only two of 27 individuals from that lineage found in the southern region (Morgan County and Rich County, Utah) of the current range (Fig. 1B, blue). This NEL contains 21 unique haplotypes from 27 individuals and a mean pairwise distance of 5.62 ± 2.78. The northwest lineage (NWL) was found historically north of the SRP in the Lost Rivers Mountains (Fig. 1B, purple) and is represented by only three individuals with two unique haplotypes and a mean pairwise distance of 0.76 ± 0.67.

### *Demographic analyses*

The demographic analyses showed evidence for recent expansion in the SL and the NEL. For example, the haplotype network exhibited a star phylogeny indicating recent population expansion in the SL (Fig. 1A, yellow). Fu's test of neutrality also produced significant $F_S$ values

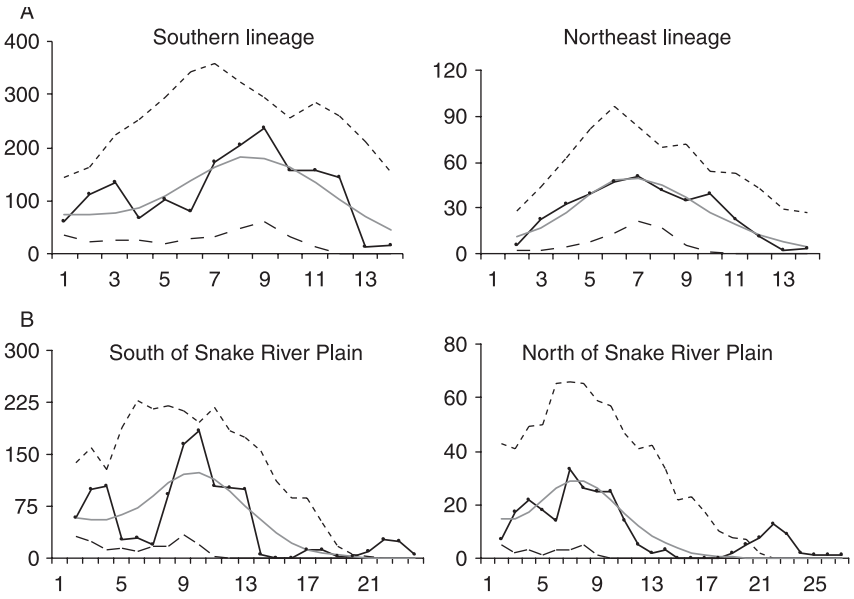


**Fig. 6** Mismatch distributions by lineage association (A) and by geography (B). Black continuous lines correspond to observed frequencies of pairwise nucleotide differences, broken lines represent upper and lower bounds for the 95% confidence intervals around the observed distribution ($\alpha = 0.05$), and the grey solid lines represent the expected frequencies under a sudden expansion model.

($F_S = -1.92$, $P < 0.001$) further supporting the finding of recent expansion within the SL. While recent expansion is much less apparent from visual inspection of the NEL haplotype network (there is no obvious star phylogeny), Fu's $F_S$ were also significant for the NEL ($F_S = -1.53$, $P < 0.001$). The mismatch distributions for the NEL and SL were both unimodal and fall well within the 95% confidence limits showing recent population expansion in each lineage (Fig. 6A). As expected, the mismatch distribution of all haplotypes divided into groups either north or south/southeast of the SRP based entirely on geography (vs. by lineage) show a bimodal distribution because of the presence of gene flow to the east of the Salt River and Teton Mountains along the Idaho–Wyoming border (Fig. 6B). This also explains why Fu's $F_S$ is significant for the haplotypes found east of the SRP, suggesting expansion, but the mismatch distribution is very 'ragged', suggesting stasis. The individuals sampled southeast of the SRP are not reciprocally monophyletic but made up of several rare haplotypes derived from both the SL and NEL, indicative of admixture.

### *Divergence times*

A likelihood ratio test on the D-loop data set did not reject clock-like behaviour (2*deltaLnL = 88.02; $P > 0.10$). Comparison of distances based on the D-loop and cytochrome *b* for the same pairwise groupings indicates that the intraspecific D-loop distances increase linearly with time, at a 7.5 times faster rate. Thus, we estimated an 11.5% per million-year divergence rate from a previously proposed *Spermophilus* cytochrome *b* rate (see Methods). Application of this rate puts the deepest divergence within all extant *S. armatus* lineages at 0.58 million years. The separation of the two northern lineages (NWL and NEL) from the southern (SL) lineage occurred relatively early in the history of *S. armatus* (0.43 Ma), as did the initial divergence within the northern Snake River between the NWL and NEL (0.33 Ma). We estimated a relatively recent age for the common ancestor of the majority of haplotypes found in the Yellowstone Plateau (0.11 Ma), which was completely covered with ice at the end of the Pleistocene. Expansion of an Idaho population south of the Snake River Plain (12 000 years ago) and northward expansion of southern haplotypes (13 500 years ago) both are estimated to have occurred near the end of the Pleistocene. The faster marker lacks resolution for evidence of Holocene expansion but the presence of identical haplotypes across the Bear River Range and Wasatch Range, parts of which were glaciated in the Pleistocene (Laabs *et al*. 2006), does not conflict with this notion.

## Discussion

Our analyses of two linked mitochondrial markers provided us with a multifaceted look into the evolution of *Spermophilus armatus.* The deepest genetic divergence within *S. armatus* is approximately 580 000 years ago. This follows the cataclysmic explosion of the Lava Creek caldera at ~640 000 years ago (Lanphere *et al*. 2002) which formed a massive caldera in what is now the middle of the species range and may explain the low maximum diversity within this species. The Lava Creek volcanic eruption and ash-fall dumped between 1 m and 10 m of ash over most of the western USA (Izett & Wilcox 1982; Perkins & Nash 2002). Smaller ash-fall than the Lava Creek eruption is known to have caused extreme population fragmentation and loss of genetic diversity in some modern rodent popu-lations (Gallardo *et al*. 1995).

A second, relatively deep divergence (0.43 Ma) between the NWL, NEL, and SL falls within local glacial cycles beginning with the Sacajawea Ridge (0.6 Ma) (Pierce 2004) and ending with the initiation of Bull Lake glaciation (Fullerton *et al*. 2004). These glacial cycles caused repeated separation of intermontane basins within the present range of *S. armatus*. Late Pleistocene glaciation (18 000 years ago) of the Yellowstone plateau (Pierce 2004) and other high montane regions within the species range did not cause appreciable divergence within the lineages. However, movement since deglaciation, about 14 000 years ago, has caused admixture along the eastern front of the continental divide near the Idaho–Wyoming border. Recolonization of the previously glaciated montane terrains came from two genetically distinct lineages (NEL and SL) and admixture between these lineages occurred exclusively east or south of the SRP at the southern edge of the Yellowstone Plateau leading into the Wind River and Salt River Range and at the confluence of the Bear River Range and Wasatch Mountains. The NEL shows evidence of recent (13 000 years ago) population expansion, consistent with population size increase since the terminal Pleistocene. Wholesale postglacial recolonization of the northern range of the GYE in the Yellowstone Plateau is demonstrated here to be from both the northeast and southern lineages, with coalescence times for both lineages significantly preceding the terminal Pleistocene. Admixture of distinct genetic lineages in the Yellowstone Plateau and Wind River Range has been found (Hadly *et al*. 2004; Hadly *et al*. unpublished findings) also in montane and long-tailed voles (*Microtus montanus* and *M. longicaudus*). Gene flow of *S. armatus* into Idaho immediately south of the SRP has been restricted to populations solely derived from the SL lineage, which shows evidence of the most recent population expansion. The SRP thus is an obvious barrier to north–south migration in the Uinta ground squirrel and is presently unoccupied by the species.

Finally, very recently (< 50 BP), the populations representing the NWL, found only in eastern Idaho north of the SRP in the Lost River drainage, have become locally extirpated (Madison *et al*. 2004). The NWL is not represented in any extant populations that we have sampled from throughout the species range today; thus, it is likely extinct. Local extirpation north of the SRP near Craters of the Moon National Park has also been observed in other species. *Spermophilus mollis artemisiae* and *S. elegans aureus,* both of which occupied the same region, are hypothesized to have been extirpated due to habitat degradation and agricultural conversion (Zegers 1984; Yensen & Sherman 2003). It is possible that the NWL of *S. armatus* was once more widespread north of the Snake River Plain. We aim to further test this hypothesis by using a phylochronologic approach from subfossils described from two caves at the northern edge of the species range (Waterfall Locality, Lamar Cave), as well as by increased modern field sampling from other localities within the current species range.

### *Cautionary notes*

The consistency of tracing shallow genealogical events to Quaternary environmental events in the Central Rocky Mountains is convincing, especially when considering extensive evidence for genetic subdivisions linked to Pleistocene climate changes elsewhere (the Pacific Northwest, Northern Rocky Mountains) (e.g. Demboski & Sullivan 2003; Eddingsaas *et al*. 2004). Nonetheless, we point out that the scenario depicted above is the maternal side of the total story that is likely more complex given strong male-biased dispersal in *S. armatus*. A nuclear gene phylogeographic investigation will prove more difficult, but not impossible (Zhang & Hewitt 2005), if based mostly on museum specimens. Also, the divergence times presented here are current best estimates, yet still approximate because of the paucity of ground squirrel fossils that can be linked to extant species. Additionally, the estimates of the tectonic and climatic events we describe are not necessarily precise because few are within the time period spanned by radiocarbon dating. Caution also must be advised because we pooled samples from different years (1920–2003) to represent a single phylogeographic story. If gene flow is extremely rapid in species with a small range, phylogeographic structure could theoretically vary over short time periods. Over periods spanning thousands of years, recent ancient DNA research indicated that current phylogeography does not always paint an entire picture of species history, and that ancient gene flow could have left different traces in the past not reflected today (Barnes *et al*. 2002; Hadly *et al*. 2004; Hofreiter *et al*. 2004). Although over a period of less than a century we have observed extirpation of an entire lineage, phylogeographic turnover is not readily apparent in our sampling of the other lineages from 1920 to 2003. Our data instead support a long-term geographic separation of haplotypes across the SRP, with recent admixture of the two largest lineages (NEL, SL) occurring to the east of the SRP where the mountains run in north–south direction.

### *Conclusions*

We report low levels of maximum intraspecific genetic diversity in the cytochrome *b* sequences of Uinta ground squirrels, suggesting significant loss of diversity consistent with the last cataclysmic eruption of the Yellowstone hotspot. Using a faster-evolving marker, we recovered shallow-time phylogeographic resolution and found that the deepest split within the species is consistent with advance of the Sacagawea Ridge glaciation. Screening of this faster marker across a reasonably large set of

specimens allowed us to further recover a demographic signal of expansion in two of the three lineages consistent with Late Pleistocene deglaciation and subsequent gene flow into some areas but not others. Our two hypotheses were confirmed: (i) the SRP was shown to be a major geographic barrier, and (ii) we found significant genetic diversity in several central montane 'islands' (the Yellowstone Plateau, Wind River Range, Wasatch-Uinta Range), which argues against a unidirectional postglacial expansion from the south. Finally, use of museum skins in a diachronic way allowed us to document the historic extirpation of a third, divergent lineage during the past several decades, probably due to agricultural conversion of ground squirrel habitat.

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## References

Arbogast B, Browne R, Weigl P (2001) Evolutionary genetics and Pleistocene biogeography of North American tree squirrels (*Tamiasciurus*). *Journal of Mammalogy*, **82**, 302–319.

Avise JC, Arnold J, Ball RM *et al*. (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecological Systematics*, **18**, 489–522.

Barnes I, Matheus P, Shapiro B, Jensen D, Cooper A (2002) Dynamics of Pleistocene population extinctions in Beringian brown bears. *Science*, **295**, 2267.

Brant SV, Orti G (2003) Phylogeography of the Northern short-tailed shrew, *Blarina brevicauda* (Insectivora: Soricidae): past fragmentation and postglacial recolonization. *Molecular Ecology*, **12**, 1435–1449.

Brunsfield SJ, Sullivan J, Soltis DE, Soltis PS (2001) Comparative phylogeography of northwestern North America: a synthesis. In: *Integrating ecology and evolution in a spatial context* (eds Silvertown J, Antonovics J), pp. 319–339. Blackwell Science Ltd, Oxford.

Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

DeChaine EG, Martin AP (2005) Marked genetic divergence among sky island populations of *Sedum lanceolatum* (Crassulaceae) in the Rocky Mountains. *American Journal of Botany*, **92**, 477–486.

Demboski J, Sullivan J (2003) Extensive mtDNA variation within the yellow-pine chipmunk, *Tamias amoenus* (Rodentia: Sciuridae), and phylogeographic inferences for northwest North America. *Molecular Phylogenetics and Evolution*, **26**, 389–408.

Eddingsaas AA, Jacobsen BK, Lessa EP, Cook JA (2004) Evolutionary history of the arctic ground squirrel (*Spermophilus parryi*) in Nearctic Beringia. *Journal of Mammalogy*, **85**, 591–600.

Eshelman BD, Sonnemann CS (2000) *Spermophilus armatus*. *Mammalian Species*, **637**, 1–6. American Society of Mammalogists.

Excoffier LGL, Schneider S (2005) ARLEQUIN, version 3.1: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Fu X (1997) Statistical neutrality of mutations against polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology and Systematics*, **34**, 397–423.

Fullerton DS, Colton RB, Bush CA (2004) Limits of mountain and continental glaciation east of the Continental Divide in northern Montana and north-western North Dakota, USA. In: *Quaternary Glaciations—Extent and Chronology* (eds Ehlers J, Gibbard PL). Part II North America, *Developments in Quaternary Science*, **2**, 131–145.

Gallardo M, Kohler N, Araneda C (1995) Bottleneck effects in local populations of fossorial *Ctenomys* (Rodentia, Ctenomyidae) affected by vulcanism. *Heredity*, **74**, 638–646.

Hadly EA, Ramakrishnan U, Chan YL *et al*. (2004) Genetic response to climatic change: insights from ancient DNA and phylochronology. *Plos Biology*, **2**, 302–319.

Halffter G (1987) Biogeography of the Montane Entomofauna of Mexico and Central America. *Annual Review of Entomology*, **32**, 95–114.

Hall ER, Kelson KR (1959) *The Mammals of North America*. The Ronald Press Co, New York.

Harrison RG, Bogdanowicz SM, Hoffmann RS, Yensen E, Sherman PW (2003) Phylogeny and evolutionary history of the ground squirrels (Rodentia: Marmotinae). *Journal of Mammalian Evolution*, **10**, 249–276.

Hewitt GW (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London B*, **359**, 183–195.

Hofreiter M, Serre D, Rohland N *et al*. (2004) Lack of phylogeography in European mammals before the last glaciation. *Proceedings of National Academy of Sciences, USA*, **101**, 12963–12968.

Irwin DM, Kocher TD, Wilson AC (1989) Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution*, **32**, 128–144.

Izett GA, Wilcox RE (1982) Map showing localities and inferred distribution of the Huckleberry Ridge, Mesa Falls, and Lava Creek ash beds (Pearlette family ash beds) of Pliocene and Pleistocene age in the western United States and southern Canada: 000. *US Geological Survey Miscellaneous Investigations Series Map* I-1325, scale 1: 4 000 000.

Johns GC, Avise JC (1998) A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. *Molecular Biology and Evolution*, **15**, 1481–1490.

Johnson JB (2002) Evolution after the flood: phylogeography of the desert fish Utah chub. *Evolution*, **56**, 948–960.

Knowles LL (2000) Tests of Pleistocene speciation in montane grasshoppers from the sky islands of western North America (Genus *Melanoplus*). *Evolution*, **54**, 1337–1348.

Laabs BJC, Plummer MA, Mickelson DM (2006) Climate during the Last Glacial Maximum in the Wasatch and southern Uinta Mountains inferred from glacier modeling. *Geomorphology*, **75**, 300–317.

Lamb T, Jones TR, Wettstein PJ (1997) Evolutionary genetics and phylogeography of tassel-eared squirrels (*Sciurus aberti*). *Journal of Mammalogy*, **78**, 117–133.

Lanphere MA, Champion DE, Christiansen RL (2002) Revised ages for tuffs of the Yellowstone Plateau volcanic field: assignment of the Huckleberry Ridge Tuff to a new geomagnetic polarity event. *GSA Bulletin*, **114**, 559–568.

Lessa EP, Cook JA, Patton JL (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceeding of National Academy of Sciences, USA*, **100**, 10331–10334.

Madison E, Rodhouse T, Garret L (2004) *2003 Mammal Inventory, Craters of the Moon National Monument and Preserve*. Report for subagreement no. 20 to cooperative agreement no. CA9000-95–018. University of Idaho and National Park Service, Upper Columbia Basin Network, University of Idaho and Department of Wildlife and Fisheries Resources, Moscow, Idaho.

Masta SE (2000) Phylogeography of the jumping spider *Habronattus pugillis* (Araneae: Salticidae): recent vicariance of sky island populations? *Evolution*, **54**, 1699–1711.

Mercer JM, Roth VL (2003) The effect of Cenozoic global change on squirrel evolution. *Science*, **299**, 1568–1572.

Perkins ME, Nash BP (2002) Explosive silicic volcanism of the Yellowstone hotspot: the ash fall tuff record. *GSA Bulletin*, **114**, 367–381.

Pierce KL (1979) History and dynamics of glaciation in the northern Yellowstone National Park area. *US Geological Survey Professional Paper*, **729F**, 91.

Pierce KL (2004) Pleistocene glaciations of the Rocky Mountains. In: *The Quaternary Period in the United States* (eds Gillespie AR, Porter SC, Atwater BF), pp. 63–76. Elsevier, Amsterdam.

Pierce KL, Morgan LA (1992) The track of the Yellowstone hop spot—volcanism, faulting and uplift. *Regional Geology of Eastern Idaho and Western Wyoming*, pp. 1–53. Geological Society of America Memoir 179.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Riddle BR (1996) The molecular phylogeographic bridge between deep and shallow history in continental biotas. *Trends in Ecology and Evolution*, **11**, 207–211.

Rogers AR, Harpending HC (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

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

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Smith RB, Siegel LJ (2000) *Windows into the Earth*. Oxford University Press, New York.

Swofford DL (2003) PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts.

van Tuinen M, Ramakrishnan U, Hadly EA (2004) Investigating the effect of environmental change on biotic evolution: genetic contributions, current work and future developments, *Philosophical Transactions of the Royal Society of London: Physical, Mathematical and Engineering Sciences*, **362**, 2795–2820.

Waltari E, Cook JA (2005) Historical demographics and phylogeography of arctic hares (*Lepus*): genetic signatures test glacial refugia hypotheses. *Molecular Ecology*, **14**, 3005–3016.

Whorley J, Alvarez-Castaneda ST, Kenagy J (2004) Genetic structure of desert ground squirrels over a 20-degree-latitude transect from Oregon through the Baja California peninsula. *Molecular Ecology*, **13**, 2709–2720.

Yensen E, Sherman PW (2003) *Ground-Dwelling Squirrels of the Pacific Northwest*. US Fish and Wildlife Service, Boise, Idaho.

Zegers DA (1984) *Spermophilus elegans*. *Mammalian Species*, **214**, 1–7. American Society of Mammalogists.

Zhang DX, Hewitt GM (2005) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology*, **12**, 563–584.

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Kim O'Keefe is interested in how the ecological and evolutionary characteristic of a species affect its response to climate at multiple spatial and temporal scales. She is currently investigating how climate influences population dynamics and genetic structure of ground squirrels. Marcel van Tuinen is assistant professor and curator of the animal frozen tissue bank at UNC Wilmington. His research program focuses broadly on birds and mammals and encompasses the use of ancient DNA for historical population genetic inquiries, fossils for molecular clock calibration, and phylogenomic tools to investigate the tempo and mode of aquatic bird evolution. Uma Ramakrishnan is interested in how genetic variation is partitioned through space and time, and how such variation allows us to understand past demographic processes. She is currently investigating genetic variation and its partitioning for mammals in the Indian subcontinent. Elizabeth A. Hadly studies the ecology and evolution of vertebrates using both fossil and modern assemblages from North America, South America and most recently, India. Her investigations focus on the response of animals to climatic change using genetic, morphological, community, and geochemical analyses.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1** Museum and specimen numbers, sampling localities, and GenBank Accession numbers for the control region (D-loop) and cytochrome *b* (cyt *b*)

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2008.03671.x
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