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## Population Stability of the Northern Desert Nightsnake (*Hypsiglena chlorophaea deserticola*) during the Pleistocene

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**ABSTRACT.**—Phylogeographic studies that seek to understand how a species responded to past climate change are largely lacking for the Great Basin of North America. Here we investigate the response of the Northern Desert Nightsnake (*Hypsiglena chlorophaea deserticola*) to the recession of the Cordilleran glacier at the end of the Pleistocene. Using mtDNA markers, we inferred the lineage age and historical demography of this widespread desert taxon. We find that *H. c. deserticola* maintained a stable population size throughout the late Pleistocene despite ice-sheet advance and retreat. These conclusions agree with other phylogeographic studies that show stable or growing population sizes throughout the Pleistocene as well as fossil evidence that sheds light on the historical distributions of snakes, suggesting that snakes have been adaptable to historical climatic fluctuations.

The Pleistocene is characterized by drastic changes in both global and regional climates (Lomolino et al., 2006). The most notable events of this epoch were the numerous shifts from glacial to interglacial cycles. During glacial periods, massive sheets of ice covered areas from the Arctic down to 45°N in North America (Fig. 1; Waitt and Thorsen, 1983). Even areas far removed from glaciers were influenced drastically in terms of climate such that at glacial maxima, shifting winds pushed saturated oceanic air masses to the interior of North America causing increased rainfall (Lomolino et al., 2006). The Great Basin is no exception given that pluvial lakes began to form during this period (Fig. 1; Lomolino et al., 2006). As the climate changed again to the present conditions, these lakes began to dry and most had evaporated by 10 kya (Lomolino et al., 2006).

Fluctuations in the extent of the glacial front and local climate changes are expected to have had differential impacts on population sizes and distribution ranges of resident species. Three distinct responses by taxa have been hypothesized for this period of time (Hewitt, 2000; Lomolino et al., 2006): 1) species tracked suitable habitat and dispersed to optimal environments (Wells and Jorgensen, 1964); 2) species remained in place and adapted to altered local environments (King et al., 2009); or 3) species experienced a range reduction and eventual extinction (Jackson and Weng, 1999).

Studies examining the demographic histories of biota within the Great Basin region are surprisingly rare when compared to those that examine organisms of northwestern and eastern North America (Soltis et al., 2006; Shafer et al., 2010). Northern Desert Nightsnakes (*Hypsiglena chlorophaea deserticola* Tanner, 1944) are small (<66 cm total length), nocturnal snakes that feed on a variety of small squamate reptiles, their eggs, anurans, and small mammals (Weaver, 2010). This species is a geographically widespread dipsadine with a distribution that encompasses both historically glaciated and unglaciated areas (Weaver, 2008), including the pluvial basin regions, thus providing an opportunity to examine the effects of the climatic cycles of the Pleistocene in this region.

We investigated the historical demography of *Hypsiglena chlorophaea deserticola* to determine what effects the Cordilleran Ice Sheet expansion had on an ectothermic Great Basin taxon. This physiogeographic region is of particular interest because of the lack of phylogeographic studies, coupled with the commonly reported heterogeneous responses to climatic change found across other North American taxa (Soltis et al., 2006; Shafer et al., 2010). More specifically, we address hypotheses regarding population expansion following glacial retreat, as it is expected that northward expansion would have followed the timing of this retreat closely. To test this we constructed a time-calibrated phylogeny using mitochondrial DNA and fossil data. Under this framework we tested for signatures and timing of population expansion.

### MATERIALS AND METHODS

**Laboratory and Sequencing Protocols.**—We sampled 36 individuals throughout the range of *H. c. deserticola* (Fig. 2; Appendix 1). Genomic DNA was extracted from ethanol-preserved liver, muscle, or tail-tip tissues using Chelex beads (Bio-Rad). An approximately 900-base pair segment of the hypervariable region of the mitochondrial genome, NADH dehydrogenase subunit 5 (ND5) (Mulcahy et al., 2011), was amplified via polymerase chain reaction (PCR). ND5 was amplified using primers designed specifically for use with *Hypsiglena*: *HypsigLeu2f.1* 5'-TGG TGC AAA TCC AAG TGG TA-3' and *HypsoIdNad5r.1* 5'-AGG AGC CKG AGC ATA GRA ATA-3' based on complete mt-genome data (Mulcahy et al., 2011). The PCR profiles were as follows: initial denature of 94°C for 5 min followed by 30 cycles of 1 min melt at 94°C, annealing temperature of 51°C for 1 min, and an elongation temperature of 72°C for 2 min with a final elongation of 72°C for 5 min. The PCRs were conducted in 25- $\mu$ l reactions with 5  $\mu$ l of 10 $\times$  buffer, 0.5  $\mu$ l dNTPs (8  $\mu$ M), 2  $\mu$ l MgCl<sub>2</sub> (25 mM), 1.25  $\mu$ l of each primer (10 mM), 0.125  $\mu$ l Taq, and 2  $\mu$ l of template DNA. Sequences were examined and aligned by eye in MacClade v4.08 (Maddison and Maddison, 2005). Sequences were deposited in GenBank (Appendix 1). The appropriate model of nucleotide substitution was selected using jModelTest version 0.1 (Posada, 2008).

**Divergence Time Estimation.**—A Bayesian relaxed-clock method of dating was used to infer the timing of origin of *H. c. deserticola* and was implemented in the program BEAST v1.6.1 (Drummond et al., 2006; Drummond and Rambaut, 2007). This method

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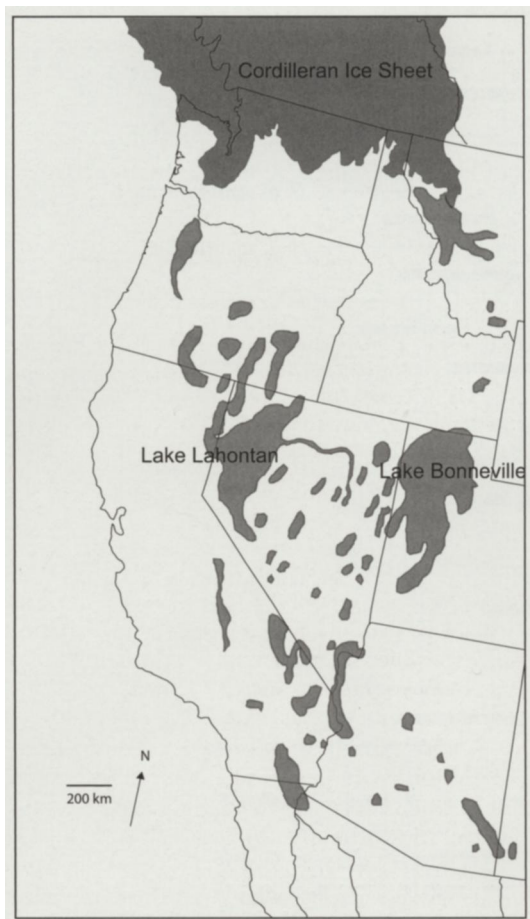


FIG. 1. Extent of the Cordilleran Ice Sheet and pluvial lakes at their maximum extent during the Pleistocene.

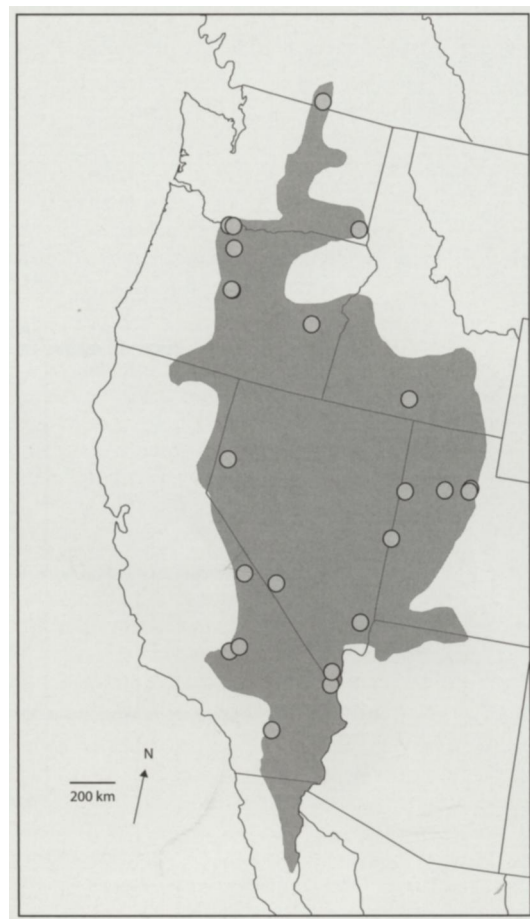


FIG. 2. Geographic distribution of *Hypsiglena chlorophaea deserticola* (Mulcahy, 2008) showing sampling localities. Additional information is listed in Appendix 1.

requires a priori assumptions on the age of at least one clade, calibrated using a fossil constraint or geological event. A pre-existing ND4 mtDNA dataset from GenBank (Appendix 2) was used to construct a time-calibrated phylogeny that included representatives of all taxonomic units suggested by Mulcahy (2008) for the genus *Hypsiglena* and a number of dipsadine outgroup taxa. These additional taxa allowed the use of late Miocene fossils from North America that are informative for dating the speciation events of interest. Fossil calibrations of this chronogram are as follows:

(1) The divergence between *Heterodon platirhinos* (Eastern Hog-nosed Snake) and *Heterodon simus* (Southern Hog-nosed Snake) was constrained with an offset of 5.28 million years ago (Mya) and a lognormal standard deviation of 0.45. This gave a median age of 6.28 Mya and a 95% credible interval ranging between 5.75–7.37, corresponding to the oldest known fossil *H. platirhinos* of the Late Miocene from Nebraska (Holman, 2000).

(2) The root of *Diadophis punctatus* (Ring-necked Snake) was constrained following Fontanella et al. (2008) and Holman (2000). This node was given a calibration of 7.5 Mya with a lognormal standard deviation of 0.29 such that the 95% credible interval ranged from 4.55–12.1 Mya.

We ran this analysis in BEAST for 35 million generations of a single MCMC chain, sampled every 1,000 generations, starting with a random tree under a Yule process of speciation; 10% of the initial samples were discarded as burn-in. The sequences were analyzed for the best-fit nucleotide substitution model using jModelTest v0.1 (Posada, 2008). Convergence of the chain

was checked with Tracer v.1.5 (Rambaut and Drummond, 2007) by checking for unimodal distributions and ESS samples greater than 200 (Drummond et al., 2006). The dates recovered from this analysis were then used in timing the coalescence inferences to test for population growth using the more-variable ND5 data set.

*Population Structure and Historical Demographics.*—Historical demography of this group was analyzed using three approaches. First, Tajima's  $D$  was calculated because it allows for the rejection of a null hypothesis; if a population has been stable through time this statistic is expected to be zero (Tajima, 1989). Following this assumption, negative values are expected if a population has experienced growth, as rare alleles are more numerous than expected and a positive value will be recovered if rare alleles have been eliminated following a bottleneck (Tajima, 1989). The significance of Tajima's  $D$  was estimated with 1,000 simulations in DnaSP v5.10 (Librado and Rozas, 2009) using the ND5 sequence data. A mismatch distribution analysis (Harpending et al., 1998) was conducted under the assumption of selective neutrality to test for population growth. Under the assumptions of this model, a population that has experienced recent, rapid expansion is expected to show a smooth, unimodal distribution whereas a stationary population will show ragged or multimodal distribution (Harpending et al., 1998). The significance of this distribution was assessed using the raggedness statistic,  $r$  (Harpending, 1994), and the  $R_2$  statistic (Ramos-Onsins and Rozas, 2002) in DnaSP v5.10.

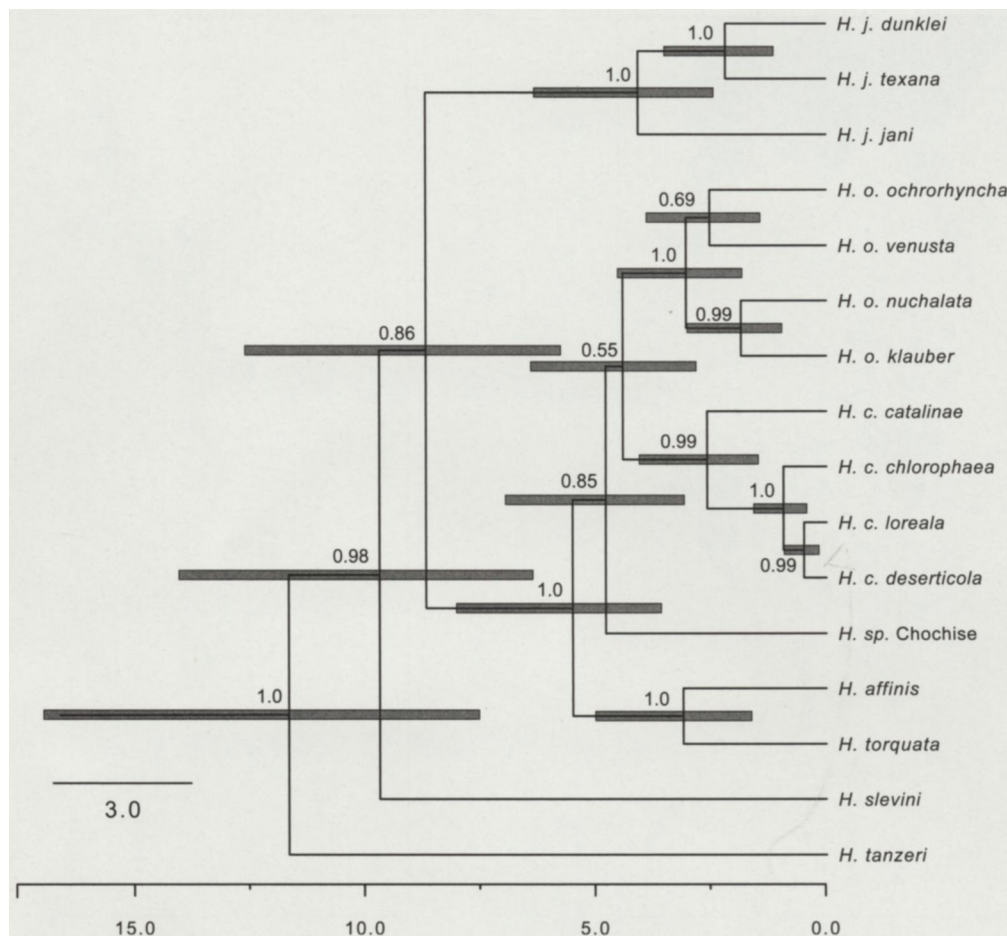


FIG. 3. Bayesian consensus tree based on ND4 sequence and fossil data. Bayesian posterior probability support values are shown above or below each node. Gray bars indicate the 95% credible interval of estimated divergence ages.

The historical population dynamics of this subspecies were also examined by constructing a Bayesian skyline plot (BSP) implemented in the program BEAST v1.6.1 (Drummond et al., 2005; Drummond and Rambaut, 2007). This genealogical method allows for an estimation of effective population size through time and does not require prior assumptions of specific demographic models. The appropriate model of nucleotide substitution (HKY + I +  $\Gamma$ ) for the ND5 data set was determined using jModelTest v0.1 (Posada, 2008) and applied in this analysis. We applied 15 grouped coalescent intervals (m), where genealogies and model parameters were sampled every 1,000 iterations for 50 million generations, with 10% of the initial samples discarded as burn-in. The resulting demographic plot was visualized in the program Tracer v1.5 (Rambaut and Drummond, 2007).

## RESULTS

**Phylogenetic Reconstruction and Divergence Dating.**—The phylogenetic relationships within the genus *Hypsiglena* from our analysis differed from previous analyses (Mulcahy, 2008; Mulcahy and Macey, 2009) in the placement of the yet-undescribed Cochise clade (Mulcahy, 2008) but did correspond with a recently published concatenated multilocus phylogeny (Fig. 3; Mulcahy et al., 2011). In addition to our recovered node, ages differed from the estimated dates found by Mulcahy and Macey (2009), who used the geological event of the initial

separation of the Baja California Peninsula from mainland North America to calibrate the chronogram. The fossil calibrations used in our analysis cannot refute either of the hypothesized calibration points for the separation of Baja California at 7.5–8.2 Mya or 12–14 Mya, as our recovered 95% CI for this node ranged from 6.36–14.06 Mya. At all nodes our recovered dates were younger; however, the confidence intervals overlap. The estimated date for the most recent common ancestor of *H. c. deserticola* is ca. 930 kya in the late Pleistocene; this date was used as a root time for examining demographics with the BSP.

**Historical Demography.**—The ND5 dataset revealed 13 distinct haplotypes with a remarkable uniformity in sequence composition in the northern extent of the range of *H. c. deserticola*. As noted by Mulcahy (2008), the same haplotype recovered from the Salt Lake region of Utah and Pyramid Lake from Nevada was also the same haplotype from the entire Pacific Northwest. This lack of genetic diversity in the northern Great Basin and Pacific Northwest is in contrast to the much-more diverse haplotypes recovered from the southern Great Basin and Mojave Deserts.

The Tajima's *D* value recovered was negative and significant ( $-2.067$ ,  $P < 0.05$ ), indicative of a recent population expansion. The graph of the mismatch distribution indicates a ragged distribution (Fig. 4). Both the raggedness statistic *r* and the Ramos-Onsins and Rozas  $R_2$  statistic do not support the null hypothesis that this lineage underwent a recent expansion in population size (0.061 and 0.072, respectively). After running the BSP analysis in BEAST v1.6.1 it was confirmed that the runs had converged, as the effective sample sizes of all parameters



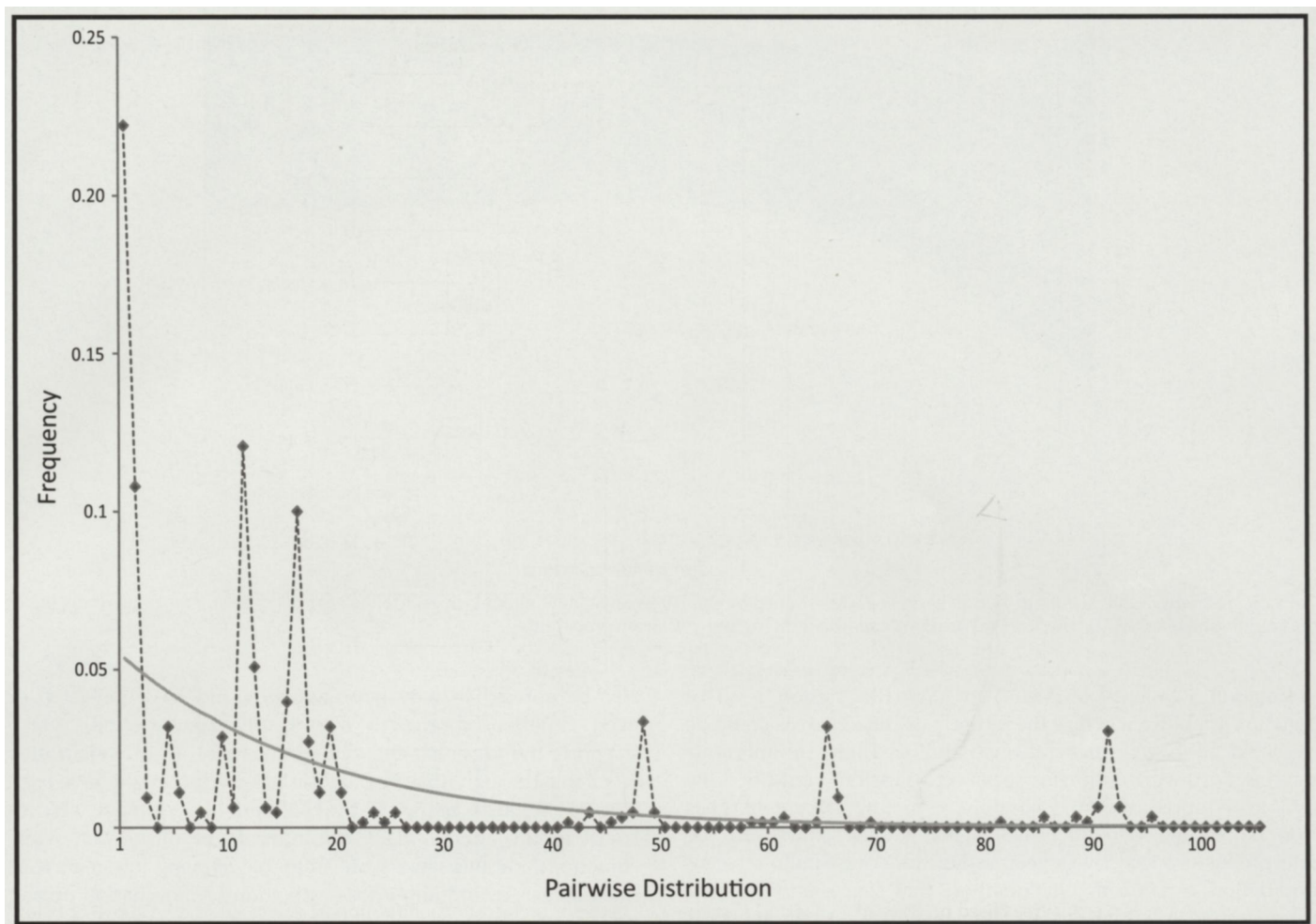


FIG. 4. Mismatch distribution portraying the demographic history of *Hypsiglena chlorophaea deserticola*. Diamonds represent observed distributions of pairwise differences and the solid line represents the expected distribution under a model of population expansion.

were greater than 200 and had uniform peaks. The mean estimate of the BSP (Fig. 5) reveals some population fluctuation through the late Quaternary; however, these patterns cannot be rejected from a stable population size through time as reflected in the 95% highest posterior probability.

#### DISCUSSION

Population expansion following the recession of the extensive ice sheets of the Pleistocene is largely an untested assumption of Holarctic taxa (Hewitt, 2000). The results from our analyses indicate that the Desert Nightsnake has had a stable, effective population size throughout the end of the Pleistocene and into the Holocene, irrespective of the extent of the Cordilleran Ice Sheet. The mismatch distribution, based on the mtDNA locus ND5, is ragged or multimodal and therefore not supportive of a hypothesis of population expansion. Instead, a ragged or multimodal distribution is often taken as support that the lineage under investigation has been stable, that the lineage was once more-widespread and has since experienced a contraction, that the population is influenced by migration, or that the population is subdivided (Rogers and Harpending, 1992; Harpending et al., 1998). The median line of the BSP demonstrates a relatively stable effective population size throughout the mid to late Pleistocene; however, the real median value could fluctuate between the confidence intervals.

Although this skyline reconstruction is based on a single locus, simulation studies have shown that one locus is sufficient in detecting general trends in population growth or decline. However, multiple unlinked loci could be used to elucidate further the finer details of this species' demographic history by reducing the error in both the median population size estimate and the 95% credible interval (Heled and Drummond, 2008). The mean node dates recovered within the genus *Hypsiglena* are younger than those found by other researchers (Mulcahy and Macey, 2009); this result should also be further investigated with multiple unlinked loci because of the nature of branch-length heterogeneity of gene trees (Edwards, 2009).

It should be noted that not all of the methods used to infer population size change are in agreement. Although not an explicit test, the observed geographic distribution of haplotype diversity, where only one haplotype is found north of the Salt Lake area of Utah, is suggestive of a population expansion with allele surfing (Klopfstein et al., 2006; Excoffier and Ray, 2008). Allele surfing occurs when populations that have recently expanded in range size because of favorable climatic change lead to the spread of rare alleles by means of a series of local founder effects. These founder effects result in bottlenecks where genetic diversity is decreased at the front of the range expansion (Klopfstein et al., 2006). This results in "surfing alleles" reaching high frequencies and occupying a large area of the species range, such that an expansion from a source

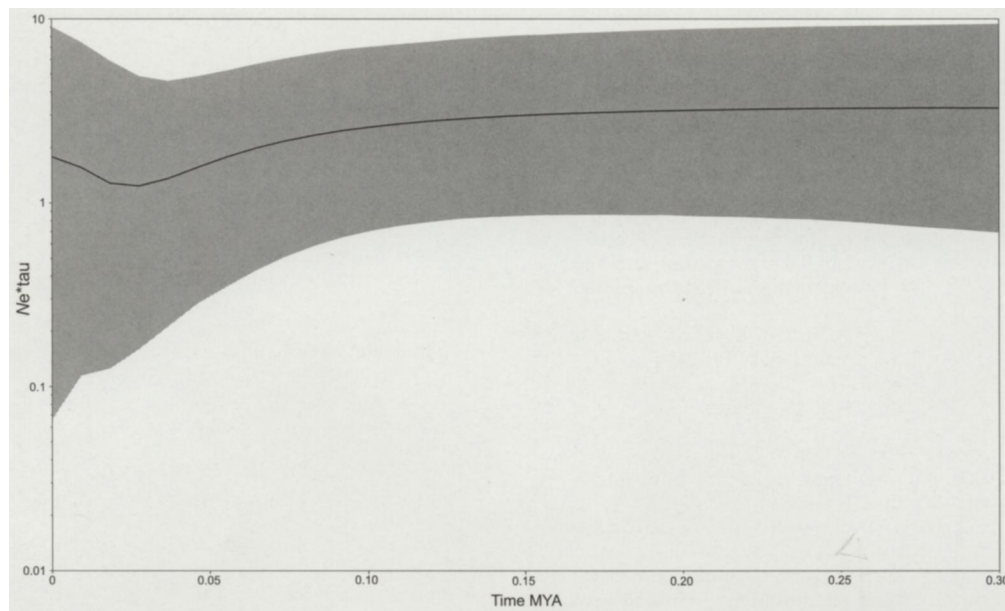


FIG. 5. Demographic history of *H. c. deserticola* as determined by the Bayesian skyline plot; black center line represents the mean value of the log of the population size ( $N_e \cdot \tau$ ), shaded region represents the 95% highest posterior probability.

population would be expected to have the highest level of genetic variability whereas the recently colonized areas could be expected to have much less variability. Therefore migrants moving northward following ice sheet recession would become established, preceding and possibly restricting other genotypes from spreading (Hewitt, 2000). This pattern could also be interpreted as a selective sweep rather than one due to a recent population expansion. One method that is commonly used when testing hypotheses regarding historical demographics is the calculation of the summary statistic Tajima's  $D$ . In our analysis the recovered Tajima's  $D$  value is significantly negative, which is indicative of a population expansion or a selective sweep. Based on the observed geographic distribution of haplotypes and Tajima's  $D$ , we cannot differentiate between population growth or a selective sweep acting on the mitochondria. Although our results may be considered at odds with one another, simulation studies have shown that effective population sizes that are derived from a genealogical tree (e.g., BSP) are much more robust than those that are estimated from methods that use pairwise or segregating sites (e.g., Tajima's  $D$ ) (Felsenstein, 1992); we therefore draw our conclusions largely based on the interpretation of the genealogical results, that of population stability.

The result of population persistence, based on BSP and the mismatch distribution, throughout the Quaternary regardless of glacial ice sheet movements and climatic fluctuations is not novel to this study. Similar patterns have been noted for two other colubrid snakes found in regions similar to those of *H. c. deserticola*. The Great Basin lineage of *Diadophis punctatus* was determined to have a stable population size through time using analogous methods to those implemented in the present study (Fontanella et al., 2008). Additionally, the western lineage of *Coluber constrictor* (North American Racer), a taxon of similar age to *H. c. deserticola*, was found to be a continually expanding population irrespective of glacial cycles (Burbrink et al., 2008). Paleoherpetologists have noted that North American snakes experienced an evolutionary stasis during the Pleistocene with no apparent extinction or speciation (Holman, 1995, 2000). Proposed hypotheses for how this may have occurred could

also be applied to why populations would have persisted or even experienced growth during this time period, which supports the idea that the climatic niche of an organism may be pliable through time. Population persistence might have been possible because many of these desert species were able to adapt to the altered plant communities of this epoch while climatic conditions were more equitable. Having low metabolic rates, which would allow for aestivation or hibernation during periods of adverse weather, could also be potentially advantageous. The possibility that reptile species are highly adaptable to climatic fluctuations is an interesting one that deserves more attention, yet some results suggest that seasonal activity patterns of snakes may be highly conserved along a latitudinal gradient (Sperry et al., 2010). Additionally, the high reproductive potential of many smaller reptiles would be favorable during climatic changes. It would be of interest to examine the demographic histories and phylogeographic structure of other ectothermic vertebrates that shared the geographic distributions of *H. c. deserticola* and that are often associated with this taxon (e.g., *Plestiodon skiltonianus* [Western Skink], Weaver, 2010 and *Uta stansburiana* [Common Side-blotched Lizard], Rodriguez-Robles et al., 1999). Such comparative studies will ultimately allow for broader conclusions to be drawn on community composition through time in the face of historical climate change (e.g. Stone et al., 2012).

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APPENDIX 1. Sampling localities for *Hypsiglena chlorophaea deserticola* used in this study.

Population	State-Province <sup>a</sup>	County-District	Locality	Museum voucher no. <sup>b</sup>	Latitude	Longitude	GenBank no.
1	WA	Whitman	Snake River	EAM172	46.6344	-117.378	JX305731
2	WA	Whitman	Snake River	EAM173	46.6344	-117.378	JX305732
3	WA	Whitman	Snake River	EAM174	46.6344	-117.378	JX305733
4	WA	Whitman	Wawawai	EAM175	46.6344	-117.378	JX305734
5	WA	Whitman	Wawawai	EAM176	46.6344	-117.378	JX305735
9	WA	Kittitas	near Ellensburg	EAM177	47.0345	-120.3914	JX305736
10	WA	Kittitas	near Ellensburg	EAM178	47.0345	-120.3914	JX305737
15	WA	Columbia	Tucannon River Rd.	EAM179	46.5035	-117.9994	JX305738
19	WA	Asotin	Asotin Creek Rd.	EAM180	46.3	117.25	JX305739
20	British Columbia	Okanagan-Similkameen		EAM181	49.029	-119.417	JX305740
22	CA	Stanislaus	Del Puerto Caynon Rd., 5.7 mi W of 15	CAS 225302	37.47	-121.13	JX305741
23	CA	San Bernardino	Havasu Lake Rd., junction US HWY 95	CAS 228100	34.84	-114.61	JX305742
24	CA	San Bernardino County	Havasu Lake Rd., junction US HWY 96	CAS 228097	34.84	-114.61	JX305743
25	CA	San Bernardino County	Havasu Lake Rd., junction US HWY 97	CAS 228098	34.84	-114.61	JX305744
26	UT	San Juan	Needles Overlook Rd.	CAS 229223	37.87	-109.34	JX305745
27	CA	Kern	Piute Mtns, County Rd. 501	CAS 219685	35.47	-118.24	JX305746
29	NV	Nye	HWY 267, NE CA/NV state line	CAS 223437	37.2	-117.1	JX305747
30	NV	Clark	Hiko Springs, near HWY 163	CAS 229952	35.17	-114.68	JX305748
31	NV	Clark	HWY 170, E New Gold Butte Rd.	CAS 223373	36.76	-114.14	JX305749
33	UT	Millard	S of Garrison, Snake Valley	CAS 223414	38.93	-114.02	JX305750
34	ID	Cassia	Rock Creek, S of Twin Falls	UTA R-51097	42.53	-114.36	JX305751
35	NV	Washoe	Pyramid Lake	UNR 07721	39.94	-119.6	JX305752
36	CA	Inyo	1 mi E Tollhouse Spring	MVZ Herp 164933	37.25	-118.18	JX305753
37	NM	Hidalgo	HWY 9, 3.6 mi E HWY 80	MVZ Herp 226235	31.94	-108.98	JX305754
38	Baja California	N/A	39.9 mi H jct. road to Bahia de Los Angeles	MVZ Herp 236389	29.48	-114.46	JX305755
39	UT	Salt Lake	Mill Creek Canyon, Mill Creek Rd., 4 mi S of St Rt. 190	MVZ Herp 241610	40.7	-111.72	JX305756
40	CA	Kern	Freeman Canyon, 4.5 mi W of CA 14 on CA 178	MVZ Herp 245879	35.64	-117.97	JX305757
41	UT	Tooele	S Willow Canyon Rd., 1.9 mi W of Mormon Trail, Stansbury Mts.	MVZ Herp 235921	40.51	-112.52	JX305758
42	UT	Salt Lake	Big Cottonwood Canyon, State Rt. 190, 3.3 mi E of State Rt. 210	MVZ Herp 241609	40.63	-111.74	JX305759
43	OR	Malheur	HWY 20, E of Juntura, OR	EAM182	43.77	-118.03	JX305760
44	OR	Crook	HWY 27	EAM183	44.13	-120.8	JX305761
45	OR	Crook	HWY 27	EAM184	44.14	-120.83	JX305762
46	WA	Klickitat	Columbia River Gorge	EAM185	45.7	-121.41	JX305763
47	WA	Klickitat	Columbia River Gorge	EAM186	45.71	-121.27	JX305764
48	UT	Tooele	Road to Ibapah	MVZ 241611	40.325	-114.03146	EU728587

<sup>a</sup> WA = Washington; CA = California; UT = Utah; NV = Nevada; ID = Idaho; NM = New Mexico; OR = Oregon.

<sup>b</sup> EAM = Edward A. Myers Collection; CAS = California Academy of Science; MVZ = Museum of Vertebrate Zoology; UNR = University of Nevada, Reno; UTA = University of Texas at Arlington.



APPENDIX 2. Species and GenBank accession numbers used to estimate divergence dates.

Species	GenBank no.
<i>Hypsiglena</i> sp. 1 DGM-2008	EU363186.1
<i>Contia tenuis</i>	AF258879.1
<i>Diadophis punctatus</i>	EU193950.1
<i>Diadophis punctatus</i>	EU194060.1
<i>Farancia abacura</i>	DQ902307.1
<i>Hypsiglena affinis</i>	EU363055.1
<i>Hypsiglena chlorophaea catalinae</i>	EU363107.1
<i>Hypsiglena chlorophaea chlorophaea</i>	EU363167.1
<i>Hypsiglena chlorophaea deserticola</i>	EU363154.1
<i>Hypsiglena chlorophaea loreala</i>	EU363166.1
<i>Hypsiglena jani dunklei</i>	EU363057.1
<i>Hypsiglena jani jani</i>	EU363059.1
<i>Hypsiglena jani texana</i>	EU363100.1
<i>Hypsiglena ochrorhyncha klauberi</i>	EU363209.1
<i>Hypsiglena ochrorhyncha nuchalata</i>	EU363193.1
<i>Hypsiglena ochrorhyncha ochrorhyncha</i>	EU363218.1
<i>Hypsiglena ochrorhyncha venusta</i>	EU363212.1
<i>Hypsiglena slevini</i>	EU363043.1
<i>Hypsiglena tanzeri</i>	EU363044.1
<i>Hypsiglena torquata</i>	EU363054.1
<i>Heterodon platirhinos</i>	AF402659.1
<i>Heterodon simus</i>	DQ902310.1
<i>Leptodeira punctata</i>	EF078577.1
<i>Pseudoleptodeira latifasciata</i>	EF078582.1
<i>Sibon sartorii</i>	EF078588.1