Molecular Ecology (2004) **13**, 2709–2720 doi: 10.1111/j.1365-294X.2004.02257.x

Genetic structure of desert ground squirrels over a 20-degree-latitude transect from Oregon through the Baja California peninsula

JOSHUA R. WHORLEY,* S. TICUL ALVAREZ-CASTAÑEDA‡ and G. J. KENAGY*
**Burke Museum and Department of Biology, University of Washington, Seattle, USA; ‡Centro de Investigaciones Biológicas del Noroeste, La Paz, BCS, México*

Abstract

The genetic structure of populations over a wide geographical area should reflect the demographic and evolutionary processes that have shaped a species across its range. We examined the population genetic structure of antelope ground squirrels (*Ammospermophilus leucurus*) across the complex of North American deserts from the Great Basin of Oregon to the cape region of the Baja California peninsula. We sampled 73 individuals from 13 major localities over this 2500-km transect, from 43 to 22° north. Our molecular phylogeographical analysis of 555 bp of the mitochondrial cytochrome *b* gene and 510 bp of the control region revealed great genetic uniformity in a single clade that extends from Oregon to central Baja California. A second distinct clade occupies the southern half of the peninsula. The minimal geographical structure of the northern clade, its low haplotype diversity and the distribution of pairwise differences between haplotypes suggest a rapid northward expansion of the population that must have followed a northward desert habitat shift associated with the most recent Quaternary climate warming and glacial retreat. The higher haplotype diversity within the southern clade and distribution of pairwise differences between haplotypes suggest that the southern clade has a longer, more stable history associated with a southern peninsular refugium. This system, as observed, reflects both historical and contemporary ecological and evolutionary responses to physical environmental gradients within genetically homogeneous populations.

Keywords: adaptation, demography, geographical evolution, historical biogeography, mitochondrial DNA, phylogeography

Received 7 February 2004; revision received 18 May 2004; accepted 18 May 2004

Introduction

Identifying and interpreting patterns of intraspecific geographical variation provides an ongoing forum for discussion about the relative roles of ecological and evolutionary processes in generating diversity. Darwin (1859) observed that phenotypic variation often coincides with geographical distance. Mayr (1956) suggested that ecogeographical variation has an adaptive basis and may represent a response to clinal variation in environmental conditions across populations. Bergmann's rule (Bergmann 1847), as originally applied intraspecifically to homeotherms, serves as a familiar example of a geographical pattern in adaptation; this 'ecogeographical rule' explains larger body size at higher latitude as a response to colder climatic conditions. This suggests that temperature or other environmental characteristics that vary systematically over a geographical gradient could, in principle, generate adaptive variation. The evolutionary response of populations to an environmental gradient may occur either by genotypic adaptation or by evolution of phenotypic plasticity (Via 1994). Mayr (1963) argued and others have demonstrated (Kirkpatrick & Barton 1997) that gene flow should swamp adaptation in such systems. However, other theoretical treatments suggest that selective clines along geographical gradients might maintain adaptive genetic and phenotypic variation (Haldane 1948; Fisher 1950; Kimura & Maruyama 1971) and even promote speciation (Endler 1977).

Although environmental gradients may drive local adaptive evolutionary processes, geological and climatological changes can mould more complex patterns of genetic

Correspondence: J. R. Whorley. Fax: 1 206-685-3039; E-mail: jwhorley@u.washington.edu

diversification over a broader regional scale and a longer time scale (Conroy & Cook 2000; Riddle *et al*. 2000; Arbogast & Kenagy 2001; Matocq 2002). Changes in population size, levels of ancestral polymorphism and patterns of gene flow can shape the genetic structure of populations and thereby influence their ability to adapt to different environmental settings. As these demographic processes are also the primary modulators of neutral variation, we can better understand population and species-level responses to historical changes of habitat and topography by examining variation at mitochondrial DNA (mtDNA) loci. We use gene geneaologies, which we conceive of as correlated to, but not absolute indicators of population history (Edwards & Beerli 2000) to infer past levels of population connectivity. This phylogeographical approach (Avise 2000) has been applied to a variety of vertebrate taxa, both individual species (Riddle 1995; Zamudio *et al*. 1997; Ashton & de Queiroz 2001) and regional biotas (Zink 1996; da Silva & Patton 1998; Riddle *et al*. 2000). We also employ methods that allow estimation of relative times of population separation and divergence within species with greater accuracy, taking into account multiple sources of variance. By tracing demography and historical patterns of gene flow one can begin to assess the potential for adaptive divergence in allopatry (Zheng *et al*. 2003).

To explore the evolution of populations in response to environmental variation, we chose a small mammalian species with an extensive geographical range that includes a lengthy north–south gradient across the four desert ecosystems of western North America (Shreve 1942; MacMahon & Wagner 1985). This latitudinal gradient contains variation in temperature, periodicity and abundance of rainfall, and vegetation types. Thus it represents a good system for investigating the connectivity of populations and the potential for adaptive differentiation in allopatry. The antelope ground squirrel, *Ammospermophilus leucurus*, covers a range of latitude as great as nearly any other North American mammal, and it is the most widely distributed desert rodent of the family Sciuridae in North America (Hall 1981). It ranges across the complex of North American deserts from the Great Basin Desert in southeastern Oregon, at 43° north, southward through the Mojave and Sonoran Deserts, and into the Peninsular Desert of Baja California to the cape region, at 22° north.

To assess the potential for local adaptation and to recover details of population history, we obtained samples of *A. leucurus* over a 2200-km transect crossing its geographical range. We examined geographical variation in genetic structure using mtDNA sequences, both the coding cytochrome *b* gene and the noncoding control region. We use these data to provide insights into how historical changes in climate and habitat distribution may have influenced genetic structure across the vast environmental gradient that spans the north–south axis of this species' geographical range. We investigated the alternative hypotheses that conspicuous genetic variation would be associated either with a gradient of ecosystem characteristics or with vicariance tied to historical barriers that may have intervened across parts of the transect.

Materials and methods

Sampling

We obtained tissues of 73 specimens from 46 localities in western North America, spanning nearly 20° latitude. We consolidated the specific localities into 13 groups ('areas') based on ranges of ~1° latitude (Table 1, Fig. 1). Tissue samples are associated with voucher specimens from the following museums: Burke Museum, University of Washington (UWBM); Colección de Mamíferos, Centro de Investigaciones Biológicas (CIB); Museum of Southwestern Biology, University of New Mexico (UNM); and U.S. National Museum, Smithsonian Institution (USNM).

Laboratory techniques

DNA was extracted from liver or kidney tissue using the DNeasy Tissue Kit (Qiagen). We used the polymerase chain

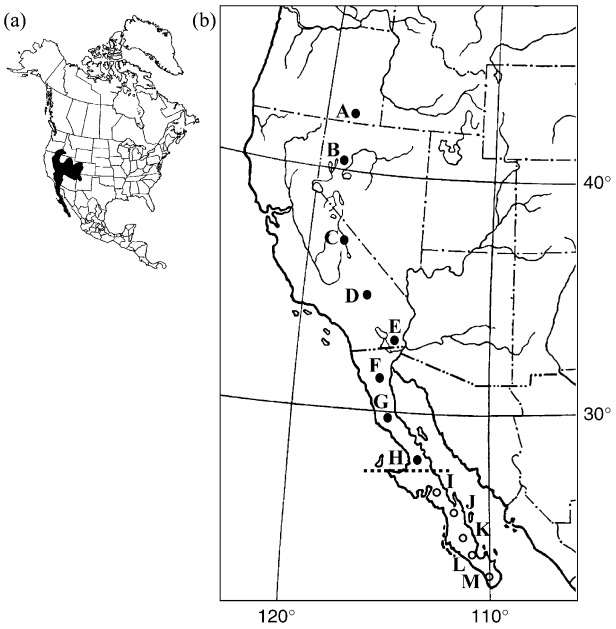

Fig. 1 Geographic range of *Ammospermophilus leucurus* and north–south transect of 13 general locality areas. (a) Geographic distribution map modified after Hall (1981). (b) The 13 locality areas (labelled A–M) represent clusters of the 46 specific localities that fall within a range of about one degree latitude (Table 1). A geographical break in genetic structure is indicated by the dashed line midway down the Baja California peninsula, separating two mtDNA clades (black vs. white circles), as demonstrated by data presented in Fig. 2.

Table 1 Locality areas (n = 13), specific localities (n = 46) and specimens (n = 73) of *Ammospermophilus leucurus* in this study

Locality area	Locality no.	State	Locality county or local name	Latitude	Longitude	Specimen numbers
A	1	OR	Harney County	42°17′-N	118°39′-W	UWBM 74589
A	2	OR	Harney County	42°17′-N	118°40′-W	UWBM 74591
A	3	OR	Harney County	42°16′-N	118°40′-W	UWBM 74554, UWBM 74561
A	4	OR	Harney County	42°15′-N	118°40′-W	UWBM 74556, UWBM 74559
A	5	OR	Harney County	42°14′-N	118°39′-W	UWBM 74587
B	6	NV	Pershing County	40°11′-N	118°25′-W	UWBM 74597, UWBM 74598, UWBM 74599
B	7	NV	Pershing County	40°09′-N	118°24′-W	UWBM 74594, UWBM 74595, UWBM 74596
B	8	NV	Pershing County	40°08′-N	118°24′-W	UWBM 74593
C	9	CA	Inyo County	37°12′-N	118°15′-W	UWBM 74607, UWBM 74615
C	10	CA	Inyo County	37°12′-N	118°15′-W	UWBM 74624
C	11	CA	Inyo County	37°11′-N	118°14′-W	UWBM 74600, UWBM 74602, UWBM 74605
C	12	CA	Inyo County	37°11′-N	118°15′-W	UWBM 74611
D	13	CA	San Bernardino County	34°40′-N	116°42′-W	UWBM 74626
D	14	CA	San Bernardino County	34°40′-N	116°42′-W	UWBM 74628, UWBM 74631, UWBM 74633
D	15	CA	San Bernardino County	34°40′-N	116°42′-W	UWBM 74636
D	16	CA	San Bernardino County	34°40′-N	116°42′-W	UWBM 74637
D	17	CA	San Bernardino County	34°36′-N	116°45′-W	UWBM 74629
E	18	CA	Imperial County	32°50′-N	114°51′-W	UWBM 74657
E	19	CA	Imperial County	32°49′-N	114°51′-W	UWBM 74645
E	20	CA	Imperial County	32°49′-N	114°49′-W	UWBM 74649, UWBM 74653
E	21	CA	Imperial County	32°48′-N	114°50′-W	UWBM 74639
E	22	CA	Imperial County	32°48′-N	114°50′-W	UWBM 74641
E	23	CA	Imperial County	32°48′-N	114°47′-W	UWBM 74655
F	24	BC	Valle de La Trinidad	31°17′-N	115°37′-W	UNM 40911, UNM 40913, UNM 40915
F	25	BC	Valle de La Trinidad	31°14′-N	115°37′-W	UNM 40923
F	26	BC	San Felipe	31°10′-N	114°56′-W	UNM 40140
G	27	BC	Rancho Sta. Catarina	29°43′-N	115°08′-W	UNM 42893
H	28	BC	Mission de San Borja	28°42′-N	113°55′-W	UNM 42833
H	29	BC	Punta Prieta	28°40′-N	114°10′-W	CIB 2875
H	30	BC	Rosarito	28°40′-N	113°59′-W	UNM 42767
I	31	BCS	Santa Agueda	27°16′-N	112°18′-W	USNM 531445
J	32	BCS	Canipole	26°30′-N	111°39′-W	USNM 531429
J	33	BCS	Comandu Viejo	26°17′-N	111°48′-W	USNM 531430
J	34	BCS	Loreto	25°58′-N	111°22′-W	CIB 6099, CIB 6100
K	35	BCS	Puerto Lopez Mateos	25°14′-N	110°58′-W	CIB 6101
K	36	BCS	Ciudad Constitución	24°59′-N	111°36′-W	CIB 6102, CIB 6103, CIB 6104, CIB 6107, CIB 6108, CIB 6109, CIB 6110
L	37	BCS	La Paz	24°13′-N	110°31′-W	CIB 104, CIB 107
L	38	BCS	Santa Rita	24°13′-N	111°30′-W	CIB 6611
L	39	BCS	La Paz	24°12′-N	110°34′-W	CIB 6125
L	40	BCS	La Paz	24°12′-N	110°34′-W	CIB 6141, CIB 6142, CIB 6143
L	41	BCS	La Paz	24°04′-N	110°37′-W	CIB 6146
L	42	BCS	Los Planes	23°57′-N	109°56′-W	CIB 5501, CIB 5502
M	43	BCS	La Burrera	23°31′-N	110°04′-W	USNM 531434
M	44	BCS	El Pulmo	23°26′-N	109°34′-W	USNM 531432, USNM 531433
M	45	BCS	Punta Lobos	23°26′-N	110°13′-W	USNM 531436
M	46	BCS	Migriño	23°01′-N	110°04′-W	CIB 6148, CIB 6149

reaction (PCR) to amplify 555 bp of the mitochondrial cytochrome *b* gene for all 73 individuals. The primers used were L14724 (5′-CGAAGCTTGATATGAAAAAC-CATCGTTG-3′), H15906 (5′-CATTTCCGGTTTACAAG-ACCAGTGTAAT-3′), L15162 (5′-GCAAGCTTCTACCAT-GAGGACAAATATC-3′; Irwin *et al*. 1991) and H15149 (5′-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′; Kocher *et al*. 1989). We ran standard PCR in a total volume of 25 μL containing 10× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1.0 μM of each primer, 1 unit of *Taq* DNA polymerase (Hoffmann-La Roche) and 1–2 μL genomic DNA. PCR were carried out in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) as follows: 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 30 s; and 72 °C for 3 min. With each round of PCR we included one negative control to check for contamination.

We also amplified 510 bp of the control region for all 73 individuals. The primers used were CTRL-L (5′-CACYWTYAACWCCCAAAGCT-3′; Bidlack & Cook 2001) and H16498 (5′-CCTGAAGTAGGAACCAGATG-3′; Kocher *et al*. 1993). PCR were run in a total volume of 25 μL containing 1× PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 1.0 μM of each primer, 1 unit of *Taq* DNA polymerase (Hoffmann-La Roche) and 1 μL genomic DNA. PCR were carried out in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) as follows: 94 °C for 45 s; 35 cycles of 94 °C for 10 s, 50 °C for 15 s and 72 °C for 30 s; and 72 °C for 3 min.

PCR products were purified for use in sequencing reactions with a QIAquick PCR Purification Kit (Qiagen). Sequencing reactions were carried out in a volume of 10 μL with the forward primers using a Big Dye Terminator Cycle Sequencing Ready Reaction Mix (Applied Biosystems). Samples were run on an ABI 377 automated sequencer (Applied Biosystems). To check the quality of obtained sequence data, in one direction, we sequenced some individuals twice and obtained identical outcomes. Sequences were aligned using SEQUENCHER 4.1 (Gene Codes Corp.). All sequences were deposited in GenBank (Accession nos AY685413–AY685558).

Phylogenetic analysis

We used the neighbour-joining (NJ) method in PAUP* 4.0b10 (Swofford 2002) to reconstruct phylogenetic relationships among individuals for both cytochrome *b* and control region genes. By applying hierarchical likelihood ratio tests with the program MODELTEST 3.06 (Posada & Crandall 1998), we determined that the Hasegawa–Kishino–Yano (HKY) model of substitution (Hasegawa *et al*. 1985) plus a gamma distribution (Γ) of rate heterogeneity across all sites provided the best fit to the cytochrome *b* data set. The estimated parameters under this model were Γ = 0.0928, transition/transversion ratio (ti/tv) = 5.1938. For the control region, we found that the HKY model plus invariable sites (*I*) and a Γ distribution of rate heterogeneity across variable sites provided the best fit. The estimated parameters under this model were Γ = 0.8382, *I* = 0.7582, ti/tv = 6.0700. We used bootstrap analyses with 1000 replicates to evaluate nodal support, as implemented in PAUP* 4.0b10. For both markers we constructed unrooted trees. As a comparison with the NJ trees, we also constructed trees using maximum likelihood methods in PAUP* 4.0b10, which yielded similar topologies and slightly longer branch lengths.

Molecular diversity

Mean nucleotide diversities within and between clades were calculated using ARLEQUIN 2.000 (Schneider *et al*. 2000). We used the substitution model of Tamura and Nei (1993) (TrN) with gamma corrections of 0.0928 for cytochrome *b* and 0.8382 for the control region. We used the TrN model of substitution instead of HKY (which is a specific case of the TrN model with one less parameter) because HKY is not available in ARLEQUIN. Mean nucleotide diversity of cytochrome *b* data between the two clades was used to estimate divergence while taking into account ancestral polymorphism using the equation for molecular diversity $D_{xy} = D - 0.5x(D_x + D_y)$ (Nei & Li 1979), where D_x and D_y are mean molecular diversities within two different clades, respectively; D is the mean nucleotide diversity between two clades, and D_{xy} is the corrected molecular diversity between two clades.

With ARLEQUIN 2.000 one directly calculates D_x and D_y, but not D. We combined the two clades into one single population (t) and calculated mean nucleotide diversity for this population (D_t) using ARLEQUIN 2.000. Then we used D_x, D_y and D_t to compute D by the following formula:

$$D_t N_t(N_t - 1)/2 = DN_xN_y + D_xN_x(N_x - 1)/2 + D_yN_y(N_y - 1)/2$$

where N_x, N_y and N_t are sample sizes of populations x, y and t, respectively, and where

$$N_t = N_x + N_y.$$

Historical population dynamics

Using ARLEQUIN 2.000 (Schneider *et al*. 2000) we calculated Fu's F_S to test for demographic expansion (Fu 1997). This test statistic evaluates the probability of observing a random neutral sample of sequences with a number of alleles similar to or smaller that the observed value. Ramos-Onsins & Rojas (2002) demonstrated that Fu's F_S is a more powerful detector of population growth than other tests of demographic expansion such as Tajima's D. Therefore, we used Fu's F_S as an indicator of growth.

We also used ARLEQUIN 2.000 to compute frequencies of pairwise differences between haplotypes (the mismatch distribution) to evaluate the hypothesis of recent population growth and to estimate growth parameters. Recent growth is expected to generate a unimodal distribution of pairwise differences (Slatkin & Hudson 1991; Schneider & Excoffier 1999). ARLEQUIN uses a nonlinear least squares approach to estimate parameters for a stepwise growth model: $\theta_0 = 2\mu N_0$ (before expansion), $\theta_1 = 2\mu N_1$ (after expansion) and $\tau = 2ut$ (time of expansion). Notice that $u = m_T\mu$ is the mutation rate for the entire DNA sequence under study, where m_T is the number of nucleotides of the sequence, and μ is the mutation rate per nucleotide (Rogers & Harpending 1992). N_0 and N_1 are effective population size of females before and after expansion, respectively.

Approximate confidence intervals for τ, θ_0 and θ_1 are obtained by a parametric bootstrap approach (1000 replicates).

The validity of the stepwise expansion model is tested using the same parametric bootstrap approach by a goodness-of-fit statistic P = (number of SSD_{sim} larger or equal to SSD_{obs})/B. SSD_{sim} is the sum of squared devi-ation (SSD) between the simulated mismatch distribution and the model expectation, SSD_{obs} is the SSD between the observed mismatch distribution and the model expectation, and B is the number of simulated samples (ARLEQUIN Version 2.000 Manual; Schneider *et al*. 2000).

To produce an additional estimate of population expansion we used FLUCTUATE 1.3 (Kuhner *et al*. 1998), based on coalescence, to generate maximum likelihood estimates of a present-day value of $\Theta(\Theta = 2\mu N_F)$ and exponential growth rate (g). Here μ is mutation rate per nucleotide, and N_F is present-day effective population size of females. FLUCTUATE 1.3 uses a Markov chain-Monte Carlo approach with Metropolis–Hastings importance sampling to make estimates of Θ and g by searching through genealogies of highest probability. The ti/tv ratio for the northern clade was set to 2.6035 and 7.3875 for cytochrome *b* and control region data, respectively, and to 14.0429 and 7.4047 for the southern clade. These values were obtained from MODELTEST 3.06. To begin the sampling on genealogies of high probability, we provided FLUCTUATE 1.3 with initial trees constructed using the programs DNADIST and NEIGHBOUR within PHYLIP 3.753c. For each population and each gene we ran 10 iterations of the program. For the cytochrome *b* data we ran 10 short chains of 1500–4000 steps each, depending on the information content of the sequences, and two long chains of 15 000 steps each. Sampling increment was set to 20. Using control region data we ran 10 short chains of 1500 steps and two long chains of 10 000 steps. This strategy resulted in low variability among intermediate estimates of Θ and g.

Results

To investigate the genetic structure of *Ammospermophilus leucurus* populations over the entire 2200-km transect, we sampled 73 individuals from 13 major locality areas from Oregon to the southern tip of the Baja California peninsula (Table 1, Fig. 1). Evidence from both the cytochrome *b* gene and the control region indicates two distinct clades over the north–south axis of the geographical range (Fig. 2). The

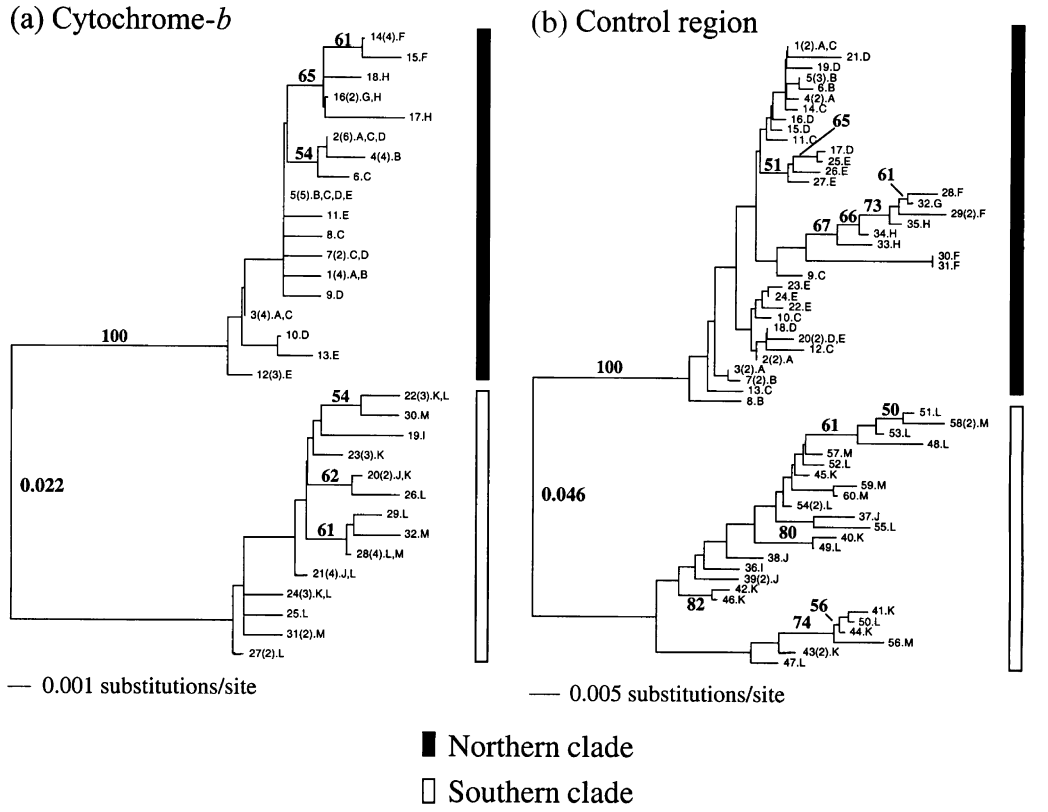

Fig. 2 Unrooted NJ trees for mtDNA sequences of the cytochrome *b* gene and control region. (a) Cytochrome *b* tree based on HKY + Γ model of substitution (α = 0.0928, ti/tv = 5.1938). (b) Control-region tree based on HKY + Γ + *I* model of substitution (α = 0.8382, p_{inv} = 0.7582, ti/tv = 6.0700). Bootstrap support of > 50% of 1000 replicates is shown in bold above branches. Branch lengths (substitutions/site) separating the two major clades are shown in bold between the branches. Numbers at each branch tip indicate haplotype number, and numbers in parentheses indicate the number of individuals sharing that haplotype. Letters at branch tips indicate locality areas of each haplotype (Table 1).

Table 2 Mean nucleotide diversity (substitutions/site ± SD) of the two *A. leucurus* clades

	Mean nucleotide diversity			
Marker	Northern clade (D_x)	Southern clade (D_y)	Between clades (D)	Corrected divergence (D_{xy})
Cytochrome *b*	0.004572 (± 0.002783)	0.004605 (± 0.002834)	0.0337	0.0291
Control region	0.018399 (± 0.009561)	0.018831 (± 0.009895)	0.0620	0.0434

genetic distances between the two clades are 0.022 substitutions per site for cytochrome *b* and 0.046 for control region. The southern 25% of the range is occupied by a relatively heterogeneous clade, whereas the northern 75% of the range contains a more homogeneous clade (locality areas in Fig. 1b). Northern clade haplotypes do not cluster geographically, except for a relatively well-supported monophyletic subtree within northern Baja California (locality areas F, G, H, Fig. 2a,b). The southern clade shows less haplotype sharing among locality areas than the northern clade. For cytochrome *b* the geographically more restricted southern clade contains 14 haplotypes among 29 individuals, whereas the more geographically expansive northern clade holds only 18 haplotypes among 44 individuals (Fig. 2a). Despite the much larger area occupied by the northern clade, only 41% of the individuals had unique haplotypes, whereas 48% of the southern clade individuals had unique haplotypes.

Mean nucleotide diversities for the cytochrome *b* gene and control region were similar within the northern and the southern clades (Table 2). For cytochrome *b*, the nucleotide diversity between the two clades was about one order of magnitude greater than that within clades (Table 2). The corrected nucleotide diversities are slightly higher than those shown in the NJ trees (Fig. 2) and closer to those generated by maximum likelihood analysis.

The F_S statistic indicated population expansion in both the northern and southern clades, with $F_S = -12.14$ ($P < 0.001$) and -11.58 ($P = 0.001$), respectively. The observed distribution of pairwise differences does not differ significantly from the simulated and modelled Poisson distributions, indicating recent demographic expansion in both clades (Fig. 3). The lack of well-supported branches within each clade (Fig. 2), as well as the preponderance of short-terminal branches, also suggests recent expansion.

To investigate historical population dynamics, we applied two models of expansion to our control region data. This allowed us to estimate effective female population size and rate or time of expansion. The exponential expansion model indicates rapid increase in population size of both clades (Table 3). The present-day Θ is larger for the southern clade than for the northern clade under this model. This indicates a larger effective population size and consequently more polymorphism. Comparison of the growth parameter *g* indicates demographic expansion of both clades,

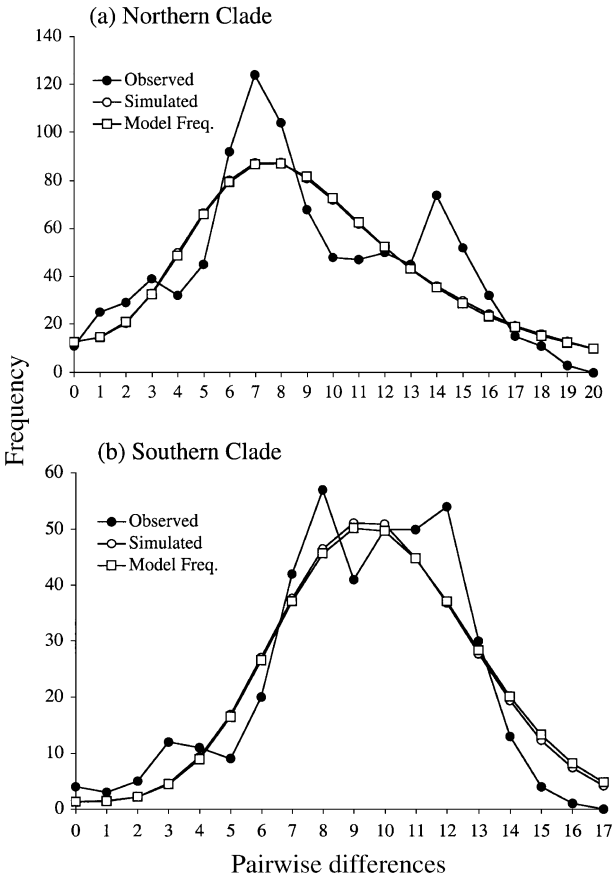

Fig. 3 Mismatch distributions (observed, simulated and stepwise-expansion model) of the two major *Ammospermophilus leucurus* clades based on sequence data for the control region. The curves represent the frequency distribution of pairwise differences for (a) the northern clade, $P = 0.76$, and (b) the southern clade, $P = 0.21$. *P*-values represent the probability that the observed pattern of pairwise differences is different from the simulated pattern based on the estimated model parameters.

and the model also estimates a more rapid expansion of the northern clade than the southern clade.

The stepwise model (Table 3) estimated periods of population expansion τ of 9.936 for the southern clade and 6.042 for the northern clade, suggesting that the northern clade expanded relatively recently, whereas the southern clade had a longer, more stable demographic history in the southern Baja California peninsula. The estimated θ_0

Table 3 Estimated parameters of population expansion for (a) exponential and stepwise (b) expansion models
(a) Exponential expansion model*

				Exponential expansion model	
Marker	Clade	No. of individuals	No. of haplotypes	$\Theta = 2uN_{FS}$	g
Cytochrome-*b*	Northern clade	44	18	0.0350 (± 0.0039)	1142.9061 (± 110.0933)
	Southern clade	29	14	0.0230 (± 0.0038)	1499.9027 (± 188.9652)
Control region	Northern clade	44	38	0.9997 (± 0.1595)	55.8318 (± 4.9328)
	Southern clade	29	25	2.4907 (± 0.4834)	29.4929 (± 3.1352)

(b) Stepwise expansion model†

		Stepwise expansion model		
Marker	Clade	$\tau = 2ut$	$\theta_0 = 2uN_0$	$\theta_1 = 2uN_1$
Cytochrome-*b*	Northern clade	2.561 (1.157, 3.256)	0.000 (0.000, 1.464)	5055.000 (32.070, 8710.000)
	Southern clade	2.716 (1.035, 3.685)	0.000 (0.000, 1.722)	4730.000 (26.367, 9902.500)
Control region	Northern clade	6.042 (2.992, 16.236)	4.203 (0.000, 13.782)	76.172 (27.156, 6388.672)
	Southern clade	9.936 (6.916, 11.857)	0.000 (0.000, 2.979)	321.250 (85.884, 7988.750)

*Population parameters under the exponential model are given as maximum likelihood estimates (± SD).
†Population parameters under the stepwise model are given as estimates (95% confidence limits).

before expansion is much smaller than after expansion (θ_1) for both clades, but the estimated θ_1, reflecting current effective population size, is much larger in the south than in the north. This indicates faster and more recent demographic expansion of the northern clade, which is remarkable considering that the northern clade extends over nearly three times the latitude as the southern clade. Interestingly, the estimated northern θ_0 is larger than the southern θ_0.

Discussion

Ammospermophilus leucurus consists of two distinct mitochondrial clades. Together, they cover the 20°-latitude axis of the species' geographical range, which represents a transect spanning several major desert ecosystems within western North America. The geographically extensive northern clade stretches from the northern edge of the Viscaíno Desert in the central Baja California peninsula to southern Oregon. The much smaller southern clade occupies only the southern peninsula. The southern clade contains a higher percentage of unique haplotypes and a greater overall haplotype diversity than the northern clade. Our genetic analysis suggests that population size in both clades has expanded recently, and that geographical expansion of the northern clade must have followed a northward desert habitat shift associated with the most recent Quaternary climate warming and habitat shift following glacial retreat. The southern mtDNA clade shows a longer expansion time than the northern clade, and the greater haplotype diversity within the southern clade also suggests a larger effective population size and longer residence time in the southern peninsula. Population demographic models also suggest that the northern clade had a higher rate of population growth than the southern clade. Estimates of gene trees and population growth support the hypothesis of rapid northward expansion of the northern clade, probably from a small population in northern Baja California. The occurrence of geographically widespread, similar haplotypes throughout the northern clade bears this out, especially north of the United States–Mexican border. The comparative study of the northern and southern population groups will be of interest for exploring possible adaptive divergence in allopatry.

Phylogenetic differentiation and population expansion

Both of our mitochondrial gene trees demonstrate the existence of two distinct clades separated by a relatively high number of mutations. No haplotypes are shared between the two clades, and within-clade divergence is low compared with between-clade divergence. The between-clade nucleotide divergence for cytochrome *b* is 0.029 and for the control region is 0.043, which accounts for 85 and 69% of the total nucleotide divergence, respectively. By comparison with other mtDNA data sets on rodents, this level of cytochrome *b* divergence falls in the middle of the range of within-species comparisons and at the low end of the range for sister species (Bradley & Baker 2001; Spradling *et al*. 2001).

The northern clade is genetically homogeneous, with short terminal branches separating haplotypes that are widespread and closely related. This tree structure also suggests either recent demographic and range expansion or high rates of gene flow relative to mutation. One exception to the general homogeneity of the northern clade is the subtree within the northern clade that consists only of haplotypes from the northern Baja California peninsula (areas F, G, H). This section of the northern clade is monophyletic and has relatively longer branches than others and higher bootstrap values separating the branches, especially on the control-region tree (Fig. 2). This suggests that the northern clade may have been restricted to northern Baja California for some time before expanding northward to fill its current range. The southern clade contains greater haplotype diversity than the northern clade, even though its latitudinal extent is only about one third as great. Relative to the northern clade, it also has longer branches separating haplotypes, suggesting that it has been evolving at demographic equilibrium within the southern peninsula for a longer period of time.

The greatest genetic break in our mtDNA phylogenies occurs at the middle of the Baja California peninsula, near the border between the states of Baja California and Baja California Sur, and near to the 28° parallel (Fig. 2). This result agrees with that of Riddle *et al*. (2000), who initially identified a similar mid-peninsular pattern of phylogeographical structuring in a number of Baja California vertebrates, including *A. leucurus*. In our study of the full latitudinal extent of *A. leucurus* populations, we found the mid-peninsular break to be the only strong genetic break over the entire transect. The structure of our observed gene tree fits a hypothesis of recent demographic and geographical expansion northward from a refugial population of small effective size in the northern peninsula. Based on emerging views of phylogeography (Avise 2000), one can expect low diversity and lack of geographical structure for mitochondrial genes in three situations that may pertain to our system: (i) recent demographic and geographical expansion from an ancestral population of small size; (ii) historically stable population size and geographical range, but with geographically variable levels of gene flow between populations; and (iii) a selective sweep, carrying to fixation only a few of the total number of mitochondrial haplotypes in the population (Bertorelle & Slatkin 1995). Unfortunately, we cannot test this last hypothesis against the first two. However, to investigate the first two hypotheses, we subjected our control-region data to treatment by two models of demographic expansion, one that assumed a sudden stepwise expansion (using ARLEQUIN, Schneider *et al*. 2000) and another that assumed exponential expansion (using FLUCTUATE, Kuhner *et al*. 1998).

Our first model uses pairwise differences between sampled DNA sequences (mismatch distribution) to analyse population history (Slatkin & Hudson 1991). Both theoretical explorations and practical analysis of human DNA sequences have demonstrated that population history leaves detectable impacts on the form of the mismatch distribution, especially when populations are not at demographic equilibrium (Di Rienzo & Wilson 1991; Schneider & Excoffier 1999). When Schneider & Excoffier (1999) extended the original method of Rogers & Harpending (1992) to include rate heterogeneity across sites, they found that reasonable estimates of both expansion time τ and initial population size θ_0 are achieved without much bias, whereas estimates of θ_1 suffer from upward bias and highly conservative 95% confidence intervals. Their estimates also reveal that the parameters of older expansions are more precisely recovered than those of more recent expansions. Such difficulties may account for our surprising estimates of a higher θ_0 for the northern clade than for the southern clade. Based on haplotype divergence and diversity, we expected that the southern clade should have the larger initial population size.

Demographic expansion should produce a unimodal distribution of pairwise differences because populations that have recently expanded show fewer coalescent events and have pairwise differences that fall in an intermediate range (Rogers & Harpending 1992). The mismatch distribution for both clades conforms to this shape quite well. Here again, such a distribution can arise by rapid spread of a selectively advantageous mtDNA haplotype (Di Rienzo & Wilson 1991; Bertorelle & Slatkin 1995) or by homoplasy due to high mutation rates at some sites (Lundstrom *et al*. 1992). A population bottleneck can also generate a unimodal distribution with elevated upper-tail probabilities (Rogers & Harpending 1992). However, because the divergence time in our system is relatively small (at most several hundred thousand years, based on the range of independent estimates of mutation rates of cytochrome *b*; Arbogast *et al*. 2002), and the sampled area is so large, homoplasy due to sequence saturation is unlikely to have generated the unimodal mismatch distributions. Selection is also unlikely to have confounded our analyses, because intraspecific selection on the mitochondrial genome may not be strong enough to promote complete replacement of unfavoured alleles over such a large geographical area. Barring the influence of these confounding factors, our observed mismatch distributions from both clades fit the simulated distributions. Therefore, we accept the hypothesis of demographic expansion of the northern clade of *A. leucurus*.

Because incorporating phylogenetic information into estimates of population growth makes more efficient use of data than analysis only of pairwise differences (Felsenstein 1992), we also used FLUCTUATE to estimate population size and growth rate. This method allows us to account explicitly for homoplasy at rapidly evolving sites. We qualitatively compared directions and magnitudes of change

of the various parameters between this method and that of ARLEQUIN. Estimates obtained using FLUCTUATE indicate that the southern clade has a larger present-day effective population size Θ than the northern clade, in agreement with estimates obtained from ARLEQUIN. The ratios of Θ for southern to northern populations are similar, with FLUCTUATE estimating 2.5 : 1 and ARLEQUIN estimating 4.2 : 1. The growth rate *g* indicates more rapid expansion of the northern clade than the southern. These estimate are robust, as multiple iterations of FLUCTUATE generated comparable estimates with sharp likelihood surfaces (data not shown). Estimates of both Θ and *g* are biased slightly upward owing to cross-correlation between the two (Kuhner *et al*. 1998), but correction of this bias does not alter our conclusion.

In the context of western North America's deserts and their history, the northern *A. leucurus* clade appears to have expanded both demographically and geographically, most recently following the last glacial maximum. Our two models of population growth estimate that the northern clade grew more rapidly and more recently than the southern clade. The contemporary western deserts and their associated biotic communities are of recent origin, and Pleistocene glaciations and climate fluctuations produced southward geographical shifts of the major North American desert habitats (Van Devender *et al*. 1987; Pielou 1991). Assuming that the northern extent of the range of *A. leucurus* has been limited by habitat and climate, as it appears to be today, we conclude that recolonization of the Great Basin desert must have occurred since the last glacial maximum. In contrast to this scenario of northward expansion into newly available habitat, the southern clade, which also shows indication of demographic expansion, has been consistently limited by the confines of the Baja California peninsula.

Historical biogeography

Our phylogenetic trees together with estimates of population growth indicate rapid expansion of the northern clade of *A. leucurus* from populations restricted to the northern half of the Baja California peninsula. Supported by mtDNA evidence from several vertebrate taxa, Upton & Murphy (1997) and Riddle *et al*. (2000) have offered an explanation of a recurrent pattern of molecular divergence between taxa of the northern and southern Baja California peninsula. They have proposed the formation of a mid-peninsular seaway ~1.5 Ma, which would have isolated formerly continuous populations of these taxa. However, habitat and ecological separation could cause disruption of gene flow within previously panmictic populations, which could also generate the observed genetic break between multiple taxa. Under the general scenario of mid-peninsular vicariance accounting for the current haplotype distributions, we see two possible explanations for the widespread but genetically homogeneous northern clade.

1 A small panmictic population was isolated immediately north of a mid-peninsular geographical barrier, and some time later that population expanded rapidly northward.
2 The populations of the entire northern peninsula have been structured since the time of a mid-peninsular barrier, whereas populations further to the north were and remain relatively unstructured.

The distributional history of fossil *Ammospermophilus* during the Pliocene and Pleistocene, in relation to climate cycles and associated habitat shifts, suggests that the first explanation is more likely. *Ammospermophilus* populations were apparently widespread in the Pliocene and early Pleistocene. An extreme southern record for *Ammospermophilus* exists in Baja California Sur from the late Pliocene (Miller 1980), and the extreme northern record is from the Pliocene of south-central Washington (Gustafson 1978). Pliocene fossil *Ammospermophilus* also occur in eastern Oregon's Juntura Basin (Black 1963). This broad fossil distribution contrasts with the pattern seen today and throughout the Pleistocene. No *Ammospermophilus* live in Washington today, and populations of *A. leucurus* barely penetrate into the extreme southeast corner of Oregon. Although *A. leucurus* fossils dating to the late Pleistocene occur in the southern Mojave Desert (Goodwin & Reynolds 1989; FAUNMAP Working Group 1994), they are notably absent from the Pleistocene record further north in the Great Basin (Grayson 1993), where, instead, the fossil record is rich with montane rodents. The most likely explanation is that habitat shifts associated with climate change pulled the northern range limits southward throughout the Quaternary, as it did for many other North American mammals (Pielou 1991). The most recent (Cordilleran) ice sheet reached its southern extreme ~18 000 years ago (Booth 1987). The northern range limit of *A. leucurus* has apparently expanded and contracted, shifting northward and southward, a number of times, during the Pleistocene. Palaeobotanical evidence corroborates these kinds of climate-driven habitat shifts; the plant species contents of packrat (*Neotoma* spp.) middens throughout western deserts indicate that much of the vegetation currently associated with Great Basin populations of *A. leucurus* was not present in the Mojave and Great Basin until at least 14 000 years ago (Van Devender *et al*. 1987).

Adaptive potential along environmental gradients

The north–south axis of *A. leucurus* in western North America represents a system that invites further investigation into intraspecific population genetics and adaptation across an environmental gradient. Theoretical treatments of genetic divergence and speciation (Felsenstein 1981) and laboratory experiments that simulate speciation (Rice & Hostert 1993) suggest that speciation can be readily achieved in allopatry, but that it requires intense, multifarious selection to develop

in sympatry. Endler (1973) and May *et al.* (1975) demonstrated that sharp, adaptive, genetic clines can develop and be maintained in the presence of gene flow, but it is unclear whether selective forces in the wild are sufficiently strong and consistent to promote divergence among wild populations (Hoekstra *et al*. 2001; Kingsolver *et al*. 2001). Although our current expectations remain that genetic divergence is more likely in allopatry than in sympatry, it is of additional interest to explore the complementary role of phenotypic adjustment in the response of a species to any environmental gradient that exists over its geographical range.

Although we conclude that the major genetic divergence between the two groups of *A. leucurus* (mtDNA clades) probably developed while populations were allopatric, it is theoretically possible that the break was promoted by selection across an environmental transition. Alternatively, divergence may have occurred in sympatry across a permeable geographical boundary. We can next ask whether the apparent historic boundary of the mid-Baja California peninsula is still being maintained, and, if so, what isolating mechanisms are currently operating. A closer inspection of genetic patterns at the mid-peninsular interface of the two clades will also be relevant to understanding the population biology of a potential zone of hybridization and the processes that lead to discordance between mtDNA and nuclear DNA. We plan to address these questions using additional molecular markers across the mid-peninsular transition.

Environmental conditions vary substantially across the desert ecosystems associated with the range of *A. leucurus*, and this apparently produces phenotypic variation. The reproductive ecology of *A. leucurus* holds a potential for analysis of life-history adaptation across a geographical range of populations. Breeding by *A. leucurus* in the relatively more predictable winter rainfall environment and floristically simple ecosystem of the northern Mojave Desert consists of a single, precisely timed annual bout with a relatively large litter (Kenagy & Bartholomew 1985). By contrast, breeding in the unpredictable summer monsoonal environment and associated floristically diverse ecosystem of the southern Baja California peninsular desert consists of a broad and protracted season of sporadic reproduction with a relatively small litter size (Kenagy *et al*. 2004). The phenotypic and genotypic components of life-history features such as these over the geographical range can be identified through further empirical study. Reznick & Travis (1996) indicate several possible patterns: a match of geographical variation in traits with the location of neutral genetic divergence, complex patterns of trait variation at sharp environmental boundaries, or gradual variation of the traits over the course of the environmental gradients. Few previous investigations have successfully addressed the complex interactions of organismal function and genetics that represent geographical adaptation (Powers & Schulte 1998). By studying genotypic and phenotypic variation in natural populations across an environmental transect one can better understand selection and adaptation over geographical space (Endler 1986).

Acknowledgements

This project was supported by the Mammalogy Program at the Burke Museum, the Graduate School Fund of the University of Washington and the University of Washington Biology Department's Snyder Award to JRW. Special thanks to Adam Augustyn and Joe Booth for field assistance in the USA and to Patricia Cortés Calva and Mayra De La Paz Cuevas for assistance in México. For provision of additional tissue samples we thank the Museum of Southwestern Biology (University of New Mexico) and the U.S. National Museum (Smithsonian Institution, Washington, D.C.). We thank Brian Arbogast, Scott Edwards, Ray Huey, Sacha Vignieri and Xiaoguang Zheng for advice on the research and manuscript, and Mary Kuhner for advice concerning FLUCTUATE.

References

Arbogast BS, Edwards SV, Wakeley J, Beerli P, Slowinski JB (2002) Estimating divergence times from molecular data on phylogenetic and population genetic time scales. *Annual Review of Ecology and Systematics*, **33**, 707–740.

Arbogast BS, Kenagy GJ (2001) Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography*, **28**, 819–825.

Ashton KG, de Queiroz A (2001) Molecular systematics of the Western rattlesnake, *Crotalus viridis* (Viperidae), with comments on the utility of the D-loop in phylogenetic studies of snakes. *Molecular Phylogenetics and Evolution*, **21**, 176–189.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.

Bergmann A (1847) Über die Verhältnesse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien*, **1**, 595–708.

Bertorelle G, Slatkin M (1995) The number of segregating sites in expanding human populations with implications for estimates of demographic parameters. *Molecular Biology and Evolution*, **12**, 887–892.

Bidlack AL, Cook JA (2001) Reduced genetic variation in insular northern flying squirrels (*Glaucomys sabrinus*) along the north Pacific coast. *Animal Conservation*, **4**, 283–290.

Black CC (1963) A review of the North American Tertiary Sciuridae. *Bulletin of the Museum of Comparative Zoology at Harvard University*, **130**, 109–248.

Booth DB (1987) Timing and processes of deglaciation along the southern margin of the Cordilleran ice sheet. In: *North America and Adjacent Oceans During the Last Deglaciation* (eds Ruddiman WF, Wright HE Jr), pp. 71–90. Geological Society of America, Boulder, CO.

Bradley RD, Baker RJ (2001) A test of the genetic species concept: cytochrome-*b* sequences and mammals. *Journal of Mammology*, **82**, 960–973.

Conroy CJ, Cook JA (2000) Phylogeography of a post-glacial colonizer: *Microtus longicaudus* (Rodentia: Muridae). *Molecular Ecology*, **9**, 165–175.

Da Silva MNF, Patton JL (1998) Molecular phylogeography and the evolution and conservation of Amazonian mammals. *Molecular Ecology*, **7**, 475–486.

Darwin C (1859) *The Origin of Species by Means of Natural Selection*, 6th edn. The Modern Library, New York.

Di Rienzo A, Wilson AC (1991) Branching patterns in the evolutionary tree for human mitochondrial DNA. *Proceedings of the National Academy of Sciences of the USA*, **88**, 1597–1601.

Edwards SV, Beerli P (2000) Gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution*, **54**, 1839–1854.

Endler JA (1973) Gene flow and population differentiation. *Science*, **179**, 243–250.

Endler JA (1977) *Geographic Variation, Speciation, and Clines. Monographs in Population Biology 10*. Princeton University Press, Princeton, NJ.

Endler JA (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.

FAUNMAP Working Group (1994) *FAUNMAP: A Database Documenting Late Quaternary Distributions of Mammal Species in the United States*. Illinois State Museum, Springfield.

Felsenstein J (1981) Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution*, **35**, 124–138.

Felsenstein J (1992) Estimating effective population size from samples of sequences: inefficiency of pairwise and segregating sites as compared to phylogenetic estimates. *Genetical Research*, **59**, 139–147.

Fisher RA (1950) Gene frequencies in a cline determined by selection and diffusion. *Biometrics*, **6**, 353–361.

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

Goodwin HT, Reynolds RE (1989) Late Quaternary Sciuridae from low elevations in the Mojave Desert, California. *Southwestern Naturalist*, **34**, 506–512.

Grayson DK (1993) *The Desert's Past. A Natural History of the Great Basin*. Smithsonian Institution Press, Washington, DC.

Gustafson EP (1978) The vertebrate faunas of the Pliocene Ringold formation, south-central Washington. *Bulletin of the Museum of Natural History University of Oregon*, **23**, 1–62.

Haldane JBS (1948) The theory of a cline. *Journal of Genetics*, **48**, 277–284.

Hall ER (1981) *The Mammals of North America*, 2nd edn. Wiley, New York.

Hasegawa M, Kishino K, Yano T (1985) Dating the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160–174.

Hoekstra HE, Hoekstra JM, Berrigan D *et al.* (2001) Strength and tempo of directional selection in the wild. *Proceedings of the National Academy of Sciences of the USA*, **98**, 9157–9160.

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution*, **32**, 128–144.

Kenagy GJ, Bartholomew GA (1985) Seasonal reproductive patterns in five coexisting California desert rodent species. *Ecological Monographs*, **55**, 371–397.

Kenagy GJ, Whorley JR, Cortés-Calva P, Alvarez-Castañeda ST (2004) Timing of reproduction in antelope ground squirrels, *Ammospermophilus leucurus*, near La Paz, Baja California Sur. In: *Contribuciones Mastozoologicas En Homenaje a Bernardo Villa* (eds Sánchez-Cordero V, Medellín RA), pp. 259–264. Universidad Autonima Nacional de Mexico, Mexico City.

Kimura M, Maruyama T (1971) Patterns of neutral polymorphism in a geographically structured population. *Genetical Research*, **18**, 125–131.

Kingsolver JG, Hoekstra HE, Hoekstra JM *et al.* (2001) The strength of phenotypic selection in natural populations. *American Naturalist*, **157**, 245–261.

Kirkpatrick M, Barton NH (1997) Evolution of a species' range. *American Naturalist*, **150**, 1–23.

Kocher TD, Conroy JA, McKaye KR, Stauffer JR (1993) Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. *Molecular Phylogenetics and Evolution*, **2**, 158–165.

Kocher TDWK, Thomas A, Meyer SV *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA*, **86**, 6196–6200.

Kuhner MK, Yamato J, Felsenstein J (1998) Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics*, **149**, 429–434.

Lundstrom R, Tavare S, Ward RH (1992) Modelling evolution of the human mitochondrial genome. *Mathematical Biosciences*, **122**, 319–335.

MacMahon JA, Wagner FH (1985) The Mojave, Sonoran, and Chihuahuan deserts of North America. In: *Hot Deserts and Arid Shrublands* (eds Evenari M *et al.*), pp. 105–202. Elsevier, Amsterdam.

Matocq DM (2002) Phylogeographical structure and regional history of the dusky-footed woodrat, *Neotoma fuscipes*. *Molecular Ecology*, **11**, 229–242.

May RM, Endler JA, McMurtrie RE (1975) Gene frequency clines in the presence of selection opposed by gene flow. *American Naturalist*, **109**, 659–676.

Mayr E (1956) Geographic character gradients and climatic adaptation. *Evolution*, **10**, 105–108.

Mayr E (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.

Miller WE (1980) The late Pliocene Las Tunas fauna from southernmost Baja California, México. *Journal of Paleontology*, **54**, 762–805.

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA*, **76**, 5269–5273.

Pielou EC (1991) *After the Ice Age: the Return of Life to Glaciated North America*. University of Chicago Press, Chicago.

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

Powers DA, Schulte PM (1998) Evolutionary adaptations of gene structure and expression in natural populations in relation to a changing environment: a multidisciplinary approach to address the million-year saga of a small fish. *Journal of Experimental Zoology*, **282**, 71–94.

Ramos-Onsins SE, Rojas J (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19**, 2092–2100.

Reznick D, Travis J (1996) The empirical study of adaptation in natural populations. In: *Adaptation* (eds Rose MR, Lauder GV), pp. 243–289. Academic Press, San Diego, CA.

Rice WR, Hostert EE (1993) Laboratory experiments on speciation: what have we learned in 40 years? *Evolution*, **47**, 1637–1653.

Riddle BR (1995) Molecular biogeography in the pocket mice (*Perognathus* and *Chaetodipus*) and grasshoper mice (*Onychomys*): the late cenozoic development of a North American aridlands rodent guild. *Journal of Mammology*, **76**, 283–301.

Riddle BR, Hafner DJ, Alexander LF, Jaeger JR (2000) Cryptic vicariance in the historical assembly of a Baja California peninsular

desert biota. *Proceedings of the National Academy of Sciences of the USA*, **97**, 14438–14443.

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

Schneider S, Excoffier L (1999) Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics*, **152**, 1079–1089.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN, Version 2.000: a Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Shreve F (1942) The desert vegetation of North America. *Botanical Review*, **8**, 195–246.

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

Spradling TA, Hafner MS, Demastes JW (2001) Differences in rate of cytochrome-*b* evolution among species of rodents. *Journal of Mammology*, **82**, 65–80.

Swofford DL (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4. Sinauer Associates, Sunderland, MA.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Upton DE, Murphy RW (1997) Phylogeny of the side-blotched lizards (Phrynosomatidae: *Uta*) based on mtDNA sequences: support for a midpeninsular seaway in Baja California. *Molecular Phylogenetics and Evolution*, **8**, 104–113.

Van Devender TR, Thompson RS, Betancourt JL (1987) Vegetation history of the desert of southwestern North America. The nature and timing in the Late Wisconsin–Holocene transition. In: *North America and Adjacent Oceans During the Last Deglaciation*, Vol. K-3 (eds Ruddiman WF, Wright HE), pp. 323–352. Geological Society of America, Boulder, CO.

Via S (1994) The evolution of phenotypic plasticity: what do we really know? In: *Ecological Genetics* (ed. Real LA), pp. 35–57. Princeton University Press, Princeton, NJ.

Zamudio KR, Jones KB, Ward RH (1997) Molecular systematics of short-horned lizards: biogeography and taxonomy of a widespread species complex. *Systematic Biology*, **46**, 284–305.

Zheng X, Arbogast BS, Kenagy GJ (2003) Historical demography and genetic structure of sister species: deermice (*Peromyscus*) in the North American temperate rain forest. *Molecular Ecology*, **12**, 711–724.

Zink RM (1996) Comparative phylogeography in North American birds. *Evolution*, **50**, 308–317.

This project is part of Joshua Whorley's PhD dissertation in Zoology at the University of Washington and was conducted at the Burke Museum. His research involves use of genetic data to understand and interpret the ecology and evolution of adaptation in natural populations. Ticul Alvarez-Castañeda leads a team of research mammalogists at the Centro de Investigaciones Biológicas del Noroeste in La Paz, focusing on the ecology, systematics and biodiversity of the northwest of Mexico. Jim Kenagy is Curator of Mammals at the Burke Museum, where he has developed a collections-based research program emphasizing population biology, historical biogeography, and evolution of mammals. He is also Professor of Zoology in the Department of Biology, where he teaches biogeography.
