Ecology, 86(8), 2005, pp. 2145–2153

MARMOTS ON GREAT BASIN MOUNTAINTOPS: USING GENETICS TO TEST A BIOGEOGRAPHIC PARADIGM

CHRIS H. FLOYD,[1,3] DIRK H. VAN VUREN,[1] AND BERNIE MAY[2]

[1]*Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, California 95616 USA*
[2]*Department of Animal Science, University of California, Davis, California 95616 USA*

Abstract. Boreal mammals in the Great Basin have long been viewed as island-bound Pleistocene relicts because they occupy island-like patches of montane habitat separated by desert lowlands that presumably are impermeable to dispersal. Recent work, however, raised the possibility that dispersal among mountain ranges is an important process in the biogeography of boreal mammals in the Great Basin. We test this proposition using genetic variation in a representative species, the yellow-bellied marmot (*Marmota flaviventris*). A total of 332 marmots was sampled from 10 ranges and genotyped at six microsatellite loci. If the intervening desert lowlands are impermeable barriers to dispersal, then there should be no relationship between genetic distance and geographic distance among mountaintop populations, and genetic diversity should be diminished because gene flow would not be available to replace alleles lost over thousands of generations of isolation. Our results did not support these predictions. There was a strong correlation between genetic and geographic distance, demonstrating an isolation-by-distance pattern, and genetic diversity was high. Our results suggest that marmot populations in the Great Basin may be linked by dispersal, providing a mechanism to replenish genetic variation lost by drift. However, global climate change over the next several decades could make the desert lowlands more difficult to traverse, eventually transforming the boreal faunas of Great Basin mountaintops into the isolated relicts they were originally portrayed to be.

Key words: *biogeography; boreal; dispersal; gene flow; Great Basin USA; habitat islands;* Marmota flaviventris; *microsatellites.*

INTRODUCTION

The equilibrium theory of island biogeography (MacArthur and Wilson 1967) has been one of the most influential models in ecology. Originally developed to explain species richness on oceanic archipelagos, the model subsequently was extended to "habitat islands" (habitat fragmented into patches) and has provided an important conceptual basis for species conservation and reserve design (Shafer 1990, Cornelius et al. 2000).

One of the first applications of the model to habitat islands was Brown's (1971) study of boreal mammal distributions in the Great Basin of western North America, where island-like mountain ranges are surrounded by "seas" of desert scrublands (see Plate 1). These habitat islands are the remnants of a once contiguous network of montane life zones present during the late Pleistocene; because of climatic warming during the Holocene, desert scrublands spread throughout the lowlands, montane habitats contracted, and mountaintop populations of boreal mammals began to dwindle and go extinct, resulting in the relatively impoverished (i.e., relaxed) fauna that exists now (Grayson 1993). Brown (1971) found that distributions of boreal mammals on Great Basin mountain ranges were incongruent with MacArthur and Wilson's model, in which a balance between immigration and extinction produces an equilibrium level of species richness. For example, geographic isolation (distance to nearest colonization source) was not related to species richness, which Brown attributed to a lack of between-range immigration. Hence Brown (1971) proposed a "nonequilibrium" model, in which the arid scrublands separating mountaintop habitats pose an insurmountable barrier to immigration in boreal mammals, resulting in extinction without recolonization since the end of the Pleistocene.

Subsequent research, however, showed that some mammals thought to be extinct on certain mountain ranges were indeed present, suggesting that extinction was overestimated or between-range dispersal (i.e., recolonization) has occurred (Lawlor 1998, Grayson and Madsen 2000). Furthermore, using updated data on species distributions, Lawlor (1998) found a much weaker relationship between mountaintop area and species richness than did Brown, suggesting that mammalian distributions cannot be explained by extinction alone, without recolonization.

Thus, the question of whether dispersal plays a significant role in the biogeography of boreal mammals

Manuscript received 4 August 2004; revised 7 December 2004; accepted 4 January 2005. Corresponding Editor: C. W. Fox.

[3] Present address: Department of Biology, Phillips Hall 330, University of Wisconsin, Eau Claire, Wisconsin 54701 USA. E-mail: floydch@uwec.edu

PLATE 1. Island-like mountain ranges of the Great Basin, Nevada, USA. Desatoya Range as seen from the Clan Alpine Range (Mt. Augusta, ~3000 m elevation), looking southeast across the Edwards Creek Valley as its narrowest point (7.6 km wide, ~1700 m elevation). Mountain ranges in the distant background are the Shoshone and Toiyabe ranges. Photo credit: C. Floyd.

in the Great Basin remains unresolved. Documenting dispersal on such a vast scale using direct methods would be impracticable (Koenig et al. 1996). However, the development of genetic markers, particularly microsatellites, has provided valuable tools to measure or detect dispersal indirectly based on models that relate population genetic structure to gene flow, a product of dispersal (Waser and Strobeck 1998). Microsatellites, tandem repeats of short nucleotide motifs (e.g., AT) usually 10–100 repeats long, are especially suitable markers because they are generally highly polymorphic, selectively neutral, and widely distributed throughout the genome (Goldstein and Pollock 1997).

In this study we used microsatellite variation in a representative species, the yellow-bellied marmot (*Marmota flaviventris*), to determine if dispersal between mountain ranges is an important process in the biogeography of boreal mammals in the Great Basin. Yellow-bellied marmots were widely distributed throughout the Great Basin in the late Pleistocene, including in the lowlands, but are now extinct on all but the highest 20–25 mountain ranges (Grayson 1993, Lawlor 1998), where they are restricted to rocky outcrops and talus in montane meadows above 2000 m elevation (Floyd 2004). Marmots have never been reported in the desert basins separating the mountain ranges, and they seldom are observed in the belt of pinyon–pine and juniper woodland that surrounds the ranges at ~2100–2400 m elevation (Grayson 1993, Floyd 2004). The most important barriers to marmot dispersal in the Great Basin are the scarcity of exposed rock formations in the basins and the lack of openness in the woodlands, two habitat features that marmots require (Floyd 2004). Marmots are known to disperse as far as 15 km (Van Vuren and Armitage 1994), but it is unknown if they will traverse desert basins.

We assumed that if between-range dispersal occurs, then it does so according to the stepping-stone model (Hutchison and Templeton 1999), an extension of Wright's (1943) isolation-by-distance concept; gene flow is most likely to occur between closely situated populations due to limits on dispersal ability, resulting in neighboring populations being more genetically similar than widely separated populations (Kimura and Weiss 1964). If gene flow and genetic drift are in equilibrium, then genetic distance (the inverse of genetic similarity) between populations will increase in a positive and monotonic fashion with the geographical distance separating the populations (Hutchison and Templeton 1999). On the other hand, if dispersal does not occur, then there should be no relationship between genetic and geographic distance (Britten et al. 1995, Hutchison and Templeton 1999), and genetic diversity within populations should be greatly reduced. Presumably, without gene flow, populations subjected to thousands of years of genetic drift would lose much of their genetic diversity and diverge without regard to the geographic distance between them (Hutchison and Templeton 1999).

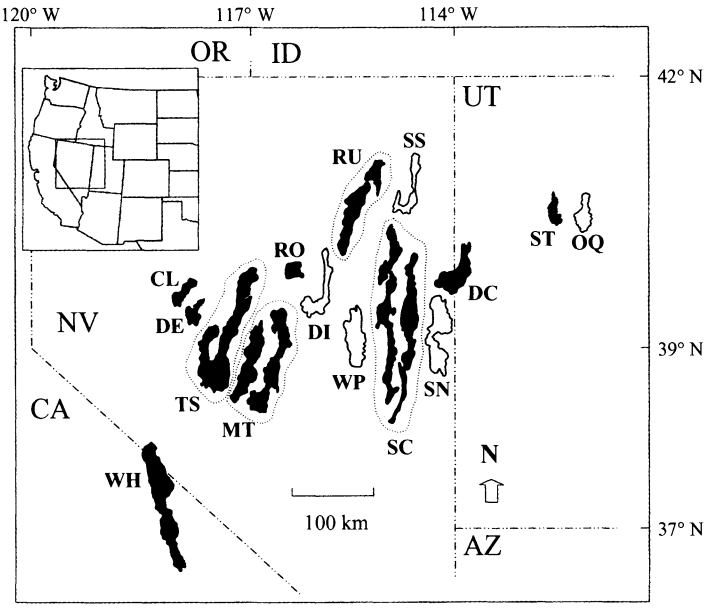

FIG. 1. Map of 10 mountain ranges (filled) in the Great Basin, where hair follicles were sampled from yellow-bellied marmots during 1999–2002, and five other ranges (open) that were not sampled. Mountain range abbreviations are: CL, Clan Alpine; DC, Deep Creek; DE, Desatoya; DI, Diamond; MT, Monitor-Toquima; OQ, Oquirrh; RO, Roberts; RU, Ruby; SC, Schell Creek-Egan-Cherry Creek; SN, Snake; SS, Spruce-Pequop; ST, Stansbury; TS, Toiyabe-Shoshone; WH, White Mountains; WP, White Pine. Ranges encircled with a dotted line are those that were lumped into the same range complex because they were too closely spaced to meet the minimum requirement for isolation (i.e., separation from nearby ranges by elevations under 2300 m that are ≥8 km wide).

METHODS

Study area and sampling

During 2000–2002, we searched for marmots on mountain ranges in Nevada, western Utah, and eastern California (Fig. 1), the same area studied by Brown (1971) and Lawlor (1998). Following Brown (1971) and Lawlor (1998), we included only mountain ranges that had at least one peak >2990 m and were separated from nearby ranges by elevations <2300 m that were ≥8 km wide. Similarly, we combined ranges that met the minimum requirement of elevation but not of separation; these were the Toiyabe and Shoshone; Monitor and Toquima; Ruby and East Humboldt; and Schell Creek, Egan, and Cherry Creek ranges. Yellow-bellied marmots were known to be recently extant on 15 of the qualifying mountain ranges (Lawlor 1998), but extensive searches failed to locate marmots on four of them, the Diamond, Oquirrh, Spruce-Pequop, and White Pine ranges, and only three marmots were found in the Snake Range (Floyd 2004), reducing our study to 10 ranges: Clan Alpine, Deep Creek, Desatoya, Monitor-Toquima, Roberts, Ruby-East Humboldt, Schell Creek-Egan-Cherry Creek, Stansbury, Toiyabe-Shoshone, and White Mountains (Fig. 1).

We sampled hair follicles from a total of 332 individuals in these 10 ranges (Table 1). Sampling hairs is relatively nonintrusive and yields DNA of a quality comparable to other tissues, such as blood (Zhang et al. 2003). For example, we sampled both hair and blood from 10 marmots in the Rocky Mountains and found identical microsatellite genotypes for both tissues (C. H. Floyd, *unpublished data*). Hair samples from the White Mountains were plucked from 111 marmots live-trapped in a separate study in 1999 (E. Stallman, C. H. Floyd, and B. May, *unpublished manuscript*). Because live trapping proved to be impractical elsewhere, most remaining samples were acquired using two other methods. One used hair snares composed of adhesive tape wrapped inversely around sticks and placed in burrow entrances in a way that forced the marmot to squeeze by, leaving hairs attached. Snares were checked 1–12 hours after placement. For marmots dwelling in talus slopes, hair snares could not be used because of the abundance of potential burrow entrances among the jumbled rocks. Hence, in talus we used an alternative method that exploited the marmots' behavior of spending much of the day lying on top of prominent rocks ("lookout posts"; Floyd 2004). Close examination of these rocks and the accompanying scat piles usually revealed numerous marmot hairs, sometimes including fur tufts (occasionally with skin attached). Because hairs exposed to sunlight are vulnerable to UV-caused DNA degradation, and shed hairs typically have lower DNA concentrations than plucked hairs (Gagneux et al. 1997), as many hairs as possible were collected at each lookout post. Hairs were put in labeled envelopes and later stored at −20°C. Additional hair samples were obtained from live-trapped marmots, marmot carcasses, and juvenile marmots captured by hand.

Sampling shed hairs presents the risk of contaminating an individual sample with DNA from another marmot, because burrows and lookout posts frequently are occupied by multiple and often closely related individuals (Armitage 1991). Thus, for shed hairs we used only one follicle per individual genotype analysis (Gagneux et al. 1997). With hair snares, contamination was unlikely because the first marmot out of the burrow usually knocked the snare out of the way.

Using shed hairs also presents the risk of inadvertently sampling an individual twice, since marmots may

TABLE 1. Summary of genetic variation within mountaintop populations of yellow-bellied marmots in the Great Basin, Western North America, analyzed across six microsatellite loci.

Mt. range	n	A	H_O	H_E
CL	10	3.7	0.62 (0.20–0.80)	0.64 (0.20–0.87)*
DC	21	3.7	0.63 (0.27–0.82)	0.71 (0.58–0.79)*
DE	23	4.2	0.82 (0.73–0.91)	0.76 (0.64–0.85)
MT	14	4.4	0.73 (0.63–0.83)	0.79 (0.72–0.84)
RO	6	3.9	0.65 (0.33–0.83)	0.74 (0.62–0.93)
RU	28	5.2	0.79 (0.59–0.93)	0.86 (0.76–0.91)*
SC	52	4.4	0.74 (0.64–0.90)	0.80 (0.71–0.88)*
ST	27	4.0	0.69 (0.32–0.95)	0.74 (0.53–0.85)*
TS	40	4.6	0.63 (0.51–0.70)	0.82 (0.76–0.86)*
WH	111	3.8	0.76 (0.68–0.86)	0.73 (0.64–0.80)

Notes: Number of individuals (n), allelic richness (A), mean observed (H_O) and unbiased (Nei 1978) estimates of heterozygosity (H_E). Ranges of values across loci are in parentheses. H_E values with asterisks denote populations with significant ($P < 0.05$) heterozygote deficiency. Abbreviations of mountain ranges are given in Fig. 2.

use more than one lookout post. Hence, in most cases we included in our analysis only one hair per burrow complex, defined as a rock outcrop or talus patch occupied by marmots ≥30 m from other burrow complexes. We selected one to six of the highest quality hairs, as determined by the presence of a well-developed follicle, from each burrow complex, then we further screened these samples by amplifying them at a single locus (Igs-6), choosing the hair that amplified best to represent the individual at that burrow complex. On a few occasions, two individuals from the same complex were included, but only if they could be discerned unambiguously via different genotypes.

Microsatellite analysis

DNA extraction.—For hair follicles taken from plucked hairs and hair snares, we used the standard Chelex method modified from Taberlet et al. (1997). Six to 12 whole marmot hairs were washed by immersing them in a 2.0-mL vial of 70% EtOH and vortexing the solution for 15 s, in order to remove debris that might inhibit DNA amplification. The hairs were then rinsed in the same manner with nanopure water, then dried and placed in a 1.5-mL vial with 600 μL of 5% Chelex 100 (Sigma Aldrich, St. Louis, Missouri, USA) solution. The vials were incubated for 8–12 h at 56°C, vortexed for 15 s, placed in a boiling water bath for 8 min, vortexed again, and then centrifuged at ~20 000 gravities (one gravity = 9.80665 m/s^2) for 3 min. The top 50 μL of supernatant was drawn off and stored at 4°C. The same procedure was conducted for single hair samples, except that after washing, the follicle end (~5 mm) of the hair was cut off and placed in only 200 μL of Chelex solution. Hair tufts collected from lookout posts were treated similarly to snare samples unless there was skin attached, in which case the sample was extracted using tissue kits (Sigma GenElute Sigma-Aldrich, St. Louis, Missouri, USA).

Microsatellite amplification.—Because there were no primers designed for microsatellite loci in yellow-bellied marmots, we examined primers developed for four related species: the alpine marmot (*M. marmota* [Klinkicht 1993, Goossens et al. 1998, Hanslik and Kruckenhauser 2000]) and three ground squirrels (*Spermophilus citellus* [Hanslik and Kruckenhauser 2000], *S. brunneus* [May et al. 1997], and *S. columbianus* [Stevens et al. 1997]). We screened 41 pairs of these primers, using high quality DNA sampled from eight yellow-bellied marmots live-trapped in a separate study in the Rocky Mountains (Floyd 2003). Polymerase chain reaction (PCR) amplifications were performed in 10 μL reactions containing 1–3 ng DNA (1–2 μL Chelex extract), 0.5 μmol/L each primer, 2 mmol/L MgCl, 200 μmol/L dNTPs, 0.1 μL BSA (10 mg/mL), 1 unit of Roche FastStart *Taq* DNA polymerase, and 1 unit of Roche FastStart *Taq* buffer (Roche, Indianapolis, Indiana, USA). Amplification conditions were an initial 95°C step for 4 min, followed by 40 cycles of 95°C for 30 s, 50–55°C for 30–35 s, and 72°C for 1 min; and then a final, 10 min, 72°C extension. PCR products were diluted with 20–30 μL of 98% formamide loading buffer, separated on 5% denaturing polyacrylamide gels, stained with fluorescent dye, and visualized with a Molecular Dynamics 595 fluorimager (Belfiore and May 2000).

Microsatellite variation.—Statistical analyses of microsatellite variation were conducted using the programs TFPGA 1.3 (Miller 1997), GENEPOP 3.4 (Raymond and Rousset 1995), and FSTAT 2.9.3 (Goudet 1995). We quantified within-mountaintop genetic diversity using FSTAT to compute allelic richness (El Mousadik and Petit 1996) and TFPGA to calculate observed heterozygosity (H_O) and Nei's (1978) unbiased estimates of average heterozygosity (H_E). We tested one-tailed probabilities of departure from Hardy-Weinberg (HW) equilibrium (heterozygote deficiency or non-amplifying alleles), using the Markov chain randomization (global) test in GENEPOP. Because our statistical analyses assume that loci are unlinked (i.e., each is an independent measure of genetic variation), we tested for linkage disequilibrium by estimating the probability of independence of genotypes for each pair of loci, using the Markov approximation in GENEPOP. Probability values ($P < 0.05$) were adjusted for multiple simultaneous tests using sequential Bonferroni tests (Rice 1989).

We tested for heterogeneity in allele frequencies among populations using the Markov chain Monte Carlo approximation in GENEPOP to estimate the one-tailed probability of allelic differentiation under the null hypothesis of no difference among populations. To quantify the overall magnitude of population differentiation, we used TFPGA to estimate F_{ST} (Weir and Cockerham 1984) and estimated standard errors and

95% confidence intervals by jackknifing and bootstrapping (10 000 iterations), respectively, over loci.

Geographic–genetic distance relationship

Geographic distance.—Distance between mountain ranges was estimated from 1:25 000 topographic maps of Nevada and Utah by first recording the two pairs of latitude/longitude coordinates that define the minimum (straight-line) distance between ranges, measured at the 2300-m contour interval encompassing each range (Lawlor 1998), and then calculating distance using Vincenty's (1975) inverse method.

Although straight-line distance is the simplest and most commonly used measure of geographic distance between mountain ranges, the measure ignores the topographical complexity of the intervening landscape (Carmichael et al. 2001, Vos et al. 2001), which often includes other primary ranges but is mostly composed of mountains and hills too small to be considered in this or previous studies. If marmots disperse between ranges, then these intervening rises might provide a more suitable route of dispersal than the basin floors, many of which were filled by pluvial lakes and marshes during cooler portions of the Holocene (Grayson 1993). Thus, as an alternative to the straight-line measure, we developed an "island-hopping" method that attempts to account for these topographical factors by summing the distances between the rises at their 2135-m elevation contour intervals, which approximate the lower woodland boundaries on Great Basin mountaintops (Lawlor 1998). The island-hopping method thus assumes that only terrain under 2135-m elevation provides substantial resistance to dispersal. Because there were many different island-hopping routes possible, we included only the route that minimized the sum of distances between islands.

Genetic distance.—To measure the relationship between genetic distance and geographic distance in mountaintop populations, we used the ISOLD (isolation-by-distance) program in GENEPOP, which evaluates the Spearman rank correlation coefficients (r) between genetic distance ($F_{ST}/1-F_{ST}$) and geographic distance (ln km) values for all pairwise comparisons and uses a Mantel (1967) permutation procedure (10^6 permutations) to determine 95% confidence intervals. To provide a more robust measure of population differentiation, we repeated the Mantel analysis with two alternative measures of genetic distance: Nei's (1972) distance, D_S, and the genotype likelihood ratio distance, D_{LR} (Paetkau et al. 1997). These two measures calculate genetic distance in different ways. D_S is a traditional measure based directly on allele frequency differences between populations and differs from F_{ST} in that it is not constrained by an upper bound and thus is more linear (Carmichael et al. 2001). D_{LR} measures differences between likelihood values, which are the expected probabilities of each individual's genotype in each population. Likelihood values are averaged over all individuals and mean values are compared between populations to obtain genetic distance. To avoid biasing D_{LR} values, we excluded the alleles of the individual being tested from its sample population when estimating allele frequencies and assigned a value of 0.01 if this removal resulted in an allele frequency of zero (Paetkau et al. 1997). The programs TFPGA and GENECLASS 1.0 (Cornuet et al. 1999) were used to calculate D_S and D_{LR} values, respectively.

In the Roberts and Clan Alpine ranges, we were able to obtain samples from only 6 and 10 individuals, respectively, but these samples may represent most or all of the marmots living there (Floyd 2004). Because sample sizes from the Roberts and Clan Alpine ranges were so much smaller than from any of the others, we repeated our analyses with a data set that excluded these ranges. Similarly, for the White Mountains data, where sample sizes were unusually high, we repeated our analyses using 50 samples randomly chosen from the 111 samples.

Results

Microsatellite variation

Of 41 microsatellite primer pairs screened, five were monomorphic and 30 produced poor amplification in the PCR (i.e., allele banding patterns could not be reliably or consistently interpreted). Six primer pairs revealed loci that were polymorphic and consistently interpretable: SGS 14 and SGS 17 (Stevens et al. 1997); Bibl 4, 18, and 36 (Goossens et al. 1998); and Igs-6 (May et al. 1997). Total number of alleles per locus over all mountain ranges ranged 10–15, and overall observed heterozygosity (H_O) ranged 0.68–0.75. Nei's (1978) unbiased heterozygosity (H_E) estimates were substantially higher, ranging 0.82–0.88. Mean overall H_O was 0.73 and H_E was 0.86. Hardy-Weinberg global tests revealed a significant ($P < 0.05$) heterozygote deficiency at four of the six loci and a heterozygote deficiency overall. Averaged across all loci, the Ruby Range had the highest heterozygosity (H_E) at 0.86 and the Clan Alpine Range the lowest at 0.64. The Ruby Range also had the highest allelic diversity with a mean of 5.2 alleles per locus, while the Deep Creek Range had the lowest, at 3.7 (Table 1).

Linkage disequilibrium.—Our test for independence of loci revealed significant linkage disequilibrium (LD) in all locus-by-locus comparisons when all populations were examined simultaneously ($P < 0.05$). Analyzed for each population, however, LD was found in only 14 of 145 pairwise locus combinations ($P < 0.0004$), and these were concentrated in six mountain ranges: Toiyabe-Shoshone, Monitor-Toquima, Schell Creek-Egan-Cherry Creek, Ruby-East Humboldt, Stansbury, and Deep Creek Ranges. In all ranges except the Deep Creek, samples were obtained from different drainages >1 km apart; thus, it seems likely that inadvertent mixing of subpopulations within ranges caused much of

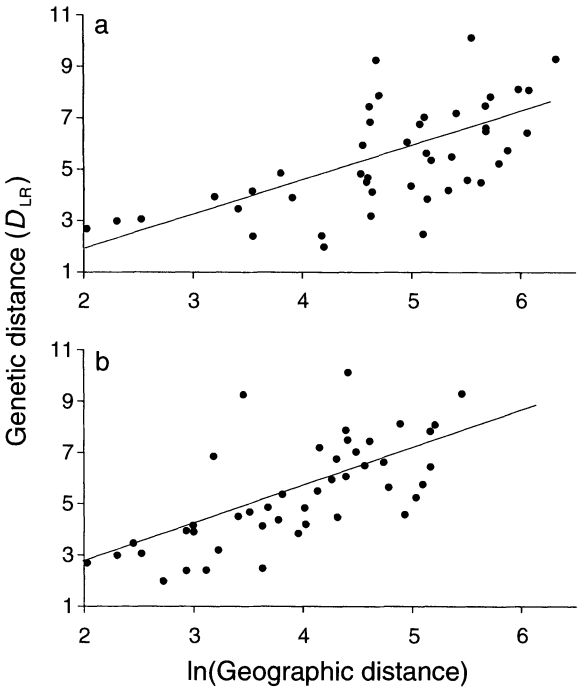

FIG. 2. Relationship between genetic distance and geographic distance among 10 mountaintop populations of yellow-bellied marmots sampled in the Great Basin during 1999–2002. Log-transformed geographic distance (originally measured in kilometers) was estimated using the (a) straight-line and (b) island hopping methods, and genetic distance was estimated using the likelihood measure, D_{LR}. Correlation coefficients (r) between genetic and geographic distance were estimated using Mantel tests: (a) $r = 0.62$, $P < 0.01$; (b) $r = 0.70$, $P < 0.01$. Similar but weaker relationships between genetic and geographic distance were revealed with two other genetic distance measures, D_S and F_{ST}.

the linkage disequilibrium and heterozygote deficiencies in our data set (see *Discussion*).

Population differentiation.—Comparisons of allele frequency distributions for each locus revealed strong genetic differentiation among mountain ranges ($P < 0.001$; SE < 0.001). Jackknife estimates of F_{ST} averaged 0.13 ± 0.01 SE, and bootstrap estimates were between 0.12 and 0.15 (95% CI). F_{ST} estimates for each pair of populations ranged 0.05–0.23.

Geographic–genetic distance analysis

Isolation by distance.—Mantel tests revealed a strong pattern of isolation-by-distance among all 10 mountain ranges, and this held for all three genetic distance measures (F_{ST}, D_{LR}, and D_S) and for both measures of geographic distance ($r = 0.50$–0.70, $P < 0.01$). Using the island-hopping geographic distance measure produced a stronger isolation-by-distance relationship than did the straight-line measure (Fig. 2). The highest correlation between genetic and geographic distance was revealed with the likelihood measure, D_{LR} ($r = 0.62$–0.70, $P < 0.01$; Fig. 2), and the weakest with Nei's D_S ($r = 0.50$–0.54, $P < 0.01$).

Analyses of abridged data sets.—Using a randomly chosen subset of 50 individuals from the White Mountains did not appreciably change the results of any of our analyses. However, removing the Clan Alpine and Roberts ranges (with only 10 and 6 samples, respectively) from the analysis substantially increased the isolation-by-distance correlation for D_{LR} (from 0.70 to 0.82) and similarly for the other two genetic distance measures.

DISCUSSION

For more than three decades boreal mammals on Great Basin mountaintops have been viewed as island-bound Pleistocene relicts, the survivors of a once widely distributed fauna that was fragmented by the spread of desert scrublands during the Holocene and forced to recede into high elevation refugia (Brown 1971, Lawlor 1998). If arid lowlands in the Great Basin are indeed impermeable barriers to dispersal, then the genetic structure of marmot populations should reflect ca. 7000–10 000 years (~1500–2200 marmot generations; Schwartz et al. 1998) of isolation on mountaintops. Completely isolated populations are expected to exhibit no relationship between genetic and geographic distance (Hutchison and Templeton 1999). However, we found a strong pattern of isolation-by-distance, with genetic distance monotonically increasing with geographic distance. Additionally, using the island-hopping method of measuring geographic distance strengthened the correlation compared with the straight-line distance approach, suggesting that the most likely route of dispersal between ranges is the one that minimizes the sum of distances between intervening hills, rather than across desert basins. Although probably not optimal marmot habitat, the intervening areas of raised topography usually possess exposed boulders and talus that may provide cover during dispersal (C. H. Floyd, *personal observation*). These corridors may have been even more favorable for dispersal during relatively cold periods of the Holocene, such as the Little Ice Age (AD 1300–1850), when treeline was as much as 180 m lower than it is now (Munroe 2003).

Isolated populations also are expected to exhibit lower genetic diversity than connected populations (Frankham 1997, Eldridge et al. 1999). For example, Kodiak Island brown bears (*Ursus arctos*) known to be completely isolated since the Pleistocene exhibited markedly reduced heterozygosity ($H_E = 0.27$) relative to mainland populations >35 km distant ($H_E = 0.68$–0.78; Paetkau et al. 1998). However, we did not find reduced heterozygosity in Great Basin marmot populations. Measured at five microsatellite loci shared between studies, heterozygosity in the Great Basin ($H_O = 0.73$, $H_E = 0.86$) was similar to that found in colonies of yellow-bellied marmots in the Rocky Mountains ($H_O = 0.78$, $H_E = 0.81$; Floyd 2003), where marmot habitat is continuous and colonies are known to be connected by dispersal (Armitage 1991).

Our results are consistent with the hypothesis that genetic differentiation has reached equilibrium between the opposing forces of genetic drift and gene flow, indicating that dispersal does indeed occur among Great Basin ranges. However, interpreting isolation-by-distance is complicated by the fact that present-day population structure can reflect past processes, as well as ongoing effects of genetic drift and gene flow (Goodman et al. 2001, Turgeon and Bernatchez 2001). Hence, our results could conceivably be a pattern left over from the late Pleistocene, when a contiguous network of montane habitat stretched across the Great Basin (Grayson 1993). However, in the absence of gene flow, this would require that mountaintop populations remained consistently large enough throughout the Holocene to prevent genetic drift from erasing the late Pleistocene pattern (Turgeon and Bernatchez 2001). We consider this scenario unlikely. Marmot populations in some of the ranges we studied appeared to be <20 individuals, and even in ranges where marmots were more common, such as the Desatoya, Schell Creek, and Toquima ranges, effective population size likely was far under 500 individuals (generally thought to be the level below which heterozygosity in isolated populations is rapidly lost; Frankham et al. 2002). Furthermore, it seems likely that still lower population sizes (and perhaps even extinctions) occurred in these and other ranges during the hottest and driest part of the Holocene (the Hypsithermal), ca. 4500–7000 years ago (Grayson 1993).

Deviations from Hardy-Weinberg expectations

Observed values of heterozygosity were consistently lower than Nei's (1978) unbiased, "expected" measure, and HW tests revealed a significant deficit of heterozygotes at four of six loci. This finding might indicate allelic dropout (i.e., the failure of an allele to amplify at a heterozygous locus, due to very low concentrations of template DNA), as has been found in other studies that used single hair follicles (Gagneux et al. 1997). To determine whether allelic dropout caused heterozygote deficiency, we grouped individuals in our data set according to hair sample type: direct (plucked from carcasses and captured marmots), snared, and shed (single hair follicles); then we randomly selected 20 marmots from each group and calculated observed (H_O) and expected heterozygosity (H_E). If the heterozygote deficit is a product of allelic dropout due to low DNA concentrations, then H_O and H_E should be higher in the direct and snared groups than in the shed group, because plucked hairs typically have higher concentrations than shed hairs (Vigilant 1999), and multiple hairs per individual were used in the direct/snared groups. These results suggested no evidence of allelic dropout. Mean heterozygosity was similar for all groups: $H_O/H_E = 0.71/0.85$ for shed hairs, 0.74/0.86 for direct, and 0.73/0.84 for snared samples.

A heterozygote deficit can also be produced when different populations are unintentionally mixed (Wahlund 1928, Hartl and Clark 1997). Thus, some of the mountaintop "populations" in our study may have been actually composed of genetically differentiated subpopulations. This phenomenon will also lead to a statistical deviation from linkage equilibrium (Hartl and Clark 1997, Goossens et al. 2001). Indeed, when all 10 populations were analyzed simultaneously, there were statistically significant levels of linkage disequilibrium (LD). Because our statistical tests assume that each locus is an independent measure of genetic variation in populations, any statistical significance here must thus be interpreted cautiously. LD might also reflect an actual physical association between loci on chromosomes (Hartl and Clark 1997), but we believe that population mixing is the most likely explanation for the observed correlation between loci. In the Clan Alpine, Roberts, Deep Creek, and White Mountains ranges, where marmots were sampled from a single location (i.e., usually within one drainage, where distance between adjacent samples was <1 km), fewer than 2% (1/55) of pairwise locus comparisons were in LD. In the other six ranges, where samples were taken from at least two locations, 14% (13/90) of pairwise locus comparisons showed significant LD.

To further examine the influence of population mixing on LD, we reanalyzed our results by partitioning mountain ranges into subpopulations based on different sample locations, most of which were in separate drainages. This reanalysis also entailed separating different mountain ranges that had been previously lumped (Monitor-Toquima, Ruby-East Humboldt, Schell Creek-Egan-Cherry Creek, Toiyabe-Shoshone). The results were greatly reduced rates of LD, further supporting our contention that linkage disequilbrium was caused by the Wahlund effect; less than 3% (6/250) of total pairwise comparisons were in significant LD, and the percentage of pairwise comparisons averaged over populations dropped from 100% to 40% (6/15). Also, the number of loci with significant heterozygote deficiency dropped from four to two.

Conservation implications

Recent studies of global climate change have predicted a 400–600 m increase in the altitudinal range margins of vegetation zones over the next several decades (Hill et al. 2002, Kullman 2002). As a result, the area available to boreal populations is expected to shrink as their habitats shift upslope (Orlóci 1994, Gottfried et al. 1999). Mountaintop populations in the Great Basin may be especially vulnerable, being small and insular, relative to those in massifs like the Rocky Mountains (MacDonald and Brown 1992). The results of one predictive model indicate that a warming of 3°C in the Great Basin will cause local extinctions of several boreal mammals and some mountain ranges will lose upward of 50% of their present faunas (McDonald

and Brown 1992). The model, however, assumes no dispersal between mountain ranges, a premise not supported by the results of our study. In this regard our results suggest a more favorable outlook than the model's alarming predictions.

Nevertheless, with global climate change the desert lowlands may become even more unfavorable for dispersal, further reducing the probability that immigrants will rescue a declining population by boosting population size or restoring genetic variation (Brown and Kodric-Brown 1977, Johnson et al. 2003) or recolonizing extinct sites (Grayson and Madsen 2000). Climate change, thus, may ultimately transform the Great Basin boreal mammal faunas into the island-bound, Pleistocene relicts originally portrayed by Brown (1971).

Acknowledgments

The American Society of Mammalogists, American Museum of Natural History, Sigma Xi, and UC Davis Jastro Shields Research Awards provided financial support for this study. We are extremely grateful to R. Miller, T. Platt, and S. Coppeto for their assistance in Nevada; T. Jenkins, C. Conway, and other members of the Genomic Variation Laboratory for assistance in the laboratory; and B. Stallman for providing samples from the White Mountains. Thanks to D. Kelt, J. Eadie, and two anonymous reviewers, for providing valuable input on earlier drafts of this paper. We also thank the following individuals and organizations for providing valuable information or other assistance: C. Baughman, P. Bradley, L. Crabtree, J. Casey, R. Sterner, E. Beever, D. Tautz, and K. Heister; and the Nevada Division of Wildlife, USDA National Forest Service, U.S. National Park Service, and the U.S. Bureau of Land Management. This work could not have been accomplished without the wide-ranging support of P. Floyd and S. Floyd.

Literature Cited

Armitage, K. B. 1991. Social and population dynamics of yellow-bellied marmots: results from long-term research. Annual Review of Ecology and Systematics **22**:379–407.

Belfiore, N. M., and B. May. 2000. Variable microsatellite loci in red swamp crayfish, *Procambarus clarkii*, and their characterization in other crayfish taxa. Molecular Ecology **9**:2231–2234.

Britten, H. B., P. F. Brussard, D. D. Murphy, and P. R. Ehrlich. 1995. A test of isolation-by-distance in central Rocky Mountain and Great Basin populations of Edith's checkerspot butterfly (*Euphyryas editha*). Journal of Heredity **86**: 204–210.

Brown, J. H. 1971. Mammals on mountaintops: nonequilibrium insular biogeography. American Naturalist **105**:467–478.

Brown, J. H., and A. Kodric-Brown. 1977. Turnover rates in insular biogeography: effect of immigration on extinction. Ecology **58**:445–449.

Carmichael, L. E., J. A. Nagy, N. C. Larter, and C. Strobeck. 2001. Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. Molecular Ecology **10**:2787–2798.

Cornelius, C., H. Cofré, and P. A. Marquet. 2000. Effects of habitat fragmentation on bird species in a relict temperate forest in semiarid Chile. Conservation Biology **14**:534–543.

Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics **153**: 1989–2000. ⟨http://www.montpellier.inra.fr/CBGP/softwares/⟩

Eldridge, M. D. B., J. M. King, A. K. Loupis, P. B. S. Spencer, A. C. Taylor, L. C. Pope, and G. P. Hall. 1999. Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. Conservation Biology **13**:531–541.

El Mousadik, A., and R. J. Petit. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. Theoretical and Applied Genetics **92**:832–839.

Floyd, C. H. 2003. Ecological genetics of dispersal and mating system in populations of yellow-bellied marmots, *Marmota flaviventris*. Dissertation. University of California, Davis, California, USA.

Floyd, C. H. 2004. Marmot distribution and habitat associations in the Great Basin. Western North American Naturalist **64**:471–481.

Frankham, R. 1997. Do island populations have less genetic variation than mainland populations? Heredity **78**:311–327.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge, UK.

Gagneux, P., C. Boesch, and D. S. Woodruff. 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Molecular Ecology **6**:861–868.

Goldstein, D. B., and D. D. Pollock. 1997. Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. Journal of Heredity **88**:335–342.

Goodman, S. J., H. B. Tamate, R. Wilson, J. Nagata, S. Tatsuzawa, G. M. Swanson, J. M. Pemberton, and D. R. McCullough. 2001. Bottlenecks, drift and differentiation: the population structure and demographic history of sika deer (*Cervus nippon*) in the Japanese archipelago. Molecular Ecology **10**:1357–1370.

Goossens, B., L. Chikhi, P. Taberlet, L. P. Waits, and D. Allainé. 2001. Microsatellite analysis of genetic variation among and within Alpine marmot populations in the French Alps. Molecular Ecology **10**:41–52.

Goossens, B., L. Graziani, L. P. Waits, E. Farand, S. Magnolon, J. Coulon, M.-C. Bel, P. Taberlet, and D. Allainé. 1998. Extra-pair paternity in the monogamous Alpine marmot revealed by nuclear DNA microsatellite analysis. Behavioral Ecology and Sociobiology **43**:281–288.

Gottfried, M., H. Pauli, K. Reiter, and G. Grabherr. 1999. A fine-scaled predictive model for changes in species distribution patterns of high mountain plants induced by climate warming. Diversity and Distributions **5**:241–251.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate *F*-statistics. Journal of Heredity **86**:485–486. ⟨http://www2.unil.ch/izea/softwares/fstat.html⟩

Grayson, D. K. 1993. The desert's past: a natural prehistory of the Great Basin. Smithsonian Institution Press, Washington, D.C., USA.

Grayson, D. K., and D. B. Madsen. 2000. Biogeographic implications of recent low-elevation recolonization by *Neotoma cinerea* in the Great Basin. Journal of Mammalogy **81**:1100–1105.

Hanslik, S., and L. Kruckenhauser. 2000. Microsatellite loci for two European sciurid species (*Marmot marmota*, *Spermophilus citellus*). Molecular Ecology **9**:2163–2165.

Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics. Sinauer Associates, Sunderland, Massachusetts, USA.

Hill, J. K., C. D. Thomas, R. Fox, M. G. Telfer, S. G. Willis, J. Asher, and B. Huntley. 2002. Responses of butterflies to twentieth century climate warming: implications for future ranges. Proceedings of the Royal Society of London Series B **269**:2163–2171.

Hutchison, D. W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution **53**:1898–1914.

Johnson, J. A., J. E. Toepfer, and P. O. Dunn. 2003. Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. Molecular Ecology **12**:3335–3347.

Kimura, M., and G. H. Weiss. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics **49**:561–576.

Klinkicht, M. 1993. Untersuchungen zum Paarungssystem des Alpenmurmeltiers, *Marmota m. marmota* mittels DNA fingerprinting. Dissertation. University of Munich, Munich, Germany.

Koenig, W. D., D. Van Vuren, and P. N. Hooge. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends in Ecology and Evolution **11**: 514–517.

Kullman, L. 2002. Rapid recent range-margin rise of tree and shrub species in the Swedish Scandes. Journal of Ecology **90**:68–77.

Lawlor, T. E. 1998. Biogeography of Great Basin mammals: paradigm lost? Journal of Mammalogy **79**:1111–1130.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, New Jersey, USA.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research **27**:209–220.

May, B., T. A. Gavin, P. W. Sherman, and T. M. Korves. 1997. Characterization of microsatellite loci in the northern Idaho ground squirrel *Spermophilus brunneus brunneus*. Molecular Ecology **6**:399–400.

McDonald, K. A., and J. H. Brown. 1992. Using montane mammals to model extinctions due to global change. Conservation Biology **6**:409–415.

Miller, M. P. 1997. Tools for population genetic analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. ⟨http://bioweb.usu.edu/mpmbio/⟩

Munroe, J. S. 2003. Estimates of Little Ice Age climate inferred through historical rephotography, northern Uinta Mountains, U.S.A. Arctic, Antarctic, and Alpine Research **35**:489–498.

Nei, M. 1972. Genetic distance between populations. American Naturalist **106**:283–292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**:583–590.

Orlóci, L. 1994. Global warming: the process and its anticipated phytoclimatic effects in temperate and cold zones. Coenose **9**:69–74.

Paetkau, D., G. F. Shields, and C. Strobeck. 1998. Gene flow between insular, coastal and interior populations of brown bears in Alaska. Molecular Ecology **7**:1283–1292.

Paetkau, D., L. P. Waits, P. L. Clarkson, L. Craighead, and C. Strobeck. 1997. An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. Genetics **147**:1943–1957.

Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248–249. ⟨http://wbiomed.curtin.edu.au/genepop/⟩

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution **43**:223–225.

Schwartz, O. A., K. B. Armitage, and D. Van Vuren. 1998. A 32-year demography of yellow-bellied marmots *Marmota flaviventris*. Journal of Zoology, London **246**:337–346.

Shafer, C. L. 1990. Nature reserves: island theory and conservation practice. Smithsonian Institution Press, Washington, D.C., USA.

Stevens, S., J. Coffin, and C. Strobeck. 1997. Microsatellite loci in Columbian ground squirrels *Spermophilus columbianus*. Molecular Ecology **6**:493–495.

Taberlet, P., J.-J. Camarra, S. Griffin, E. Uhres, O. Hanotte, L. P. Waits, C. Dubois-Paganon, T. Burke, and J. Bouvet. 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Molecular Ecology **6**:869–876.

Turgeon, J., and L. Bernatchez. 2001. Clinal variation at microsatellite loci reveals historical intergradation between glacial races of *Coregonus artedi* (Teleostei: Coregoninae). Evolution **55**:2274–2286.

Van Vuren, D., and K. B. Armitage. 1994. Survival of dispersing and philopatric yellow-bellied marmots: what is the cost of dispersal? Oikos **69**:179–181.

Vigilant, L. 1999. An evaluation of techniques for the extraction and amplification of DNA from naturally shed hairs. Biological Chemistry **380**:1329–1331.

Vincenty, T. 1975. Direct and inverse solutions of the geodesics on the ellipsoid with applications of nested equations. Survey Review **22**:88–93. ⟨http://www.auslig.gov.au/geodesy/datums/distance.htm⟩

Vos, C. C., A. G. Antonisse-De Jong, P. W. Goedhart, and M. J. M. Smulders. 2001. Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). Heredity **86**:598–608.

Wahlund, S. 1928. Zusammensetzung von populationen und korreletionser cheinungen vom standpunkt der vererbungslehre aus betrachtet. Hereditas **11**:65–106.

Waser, P. M., and C. Strobeck. 1998. Genetic signatures of interpopulation dispersal. Trends in Ecology and Evolution **13**:43–44.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution **38**:1358–1370.

Wright, S. 1943. Isolation by distance. Genetics **28**:114–138.

Zhang, Y.-G., D.-Q. Li, L.-Q. Rao, Q.-M. Xiao, and D. Liu. 2003. Identification of polymorphic microsatellite DNA loci and paternity testing of Amur tigers. Acta Zoologica Sinica **49**:118–123.