Conservation Genetics (2005) 6:759–774
DOI 10.1007/s10592-005-9035-3

Genetic diversity and population divergence in fragmented habitats: Conservation of Idaho ground squirrels

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Received 12 October 2004; accepted 24 April 2005

Key words: genetic diversity, ground squirrels, habitat fragmentation, microsatellites, *Spermophilus brunneus*

Abstract

The Idaho ground squirrel, which consists of a northern (*Spermophilus brunneus brunneus*) and a southern subspecies (*S. b. endemicus*), has suffered from habitat loss and fragmentation, resulting in a reduction in both numbers and geographic range of the species. The northern Idaho ground squirrel (NIDGS) is listed as a threatened subspecies under the Endangered Species Act, and the southern Idaho ground squirrel (SIDGS) is a candidate. Because Idaho ground squirrel populations are small and often isolated, they are susceptible to inbreeding and loss of genetic diversity through drift. This research evaluates levels of genetic diversity and patterns of population divergence in both subspecies of Idaho ground squirrels. We hypothesized that NIDGS would exhibit lower genetic diversity and greater population divergence due to a longer period of population isolation relative to most SIDGS populations. Genetic diversity and divergence were quantified using 8 microsatellite loci. Contrary to expectations, SIDGS populations exhibited consistently lower levels of microsatellite diversity. Additionally, NIDGS exhibited only modest divergence among populations, while divergence levels among SIDGS populations were highly varied. Preliminary evaluations of mitochondrial DNA diversity and structure revealed lower diversity in NIDGS and some differences in gene flow that warrant further study. Based on our results, we suggest different management strategies for the two subspecies. Habitat restoration appears to be the most desirable conservation strategy for NIDGS populations. In contrast, low genetic diversity observed in SIDGS may warrant supplementation of isolated populations through translocations or captive breeding to mitigate further loss of genetic variability.

Introduction

Deterioration of genetic diversity is a growing concern in the conservation of a variety of declining species (Frankham et al. 2002; Garner et al. 2005). Genetic diversity has been linked to individual fitness (Coulsen et al. 1998; Coltman et al. 1999; Cassinello et al. 2001) and to the ability of populations to adapt to environmental change (Lande 1988; Lacy 1997). Ultimately, loss of genetic variability increases probability of extinction (Boyce 1992; Hedrick 1995; Reed and Frankham 2003). Small populations are particularly vulnerable to inbreeding and loss of genetic diversity through genetic drift (Templeton and Read 1994). Furthermore, habitat fragmentation

also threatens many species (Lacy 1988; Clarke and Young 2000) by decreasing gene flow among populations and intensifying problems associated with inbreeding and genetic drift.

Levels of genetic diversity within and divergence among populations are expected to vary with degree of fragmentation. As geographic distance between fragments or time since isolation increases, genetic diversity within small populations is expected to decrease, and populations will diverge (Frankham et al. 2002). Inversely, gene flow counteracts both differentiation and the loss of genetic diversity. In continuous or stepping-stone models of dispersal, genetic distances and geographic distances among populations are expected to correlate (Wright 1943; Kimura and Weiss 1964), unless barriers to dispersal interrupt gene flow (Hellberg 1995). Hence, measures of gene flow and population divergence, such as F_{ST} (Wright 1969), indicate relative degree of isolation. In small, fragmented populations, both genetic diversity and degree of divergence from other populations are frequently used to assess genetic risk.

The Idaho ground squirrel (*Spermophilus brunneus*), which belongs to a group of small-eared, western United States (U.S.) ground squirrels, has undergone severe declines and increasing isolation of populations due to loss and fragmentation of habitat. This species consists of a northern (*S. brunneus brunneus*) and a southern subspecies (*S. b. endemicus*), which are distinct geographically, morphologically and behaviorally (Yensen 1991), and which may represent two distinct species (Yensen and Sherman 1997). The northern Idaho ground squirrel (NIDGS), found in Adams and Valley Counties of west-central Idaho, was listed as threatened under the U. S. Endangered Species Act (ESA) in April, 2000 (U.S. Fish and Wildlife Service 2000). The total population of NIDGS was estimated at 5000 individuals in 1985 and is currently estimated at 700 individuals (Evans-Mack 2003). The southern Idaho ground squirrel (SIDGS) occurs in Gem, Payette, and Washington Counties, Idaho, and is a candidate for listing under the ESA. The southern subspecies has been greatly reduced in number and restricted in geographic range within the last fifteen to twenty years (Yensen 1999), decreasing from an estimated 40,000 individuals in the early 1980s to 2000–4500 in 2001 (Yensen 2001).

Habitat alterations, including changes in natural fire cycles, have impacted both subspecies of Idaho ground squirrels. The NIDGS has suffered from loss of habitat largely due to fire suppression and consequent forest encroachment into meadows (Haak 2000). Conifer invasion into former meadow habitats has been ongoing in Adams County for at least 50 years (U.S. Forest Service and U.S. Fish and Wildlife Service 1999), and subsequent loss or reduction of dispersal corridors has isolated populations of NIDGS (Gavin et al. 1999). In contrast, invasion of non-native grasses into the lower-elevation SIDGS habitat has increased the intensity and frequency of fires, preventing reestablishment of native sage-steppe species (Yensen 1991; Yensen et al. 1992). While a few SIDGS populations may have been isolated in the 1980s, most SIDGS populations have become isolated only recently, presumably within the last 5–20 years (E. Yensen, pers. comm.), and some populations may remain connected.

Because Idaho ground squirrel populations are small and often isolated, they are susceptible to inbreeding and loss of genetic diversity through drift. Gavin et al. (1999) documented allozyme divergence among populations of NIDGS and attributed this pattern to genetic drift. However, allozymes cannot quantify genetic diversity and recent gene flow on the fine scale necessary to address many conservation concerns (Haig 1998; Hughes and Queller 1993). Furthermore, population genetic structure of SIDGS had not been analyzed previously. Therefore, the objective of this study was to evaluate levels of microsatellite diversity and patterns of population divergence in both subspecies of the Idaho ground squirrel. Because Idaho ground squirrels may exhibit male-biased dispersal and female philopatry, we also conducted preliminary analyses of mitochondrial DNA (mtDNA) to evaluate whether matrilineal structure may be present and to assess the need for further mitochondrial analyses. We hypothesized that genetic diversity within populations would be lower and genetic divergence among populations would be greater in NIDGS due to their longer period of population isolation. Genetic data provided by our research will assist in evaluating conservation options for Idaho ground squirrels, including translocations, captive breeding, and habitat restoration.

Methods

Sample collection and DNA extraction

During February–June of 2002, we collected 266 samples from seven populations of NIDGS and 201 samples from eight populations of SIDGS. Our 15 study populations spanned the entire distribution of each subspecies and included most of the known persisting populations (Figure 1). Geographic distances averaged 12.3 km (max: 22.4 km; min: 2.6 km) among the 7 NIDGS populations and 32.0 km (max: 68.2 km; min: 4.4 km) among the 8 populations of SIDGS. Samples were collected in collaboration with researchers at Boise State University (BSU) and with ongoing population monitoring by the Idaho Department of Fish and Game (IDFG). Eight populations monitored by BSU and IDFG were trapped intensively, with attempts to collect samples from all adult squirrels in each population. Those eight populations were: Summit Gulch, Tree Farm, Cold Springs, Squaw Butte, Bissel Creek, Sand Hollow, Clay Peak, and Holland Gulch (Figure 1). The other seven populations were sampled non-exhaustively; however, a minimum of 10 animals was trapped from each population.

Because of the difficulty of obtaining sufficient blood from Idaho ground squirrels, as well as our desire to avoid highly invasive tissue or blood sampling in a threatened species, plucked hair was used as the source for DNA in this study. The reliability of multiple plucked hairs as a DNA source for genotyping has been demonstrated in previous research (Goossens et al. 1998). Approximately 50–60 hairs were plucked from the tail of each squirrel and placed in paper envelopes, and the envelopes were stored in silica desiccant. DNA was extracted from 10 to 15 hairs per individual using the Qiagen tissue kit (Qiagen Co., USA) in a room dedicated to low-quantity DNA samples. Each batch of 10–18 extractions

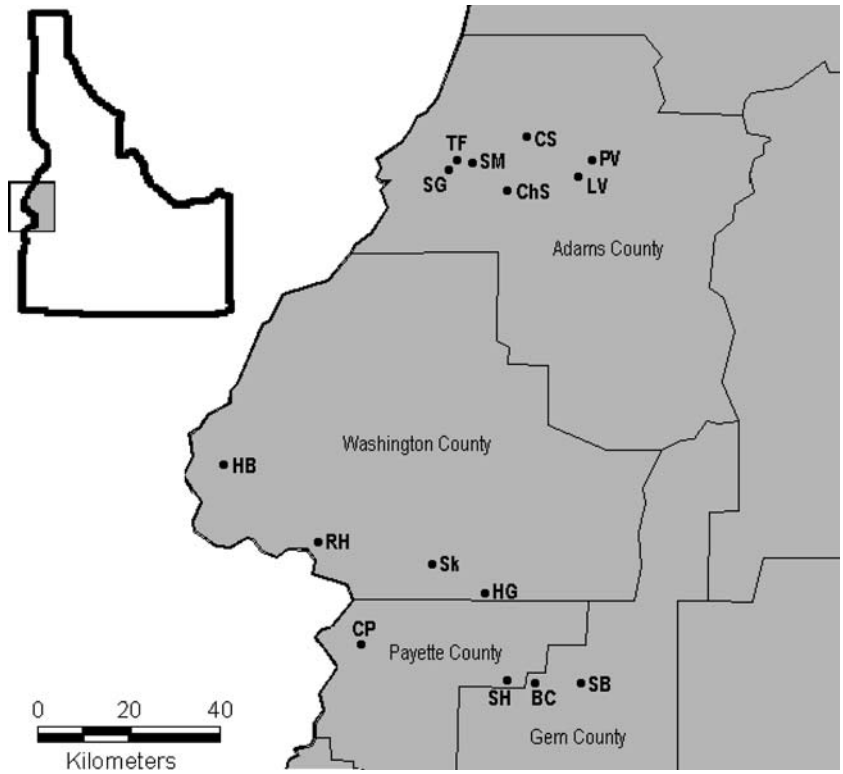

Figure 1. Locations of study populations of Idaho ground squirrel in western Idaho, USA. Northern Idaho ground squirrel populations: Cold Springs (CS), Tree Farm (TF), Summit Gulch (SG), Squirrel Manor (SM), Chipmunk Springs (ChS), Lost Valley (LV), and Price Valley (PV). Southern Idaho ground squirrel populations: Henley Basin (HB), Rolling Hills/Hillcrest (RH), Skow (Sk), Holland Gulch (HG), Clay Peak (CP), Sand Hollow (SH), Bissel Creek (BC), and Squaw Butte (SB).

contained a blank sample to serve as a negative control.

Microsatellite analyses

Eight microsatellite loci were amplified for each sample by the Polymerase Chain Reaction (PCR). Four of these loci were isolated in Columbian ground squirrels (*S. columbianus*, Stevens et al. 1997), one had been previously isolated for NIDGS (May et al. 1997), and three novel loci were isolated for SIDGS (Garner 2004; Table 1). Loci GS3 and GS17 were amplified together with the following reaction conditions: 1.88 mM $MgCl_2$, 1 unit Amplitaq Gold Polymerase (Applied Biosystems, USA), 1X Amplitaq Gold Buffer, 0.75 mM of each dNTP, 0.75 μM of each primer, and 2 μl DNA in a total reaction volume of 20 μl. The PCR profile consisted of 35 cycles (95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min), with an initial denaturation step of 95 °C for 5 min and final extension at 72 °C for 5 min. Loci GS12, GS26, and IGS-110b were amplified together, as above, but with 1.5 μM of each GS12 primer, and an annealing temperature of 53 °C. The two reactions were multiplexed (1:1) for loading of all five loci in one gel lane. A second multiplex consisted of loci B109, B126, and D117 amplified together, with reaction conditions and PCR profile as in multiplex 1, but with 2 mM $MgCl_2$ and an annealing temperature of 54 °C. Forward primers were flourescently labeled for analysis on an ABI 377 automatic DNA sequencer (Applied Biosystems), and the computer programs Genotyper 2.5 and Genescan version 3.1.2 (Applied Biosystems) were used for genotyping the samples. All batches of PCR reactions contained negative controls to screen for contamination. For each locus, an average of 21% (range: 13–27%) of all samples were amplified multiple times to assess the rate of genotyping errors (i.e. the percentage of genotyping errors detected in repeated PCRs for each locus). Samples with ambiguous or unique genotypes were reamplified until the genotype could be verified, and samples amplified repeatedly with ambiguous or conflicting results were not assigned a genotype.

Preliminary mitochondrial sequencing

For a subset of samples, we sequenced a 558 bp portion of the mitochondrial genome, consisting of the last 84 bp of the cytochrome b gene and 474 bp of the control region. Primers used were:

Table 1. Microsatellite loci used for analyses of diversity and divergence in the Idaho ground squirrel

Locus	Species of origin	Repeat unit	Primer sequences		Annealing T (°C)	Size range (bp)	No. of Alleles		
GS3	CGS	TG	F	GTTAAGTGTGTATGATGTGGA	50	115–117	2	N	2
			R	TCACCTAAAGAAGTGTCGTAT				S	2
GS12	CGS	TG	F	CCAAGAGAGGCAGTCGTCCAG	53	144–166	11	N	9
			R	TCAGAGCAGAGCACTTACAGA				S	8
GS17	CGS	TG	F	CAATTCGTGGTGGTTATATC	50	149–167	10	N	8
			R	CTGTCAACCTATATGAACACA				S	8
GS26	CGS	TG	F	CCCAGGGACCACATAGGAGGTA	53	106–124	7	N	7
			R	AGGACTGGGGTTGTAGGTGAGT				S	4
IGS-110b	NIDGS	TGC	F	CCATGGAAGCATGTCTGGTG	53	132–153	6	N	6
			R	TGCTTCCTGATTTCAAAGTTGC				S	2
B109	SIDGS	GA	F	TGTACAAGCGGATAAGTTTTGG	54	211–233	10	N	8
			R	TGTAACTGTCCTCTCGACTAAG				S	9
B126	SIDGS	GA	F	AGGTGGCTTAGTGGTCTAGTG	54	168–192	12	N	8
			R	AGTACCAACAACCACTATCCTC				S	9
D117	SIDGS	TAGA	F	TTCTTTGGGTTCAGCGATAG	54	226–246	6	N	5
			R	GGCTTTCCAAGATGTCAATC				S	5

Species of origin: CGS = Columbian ground squirrel (Stevens et al. 1997), NIDGS = Northern Idaho ground squirrel (May et al. 1997), SIDGS = Southern Idaho ground squirrel (this study). Allele number is reported across subspecies as well as for the northern (N) and southern (S) subspecies separately.

L15774, CGACGTTGTAAAACGACGGCCAG TACATGAATTGGAGGACAACCAGT and H1 6498, GGAAACAGCTATGACCATGATTACG CCTGAACTAGGAACCAGATG (Paetkau and Strobeck 1996). PCR consisted of 55 cycles (95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min), with an initial denaturation step of 95 °C for 5 min and final extension at 72 °C for 5 min. PCR products were cleaned with ExoSAPit (US Biochemical Corp.) , sequenced in the forward direction with Big Dye kit version 3.0 (Perkin Elmer), and analyzed on an ABI 377 automatic sequencer according to the manufacturers' protocols.

Statistical analyses

Tests for Hardy–Weinberg equilibrium were completed with the program GENEPOP (available at http://wbiomed.curtin.edu.au/genepop/; Raymond and Rousset 1995), linkage equilibrium was tested using the program FSTAT (Goudet 1995, 2002), and a standard Bonferroni correction was applied for both sets of tests (Ott and Longnecker 2001). Allelic diversity (alleles per locus) and observed and expected heterozygosities for each population were calculated in GENEPOP. Because allelic diversity is strongly affected by sample size (Petit et al. 1998), we calculated allelic richness for each population, based on a sample size of 10, using the program FSTAT. We used a Mann–Whitney test to contrast differences in heterozygosity and allelic richness between subspecies and a Kruskal–Wallis test to evaluate differences in diversity levels among populations within each subspecies (Systat Software Inc. 2002). To evaluate inbreeding, we also calculated *f*is values for each population using FSTAT.

We quantified genetic divergence among populations in three ways. First, Weir and Cockerham's (1984) F_{ST} statistic, θ, was calculated in GENEPOP. Second, Nei's standard genetic distance, D_S (Nei 1972, 1978), was calculated using the ''gnkdst'' program in the Dispan package (Ota 1993). Finally, an assignment test (Paetkau et al. 1995) was conducted using the ''Doh assignment test calculator'' (Brzustowski 2002), to detect possible migrants between populations. Following calculation of divergence measures, groups of similar populations were tested for significant divergence from other groups, using hierarchical Analysis of Molecular Variance (AMOVA) in the program Arlequin (Schneider et al. 2000).

Slatkin's (1993) isolation-by-distance model was used to assess correlation between genetic and geographic distances, and significance was evaluated in GENEPOP using a Mantel test with 1000 permutations. F_{ST} was linearized with the ratio F_{ST} /(1−F_{ST}), as in Rousset 1997), and regressed against the natural logarithm of geographic distance.

Four of the eight microsatellite loci used in our study were developed for Columbian ground squirrels (Stevens et al. 1997) and four were designed for Idaho ground squirrels. Microsatellite loci may exhibit lower variability when amplified using primers developed in other species, a phenomenon know as ''ascertainment bias'' (Primmer et al. 1996; Webster et al. 2002). Therefore, to test for ascertainment bias, we calculated heterozygosity and allelic richness independently for primers isolated in each species and used paired *t*-tests to assess differences between the two primer sets.

Mitochondrial sequences were aligned using Sequencher 4.1 (Gene Codes Corporation), and duplicate haplotypes within each population were pruned. Sequences were analyzed with the program PAUP 4.0b10 (Swofford 2002). Heuristic searches were conducted using both parsimony and maximum likelihood optimality criteria, using PAUP default settings. Distance method neighbor-joining defaults also were used to construct a neighbor-joining tree. Nucleotide diversity (π), haplotype diversity (h), and average number of pairwise differences within and between populations were calculated in Arlequin.

Results

Microsatellite analyses

Microsatellite amplification was successful for all 467 samples. Error rates ranged from 0.68% (locus IGS-110b) to 2.65% (locus GS17) per locus (and averaged 1.64%). We tested for Hardy–Weinberg equilibrium and evaluated linkage equilibrium to assess independence of loci. Following a Bonferroni correction, only one population (Sand Hollow) was out of Hardy–Weinberg equilibrium at only one locus (B109), showing an excess of

homozygotes. There was no evidence that any locus was out of equilibrium consistently; therefore we believe the frequency of allelic dropout or false alleles is very low for these loci. No tests of linkage equilibrium were rejected at the global $P < 0.05$ level after a Bonferroni correction, and therefore we assumed independence of loci.

Contrary to anticipated results, NIDGS exhibited higher levels of genetic diversity than SIDGS. NIDGS exhibited an average expected heterozygosity of 0.58 and average allelic richness of 3.61, while SIDGS had an average expected heterozygosity of 0.43 and average allelic richness of 3.02 (Table 2). Differences between subspecies were significant for both heterozygosity (Mann–Whitney $U = 56$, $P = 0.001$), and allelic richness (Mann–Whitney $U = 49$, $P = 0.015$). Even when low-diversity, peripheral populations of SIDGS (Henley Basin, Rolling Hills, and Squaw Butte) were removed, the heterozygosity difference between subspecies remained significant (adjusted SIDGS mean $= 0.49 \pm 0.006$; Mann–Whitney $U = 35$, $P = 0.004$).

Within each subspecies, differences in diversity across all populations were not statistically significant. Within NIDGS, there were no significant differences among populations for either heterozygosity (Kruskal–Wallis $= 0.49$, $P = 0.998$) or allelic richness (Kruskal–Wallis $= 0.516$, $P = 0.998$). Similarly, there were no significant differences among populations of SIDGS for either heterozygosity (Kruskal–Wallis $= 7.513$, $P = 0.378$) or allelic richness (Kruskal–Wallis $= 11.792$, $P = 0.108$). Although not statistically significant, lower levels of genetic diversity were noted in some populations within each subspecies. For example, within SIDGS, the populations at Henley Basin and Squaw Butte both exhibited relatively low levels of genetic diversity (Table 2). Regardless, *f*is values were not significant (global $P > 0.05$) for any populations, indicating a lack of inbreeding.

Patterns of genetic differentiation among populations differed between the subspecies. We used three measures to evaluate genetic divergence among populations: F_{ST}, D_S, and assignment tests. All three measures produced similar results in both subspecies. Pairwise F_{ST} (θ) values indicated low to moderate relative levels of differentiation between populations of NIDGS and low to high levels of genetic differentiation between SIDGS populations (Table 3). Nei's genetic distance statistics, D_S, revealed a similar pattern (Table 3). Assignment tests for SIDGS were consistent with θ values, indicating exchange of individuals (i.e. high mis-assignment rates) among populations that exhibited low levels of divergence, and no exchange with the two most isolated populations, Henley Basin and Rolling Hills (Table 4). For the NIDGS, assignment tests indicated rates of exchange that were relatively lower (9.68–23.08% mis-assigned) than documented between the non-isolated SIDGS populations (12.12–50.91%); these patterns also were generally consistent with θ values.

Differentiation between the two subspecies was moderate to high. Across subspecies, pairwise F_{ST} values between populations ranged from 0.20 to 0.46 (mean $= 0.29 \pm 0.008$; Table 3). In two cases (Henley Basin and Rolling Hills), relative levels of differentiation between populations of SIDGS were as high as differentiation between the subspecies.

Because high levels of differentiation were observed between SIDGS populations in the northern and central portions of their distribution, we tested for significant divergence between these two groups of populations. The Rolling Hills and Henley Basin populations (RH-HB) were designated as one group because of their high divergence from more southerly groups. The second group consisted of the remaining 6 populations. A hierarchical AMOVA, which examines the partitioning of genetic variation among populations versus between groups, indicated that the two designated groups were significantly differentiated. Groups accounted for 19.9% of the variability ($P < 0.000$), while variation partitioned within groups and within populations was 9.5 and 70.7%, respectively. There also was support for designating three groups: Henley Basin, Rolling Hills, and the remaining 6 populations (22.6%, $P < 0.000$). Hence, SIDGS populations can be grouped into two distinct genetic complexes, although the RH-HB complex may be further subdivided.

While NIDGS populations did not exhibit isolation by distance (Mantel test $P > 0.1$), populations of SIDGS did (Mantel test $P < 0.005$). However, when the most isolated populations of SIDGS (Henley Basin and Rolling Hills) were excluded, the relationship disappeared ($P > 0.2$). Therefore, no strong correlation between genetic and geographic distances was apparent in either subspecies.

We tested for ascertainment bias by contrasting diversity values observed for Columbian and Idaho

Table 2. Northern and southern Idaho ground squirrel genetic diversity

Northern Idaho ground squirrel

Locus	Summit Gulch				Tree Farm				Squirrel Manor				Cold Springs			
	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e
GS3	31	2	2.00	0.39	56	2	2.00	0.49	18	2	2.00	0.50	26	2	2.00	0.51
GS12	31	5	4.24	0.74	61	7	5.50	0.72	18	6	5.58	0.75	26	6	5.37	0.74
GS17	30	4	3.95	0.66	61	5	4.72	0.74	18	4	3.81	0.68	24	3	2.98	0.58
GS26	31	4	3.39	0.58	60	4	2.51	0.21	17	3	2.59	0.35	26	4	3.00	0.38
IGS-110b	31	2	1.97	0.23	60	4	2.58	0.23	18	4	3.97	0.68	26	2	1.92	0.18
B109	31	6	4.36	0.55	61	5	3.75	0.52	18	6	4.66	0.64	24	5	4.60	0.72
B126	31	6	4.81	0.76	61	5	4.35	0.74	18	5	4.11	0.69	26	5	4.85	0.78
D117	31	5	4.72	0.73	61	4	3.50	0.63	18	4	3.81	0.66	26	4	3.78	0.71
Ave			3.68	0.58			3.61	0.54			3.81	0.62			3.56	0.57
SE			0.40	0.07			0.43	0.08			0.40	0.05			0.46	0.07

Locus	Chipmunk Springs				Lost Valley				Price Valley			
	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e
GS3	10	2	2.00	0.44	21	2	2.00	0.40	31	2	2.00	0.47
GS12	10	5	5.00	0.74	23	5	4.65	0.69	32	6	4.84	0.72
GS17	10	4	4.00	0.70	23	4	3.42	0.55	32	3	2.51	0.32
GS26	10	2	2.00	0.34	23	2	2.00	0.41	32	2	2.00	0.40
IGS-110b	10	2	2.00	0.10	23	4	3.78	0.66	32	5	4.64	0.63
B109	10	4	4.00	0.75	23	4	3.66	0.58	31	5	4.94	0.79
B126	10	3	3.00	0.53	23	5	4.30	0.69	32	4	3.31	0.66
D117	10	5	5.00	0.73	23	5	4.80	0.78	32	5	4.89	0.79
Ave			3.38	0.54			3.58	0.59			3.64	0.60
SE			0.46	0.08			0.38	0.05			0.47	0.06

NIDGS		
	AR_{10}	H_e
Ave	3.61	0.58
SE	0.05	0.01

SIDGS		
	AR_{10}	H_e
Ave	3.02	0.43
SE	0.23	0.03

Southern Idaho ground squirrel

Locus	Squaw Butte				Bissel Creek				Sand Hollow				Clay Peak			
	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e
GS3	33	1	1.00	0.00	55	2	1.33	0.04	21	2	1.87	0.14	24	1	1.00	0.00
GS12	33	2	1.90	0.17	55	6	3.67	0.54	21	4	3.86	0.64	24	5	3.80	0.63
GS17	30	4	3.95	0.71	55	7	5.19	0.63	21	4	3.47	0.59	23	4	3.42	0.65
GS26	33	2	1.97	0.24	54	3	2.88	0.49	21	3	2.97	0.48	24	3	2.75	0.30
IGS-110b	33	2	1.90	0.17	55	2	1.92	0.20	21	2	1.87	0.14	24	1	1.00	0.00
B109	33	4	3.18	0.42	54	8	5.26	0.57	21	5	3.94	0.59	24	6	4.47	0.61
B126	28	5	3.94	0.68	45	6	5.05	0.77	19	6	5.04	0.76	22	5	4.92	0.80
D117	33	4	2.82	0.55	54	5	4.94	0.79	21	4	3.41	0.51	24	5	4.61	0.75
Ave			2.58	0.37			3.78	0.50			3.30	0.48			3.25	0.47
SE			0.38	0.09			0.56	0.09			0.38	0.08			0.55	0.12

Locus	Holland Gulch				Skow				R.Hills/H.Cem.				Henley Basin			
	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e	n	A	AR_{10}	H_e
GS3	11	1	1.00	0.00	19	1	1.00	0.00	82	1	1.00	0.00	21	1	1.00	0.00
GS12	11	3	3.00	0.66	19	4	3.43	0.49	82	5	4.32	0.64	21	3	2.48	0.52
GS17	11	4	4.00	0.69	19	5	4.43	0.72	82	4	2.81	0.32	21	2	1.48	0.05
GS26	11	4	3.91	0.57	19	3	2.96	0.46	79	3	2.94	0.58	21	2	2.00	0.32
IGS-110b	11	1	1.00	0.00	19	2	1.53	0.05	82	1	1.00	0.00	21	1	1.00	0.00
B109	11	5	4.99	0.71	19	6	5.05	0.79	82	3	2.74	0.48	21	3	2.99	0.59
B126	11	5	4.81	0.65	16	4	3.62	0.66	82	4	3.34	0.56	21	2	2.00	0.42
D117	11	5	4.99	0.68	19	5	4.89	0.78	81	3	2.98	0.60	21	2	1.48	0.05
Ave			3.46	0.50			3.36	0.50			2.64	0.40			1.80	0.24
SE			0.59	0.11			0.53	0.11			0.40	0.09			0.25	0.09

Sample size (n), alleles per locus (A), allelic richness based on a sample size of 10 (AR_{10}), and expected heterozygosity (H_e) are reported for each population.

Table 3. Pairwise F_{ST} values (below diagonals) and Nei's standard genetic distances (above diagonals) between populations for both northern and southern Idaho ground squirrels

	NIDGS							*SIDGS*							
Population	**SG**	**TF**	**SM**	**CS**	**ChS**	**LV**	**PV**	**SB**	**BC**	**SH**	**CP**	**HG**	**Sk**	**RH**	**HB**
Summit Gulch (SG)		0.18	0.21	0.11	0.24	0.30	0.23	0.52	0.47	0.48	0.43	0.46	0.43	0.41	0.42
Tree Farm (TF)	0.12		0.14	0.09	0.24	0.39	0.25	0.64	0.56	0.59	0.49	0.54	0.49	0.53	0.52
Squirrel Manor (SM)	0.12	0.10		0.12	0.33	0.28	0.16	0.49	0.51	0.60	0.51	0.57	0.48	0.61	0.51
Cold Springs (CS)	0.07	0.07	0.07		0.20	0.25	0.15	0.45	0.44	0.50	0.41	0.46	0.37	0.55	0.58
Chipmumk Springs (ChS)	0.14	0.16	0.17	0.12		0.30	0.32	0.51	0.38	0.37	0.28	0.35	0.34	0.60	0.58
Lost Valley (LV)	0.15	0.21	0.14	0.14	0.16		0.10	0.64	0.60	0.70	0.56	0.55	0.48	0.63	0.65
Price Valley (PV)	0.13	0.15	0.09	0.09	0.17	0.06		0.53	0.46	0.48	0.42	0.46	0.41	0.62	0.64
Squaw Butte (SB)	0.32	0.36	0.31	0.30	0.36	0.36	0.32		0.06	0.15	0.12	0.17	0.10	0.41	0.39
Bissel Creek (BC)	0.25	0.29	0.25	0.24	0.23	0.28	0.24	0.08		0.04	0.04	0.05	0.06	0.37	0.41
Sand Hollow (SH)	0.25	0.30	0.27	0.26	0.23	0.30	0.24	0.17	0.04		0.04	0.10	0.11	0.42	0.46
Clay Peak (CP)	0.24	0.28	0.26	0.24	0.20	0.28	0.23	0.14	0.04	0.04		0.06	0.05	0.34	0.38
Holland Gulch (HG)	0.23	0.28	0.25	0.24	0.22	0.26	0.23	0.18	0.04	0.09	0.07		0.06	0.35	0.47
Skow (Sk)	0.23	0.26	0.23	0.21	0.21	0.24	0.21	0.12	0.05	0.10	0.05	0.06		0.25	0.38
Rolling Hills/Hillcrest (RH)	0.29	0.33	0.35	0.34	0.37	0.36	0.35	0.35	0.28	0.32	0.28	0.29	0.23		0.20
Henley Basin (HB)	0.33	0.36	0.38	0.39	0.46	0.41	0.39	0.42	0.33	0.40	0.37	0.43	0.37	0.26	

0.05-0.14 low differentiation
0.15-0.24 moderate differentiation
≥0.25 high differentiation

F_{st} values indicating relative levels of differentiation, as suggested by publishing guidelines (Frankham et al., 2002; Hartl and Clark, 1997), are indicated by shading

Table 4. Results of assignment tests for populations of northern and southern Idaho ground squirrels. Assignments are from the population on the left (capture population) to populations across the top row. Also reported is the percentage of individuals assigned to populations other than the capture population (% mis-assigned)

Northern Idaho ground squirrel

	SG	TF	SM	CS	ChS	LV	PV	% mis-assigned
SG	28	2	1	0	0	0	0	9.68%
TF	0	55	2	3	1	0	0	9.84%
SM	0	2	14	0	0	1	1	22.22%
CS	0	2	2	20	0	2	0	23.08%
ChS	0	1	0	0	8	1	0	20.00%
LV	0	0	1	0	0	18	4	21.74%
PV	0	0	0	1	0	4	27	15.63%

Southern Idaho ground squirrel

	SB	BC	SH	CP	HG	Sk	RH	HB	% mis-assigned
SB	29	3	0	0	0	1	0	0	12.12%
BC	4	27	8	9	4	3	0	0	50.91%
SH	0	4	15	1	0	1	0	0	28.57%
CP	0	2	2	16	1	3	0	0	33.33%
HG	0	4	0	0	6	1	0	0	45.45%
Sk	0	1	1	4	0	13	0	0	31.58%
RH	0	0	0	0	0	0	82	0	0.00%
HB	0	0	0	0	0	0	0	21	0.00%

ground squirrel microsatellite primers. Heterozygosity values calculated for the four Columbian ground squirrel primers ($H_e = 0.449 \pm 0.027$) were significantly lower than for Idaho ground squirrel primers ($H_e = 0.551 \pm 0.03$; n = 15; paired $t = -4.677$; $P = 0.000$). Similarly, allelic richness was significantly lower for Columbian ground squirrel primers (AR = 2.963 ± 0.127) than for Idaho ground squirrel primers (AR = 3.644 ± 0.180; paired $t = -5.337$; $P = 0.000$). In these analyses, Idaho ground squirrels were treated as one species. However, percent reduction in heterozygosity was similar for both subspecies when evaluated independently using Columbian ground squirrel primers (16 and 19% for NIDGS and SIDGS, respectively). Hence, ascertainment bias was observed for Columbian ground squirrel primers used in this study, and the pattern was consistent across subspecies of Idaho ground squirrels.

Preliminary mitochondrial analyses

A portion of the mitochondrial cytochrome b gene (84 bp) and control region (474 bp) was sequenced for a total of 56 samples (21 NIDGS and 35 SIDGS). Samples from the Chipmunk Springs population did not produce long enough sequences to be informative in the analyses, but all other populations were represented by two to five sequences ≥400 bp (≥350 bp control region) in length. A total of 558 bp (474 bp control region) were used for the phylogenetic analyses in PAUP, while 480 bp (404 bp control region) were used for the measures of diversity, because Arlequin requires equal sequence lengths.

In marked contrast to patterns observed with microsatellites, NIDGS exhibited lower mitochondrial diversity than SIDGS. A total of three haplotypes were identified in NIDGS, as opposed to nine in SIDGS (Table 5). There were no haplotypes shared between the two subspecies. Additionally, because each population of NIDGS was fixed for one haplotype, that subspecies exhibited lower mean haplotype diversity and nucleotide diversity than SIDGS (Table 5).

Unlike mtDNA diversity levels, patterns of mtDNA divergence among populations were generally consistent with those observed for

Table 5. Results of preliminary analysis of mitochondrial diversity in northern and southern Idaho ground squirrels

Population	n	Haplotype	*h*	SE	π	SE
Northern Idaho ground squirrel						
Tree Farm	2	J	0.00	0.00	0.0000	0.0000
NIDGS1[a]	14	K	0.00	0.00	0.0000	0.0000
Lost Valley	5	L	0.00	0.00	0.0000	0.0000
Southern Idaho ground squirrel						
Squaw Butte	3	I	0.50	0.27	0.0042	0.0035
	1	A				
Bissel Creek (N)	4	C	0.70	0.22	0.0033	0.0027
Bissel Creek	1	D				
Bissel Creek (S)	1	I				
Sand Hollow[b]	2	C	0.67	0.20	0.0014	0.0016
	2	D				
	1	I				
Clay Peak	4	E	0.00	0.00	0.0000	0.0000
Holland Gulch	4	A	0.40	0.24	0.0008	0.0011
	1	B				
Skow	2	A	0.67	0.20	0.0042	0.0035
	2	F				
Rolling Hills	4	G	0.00	0.00	0.0000	0.0000
Henley Basin	3	H	0.00	0.00	0.0000	0.0000
Average for SIDGS			**0.37**	0.11	**0.0017**	0.0007

Sample sizes (n), haplotypes, haplotype diversity (h), and nucleotide diversity (π) are reported.
[a]The NIDGS1 haplotype was fixed in the Cold Springs, Price Valley, Summit Gulch, and Squirrel Manor populations.
[b]The sequence from the Sand Hollow sample with haplotype I was too short to include in diversity estimates.

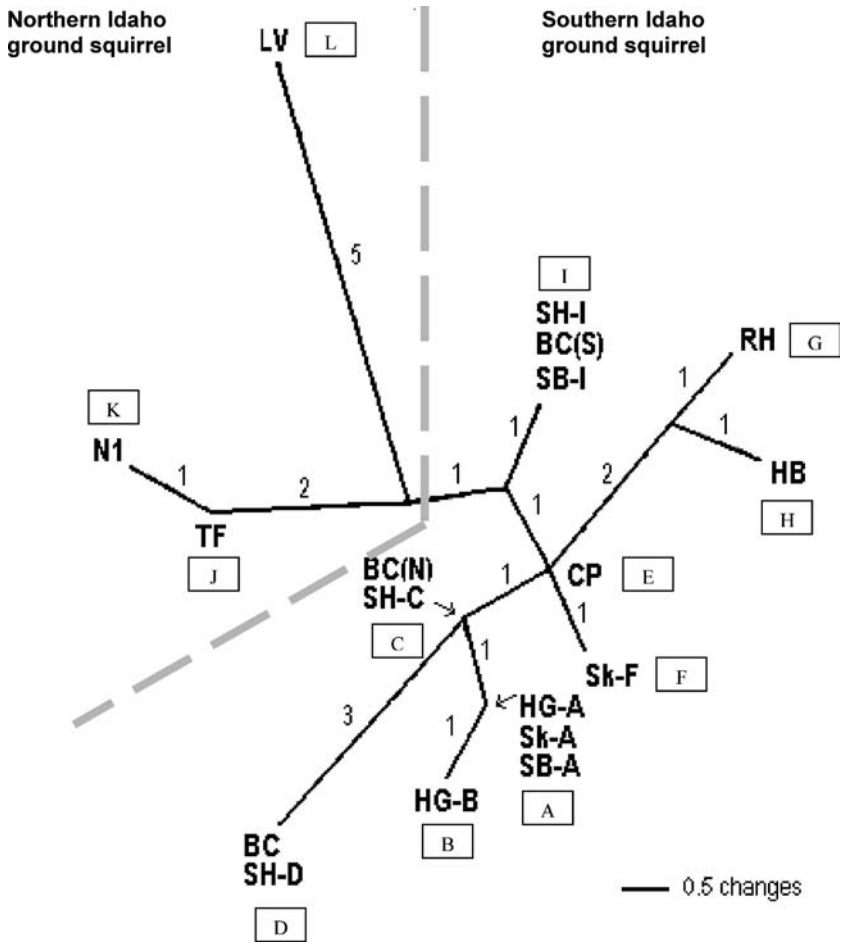

Figure 2. Preliminary phylogenetic tree generated with parsimony and maximum likelihood methods for mitochondrial sequences of northern and southern Idaho ground squirrels. Number of substitutions is shown above each line, and haplotypes are written in boxes. Population abbreviations are as in Figure 1, and population-haplotype abbreviations (e.g. SH-I, SH-C, SH-D) are used for populations containing more than one haplotype.

microsatellite data. An unrooted phylogenetic tree was created using parsimony, maximum likelihood, and distance methods, and all three methods produced the same topology (Figure 2). Within SIDGS, samples from the same population did not cluster together, with the exceptions of Clay Peak, Rolling Hills, and Henley Basin. These results suggest low divergence among the central SIDGS populations, a pattern that is consistent with results of microsatellite analyses. One important disparity between the two genetic marker types was noted in the Lost Valley population of NIDGS, which was fixed for a mtDNA haplotype as different from other haplotypes in its own subspecies (6–7 substitutions) as from SIDGS haplotypes (5.5–9 substitutions).

Discussion

Because loss of genetic diversity can impact fitness and evolutionary potential, the maintenance of genetic diversity is a conservation priority for rare or declining species. Understanding patterns of diversity and divergence in remaining populations is important for designing management strategies to mitigate for the loss of genetic diversity. This is particularly true in fragmented habitats, where natural gene flow may be interrupted and populations become simultaneously small and isolated. Both subspecies of Idaho ground squirrels occur in fragmented habitats, and both face genetic and demographic challenges due to their rapid decline in numbers. For Idaho ground squirrels, knowl-

edge about genetic differences among populations is essential for effective conservation planning for both subspecies. Additionally, we contrasted patterns of persisting variability between the subspecies to assess potential processes underlying current genetic patterns.

Microsatellite diversity and divergence

Based on conservation status and presumed time since isolation, we hypothesized that NIDGS would exhibit lower levels of genetic diversity within populations and greater divergence among populations. However, observed patterns differed from those expected, in terms of both genetic diversity and divergence. The most striking result in this study was the difference in levels of microsatellite diversity between the two subspecies of Idaho ground squirrels. In contrast to predictions, SIDGS populations exhibited consistently lower levels of microsatellite diversity than NIDGS populations. Additionally, the SIDGS exhibited a more complex pattern of divergence than predicted; we observed low divergence among centrally located populations of SIDGS and high divergence of the peripheral SIDGS populations, in contrast to only moderate levels of divergence among NIDGS populations.

A number of different processes may potentially explain the patterns of microsatellite diversity and divergence that we observed in the Idaho ground squirrel species. One plausible explanation for lower microsatellite diversity in SIDGS is that those populations may have been isolated for longer periods of time than previously believed. Alternatively, plague or other disease, widespread poisoning campaigns in the 1930s and '40s (E. Yensen, pers. comm.), or other factors could have caused a subspecies-wide bottleneck prior to isolation of individual populations. In fact, connectivity could have contributed to population declines in the case of disease, by increasing transmission of pathogens or parasites among populations (Yensen and Sherman 2003). The degree of differentiation among populations can help decipher which of these two explanations is most likely; high differentiation would indicate that isolation has been a major factor in the decline of genetic diversity. Two SIDGS populations (Henley Basin and Rolling Hills) exhibited a high degree of differentiation from all other populations. These two are the most geographically isolated, occurring on or near the periphery of current SIDGS distribution (see Figure 1). However, other populations, which are less geographically isolated, exhibited low to moderate differentiation based on F_{ST} values; several population pairs in the central portion of SIDGS range exhibited high gene flow and no significant differentiation, yet still had relatively low heterozygosity levels. In fact, when the lowest-diversity, peripheral populations (Henley Basin, Rolling Hills, and Squaw Butte) were excluded, central SIDGS populations still exhibited significantly lower heterozygosity than did NIDGS populations. Therefore, a subspecies-wide bottleneck, followed by isolation of some populations, is a likely explanation for the observed patterns of microsatellite diversity and divergence in SIDGS.

Populations on the periphery of the SIDGS distribution exhibited particularly low levels of microsatellite diversity, as well as high divergence. This may be due in part to geographical features that increase isolation and subsequent loss of genetic diversity in peripheral populations. For example, the Weiser River, which separates Henley Basin and Rolling Hills from other populations, may act as a barrier to gene flow. Similarly, the higher elevation of Squaw Butte may decrease gene flow into that population. Additionally, populations on the extremes of species ranges may exhibit lower genetic diversity than core populations because gene flow is not occurring from multiple directions (Schwartz et al. 2003), or because effective population sizes are expected to be smaller in peripheral than in core populations (Schwartz et al. 2003; Vucetich and Waite 2003). Low diversity levels on the periphery of the species distribution have been observed in other mammals, such as wolverines (*Gulo gulo*; Kyle and Strobeck 2002), grizzly bears (*Ursus arctos*; Paetkau et al. 1998), and lynx (*Lynx canadensis*; Schwartz et al. 2003). In SIDGS, the Squaw Butte population, as well as the extreme examples of Henley Basin and Rolling Hills, are consistent with this pattern.

Although NIDGS populations exhibited relatively high levels of microsatellite diversity, patterns of divergence suggested a moderate degree of isolation among populations when compared with central SIDGS populations. For example, assignment tests revealed apparently lower levels of

exchange occurring among NIDGS populations (15.4% assigned to other populations) than among central SIDGS populations (35% excluding the two peripheral populations). Despite moderate divergence among populations, we did not observe isolation by distance in NIDGS. This is somewhat surprising, given that Gavin et al. (1999) documented isolation by distance for NIDGS using allozymes. However, the five polymorphic allozyme loci used in their study were biallelic, and microsatellites represent a more detailed survey of genetic diversity.

Additionally, Gavin et al. (1999) sampled a different subset of populations, which covered a slightly more extensive range north to south than was sampled in the current study; the geographic range of NIDGS populations included in our analysis was approximately 22 km across, as opposed to distances as great as 68 km between the most extreme populations of SIDGS. Lastly, the montane meadows inhabited by NIDGS were likely historically more disjunct than the sage-steppe habitats of SIDGS. Barriers, such as forest habitats, may confound the isolation-by-distance effect, as genetic relationships among populations are not expected to fit an isolation-by-distance model in the presence of barriers to dispersal (Hellberg, 1995).

An understanding of genetic patterns within and between subspecies of Idaho ground squirrels helps define their current conservation status from a genetic perspective. NIDGS exhibited low to moderate divergence at microsatellite loci among all populations but appeared to have tolerated some degree of isolation without losing genetic diversity. However, because of ongoing forest encroachment, NIDGS are more isolated now than in the past (Gavin et al., 1999), which may continue to negatively impact gene flow and genetic diversity unless connectivity among populations is restored. In contrast, SIDGS exhibited low microsatellite diversity in all populations and appeared to have undergone a subspecies-wide bottleneck. Populations in the central portion of SIDGS distribution remain connected, while peripheral populations have experienced isolation and further loss of genetic diversity.

Ascertainment bias

When making inferences about genetic diversity across species, it is important to consider the possible effects of ascertainment bias (Garner et al. 2005). Within our study, both heterozygosity and allelic richness were lower when only primers from Columbian ground squirrels were considered, indicating an ascertainment bias. Although Idaho ground squirrels were considered one species in this analysis, the trend was consistent across both subspecies. Because of ascertainment bias, caution is urged when comparing diversity levels in Idaho ground squirrels to ''typical'' diversity levels reported for other sciurids (family mean 0.62; Garner et al. 2005). However, a contrast between the two subspecies indicates that microsatellite diversity levels in NIDGS are reasonably high, while diversity levels in SIDGS populations are low, suggesting the need for mediation in some SIDGS populations.

Preliminary mitochondrial analyses

Because many mammals, including ground squirrels, exhibit male-biased dispersal and female philopatry (Sherman and Morton 1984), genetic structure based on maternally inherited mtDNA may differ from patterns detected by nuclear loci (Moritz 1994; Paetkau, et al. 1997). For this reason, we conducted preliminary analyses of a portion of the mitochondrial genome on a subset of samples, to evaluate whether different matrilineal patterns may be present in the Idaho ground squirrel and to assess the need for further mitochondrial analyses.

In contrast to diversity levels detected with microsatellites, mtDNA haplotype diversity was lower in NIDGS than in SIDGS. For NIDGS, possible explanations include a founder event during northward expansion, which occurred long enough in the past that microsatellite diversity has rebounded. Indeed, because of their differing mutation rates, mitochondrial and microsatellite markers resolve genetic structure at different time depths (Bossart and Prowell 1998; Crandall et al. 2000). Though preliminary, the differing trends observed for the two marker types in NIDGS suggest an ancient bottleneck or founder event, rather than a recent reduction of genetic diversity. Reduced mtDNA diversity relative to microsatellite diversity may also reflect male-biased dispersal during northward expansion. Regardless, no haplotypes were shared between the subspecies, which suggests a significant period of isolation. Despite the

higher mitochondrial diversity of SIDGS relative to NIDGS, mitochondrial variability in SIDGS was only moderate to low when compared to other mammals (Simonsen et al. 1998; Rosel et al. 1999; Vilà et al. 1999; Wilson et al. 2000; Girman et al. 2001; Tatsuo et al. 2001). Therefore, the diversity levels observed for SIDGS in the preliminary mtDNA study do not necessarily contradict low levels of diversity observed for microsatellite loci.

Population divergence and phylogenetic relationships suggested by our preliminary mtDNA analyses also did not differ widely from patterns of divergence observed with microsatellites, with the exception of the Lost Valley population of NIDGS, which was highly diverged from other populations. Additionally, some populations (such as Tree Farm and Clay Peak) that showed limited gene flow with other populations based on microsatellites did not share mitochondrial haplotypes with other populations, suggesting that female gene flow is more restricted than male gene flow. However, sample sizes were small, and a more thorough investigation of mitochondrial diversity and structure in Idaho ground squirrels is warranted.

Conservation implications

Recent conservation efforts for Idaho ground squirrels have included translocations, captive breeding, and habitat restoration (Haak 1999; U.S. Fish and Wildlife Service 2003). Results of our genetic analyses can aid in assessing the appropriateness of each of these conservation options for Idaho ground squirrels and can provide valuable baseline genetic data for monitoring Idaho ground squirrel recovery.

Given that the two Idaho ground squirrel subspecies display striking morphological and behavioral differences (Yensen 1991) over a relatively small geographical area, local adaptation within subspecies also is probable. Genetic mitigation of small populations often can become a balancing act between maintaining genetic diversity and safeguarding unique local adaptations. In several species, including bobwhite quail (*Colinus virginianus*) and a number of fishes, translocation of wild or captive individuals has contributed to decreased fitness in recipient populations (Storfer 1998). Therefore, translocation or supplementation through captive breeding should be planned so as to minimize mixing of highly differentiated populations. Furthermore, when genetic diversity levels within populations or natural gene flow rates among them are already high, actions that artificially boost gene flow may be unnecessary and possibly even detrimental to overall genetic diversity in the species.

In general, NIDGS exhibited high levels of microsatellite diversity and only low to moderate differentiation among populations. Therefore, widespread measures to induce gene flow artificially with translocations among NIDGS populations may not be warranted at this time. Furthermore, captive breeding may not be advisable for NIDGS currently, because of genetic risks inherent in such programs, such as adaptation to captivity and fixation of alleles that may be deleterious in the wild (Briscoe et al. 1992; Lynch and O'Hely 2001; Gilligan and Frankham 2003). NIDGS populations have responded positively to habitat treatments (U.S. Fish and Wildlife Service 2003), and habitat restoration appears to be the most desirable conservation strategy for most NIDGS populations from a genetic standpoint, as well.

In contrast to NIDGS, SIDGS exhibited relatively low levels of microsatellite diversity, high levels of divergence among isolated populations, and low divergence among central populations. Habitat restoration is less likely to successfully connect SIDGS populations, due to the pervasive invasion of exotic weeds in the SIDGS range (Yensen 1991), as well as to long distances and potential barriers isolating peripheral populations. Therefore, translocations or supplementation with captive breeding may become important management tools for maintaining genetic diversity in some SIDGS populations. Additionally, the populations of SIDGS sampled in this study may represent two genetic complexes, one in the northern (Rolling Hills and Henley Basin) and one in the central and southern portions of the subspecies range (including the remaining 6 populations). Low-level exchange of individuals, when deemed necessary to maintain genetic variability, would be best kept within genetic complexes.

Conservation efforts for Idaho ground squirrels will be more likely to succeed if patterns of genetic diversity and population divergence are considered. Observed patterns in Idaho ground squirrels suggest two different management strategies for the two subspecies. Habitat restoration should continue to be a conservation priority for NIDGS,

while low microsatellite diversity in peripheral populations of SIDGS may warrant more direct management, such as translocations or captive breeding. Ultimately, the persistence and full evolutionary potential of both Idaho ground squirrel subspecies may be compromised unless the genetic component of their diversity is conserved.

Acknowledgments

This research was funded by the Idaho Department of Fish and Game through the Wildlife Conservation and Restoration Program, the Bureau of Land Management through the Challenge Cost Share Program, Idaho Power Company, and American Society of Mammalogists Grants-in-Aid of Research. Funding for A. Garner was provided by the Berklund Foundation, University of Idaho, and the Idaho Department of Fish and Game. We also thank the U.S. Fish and Wildlife Service, the U.S. Forest Service, R. Howard, B. Haak, J. Rohlman, L. Schultz, and B. Gould for logistical support in the field. Thanks also to E. Yensen, S. Brunsfeld, and two anonymous reviewers for comments on the manuscript, and to J. Clark, K. Russell, C. Harris, T. Holthuijzen, and D. Evans-Mack. Assistance with sample collection was provided by J. Munger, J. Barrett, D. Collins, D. Sparber, J. Kissler, J. Lee, K. Mallea, R. Collier, M. Collie, K. Lauden, and J. Beals. We appreciate the cooperation of landowners M. Soulen and J. Hinson, J. Dyer and F. Anderson, J. Little, A. Brailsford, P. Skow, and B. Mack, as well as the Rolling Hills Golf Course and Hillcrest Cemetery management. Assistance with DNA extraction was provided by S. Bajimaya, B. Nichols, and C. Holmberg, and mitochondrial sequencing was completed by C. Anderson. We also thank E. Strand for assistance with Arcview and D. Onorato for assistance with Arlequin. Novel microsatellite loci for the southern Idaho ground squirrel were isolated by Genetic Identification Services, Chatsworth, CA.

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