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RESEARCH ARTICLE

Bottlenecks and rescue effects in a fluctuating population of golden-mantled ground squirrels (*Spermophilus lateralis*)

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Abstract Mammal species characterized by highly fluctuating populations often maintain genetic diversity in response to frequent demographic bottlenecks, suggesting the ameliorating influence of life history and behavioral factors. Immigration in particular is expected to promote genetic recovery and is hypothesized to be the most likely process maintaining genetic diversity in fluctuating mammal populations. Most demographic bottlenecks have been inferred retrospectively, and direct analysis of a natural population before, during, and after a bottleneck is rare. Using a continuous 10-year dataset detailing the complete demographic and genetic history of a fluctuating population of golden-mantled ground squirrels (*Spermophilus lateralis*), we analyzed the genetic consequences of a 4-year demographic bottleneck that reduced the population to seven adult squirrels, and we evaluated the potential "rescue effect" of immigration. Analysis of six microsatellite loci revealed that, while a decline in allelic richness was observed during the bottleneck, there was no observed excess of heterozygosity, a characteristic bottleneck signature, and no evidence for heterozygote deficiency during the recovery phase. In addition, we found no evidence for inbreeding depression during or after the bottleneck. By identifying immigrants and analyzing their demographic and genetic contributions, we found that immigration promoted demographic recovery and countered the genetic effects of the bottleneck, especially the loss of allelic richness. Within 3 years both population size and genetic variation had recovered to pre-bottleneck levels, supporting the role of immigration in maintaining genetic variation during bottleneck events in fluctuating populations. Our analyses revealed considerable variation among analytical techniques in their ability to detect genetic bottlenecks, suggesting that caution is warranted when evaluating bottleneck events based on one technique.

Keywords Population bottleneck · Immigration · Rescue effect · Genetic diversity · Golden-mantled ground squirrels · *Spermophilus lateralis*

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Introduction

Demographic bottlenecks can reduce genetic diversity, promote inbreeding depression (Nei et al. 1975; Frankham 1995), and hinder a population's ability to adapt to environmental changes (Frankham et al. 1999; Spielman et al. 2004; Hale and Briskie 2007). All of these conditions are expected to increase a population's risk of extinction (Frankel and Soulé 1981; Saccheri et al. 1998), although questions over the relative importance of genetic diversity to extinction risk in natural populations remain (Lande 1993; Keller et al. 2001; Reed and Frankham 2003; Spielman et al. 2004).

Some mammalian species, particularly those that are characterized by highly fluctuating or cyclical population dynamics, maintain high levels of genetic diversity in the

face of frequent population bottlenecks (Burton et al. 2002; Ehrich and Jorde 2005; Berthier et al. 2006; Redeker et al. 2006; Busch et al. 2007). The development of sound conservation strategies for such species, and in particular evaluating the extent to which genetic concerns should factor into such strategies, requires a better understanding of these demographic and genetic patterns. However, due to a lack of long-term demographic data for many species, past demographic trends are often inferred from a snapshot of genetic data. More empirical studies of natural populations are needed to investigate the genetic signatures of known demographic bottlenecks, clarify why some populations suffer no apparent loss of genetic diversity following bottlenecks, and update our theoretical predictions to reflect inputs from the variable life histories and social structures within species.

Population genetic models, including those used to detect bottlenecks, are often based on simplifying assumptions of randomly mating, closed populations with non-overlapping generations. However, the life histories and social structures within species can vary widely and many are characterized by non-random patterns of mating, inbreeding avoidance, and sex-biased dispersal. All of these factors can dampen the expected genetic consequences of demographic bottlenecks (Busch et al. 2007; Kramer and Sarnelle 2008). The process of dispersal and immigration, in particular, is expected to increase the rate of genetic recovery in post-bottleneck populations and is thought to be the most likely process maintaining high genetic diversity in cyclic or highly fluctuating mammalian populations (Berthier et al. 2006).

Direct comparisons of a natural population before, during, and after a bottleneck are rare, and few studies have both detailed genetic data and demographic information on known native and immigrant individuals during a bottleneck period (Hoelzel et al. 2002; Busch et al. 2007). Here we present the results of a long-term study, during which we followed a population of golden-mantled ground squirrels (*Spermophilus lateralis*) through a natural demographic bottleneck event. Our study is noteworthy in that we were able to monitor both the complete demographic history of this population (i.e., the population was enumerated completely, rather than sampled), and we were able to analyze annual changes in genetic structure and diversity over a 10-year period. During this time, a 4-year demographic bottleneck provided the opportunity to directly evaluate the effects of a bottleneck on the genetic structure of a natural population, and further, to evaluate potential "rescue effects" (Brown and Kodric-Brown 1977) of immigrants on a severely reduced breeding population.

We compare estimates of genetic diversity before, during, and after the bottleneck to test for expected bottleneck signatures (reduced allelic diversity and an initial excess in heterozygosity) (Cornuet and Luikart 1996; Garza and Williamson 2001). In addition, we test for a rescue effect by estimating allelic richness, heterozygosity, and inbreeding (F_{IS}) with and without immigration. Our results support the idea that immigration is vital for maintaining high levels of genetic diversity in highly stochastic populations and illustrate the extent to which immigration contributes to population recovery. Our study also allows us to use empirical data to evaluate the effectiveness of different analytical techniques in detecting genetic bottlenecks—as we show, results can vary, highlighting the need for caution.

Methods

Study site and species

The study was conducted at the Rocky Mountain Biological Laboratory (2900 m elevation), located in the East River Valley, Gunnison County, Colorado (38°58′N, 106°59′W). Vegetation in the valley is a mosaic of subalpine meadows, aspen (*Populus tremuloides*) woodlands, and spruce (*Picea* spp.) forests. *S. lateralis* favor more open habitats (Shick et al. 2006), and in our study area they preferred meadows and avoided aspen woodlands and spruce forests (K. M. Ip, unpublished ms.). The 13-ha study site was a subalpine meadow bordered by the East River to the west and Copper Creek to the south, which formed barriers to dispersal, and aspen woodlands to the north and east that were uninhabited by squirrels. The population of *S. lateralis* in the study area was separated from the nearest localities supporting other ground squirrel populations by >1000 m to the west, 1875 m to the south, 300 m to the north, and 250 m to the east. Our study population occupied geographically distinct habitat. Dispersal events typically involve moves of less than 250 m, however, some can exceed 1000 m (B. R. Jesmer, unpublished ms.). Thus, while the focal population's demographic trajectory was largely independent, there was potential for gene flow between it and other populations.

Spermophilus lateralis is a small (ca. 200 g), hibernating squirrel that is considered asocial (Ferron 1985). The species is strictly diurnal and is readily observable when aboveground; it seeks shelter in underground burrows. At our study site, squirrels emerged from hibernation in May, young of the year typically appeared aboveground during late June through mid-July, and all squirrels entered hibernation during late August and early September. For the species as a whole, age of reproductive maturity is 1 year, females produce 1–2 litters per year with an average litter size of 5.2 offspring, and generation time is 2.45 years (Bronson 1979; Millar and Zammuto 1983).

We censused the squirrel population over a 10-year period (1996–2005) during late May and early June of each

year by live-trapping all squirrels within the study site. Trapped squirrels were weighed, sexed, marked with numbered ear tags and a set of distinct black dye marks added to their pelage. Reproductive status of adult females was indicated by swollen nipples during June, and subsequently confirmed when a litter emerged from the female's burrow. Trapping, combined with visual observations to detect unmarked squirrels, was continued until all squirrels in the study area were trapped and marked. Juveniles born in the study area were identified by trapping and marking them within 1–3 days after they first emerged from their natal burrow. Observations of squirrels throughout the study area were conducted almost daily from early June through late August, allowing identification of immigrants, which were then trapped and marked. DNA samples were collected from all trapped squirrels by plucking 10–20 hairs with attached follicles from the rump. Samples were placed in sealed, paper envelopes and stored at ambient temperature until DNA extraction.

Genetic analysis

DNA was extracted and purified using the Puregene tissue extraction kit and glycogen protocol (Gentra Systems). DNA was amplified at six microsatellite loci developed for closely related sciurid species (Table 1). PCR amplifications were performed in 10 µl reactions containing 1–3 µl template DNA, 0.4 µM of each primer, 1.5–2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 µl BSA (20 mg/ml), one unit of FastStart (Roche Applied Science) or Platimun® (Invitrogen) Taq DNA polymerase, and one unit of Taq buffer. All forward primers except for Sgs 17 were 6-FAM labeled. Amplifications began with initial denaturation at 95°C for 2–4 min, followed by 35–40 cycles of 95°C for 30 s, 52°C for 30–35 s, and 72°C for 1 min; and then a final 10 min extension at 72°C. PCR products were diluted with 10 µl of 98% formamide loading buffer, separated on 5.5% denaturing polyacrylamide gels and visualized with a Molecular Dynamics 595 fluorimager (Belfiore and May 2000). The non-labeled locus, Sgs 17, was gel-stained with a Vistra Green® (Amersham Biosciences) and agarose mix prior to scanning.

Data analysis

The programs FSTAT 2.9.2 (Goudet 1995) and GENEPOP 4.0 (Raymond and Rousset 1995) were used to calculate allele frequencies and test for Hardy–Weinberg equilibrium and linkage disequilibrium. We used program HP-RARE (Kalinowski 2005) to evaluate our sampling adequacy to assess allelic richness in our population in each year. HP-RARE uses rarefaction analysis to account for differences in samples size among populations. As a baseline, we standardized sample size to the largest sample (1997, 62 genes) and then determined, using rarefaction, the expected number of alleles in samples of the observed size in each year. We contrasted observed and expected number of alleles in each year for each locus using contingency analysis.

We used two methodological approaches on three different data sets to test for a genetic bottleneck signature in our population. First, we used program BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999) to test the theoretical prediction that a population bottleneck generates a faster reduction in allelic diversity compared to heterozygosity, and this in turn generates an excess of heterozygotes in the post-bottleneck population. We contrasted all four tests provided by BOTTLENECK, focusing on the two-phase model (TPM) of mutation recommended for microsatellite loci because it is a better fit to observed allele frequency data than the infinite alleles model (IAM)

Table 1 Microsatellite loci used to investigate genetic structure in golden-mantled ground squirrels

Locus	Sequence	Total no. alleles	Species source	Citation
ST 7	F: gAATCTTgACTCCTgAgATA	10	*Spermophilus citellus*	Hanslik and Kruckenhauser (2000)
	R: CCATCTCCTGACATTTAATA			
Bib 4	F: CCTAggTTCAgTCTTCAACACA	11	*Marmota marmota*	Klinkicht (1993), Goossens et al. (1998)
	R: TggTgTTgCCATTgTTCT			
Bib 18	F: ATggTCATggAAgggAAg	8		
	R: ggCATCTTCACAgTTgATCT			
Bib 36	F: AAgAACATgAAgTCTAAACATAA	11		
	R: AAgTgAAACTTTgCCACAC			
Sgs 14	F: CAggTgggTCCATAgTgTTAC	16	*Spermophilus columbianus*	Stevens et al. (1997)
	R: TTgTgCCTCAgCATCTCTTTC			
Sgs 17	F: CAATTCgTggTggTTATATC	4		
	R: CTgTCAACCTATATgAACACA			

and single step model (SSM) (Di Rienzo et al. 1994). For the TPM, we defined 90% of mutations as following a stepwise mutation model and 10% as multistep with a variance (σ_g^2) = 12 for the geometric distribution of number of repeat units per multi-step, as recommended by Garza and Williamson (2001; and see also Hundertmark and Van Daele 2010).

In addition, we tested for a bottleneck signature using the M-RATIO method developed by Garza and Williamson (2001). This approach calculates M, the ratio of the total number of alleles to the range in allele sizes and M_C, the critical value of M determined by simulations described in Garza and Williamson (2001). A bottleneck is detected when the observed average M-ratio is lower than its critical value (defined such that 5% of the simulations fall below M_C). We used program M_P_VAL (Garza and Williamson 2001) to calculate the M-ratio (M) for our population in each year of study and to contrast the observed values of M to a distribution of M values calculated from simulated populations assumed to be at mutation-drift equilibrium. We used program CRITICAL_M (Garza and Williamson 2001) to calculate M_C given the observed sample size in each year. These simulations require three input parameters (θ, p_g, Δ_g). The parameter $\theta = 4\ N_e\mu$, where N_e = effective population size and μ = mutation rate. Effective population sizes were estimated using the sex-ratio equation, $N_e = 4\ N_m N_f/(N_m + N_f)$. A common estimate of microsatellite mutation rate = 5.0×10^{-4} mutations/generation/locus (Garza and Williamson 2001), but Busch et al. (2007) have noted that this value may be low for rodents given their accelerated rate of molecular evolution. Busch et al. (2007) estimated a mutation rate of $\mu = 0.0081$ for the banner-tailed kangaroo rat (*Dipodomys spectabilis*). We explored a range of mutation rates that bracketed this value (0.001–0.010). To provide an even more conservative test, we also used a value of $\theta = 2.0$, which would equate to $N_e = 100$ and $\mu = 0.005$ or $N_e = 1000$ and $\mu = 0.0005$. Thus, our estimates of N_e and μ encompass a wide range of plausible values. We set the percent of mutations larger than a single step $p_g = 0.10$ and the mean size of mutations larger than a single step $\Delta_g = 3.5$ following the recommendation of Garza and Williamson (2001). Each set of simulations comprised 10,000 iterations.

BOTTLENECK and M-ratio analyses were conducted on three different data sets: the data set including all adult *S. lateralis* present on the study site each year, the data set including only native adults and excluding immigrants, and a simulated dataset assuming a closed population with no immigration or emigration. Analyzing the data in this way allowed us to more closely examine the effects of increased immigration following the demographic bottleneck.

Program BOTTLESIM (Kuo and Janzen 2003) was used to create our simulated data set. BOTTLESIM simulates expected changes in observed heterozygosity H_O, expected heterozygosity H_E, total number of alleles A, and the fixation index F ($H_E - H_O/H_E$). Each iteration of BOTTLESIM begins by generating a founder population with the observed number of alleles, allele frequencies, and population size specified by the user (Kuo and Janzen 2003). The user further specifies the degree of generation overlap, the age of reproductive maturity, expected longevity, and mating system. Each subsequent year is simulated by (i) generating a list of surviving individuals, (ii) identifying those that reach reproductive maturity and generating a list of possible reproducing individuals, (iii) identifying individuals who have reached the longevity limit and replacing them with new individuals (genotypes) based on the mating system and changes in population size. Measures of genetic diversity are calculated at the end of each simulated year.

Our simulations were based on a founder population using the number of alleles, allele frequencies, and population size in the year of highest abundance prior to the demographic bottleneck (1997). We ran the model for 1,000 iterations assuming random mating, age of reproductive maturation = 1 year, expected longevity = 2 years, (E. Kneip, personal communication) and 10% generation overlap (simulations with 0 and 100% overlap did not differ substantially). BOTTLESIM allows users to generate multilocus genotypes in each successive year for populations following the observed interannual variation in abundance and based on the empirical genotypic data (Kuo and Janzen 2003). Thus, we were able to simulate expected changes in measures of genetic diversity for our population assuming no immigration or emigration and for which the population size and composition (number of females) in each year followed precisely the observed decline and recovery (Fig. 1).

Our simulated dataset also allowed us to evaluate the power of program BOTTLENECK to detect a genetic bottleneck in our study population. Using BOTTLESIM, we constructed the gentoypes of all individuals in 100 simulated populations, using the input parameters above. At the end of the simulated time period (8 years: 1997–2005), BOTTLESIM generates a genotype file from the last year of each iteration. We then analyzed each simulated population using program BOTTLENECK to determine the number of simulated populations for which a bottleneck event would have been detected (at $\alpha < 0.05$, $\alpha < 0.10$ and $\alpha > 0.10$). In other words, given the initial allele numbers and frequencies at the six loci we examined, we calculated the likelihood of detecting a genetic bottleneck in a closed population experiencing the same demographic decline and recovery as our focal population. As in our original analysis, we contrasted all four BOTTLENECK tests. Using the TPM, we defined 90% of mutations as following a stepwise mutation model and 10% as multistep with a variance

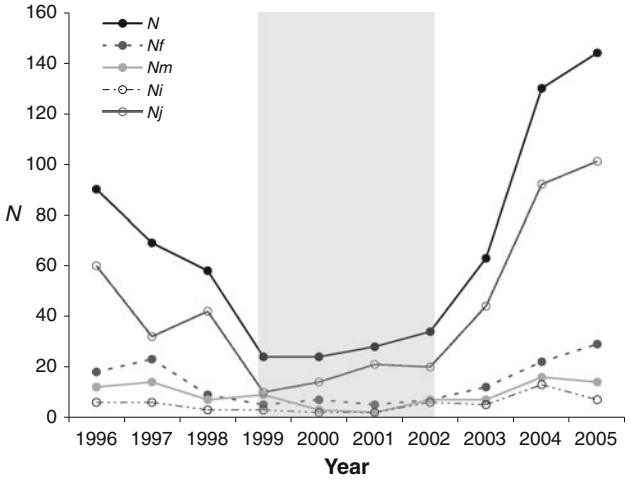

Fig. 1 Population sizes (N) of golden-mantled ground squirrels over the 10-year study, including numbers of females (N_f), males (N_m), immigrants (N_i) and juveniles (N_j). Calculation of total population size includes all adults and juveniles. Bottleneck years are indicated by the *vertical shaded area*

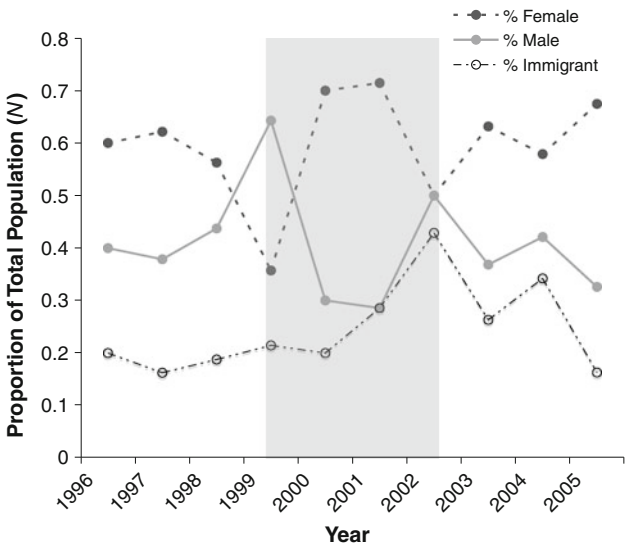

Fig. 2 Proportions of the adult golden-mantled ground squirrel population (N) represented by native resident females, native resident males, and immigrants. Bottleneck years are indicated by the *vertical shaded area*

$(\sigma_g^2) = 12$ for the geometric distribution of number of repeat units per multi-step mutation (Garza and Williamson 2001; Hundertmark and Van Daele 2010).

While our simulated populations allowed us to examine the role of dispersal and immigration in population recovery, they assumed closed populations, with no immigration or emigration. Using the native adults dataset, we were able to investigate the effects of reduced immigration and contrast this to our simulated dataset. The native adults dataset was constructed by excluding all immigrants and the recruited offspring of immigrant females. This had the effect of maintaining some rare immigrant alleles in the population through matings between resident females and transient immigrant males. For this analysis, squirrels born in the study area that did not disperse were considered natives. Immigrants were those that dispersed into the study area from elsewhere and established residency. Adult male and female immigrants, including the offspring of immigrant females recruited into the adult population of subsequent years, were identified using census data. We then could compare bottleneck signatures among our three datasets (all adults, native adults, and simulation data) and estimate the magnitude of a potential rescue effect.

Results

Demographics

We trapped and monitored 523 *S. lateralis* (159 adults) over the 10-year study. Demographic trends revealed a 4-year bottleneck during 1999–2002, when population size was reduced to ≤14 adults, reaching a low of seven adult ground squirrels (two males and five females) in 2001 (Fig. 1). An influx of immigration was apparent in 2002 and again in 2004 (Fig. 2), after which the population returned to and exceeded its pre-bottleneck size.

Genetic variation

A total of 137 adults was successfully genotyped at the six microsatellite loci. Evaluation of sampling adequacy using rarefaction analysis in program HP-Rare (Kalinowski 2005) indicated that we were successful in detecting all alleles for all loci. Differences in the observed versus expected number of alleles were either nil or <1 allele in all years. Contingency analysis revealed no significant differences in observed versus expected number of alleles at any of the six loci (all $\chi^2 < 1.00$, $P > 0.95$). Allele frequencies were normally distributed.

Focusing first on the two observed datasets (i.e., all adults and native adults), tests for Hardy–Weinberg equilibrium revealed no significant deviations within years. However, when analyzed over all years and loci, a significant deviation from Hardy–Weinberg equilibrium was apparent and expected due to the extreme fluctuation in population size over time. When all adults were analyzed, linkage disequilibrium was detected between three pairs of loci: Bib 1 St 7, Bib 18 and Sgs 14, and Bib 18 and Bib 4. There was no evidence for linkage disequilibrium in the native adult dataset.

Mean heterozygosity for all adults ranged from a low of 0.69 in 2001, a bottleneck year, to a high of 0.79 in 1997, a

pre-bottleneck year (Fig. 3). Overall, there was not much change in heterozygosity over the 10-year period and no significant excess of heterozygosity during bottleneck or post-bottleneck years. When immigrants were excluded from analysis, mean heterozygosity ranged from a low of 0.74 in 2004 to a high of 0.81 in 2003. Estimates including only native adults tended to be higher than those including all adults (0.69–0.78), especially during bottleneck years (Fig. 3).

Total allelic richness, summed over all loci and all adults, declined during the bottleneck, reaching a low of 30 alleles in 2001 compared to a high of 49 and 50 alleles in 1997 and 2004, respectively. Allelic richness was even lower when immigrants were excluded from analysis (Fig. 4).

Inbreeding, as measured by F_{IS}, the probability that two alleles in an individual are identical by descent, was consistently at or near zero. This was true for analyses including all adults and those including only natives (Fig. 5). The variance in F_{IS} was greater during bottleneck years compared to non-bottleneck years.

All reproductive adults, including natives and immigrants, were included in estimates of effective population size. Using the biased sex-ratio equation, N_e was lower than the census adult population size as expected, reaching a low of 5.7 in 2001 and a high of 32.2 by the end of the study in 2005 (Table 3).

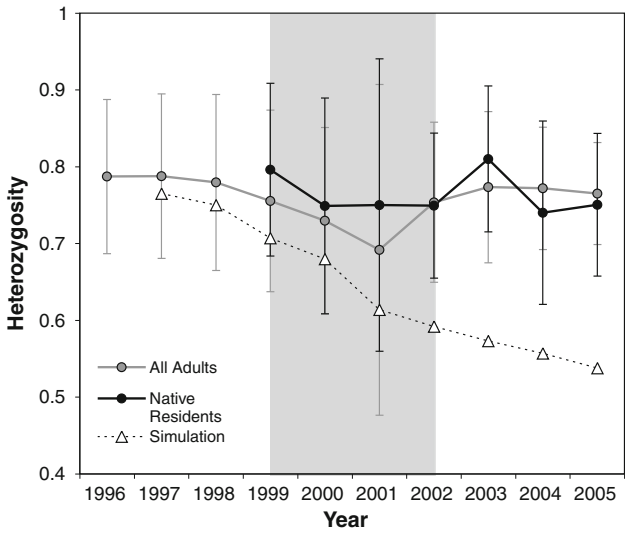

Fig. 3 Observed heterozygosity averaged over six microsatellite loci for golden-mantled ground squirrels. Estimates were calculated for the entire adult population including immigrants (*grey*) and native resident adults only (*black*). Estimates of heterozygosity for simulated populations following the same decline and recovery in the absence of immigration or emigration are indicated by the *dashed line* (*triangles*). *Error bars* represent standard deviations. Bottleneck years are indicated by the *vertical shaded area*

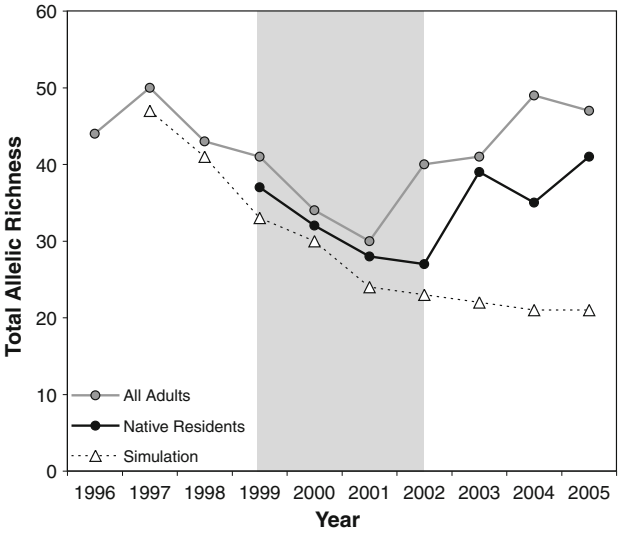

Fig. 4 Allelic richness totaled over six microsatellite loci for golden-mantled ground squirrels. Calculations were conducted for the entire adult population including immigrants (*grey*) and for native resident adults only (*black*). Estimates of allelic richness for simulated populations following the same decline and recovery in the absence of immigration or emigration are indicated by the *dashed line* (*triangles*). Bottleneck years are indicated by the *vertical shaded area*

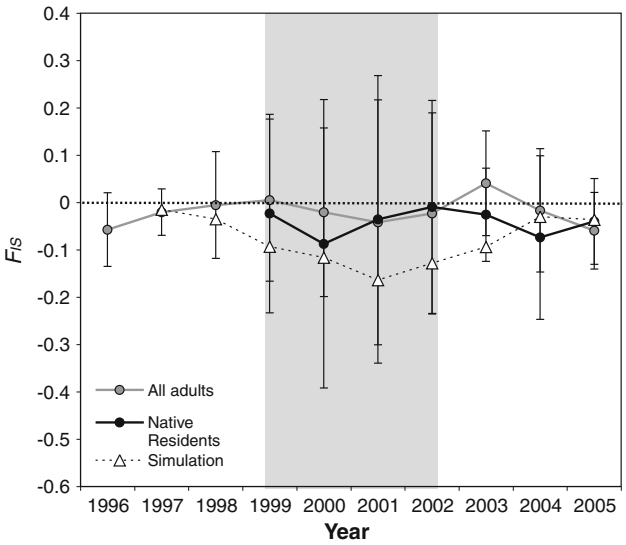

Fig. 5 Estimates of inbreeding (F_{IS}) averaged over six microsatellite loci for golden-mantled ground squirrels. Calculations were made for all adults in the population, including immigrants (*grey*) and for native resident adults only (*black*). Estimates of F_{IS} for simulated populations following the same decline and recovery in the absence of immigration or emigration are indicated by the *dashed line* (*triangles*). *Error bars* represent standard deviations. Bottleneck years are indicated by the *vertical shaded area*

Bottleneck tests

Wilcoxon tests of excess heterozygosity using the TPM in program Bottleneck were significant only in 1996 and 1997 (Table 2). In comparison, when only native adults

Table 2 Results of tests conducted with BOTTLENECK 1.2.02 for golden-mantled ground squirrels analyzed in two groups: (A) all adults and (B) native resident adults only

Year	Sign test			Standardized difference test			Wilcoxon test			Mode shift test
	IAM	TPM	SMM	IAM	TPM	SMM	IAM	TPM	SMM	
(A)										
1996	**0.044**	0.221	0.219	**0.005**	0.098	0.199	**0.008**	**0.023**	0.055	No
1997	**0.041**	0.221	0.214	**0.006**	0.176	0.413	**0.008**	**0.016**	0.078	No
1998	**0.047**	0.437	0.185	0.070	0.450	0.305	**0.008**	0.578	0.781	No
1999	**0.047**	0.182	0.198	0.125	0.277	0.092	**0.008**	0.922	0.977	No
2000	0.552	0.442	0.475	0.175	0.319	0.157	0.055	0.656	0.922	**Yes**
2001	0.227	0.102	0.076	0.356	0.072	**0.038**	0.656	0.977	0.977	No
2002	0.462	0.447	0.460	0.171	0.179	0.044	0.219	0.719	0.922	No
2003	0.219	0.538	0.521	**0.023**	0.333	0.457	**0.016**	0.344	0.422	No
2004	**0.043**	0.185	0.046	**0.021**	0.452	0.117	**0.008**	0.781	0.945	No
2005	**0.044**	0.468	0.195	**0.018**	0.430	0.080	**0.008**	0.578	0.945	No
(B)										
1996	–	–	–	–	–	–	–	–	–	–
1997	–	–	–	–	–	–	–	–	–	–
1998	–	–	–	–	–	–	–	–	–	–
1999	**0.048**	**0.043**	0.221	**0.030**	0.171	0.268	**0.008**	**0.008**	**0.016**	**Yes**
2000	0.190	0.519	0.195	0.092	0.373	0.489	**0.016**	0.281	0.656	No
2001	0.081	0.054	0.123	**0.039**	**0.046**	**0.030**	0.977	0.945	0.945	**Yes**
2002	0.520	0.645	0.570	0.140	0.465	0.470	0.078	0.422	0.500	No
2003	0.217	0.358	0.446	0.166	0.381	0.242	0.078	0.500	0.578	**Yes**
2004	0.527	0.536	0.512	0.147	0.300	0.128	0.219	0.578	0.578	No
2005	0.519	0.198	0.189	0.114	0.193	**0.025**	0.055	0.781	0.977	No

We contrasted all four tests provided by BOTTLENECK and examined three models of mutation (*IAM* infinite alleles model, *TPM* two-phase model, and *SMM* stepwise mutation model). Each entry in the table represents the probability that a bottleneck was detected. Wilcoxon test *P*-values represent one-tailed probabilities for heterozygosity excess. *P*-values less than 0.05 are indicated in bold type-face

were analyzed (1999–2005), excess heterozygosity was detected only in 1999. A mode-shift was detected in 2000, the second year of the demographic bottleneck, when all adults were included, whereas mode-shifts were detected in 1999, 2001, and 2003 when only native adults were considered.

In contrast, *M*-ratio analyses revealed significant bottlenecks in all years of study (Table 3). This was true using a wide range of mutation rates and also when using a conservative value of $\theta = 2.0$ (which assumed larger N_e and lower μ). M_C ratios ranged from 0.67 to 0.82 depending on assumptions; average observed *M*-ratios were lower than critical values in all years (Fig. 6). For analyses including all adults, average observed *M*-ratios ranged from 0.55 in 2001 (bottleneck year) to 0.72 in 1997 (pre-bottleneck year). For native resident adults, average *M*-ratios ranged from 0.53 in 2001 to 0.70 in 1999 and 2002 (Fig. 6). *M*-ratios declined markedly over the first 3 years of the demographic bottleneck (1999–2001) and then increased during the last year (2002) of the bottleneck for both native adults, and for all adults (Fig. 6).

One of the loci used in our study (Bib18) exhibited linkage disequilibrium with three other loci (St 7, Sgs 14, and Bib 4). To ensure that this did not influence our results, we repeated all BOTTLENECK and *M*-ratio analyses after excluding this locus. Our results were largely unchanged. When all adults were included, BOTTLENECK again revealed a genetic bottleneck signature in 1996 and 1997.

Simulations of our population using BOTTLESIM (Kuo and Janzen 2003) allowed us to examine the expected change in measures of genetic diversity under the assumption of no immigration or emigration, with the simulated population following the observed population decline and recovery. Measures of expected heterozygosity H_E and allelic richness A closely paralleled our observed population until 2001 (Figs. 3, 4). However, in the simulated populations, both H_E and A continued to decline after the bottleneck, in contrast to the marked increase observed in our focal population (Figs. 3, 4). Estimates of F_{IS} revealed no strong patterns, although the average value of F_{IS} for simulated populations tended to decline more strongly and then recover relative to the observed values (Fig. 5).

Table 3 Estimates of effective population size (N_e) and results of *M*-ratio analyses of golden-mantled ground squirrels based on all adult squirrels 1996–2005

Year	All adult males	All adult females	N_a	N_e	Average *M*-ratio observed	$\mu = 0.001$			$\mu = 0.005$			$\mu = 0.010$			$\theta = 2.00^{b}$		
						θ	p^{a}	M_C	θ	p	M_C	θ	p	M_C	θ	p	M_C
1996	12	18	30	28.80	0.683	0.115	0.002	0.806	0.576	0.009	0.767	1.152	0.017	0.733	2.0	0.033	0.700
1997	14	23	37	34.81	0.721	0.139	0.006	0.806	0.696	0.022	0.761	1.392	0.046	0.727	2.0	0.067	0.710
1998	7	9	16	15.75	0.656	0.063	0.001	0.817	0.315	0.002	0.787	0.630	0.005	0.757	2.0	0.022	0.691
1999	9	5	14	12.86	0.648	0.051	0.0007	0.811	0.257	0.002	0.794	0.514	0.003	0.767	2.0	0.021	0.689
2000	3	7	10	8.40	0.606	0.606	0.0004	0.817	0.168	0.0001	0.800	0.336	0.0006	0.781	2.0	0.009	0.683
2001	2	5	7	5.71	0.545	0.023	0.0000	0.817	0.114	0.0001	0.806	0.229	0.0001	0.789	2.0	0.002	0.667
2002	7	7	14	14.00	0.676	0.056	0.002	0.817	0.280	0.003	0.789	0.560	0.007	0.768	2.0	0.033	0.687
2003	7	12	19	17.68	0.620	0.071	0.0004	0.809	0.354	0.0008	0.785	0.707	0.001	0.756	2.0	0.008	0.696
2004	16	22	38	37.05	0.655	0.148	0.001	0.806	0.741	0.003	0.758	1.482	0.010	0.726	2.0	0.013	0.713
2005	14	29	43	37.77	0.588	0.151	0.0002	0.806	0.755	0.0004	0.761	1.511	0.001	0.730	2.0	0.002	0.714

N_e was estimated as $(4\ N_m N_f)/N_a$, where $N_a = N_m + N_f$. The *M*-ratio was calculated based on allele distributions for all adults in each year, where M = the number of alleles (k) divided by the range (r) in allele size (size of largest allele − size of smallest allele + 1); *M* was averaged over six loci each year. Simulations to generate critical values (M_C) at $\alpha = 0.05$ and the expected proportion of *M*-values smaller than the observed value (p) require estimates of theta (θ) = $4\ N_e\mu$, where μ = mutation rate. We examined a range of mutation rates ($\mu = 0.001$–0.01) and also used a value of $\theta = 2.0$. Each set of simulations comprised 10,000 iterations with the mean size of mutations larger than a single step $\Delta_g = 3.5$ and the percent of mutations larger than a single step $p_g = 0.10$

[a] p = the expected proportion of *M*-values smaller than the observed value of *M*, based on 10,000 iterations

[b] $\theta = 2.00$ is equivalent to $N_e = 100$ and $\mu = 0.005$ or $N_e = 1000$ and $\mu = 0.0005$

Our simulations also allowed us to evaluate the power of our BOTTLENECK tests and determine the likelihood of detecting a genetic bottleneck in a closed population experiencing the same demographic decline and recovery as our focal population. Using the Wilcoxon test and the TPM mutation model, our estimated power to detect a bottleneck was 0.55 at $\alpha < 0.05$, and 0.72 at $\alpha < 0.10$.

Discussion

Our study provided a unique opportunity to track parallel changes in the demography and genetic structure of a wild population during a natural bottleneck. In doing so, we were able to assess the consequences of the 4-year bottleneck on genetic diversity, and to evaluate the role of demographic rescue resulting from dispersal and immigration. Further, our analyses allow us to contrast several analytical techniques used by conservation geneticists to detect bottlenecks in the wild. As we found, not all measures are equally sensitive to the magnitude of changes observed in our *S. lateralis* population.

Evidence for a genetic bottleneck

The demographic bottleneck reduced the population to low numbers and persisted for 4 years, exceeding the average generation time for *S. lateralis* (2.45 years, Bronson 1979; Millar and Zammuto 1983). In the absence of immigration, we anticipated that this population would experience a potentially severe reduction of genetic diversity.

The demographic bottleneck from 1999 to 2002 was indeed accompanied by a marked decline in allelic richness that closely tracked the population decline (Fig. 4). However, BOTTLENECK did not detect any excess heterozygosity, and there was no evidence for heterozygote deficiency during the recovery phase (Fig. 3). In contrast, *M*-ratios detected genetic bottleneck signatures in all years of study, and *M*-ratios were lower in bottleneck years compared to non-bottleneck years, indicating a stronger bottleneck signature during the observed bottleneck.

How do we reconcile the different findings from these two methodologies? We draw three general inferences. First, several lines of evidence indicate that a genetic bottleneck did indeed occur, but the different methods used to test for genetic bottleneck signatures vary in their sensitivity. Sensitivity to bottleneck detection depended on the power of each test given a limited number of genetic markers, and the different sensitivities of each test to violations of model assumptions. Second, we found evidence that our *S. lateralis* population may have experienced a significant bottleneck prior to our study, which may have limited the potential to detect a subsequent bottleneck.

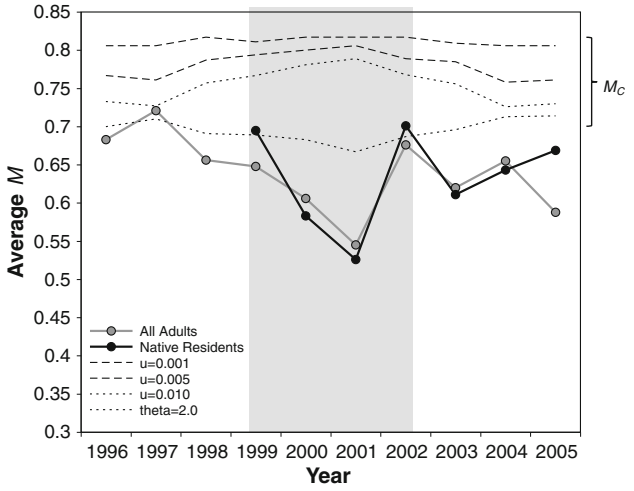

Fig. 6 Average *M*-ratios for all adult golden-mantled ground squirrels (*grey*) and native resident adults only (*black*). Critical *M* values (M_C) are indicated by the *dashed lines*, corresponding to different values of μ or θ. Bottleneck years are indicated by the *vertical shaded area*

Finally, our study provides empirical evidence that immigration played a significant role in the maintenance and recovery of genetic variation following the demographic decline. We consider each of these issues in turn.

Sensitivity of the methods

The reduction in allelic richness, but not heterozygosity, observed in our population is consistent with the theoretical expectation that allelic diversity is more sensitive to bottlenecks than heterozygosity, at least in the case of short, severe bottlenecks (Nei et al. 1975). Similar to our study, other empirical data have indicated a somewhat weak association between bottlenecks and genetic heterozygosity, concluding that allelic richness is a more sensitive measure for detecting bottlenecks (Leberg 1992). Given these findings, methods based on measures of heterozygosity (e.g., BOTTLENECK) are expected to be less sensitive to bottleneck detection. Using excess heterozygosity to detect bottlenecks has the further practical disadvantage of requiring a large sample of loci (Spencer et al. 2000). As our power analysis indicated, using the Wilcoxon test and the TPM mutation model, our estimated power to detect a bottleneck with six microsatellite loci was 0.55–0.72 depending on the alpha level assumed.

Another issue is the severity of the bottleneck in relation to demographic history. If population size fluctuates over time and the long-term N_e is low to begin with (13.4 for our study population), then reductions in census size are not expected to generate a strong reduction in N_e nor a strong bottleneck signature (Pimm et al. 1989; Cornuet and Luikart 1996). Our genetic and demographic analysis is consistent with this general scenario (Table 2; Fig. 5) and could explain why a strong bottleneck signature was not detected using BOTTLENECK.

In contrast, mode-shift tests and *M*-ratio analysis (Garza and Williamson 2001) were more successful in detecting a bottleneck. In the case of mode-shifts, this result was more evident when only native resident adults were considered (Table 2), whereas *M*-ratios clearly declined for all groups (native and immigrant) during the first three bottleneck years (Fig. 6). These results suggest that *M*-ratios provide a more sensitive indicator of genetic bottlenecks compared to the alternative methods described. This conclusion has been echoed in other studies (e.g., Cornuet and Luikart 1996; Williamson-Natesan 2005; Hundertmark and Van Daele 2010).

Both BOTTLENECK and *M*-ratio analysis assume mutation-drift equilibrium prior to the bottleneck, yet this assumption may rarely hold for fluctuating rodent populations experiencing frequent reductions in population size. Population subdivision and admixture also violate model assumptions—our population received 2–5 immigrants each year (with as many as 13 immigrants in the years following the bottleneck) indicating that it was perhaps part of a larger meta-population. Moreover, a pattern of female philopatry in golden-mantled ground squirrels may have contributed to genetic subdivision. Again, *M*-ratios appear to be both more sensitive in detecting bottleneck signatures and more robust to violations of model assumptions—other simulation studies indicate that reductions in *M*-ratios following a bottleneck are, for the most part, independent of the mutational model assumed (Garza and Williamson 2001). Our results support this idea, demonstrating a stronger and more consistent bottleneck signature from *M*-ratios compared to BOTTLENECK measures.

Evidence for a previous bottleneck

Several lines of evidence suggest that our population experienced a bottleneck event pre-dating our demographic study. Even with the limitations noted above, BOTTLENECK identified a significant excess of heterozygosity in our population at the beginning of the study (1996 and 1997; Table 2). Similarly, observed *M*-ratios were consistently lower than critical values in all 10 years and lower than Garza and Williamson's (2001) general critical *M*-ratio of 0.68, considered an upper limit for bottleneck detection based on a wide survey of data. Our observed *M*-ratios were at or below this general critical value for all years.

These results raise the possibility that bottlenecks may be more frequent than anticipated in our population. High altitude populations facing extreme environmental conditions may experience frequent demographic fluctuations, including precipitous population declines, making it difficult to detect the effects of a current bottleneck once

genetic diversity has been winnowed. This may help explain why some methods were not successful in detecting a bottleneck signature during the observed crash.

Immigration and the rescue effect

Our study illustrates the critical role that even low levels of immigration play in maintaining genetic variation in small populations. This influx of genetic variation from outlying areas, even when rare, can substantially dampen bottleneck signatures.

In our study, while there was clear evidence for a demographic rescue and maintenance of neutral genetic variation following the demographic bottleneck, it is unclear the extent to which immigration led to a genetic rescue as defined by Tallmon et al. (2004)—i.e. increased population *fitness* due to immigration. Based on estimates of F_{IS}, there was no evidence of inbreeding throughout the 10-year study and no evidence that immigration reduced levels of inbreeding relative to estimates based on the native population. Since we considered only neutral genetic variation, we are limited in our ability to comment on the fitness consequences of genetic rescue. Future work will need to address how bottlenecks influence genetic variation at loci with individual and population fitness consequences (e.g. the major-histocompatibilty complex (MHC) Hedrick et al. 2001; Smulders et al. 2003; Miller et al. 2008).

In addition to immigration, the life history and social structure of a species can also buffer populations from significant losses of genetic diversity (Berthier et al. 2006). Golden-mantled ground squirrels are characterized by a relatively high rate of reproduction (females produce 1–2 litters per year with an average litter size of 5.2) and overlapping generations, with an average estimated generation time of 2.45 years. Thus, the high reproductive potential, combined with the relatively short duration of the observed bottleneck and the buffering effects of immigration, may be critical to ensuring the persistence of these populations. Similar results have been noted in other vertebrate species that have experienced little reduction in genetic variation following a known bottleneck (Kuo and Janzen 2004; Hailer et al. 2006; Busch et al. 2007; Ortego et al. 2007).

Conclusions and conservation implications

The demographic bottleneck in *S. lateralis* clearly caused a reduction in allelic richness, and several analyses indicated that the population suffered a genetic bottleneck. However, heterozygosity remained largely unchanged during the bottleneck and returned to pre-bottleneck levels when the population recovered. We found no evidence of increased inbreeding during or after the decline. These results suggest that the principle drivers of local population extinction risk in this species are more likely to be demographic (reproduction, immigration, predation) than genetic in origin.

There was considerable variation among analytical techniques in the ability to detect evidence of a genetic bottleneck. Given a modest number of sampled loci, allelic richness and *M*-ratios appear to be the most sensitive indicators. Our observed bottleneck was not detectable using tests of excess heterozygosity under the TPM, and mode-shifts tests were only marginally effective, except when immigrants were excluded from analysis. These findings warrant caution for researchers attempting to detect bottlenecks in wild populations. In our study, we were in a unique position whereby we could identify and follow all individuals and so could precisely track the population's decline and recovery. Many field studies are not afforded such luxury (especially for species that are rare or difficult to census), hence inferences about population bottlenecks are often based on genetic assays alone. Our results indicate that such inferences should be viewed cautiously—conclusions may vary considerably depending on the analytical technique chosen.

Finally, we show that immigration can play an important role in demographic and genetic recovery following an observed population bottleneck. Despite the reduction to only five breeding females, within 3 years of the bottleneck, both population size and genetic variation recovered to pre-bottleneck levels. Interestingly, BOTTLENECK and *M*-ratio analyses both suggest that the regional meta-population may have experienced an historic bottleneck and is still in recovery. In terms of conservation management, these results suggest that local populations of *S. lateralis* may experience frequent, low-intensity bottlenecks in addition to more severe, regional bottlenecks. Clearly, sufficient levels of immigration and gene flow within the regional meta-population are critical for long-term viability of these populations and highlight the importance of maintaining connectivity in natural populations.

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