

Effective population size and genetic structure of a Piute ground squirrel (*Spermophilus mollis*) population

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Abstract: Piute ground squirrels (*Spermophilus mollis*) are distributed continuously in habitat dominated by native shrubs and perennial grasses in the Snake River Birds of Prey National Conservation Area in Idaho, U.S.A. This habitat is being fragmented and replaced by exotic annual plants, changing it to a wildfire-dominated system that provides poor habitat for ground squirrels. To assess potential effects of this fragmentation on ground squirrel populations, we combined an estimate of effective population size (N_e) based upon a demographic study with a population genetic analysis. The study area included three subpopulations separated from each other by 8–13 km. The ratio of effective population size to census number (N_e/N) was 0.57. Combining N_e/N with dispersal distances from a radio-tracking study, we calculated that neighborhood size was 62.2 ha, which included between 204 and 480 individuals. Our population genetic analysis (based on randomly amplified polymorphic DNA (RAPD) and microsatellite markers) showed relatively low levels of genetic differentiation ($\Theta_{\text{populations}} \cong 0.07\text{--}0.10$) between subpopulations and no inbreeding within subpopulations ($f = 0.0003$). These estimates of population subdivision translate into an effective migration rate ($N_e m$) of 2.3–3.3 per year, which represents a high level of gene flow. Invasion by exotics will reduce the overall productivity of the habitat, and will lead to isolation among subpopulations if favorable habitat patches become isolated.

Résumé : Les spermophiles *Spermophilus mollis* ont une répartition continue dans l'habitat dominé par les buissons indigènes et les herbes vivaces dans la zone de conservation Snake River Birds of Prey National Conservation Area, Idaho, É.-U. L'habitat est en voie de fragmentation et les plantes indigènes sont remplacées par des plantes exotiques annuelles, ce qui a transformé l'habitat en un système dominé par les feux de brousse, un milieu inadéquat pour les spermophiles. Pour mesurer les effets potentiels de cette fragmentation sur les populations de spermophiles, nous avons utilisé une estimation de la taille effective de la population (N_e), basée sur une étude démographique, combinée à une analyse génétique de la population. La zone étudiée comportait trois sous-populations séparées par une distance de 8 à 13 km. Le rapport entre la taille effective de la population et le nombre au recensement (N_e/N) a été évalué à 0,57. En combinant ce rapport aux distances de dispersion enregistrées lors d'une étude télémétrique, nous avons calculé que l'aire habitée mesurait 62,2 ha et comptait entre 204 et 480 individus. Notre analyse génétique (basée sur des marqueurs ADN polymorphe amplifié au hasard (RAPD) et des marqueurs microsatellites) a démontré qu'il y avait peu de différenciation génétique ($\Theta_{\text{populations}} \cong 0,07\text{--}0,10$) entre les sous-populations et pas de consanguinité au sein des sous-populations ($f = 0,0003$). Ces estimations de la subdivision de la population se traduisent par un taux effectif de migration $N_e m = 2,3\text{--}3,3$ par an, ce qui représente un flux de gènes important. L'invasion des plantes exotiques est appelée à réduire la productivité globale de l'habitat et risque de mener à l'isolement des sous-populations si des parcelles d'habitat favorable deviennent isolées

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Introduction

The continuity of natural populations depends on the frequency and distance of dispersal, but habitat fragmentation caused by human disturbance may result in a series of subpopulations isolated from each other by unsuitable habitat.

Predicting genetic and demographic effects of fragmentation depends upon understanding how dispersal maintains continuity. However, patterns of genetic variation within and between populations are a consequence not only of frequency and distance of dispersal, but also of the mating system (e.g., monogamous pairs versus harems, where an individual male mates with several females). The genetic effects of dispersal and mating cannot be distinguished by genetic sampling alone, yet both dispersal and mating will strongly influence how habitat fragmentation affects a population (McCauley 1991; Nunney and Campbell 1993; Hastings and Harrison 1994; Barton and Whitlock 1997). Hence, combining ecological/demographic data (survival, mating patterns, dispersal distances) with genetic data will provide a better foundation for predicting the effects of fragmentation.

One goal of conservation biology is to assess both the demography and the genetic structure of populations, while

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taking into account how habitats vary across landscapes and over time (Nunney and Campbell 1993; Holsinger 1996). Few studies have done this (Chepko-Sade et al. 1987; Koenig et al. 1996; Gaines et al. 1997), leaving few opportunities for directly comparing inferences drawn from genetic and demographic data. Templeton (1987) and Chesser (1991) have pointed out that behavioral/demographic data tend to overestimate gene flow, while genetic data can underestimate gene flow when one sex disperses more commonly (e.g., female philopatry). Successfully predicting how population fragmentation and local extinction caused by habitat loss will influence the genetics and demography of a species depends in part on prior knowledge of the spatial structure of a population. In particular, habitat fragmentation can influence effective population size (N_e), which measures a population in terms of the number of individuals that contribute to breeding and the amount of genetic variability that would be maintained in a population of size N_e (Barton and Whitlock 1997). Fragmentation will reduce the effective population size even if the overall population is of the same size. However, the reduction in effective population size will be greater if subpopulations in isolated patches undergo episodes of extinction and recolonization (Barton and Whitlock 1997).

To get a picture of how fragmentation might affect Piute ground squirrels, *Spermophilus mollis* (formerly *Spermophilus townsendii*; Hoffman et al. 1993), we conducted both demographic and genetic analyses of a population in southeastern Idaho. Using demographic data from a long-term study of ground squirrels (Van Horne et al. 1997), we estimated the effective population sizes for three subpopulations with a model for overlapping generations (Nunney 1993; Nunney and Elam 1994). Using genetic data from two classes of molecular genetic markers (randomly amplified polymorphic DNA (RAPD) and simple sequence repeats (microsatellites)) we also estimated the effective migration rate ($N_e m$) among the same subpopulations. By estimating demographic and genetic parameters from the same subpopulations simultaneously, we were able to assess the concordance of demographic and genetic measures of population structure.

Piute ground squirrels are abundant and widely distributed throughout both the shrub and grassland habitats of the Great Basin and Columbia Plateau in the western United States (Van Horne et al. 1997). This herbivorous species is small and short-lived compared with most other ground squirrels, with adults living less than 2 years, on average (Smith and Johnson 1985). Other ground squirrels are known for their cooperative social behavior, but Piute ground squirrels are defined as "non-social" even though they aggregate in burrow systems in favorable habitats (Armitage 1981). As in most ground squirrel species (Hoelkamp 1984), female Piute ground squirrels are sedentary, while juvenile males regularly disperse from their natal burrows to breed elsewhere.

Piute ground squirrel populations were not fragmented in favorable habitat within our study area (Van Horne et al. 1997). This is likely to change, however, as the habitat is invaded by exotic annual plants, which increase the extent and frequency of fires because they are highly flammable when senescent (U.S. Department of the Interior 1996; Knick and Rotenberry 2000). Native shrubs in favorable habitat are not fire-adapted and are poor colonizers of burned areas. Burned areas that contain Sandberg's bluegrass (*Poa sandbergii*), a

native perennial bunch grass, still provide adequate habitat for Piute ground squirrels (Van Horne et al. 1997). In drought years, however, only shrub habitat can support ground squirrels. Our study area was affected by a severe drought that led to low adult survival in grassland habitats, near-zero juvenile survival in all habitats, and lower adult reproduction during subsequent years (Van Horne et al. 1997). The shrub-steppe habitat of the Piute ground squirrel is becoming dominated by exotic annual plants as a result of repeated burning, and suitable ground squirrel habitat is becoming fragmented (U.S. Department of the Interior 1996; Knick and Rotenberry 2000).

Materials and methods

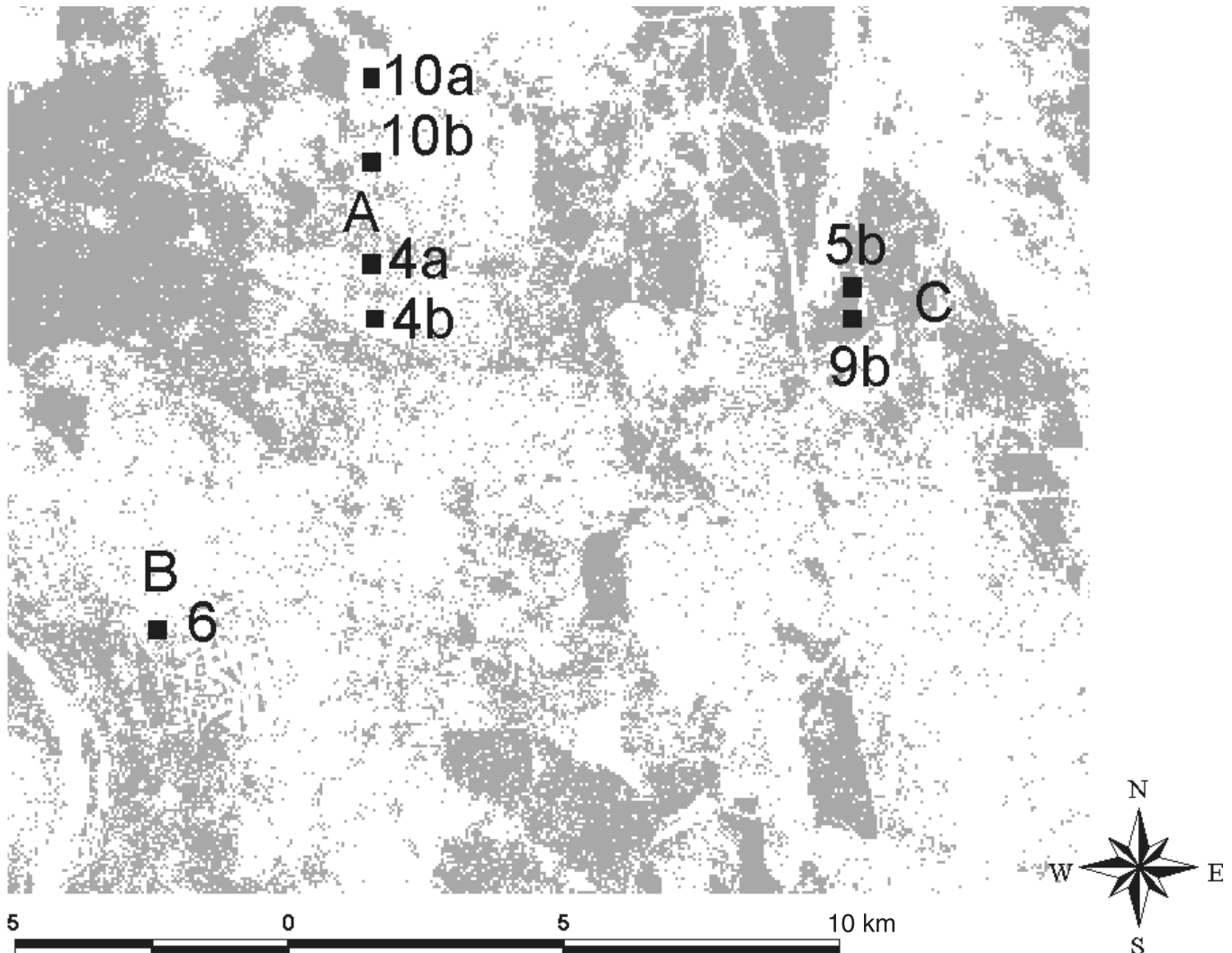
Field sites

We conducted our study on the benchlands of the Snake River Birds of Prey National Conservation Area (hereinafter Birds of Prey Area; 43°N, 116°W), 20 km south of Boise, Idaho (Fig. 1). The Birds of Prey Area covers 195 243 ha, the elevation varies between 900 and 950 m, the topography is flat except for isolated buttes, and the climate is arid (precipitation 20–30 cm/year); there are no persistent watercourses in our study area. Historically the area has supported shrub-steppe plant and animal communities, with two native species, big sagebrush (*Artemisia tridentata*) and winterfat (*Krascheninikovia lanata*), being the most common. Invasion by exotic annual grasses and forbs has increased the frequency and extent of fires, and approximately 50% of the shrub habitat in the Birds of Prey Area has burned since 1980 (Kochert and Pellant 1986). Burned areas change to dominance by grasses and forbs, including the native Sandberg's bluegrass and exotic annuals like cheatgrass (*Bromus tectorum*), Russian thistle (*Salsola iberica*), and tumbledustard (*Sisymbrium altissimum*). Besides habitat conversion, burned areas also convert to a higher percentage of disturbed bare ground, which is unsuitable habitat for ground squirrels (U.S. Department of the Interior 1996; Knick and Rotenberry 2000).

We conducted a population/demographic study from 1991 through 1994 by livetrapping and tagging squirrels at 9-ha sites (A, B, and C) during the active season for Piute ground squirrels, February–June (Fig. 1; Van Horne et al. 1997). In 1993 we also collected tissue samples from the trapped areas for genetic analysis. Areas A and B had been burned 9–12 years prior to the initiation of this study and the habitat was primarily grassland dominated by Sandberg's bluegrass. Area C had not been recently burned and was dominated by big sagebrush. Although ground squirrels in this area were continuous in distribution and formed a single population, we refer to the animals sampled in each area as a subpopulation.

The collection of samples for genetic analysis in 1993 followed a drought, when fewer than 1% of juveniles survived (Van Horne et al. 1997). Three-month cumulative rainfall in April and May of 1992 was the most sparse since precipitation records were first kept in 1860 (Van Horne et al. 1997). As is the case for most ground squirrel species, dispersal of breeding adults is uncommon (Hoelkamp 1984; Smith and Johnson 1985; Van Horne et al. 1997). Thus, the adults sampled in 1993 were a subset of animals that bred in the same localities in 1991 and earlier, and represented the genetic neighborhood as it existed in 1991. To compare genetic and demographic estimates from the same population, we estimated the effective population size from demographic data collected before the drought in 1992. No unusual weather events occurred during the 5 years prior to 1992 and ground squirrel densities before 1992 were likely to have been high and stable.

Fig. 1. Location of three study areas (A, B, and C) within the Snake River Birds of Prey National Conservation Area in southwestern Idaho. Numbered black squares indicate locations of 9-ha trapping grids for the demographic study (Van Horne et al. 1997). Samples for genetic analysis were collected adjacent to the trapping grids, and the animals collected from each area are considered a "subpopulation." Shaded areas were dominated by shrubs and the white areas by grassland.



Estimating N_e/N from population data

Major assumptions

Methods for estimating the effective population size depend on the type of population data available and the assumptions demanded by the specific model relating the population census number (N) to the effective population size (Crow and Denniston 1988; Nunney and Campbell 1993). In particular, the variance-effective size of a population is calculated from demographic data and is usually less than the number of adults in a population because a few individuals account for most of the reproduction. We used a method for estimating the ratio of variance-effective population size to the census number (N_e/N) in organisms with overlapping generations that requires minimal demographic information (Nunney 1993; Nunney and Elam 1994). The model makes a number of assumptions that are met by the biology of Piute ground squirrels. At the most basic level, we assumed that subpopulations form randomly mating groups, at least in regard to litter size and mating success. Our observations of male mating behavior and heterozygosity within subpopulations (see below) support this assumption.

Variance in reproductive success of both males and females has a large effect on the effective population size (Nunney 1993; Barton and Whitlock 1997). Estimating the effective population size requires the simplifying assumption that survival of males and females is independent of age after sexual maturity. In a 7-year study also conducted on the Birds of Prey Area, Smith and Johnson (1985) found a nearly constant survival rate of adults once animals reached maturity, although mean litter sizes vary from site to site and from year to year (Smith and Johnson 1985; Van Horne et al. 1997). The method of estimation also assumes that both male mating success and female fecundity follow a Poisson distribution within populations (i.e., variation arises because of random factors that are independent of age). Given this assumption, Nunney's (1993) method depends upon knowing the proportion of individuals that attempt reproduction. We found that during the breeding season, virtually all Piute ground squirrels older than 1 year had descended testes and swollen nipples, which indicate sexual maturity. Thus, we have evidence that nearly all individuals attempt reproduction in the year following their birth (Van Horne et al. 1997).

Table 1. Density (number/ha) of adult males, adult females, and juvenile Piute ground squirrels, *Spermophilus mollis* (N_{am} , N_{af} , and N_j , respectively), ratio of effective population size relative to population census size (N_e/N) for each area, and estimates of parameters used in its calculation (Nunney and Elam 1994) for subpopulations found in each of three areas (A–C).

Area	N_{am}	N_{af}	N_j	N_j/N_{af}	N_e/N	A_m	A_f	$I_{A_m}(=v_m)$	$I_{A_f}(=v_f)$	T	$\alpha_f = p$	x	r	$I_{b_m}(=K)$	I_{b_f}
A	4.2	9.1	67.9	7.5	0.58	1.3	1.2	0.23	0.14	1.2	0.99	7.5	0.32	0.47	0.010
B	2.0	4.2	15.5	3.7	0.53	2.0	2.4	0.50	0.58	2.2	0.98	3.8	0.32	0.48	0.020
C	1.5	5.4	16.6	3.1	0.59	1.0	1.5	0.00	0.34	1.3	0.95	3.2	0.22	0.30	0.053

Note: A_m and A_f are male and female life-spans, respectively; I_{A_i} and I_{A_m} are standardized variances of male and female life-spans, respectively ($= v_i$, survival rate); T is generation time; α_f is female reproductive success ($= p$, proportion of females that successfully breed); x is the fecundity of females who produced at least one offspring; r is the sex ratio of adults (proportion of males), I_{b_m} and I_{b_f} are standardized variances of male and female lifetime reproductive success, respectively. See the Methods for further explanation.

Male reproductive success also depends on the mating system. Here we assume the lottery polygyny model of mating: females mate once during a season, while males mate multiple times with a random number of females. The mating behavior of male Piute ground squirrels fits this model. During the mating period for Piute ground squirrels in the Birds of Prey area, we observed males running long distances and entering widely scattered burrows inhabited by breeding females. Males were nonterritorial and appeared not to discriminate with regard to location. Daily movements of males were greater than the size of our study plots (9 ha), indicating that males can range over large areas to mate. It is possible that females also mate more than once, but assuming single matings by females makes our estimates of N_e smaller than if broods were sired by several males (Nunney 1993).

Calculations and additional assumptions

We calculated the ratio of effective population size to actual population size (N_e/N) for each of the three subpopulations (Fig. 1; Van Horne et al. 1997). For areas A and B, we used the sum of individuals per hectare in calculations of demographic parameters rather than averaging estimates across sites within areas. This produced a weighted average that minimized the spurious effects of small sample sizes within individual plots. We had only one plot in area C.

Nunney’s (1993) method requires calculation of six parameters: (1) maturation time, M , (2) adult life-span, A , (3) generation time T , (4) variance in female reproductive success, I_{b_f} , (5) variance in male reproductive success, I_{b_m} , and (6) adult sex ratio, r (Table 1). Calculation of N_e/N was based upon the following (Nunney 1993; Nunney and Elam 1994):

$$\frac{N_e}{N} = \frac{4r(1-r)T}{rA_f(1+I_{A_f}) + (1-r)A_m(1+I_{A_m}) + (1-r)I_{b_m} + rI_{b_f}}$$

We assumed the mean maturation time (M) for both males and females to be 1 year, as we saw no indication of lower reproductive rates for yearling males and females than for older adults (Van Horne et al. 1997).

Our estimates of the average adult life-span for each sex, A_m and A_f , which are needed to calculate generation time (T), were based on the probability of recapturing in 1992 adults that were known to be alive at the end of the season in 1991. This estimate of survival is low because it ignores emigration, but it provides an accurate count of the animals that survive to breed at a given site. It is unlikely that we failed to capture adult breeders still present the following year. Assuming constant adult survival, v_i , the average adult life-span is $A_i = 1/(1 - v_i)$ for each sex ($i = m, f$). The standardized variance (variance/mean²) of the life-span of each sex was $I_{A_i} = (A_i - 1)/A_i$, which decreases to $I_{A_i} = v_i$ assuming age-independent survivorship. Generation time was calculated as $T = M - 1 + A_i$ (Nunney and Elam 1994).

Estimating variance in female reproductive success (I_{b_f}) depends upon average litter size (N_j/N_{af} ; Table 1), the proportion of females

that attempt to breed (p), the proportion of females that successfully breed (α_f), and the assumption of a Poisson distribution of breeding success (Nunney and Elam 1994). Given the average fecundity of all females, b_f , the mean fecundity of females that successfully breed is $x = b_f/[1 - \exp(-b_f)]$, from the Poisson distribution. Thus, the proportion of females that successfully breed is $\alpha_f = px/b_f$. If x is large (≥ 4), then $x/b_f \approx 1$, and the proportion of females that successfully breed decreases to $\alpha_f = p$. In other words, when the numbers of offspring per female are Poisson-distributed and fecundity is between 3.1 and 7.5 offspring per female (see Table 1), then few of the females that attempt to breed will be completely unsuccessful. The standardized variance I_{b_f} was calculated as $(1 - \alpha_f)/\alpha_f$.

To estimate variance in male reproductive success (I_{b_m}), we assumed a lottery polygyny breeding system. Because males are non-territorial, we assume that all males attempted to breed, so $\alpha_m = 1$. Under lottery polygyny, variance in male reproductive success will be affected by the number of females that attempt to breed, which is included by adding a parameter, K : $K = r/[(1 - r)\alpha_f]$, where r is the adult sex ratio (proportion of males in 1991) and α_f is the proportion of females that attempted to breed. The variance in male reproductive success was calculated as $I_{b_m} = K + (1 - \alpha_m)/\alpha_m$. When $\alpha_m = 1$, $I_{b_m} = K$.

Estimates of N_e/N were based upon the assumption that reproductive success was randomly distributed as a Poisson variate. To test the effects of this assumption for females, we recalculated N_e/N by assuming that a minimum number of females (p_{min}) were responsible for the juveniles observed on each site. The p_{min} value was determined from the number of juveniles on each site divided by the average maximum litter size (9), which was determined by counting embryos and uterine scars in necropsied adult females (Van Horne et al. 1997). We also tested the effect of the assumption that all males attempt to breed ($\alpha_m = 1$) on N_e/N by recalculating N_e/N with the proportion of males attempting to breed (α_m) of 0.75, 0.5, and 0.1.

Population genetic analysis

Our genetic study is based primarily upon RAPD from the polymerase chain reaction (RAPD-PCR; Williams et al. 1990). RAPD markers are abundant, dispersed throughout the genome, and segregate as Mendelian loci (Antolin et al. 1996). The advantage of this method is that when protocols are standardized, a large number of polymorphic markers can be generated quickly. As demonstrated by Nei (1978), sampling variance of population genetic estimates is more affected when there are few loci than when there are few individuals per subpopulation or few alleles per locus.

We collected material for genetic analysis by tail-clipping. When possible, we recorded the age, sex, reproductive status, and body mass of each animal. For tail-clipping we used canine nail clippers to snip a small section of the tail tip, including just enough of the tail to contain a small piece of bone. We cleaned the end of the tail with Providine surgical scrub before and after clipping, and we

also applied an antibiotic cream after clipping. Tail snips were placed individually in sterile tubes in STE buffer (0.1 M sodium chloride, 0.05 M Tris-HCl, pH 7.5, 0.001 M EDTA) for storage and were refrigerated until they were placed in freezers (-80°C).

To avoid influencing our live-trapped animals, we collected samples from unmarked animals captured on assessment trap lines perpendicular to each side of the square trapping grids in areas A–C. We also sampled tissues from animals collected within 500 m of the trapping grids at sites A and C. These animals were collected by shooting or snap-trapping; carcasses were kept frozen prior to tail-clipping for genetic analyses. Sample sizes for each area were 23 for area A, 11 for area B, and 24 for area C. The total sample was slightly male-biased (37 males, 21 females) and was composed mainly of adults (44 of 58). The sampled juveniles were likely to have been born in the area where they were collected. Although male juvenile ground squirrels disperse, most of this dispersal occurs late in the season when animals are approaching their normal hibernation/estivation mass of >200 g. We sampled animals before May 1, prior to the juvenile dispersal period, and at relatively low body masses (62–98 g).

DNA extraction

DNA was extracted from tail tissue by means of the CTAB (hexadecyltrimethylammonium bromide) procedure (Black and DuTeau 1997). Tail clips were lysed in 500 μL CTAB homogenization buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 0.02 M EDTA, 2% CTAB, 0.2% 2-mercaptoethanol) by grinding each sample with a plastic pestle (Kontes) in a 1.7-mL microcentrifuge tube until only a small bone fragment remained. In the end, the DNA pellet was resuspended in 100 μL TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), the DNA concentration was determined by spectrophotometry, and DNA was diluted to a concentration of 5 ng/ μL .

PCR amplification

RAPD-PCR was performed as described in Black et al. (1992), with 1 μL (5 ng) DNA template used in each 50- μL reaction. Each set of PCR reactions was checked for contamination using a negative control (all reagents except template DNA). Primers were either obtained from Operon Technologies, Alameda, California (primers A5, A6, A9, A20, B16, C1, C4, AM10) or were designed by N. DuTeau (*Bam*H1: 5'-ATGGATCCCG-3', *Eco*: 5'-ATGAATTCGC-3'). These primers were chosen because they amplified numerous repeatable RAPD fragments.

One microsatellite locus was also amplified using a pair of primers developed for the Idaho ground squirrel (*Spermophilus idahoensis*) by May et al. (1997). The primer pair IGS-110b (5'-CCATGGAAGCATGTCTGGTG-3', 5'-TGCTTCTGATTTCAAAGTTGC-3') amplified a variable 3-base-pair (bp) (TGC) motif in the Idaho ground squirrel. PCR conditions (i.e., nucleotide and MgCl_2 concentrations, annealing temperature 52°C) exactly followed those in May et al. (1997), using 1 μL (5 ng) DNA per reaction.

Electrophoresis

Complete details concerning visualization of RAPD-PCR by means of single-strand conformational polymorphism (SSCP) analysis, including silver-staining of gels, are given in Antolin et al. (1996) and Black and DuTeau (1997). The use of large polyacrylamide gels reveals a large number of polymorphisms per primer and, in particular, detects codominant size-fragment polymorphisms where all homozygous and heterozygous genotypes can be resolved. Briefly, PCR products (5 μL) were mixed with 2 μL loading buffer (95% formamide, 10 mM NaOH), denatured to single strands with heat and then plunged into ice to promote the formation of intra-strand complexes (i.e., SSCPs) while reducing the formation of double-stranded DNA. The products were electrophoresed on large (40 \times 50 cm), thin (0.4 mm) glycerol (5%) polyacrylamide (5% with 2% cross-linking) gels. Electrophoresis proceeded at constant volt-

age (350 V) at room temperature for 16 h (overnight). Six-millimetre shark's-tooth combs were used for loading to accommodate the 6–7 μL of sample needed for each lane.

PCR products amplified by microsatellite primer IGS-110b were visualized by silver-staining after electrophoresis in 8% acrylamide gels (Longranger 50% solution, FMC Bioproducts) with 8 M urea at room temperature at 45 W, 40 mA for 4.5 h.

Scoring RAPD-SSCP bands and microsatellites

Amplified markers were scored directly from dried gels by measuring the band mobility of known size markers (1-kilobase ladder, BRL Laboratories) run in adjacent lanes, and fitting an inverse function relating fragment size to mobility (Schaffer and Sederoff 1981). Sizes of amplified fragments were estimated from their mobilities using the mobility-size function. The RAPD-PCR method generates numerous bands, some not repeatable. All PCR reactions were repeated twice, and we scored only bands that were clearly discernible in both PCR runs.

The majority of RAPD-SSCP markers segregate as band presence / band absence polymorphisms, the band-present alleles being dominant (in the genetic sense) and the band-absent (null) alleles recessive. We identified three codominant markers after subjecting our RAPD data to RAPDL3, a program that calculates linkage disequilibrium from RAPD data (William C. Black IV, available on the Internet by anonymous ftp from <ftp://lamar.colostate.edu/pub/wcb4>). Three pairs of adjacent bands had significant disequilibrium in all three subpopulations and never showed a null genotype (i.e., bands were always present at one or both positions). Linkage disequilibrium between adjacent bands on the gel indicated that inheritance of the two markers was not independent, and these were therefore treated as codominant loci, each with a "fast" and a "slow" band-present allele. We have found similar codominant markers in other studies based on RAPD-SSCP markers (Antolin et al. 1996; Vaughn and Antolin 1998). Only these three pairs of bands showed consistent linkage disequilibrium; otherwise, linkage disequilibrium was less than 5% in all subpopulations.

Microsatellite alleles amplified by primer pair IGS-110b were also identified from their mobility on gels. Five alleles were identified, four of which (A–D) differed by a single 3-bp repeat in the range 285–300 bp. A fifth allele (E) was much shorter, 270 bp.

Genetic-data analysis

Data were analyzed using the program TFGA (version 1.3, available from Mark P. Miller, MPM2@nauvax.ucc.nau.edu). Genotype frequencies of the codominant markers (one microsatellite and three RAPD-SSCP) were tested for deviations from Hardy–Weinberg expectation using Haldane's exact probabilities. Genetic parameters for a subdivided population were estimated according to the analysis of variance of Weir and Cockerham (1984). These parameters were first defined by Wright (1978) and include the overall level of inbreeding ($F = F_{IT}$), genetic differentiation of subpopulations ($\Theta_{\text{populations}} = F_{ST}$), and levels of inbreeding within subpopulations ($f = F_{IS}$). Weir and Cockerham's (1984) procedure corrects for small and unequal sample sizes among subpopulations, and allows for estimation of confidence intervals by bootstrapping.

Allele frequencies of 32 dominant presence/absence RAPD-SSCP markers were estimated from the frequency of the recessive band-absent genotype. In each subpopulation, $q^2 = S_0/N$, where S_0 is the number of individuals having no band and N is the number of individuals in the subpopulation. Frequencies of homozygous genotypes relate directly to allele frequencies when each subpopulation has Hardy–Weinberg genotype frequencies, which we confirmed from the four codominant loci. For each locus, estimates of allele frequency, q , were corrected for bias and small sample size according to Lynch and Milligan (1994). The population subdivision parameter $\Theta_{\text{populations}}$ (Weir and Cockerham 1984) was estimated for these markers, as was the F_{ST} parameter based upon Lynch and

Table 2. Ratio of effective population size to population census number (N_e/N) for subpopulations in each of the three sampling areas, assuming that observed offspring were produced by the fewest adult females possible.

Area	$p = \alpha_f$	x	$I_{b_m} = K$	I_{b_i}	N_e/N
A	0.83	9.0	0.57	0.20	0.55
B	0.41	9.0	1.15	1.44	0.43
C	0.34	9.0	0.83	1.94	0.38

Note: Parameters that differ from those given in Table 1 are shown.

Milligan’s (1994) corrections for RAPD markers, using the program RAPDFST (available from William C. Black IV at Colorado State University).

Population parameters ($\Theta_{\text{populations}}$ and F_{ST}) were used to estimate the effective migration rate, $N_e m$, which is the average effective size of each population multiplied by the migration rate, m , between subpopulations (Wright 1978). The relationship between $\Theta(F_{ST})$ and m in the island model, assuming that populations are in migration-drift equilibrium, is $N_e m = (1 - \Theta)/(4\Theta)$ or $N_e m = (1 - F_{ST})/(4F_{ST})$.

Results

Effective population size

Estimates of parameters and effective population sizes are given in Table 1. Despite the fact that densities in the three areas ranged from 6.2 to 13.3 adults/ha, estimates of the ratio of effective population sizes to census number (N_e/N) fell in a narrow range, between 0.53 and 0.59. The N_e/N value was also insensitive to variation among the three areas in the number of offspring per female, adult survival, and life-span (Table 1).

Effective population size was sensitive to assumptions about male and female reproductive success. When effective population size was calculated by assuming that offspring were from the fewest females possible (9.0 progeny per female), we found larger differences in N_e/N values among areas (Table 2). Estimates of effective population size were reduced most in subpopulations with the greatest increase in variance in female reproductive success. Changes in female reproductive success increased variance in male reproductive success through the parameter K (i.e., male mating opportunities become restricted when fewer females breed). In area A, N_e/N was reduced by 5%, while in areas B and C it was reduced by 20 and 36%, respectively. Changing the proportion of males that attempt to breed (α_m) also increased variance in male reproductive success and reduced effective population size (Table 3). At the extreme, if only 10% of males bred, variance in reproductive success dramatically increased to >9 and N_e/N was reduced to 0.10–0.19.

We can estimate effective population size and “neighborhood size” for each subpopulation and the probability of successful dispersal between subpopulations by combining these data with the results of a parallel dispersal study carried out on the Birds of Prey Area at the same time (Olson and Van Horne 1998). Dispersal was measured by following radio-collared animals. We may calculate neighborhood size by considering the 85th percentile distance that individuals are likely to move from their place of birth to where they breed (Chepko-Sade et al. 1987). This distance, taken as the average for the 26 radio-collared males and 12 radio-collared

Table 3. Ratio of effective population size to population census number (N_e/N) for subpopulations in each of the three sampling areas, and variance in male reproductive success (I_{b_m}) when the proportion of males attempting to breed (α_m) is reduced.

Area	$\alpha_m = 1.0$	$\alpha_m = 0.75$	$\alpha_m = 0.5$	$\alpha_m = 0.1$
A				
N_e/N	0.58	0.51	0.43	0.13
I_{b_m}	0.47	0.81	1.47	9.47
B				
N_e/N	0.53	0.50	0.45	0.19
I_{b_m}	0.48	0.81	1.48	9.48
C				
N_e/N	0.59	0.50	0.38	0.10
I_{b_m}	0.30	0.63	1.30	9.30

Note: Values for $\alpha_m = 1.0$ are the same as in Table 1.

females (including both dispersers and non-dispersers) from Olson and Van Horne (1998), is 445 m. A circle with radius of 445 m has an area of 62.2 ha. Densities in our study area ranged from 6.2 to 13.3 adults/ha (Table 1). Combining subpopulation densities with estimates of N_e/N from each of the areas (Table 1) provides estimates of effective population sizes in the neighborhood, N_e , that range from 204 adults for area B (6.2 adults/ha \times 62.2 ha \times 0.53) to 480 adults for area A (13.3 adults/ha \times 62.2 ha \times 0.58).

Population genetic estimates

Within each subpopulation, genotype frequencies of the three codominant RAPD loci and the microsatellite locus did not differ from Hardy–Weinberg expectations (exact tests, all $P > 0.05$). Meeting the assumption of Hardy–Weinberg equilibrium within each subpopulation is necessary for correctly estimating allele frequencies of band present/band absent (dominant) RAPD markers (Lynch and Milligan 1994).

For the 32 dominant RAPD markers, both measures of population structure showed low but statistically significant differentiation ($\Theta_{\text{populations}}$, $F_{ST} = 0.07$), and both had confidence intervals that did not include zero (Table 4). The value for the four codominant markers was slightly greater ($\Theta_{\text{populations}} = 0.10$), but was not significantly different from zero. Also, the codominant markers showed no evidence of inbreeding within subpopulations ($f \approx 0$), as expected when there are no deviations from Hardy–Weinberg expectations. The effective migration rates ($N_e m$), ≈ 3.3 for the 32 dominant RAPD markers and 2.3 for the 4 codominant markers, indicate that there was considerable gene flow among these subpopulations.

Discussion

Our estimates of effective population size and population subdivision of the Piute ground squirrel population in the Snake River Birds of Prey National Conservation Area in Idaho complement each other: effective population size was relatively large in this continuous habitat and genetic differentiation among the three subpopulations was low. Large population size ($N_e > 200$) and (or) regular gene flow ($N_e m \approx 2.3$ – 3.3) prevent genetic drift from causing subpopulations to diverge. In addition, our genetic data show no evidence of inbreeding within subpopulations, and thus many of the

Table 4. Levels of population subdivision using 4 codominant markers and 32 dominant RAPD markers.

	F	$\Theta_{\text{populations}}$	f	F_{ST} (Lynch and Milligan 1994)
4 codominant markers				
IGS-110b	0.064	-0.003	0.066	
A6.825	0.570	0.431	0.245	
A6.900	-0.052	-0.015	-0.037	
A9.870	-0.211	-0.012	-0.197	
Total	0.095 (-0.13, 0.42)	0.095 (-0.01, 0.32)	0.0003 (-0.12, 0.14)	
32 dominant RAPD markers		0.071 (0.02, 0.13)		0.072 (0.01, 0.13)

Note: Estimates of overall levels of inbreeding ($F = F_{\text{IT}}$), population subdivision ($\Theta = F_{\text{ST}}$), and within-subpopulation inbreeding ($f = F_{\text{IS}}$) follow Weir and Cockerham (1984) or Lynch and Milligan's (1994) correction of F_{ST} for RAPD markers. Numbers in parentheses show 95% confidence intervals.

population-level assumptions needed for estimating effective population size (Nunney and Elam 1994) and gene flow based upon RAPD-PCR markers (Lynch and Milligan 1994) are met. This genetic pattern matches our field observation that adult males are nonterritorial and wide-ranging, and commonly moved in and out of burrows where females reside. We can combine our estimates of neighborhood size (N_e) with estimates of effective migration rate ($N_e m$) of 2.3–3.3 to estimate migration rates among subpopulations. Thus, we may expect that m , the probability of successful dispersal per generation between subpopulations separated by ~10 km, is between 0.005 and 0.016. The effective population size and neighborhood size for the Piute ground squirrel are considerably larger than estimates for the banner-tailed kangaroo rat (*Dipodomys spectabilis*; Waser and Elliot 1991) and white-footed mouse (*Peromyscus leucopus*; Smith and Sloan 1988). Average dispersal distances were much shorter for these species than for the Piute ground squirrel.

Estimates of N_e/N such as Nunney's (1993) are based on variance-effective population sizes and are calculated from demographic data. Estimates of variance-effective size are usually less than the number of adults in a population because a few individuals account for most of the reproduction in a population (Crow and Denniston 1988; Nunney and Elam 1994). As is seen in Tables 2 and 3, variance-effective size depends critically on the variance in reproductive success. Our estimates of N_e/N decrease if we assume that fewer females or males contributed to reproduction. However, our observations of male movements and the numbers of juveniles emerging from each burrow show that it is unrealistic to assume that few breeding adults produced large litters. Even if we relax the assumption that all males breed ($\alpha_m = 1.0$) in order to include only 50% of males ($\alpha_m = 0.5$), estimates of N_e/N are only reduced to ~0.4 (Table 3). Thus, our estimates of N_e/N are robust and represent the average for a number of mammalian species, ~0.5 (Nunney and Elam 1994).

Our population endured a bottleneck caused by drought in 1992 (Van Horne et al. 1997). The long-term effect of a drought would be a reduction in effective population size in proportion to the smallest population size during the bottleneck (the harmonic mean of N ; Crow and Denniston 1988). Over the long term, population bottlenecks may reduce genetic variability and effective population size. This reduction in genetic size of a population is known as inbreeding-effective size and results from limited genetic variability in the past

rather than from present-day influences of the mating system or variance in reproductive success. The inbreeding-effective size may be much smaller than the variance-effective size estimated from demographic and dispersal data (cf. Grant and Grant 1992). The 1992 bottleneck in the Piute ground squirrel population resulted from both reduced reproduction by adults and lower juvenile survival, rather than from lower adult survival. Thus, surviving adults provided a "genetic bridge" that served to ameliorate the effects of genetic drift during the bottleneck (Nunney and Elam 1994), and would prevent the inbreeding-effective size of the population from becoming smaller than the variance-effective population size. Our measures of genetic variation within subpopulations show no deficiency of heterozygosity immediately after the drought (Table 4), which further strengthens the conclusion that the genetic effects of the drought were small. Finally, despite lowered juvenile survival and lower reproduction, none of the subpopulations became extinct during or immediately after the drought. Thus, our estimates of $\Theta_{\text{populations}}$ (F_{ST}) are not likely to be inflated by extinction-recolonization processes (McCauley 1991; Hastings and Harrison 1994; Barton and Whitlock 1997).

The level of genetic differentiation that we observed between areas ($\Theta \sim 0.07$ –0.10) compares favorably with estimates made on similar spatial scales for other small mammals (Chesser 1983; Lidicker and Patton 1987; Waser and Elliott 1991; van Staaden et al. 1994; van Staaden 1995; Roach 1999). Two exceptions make interesting contrasts. First, Columbian ground squirrels (*Spermophilus columbianus*) in the Rocky Mountains in southern Alberta, Canada, had lower levels of genetic subdivision among populations ($F_{\text{ST}} = 0.026$) in mountain valleys separated by 20–180 km (Dobson 1994). This contrasting pattern could have arisen because of historical effects like dispersal/colonization during a short period in the past and large effective population sizes since colonization (Koenig et al. 1996). Second, northern Idaho ground squirrels (*Spermophilus brunneus brunneus*) displayed greater genetic subdivision ($F_{\text{ST}} = 0.167$) over a 30-km distance than we found in the Piute ground squirrel (Gavin et al. 1999). The Idaho ground squirrel had smaller subpopulations and a shorter dispersal distance (20–200 m) than the Piute ground squirrel, and its habitat became fragmented recently (Gavin et al. 1999).

Our sampling regime did not allow us to examine the extent to which our measures of population subdivision could

be inflated by local genetic structure resulting from “matrilineal effects” (Templeton 1987; van Staaden 1995; Dobson et al. 1998). Given that females are sedentary, we may expect that individuals collected from a single area may be closely related to each other, and that this family structure will contribute to the genetic variation among areas. In Richardson’s ground squirrel (*Spermophilus richardsoni*) and the black-tailed prairie dog (*Cynomys ludovicianus*), where local lineages were identified both by direct observation and by genetic markers, evidence of genetic differentiation between matrilineal groups could be detected on a smaller spatial scale than we have sampled (van Staaden et al. 1994; Dobson et al. 1998). It is unlikely, however, that matrilineal effects will be as strong in the Piute ground squirrel because males are nonterritorial in this species.

Our study shows how genetic data from neutral genetic markers provide insights into how much movement by individuals is likely. Although demographic data used to estimate birth rates, death rates, fertility, and effective population sizes are also critical for understanding population dynamics, genetic approaches provide the best measures of effective dispersal associated with gene flow. Understanding such movement is vital in assessing the effects of fragmentation. Despite regular dispersal by individuals, animals like Piute ground squirrels that live in continuous habitats nonetheless display isolation-by-distance population structure, where subpopulations 10 km from each other are slightly differentiated. Habitat fragmentation can have two consequences for populations with this kind of genetic structure. First, because neighborhoods of individuals are already genetically subdivided, fragmentation will quicken the pace of differentiation by further limiting gene flow and reducing effective population sizes (Hedrick and Gilpin 1997). Second, because of limited dispersal, recolonization after extinction will magnify this subdivision, as is predicted in “propagule pool” models of metapopulation structure, where recolonization is by individuals from nearby subpopulations (McCauley 1991; Hastings and Harrison 1994; Barton and Whitlock 1997). The probability of recolonization may be further reduced if Piute ground squirrels avoid vacant habitat, as do Columbian ground squirrels and black-tailed prairie dogs (Halpin 1987; Weddell 1991). The importance of habitat loss and fragmentation to adaptive evolution, and whether restricted gene flow may lead to inbreeding depression in traits that affect population growth, remain to be explored empirically (see Lande 1988; Lynch 1996). Nevertheless, the combination of genetic and demographic approaches provides critical insight into the potential effects of fragmentation.

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References

- Antolin, M.F., Bosio, C.F., Cotton, J., Sweeney, W., Strand, M.R., and Black, W.C., IV. 1996. Intensive linkage mapping in a wasp (*Bracon hebetor*) and a mosquito (*Aedes aegypti*) with SSCP analysis of RAPD markers. *Genetics*, **143**: 1727–1738.
- Armitage, K.B. 1981. Sociality as a life-history tactic of ground squirrels. *Oecologia*, **48**: 36–49.
- Barton, N.H., and Whitlock, M.C. 1997. The evolution of metapopulations. *In* *Metapopulation biology: ecology, genetics and evolution*. Edited by I. Hanski and M.E. Gilpin. Academic Press, San Diego. pp 183–210.
- Black, W.C., IV, and DuTeau, N.M. 1997. RAPD-PCR and SSCP analysis for insect population genetic studies. *In* *The molecular biology of insect disease vectors: a methods manual*. Edited by J. Crampton, C.B. Beard, and C. Louis. Chapman and Hall Publishers, New York. pp. 361–373.
- Black, W.C., IV, DuTeau, N.M., Puterka, G.J., Nechols, J.R., and Pettorini, J.M. 1992. Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids. *Bull. Entomol. Res.* **82**: 151–159.
- Chepko-Sade, B.D., Shields, W.M., Berger, J., Halpin, Z.T., Jones, W.T., Rogers, L.L., Rood, J.P., and Smith, A.T. 1987. The effects of dispersal and social structure on effective population size. *In* *Mammalian dispersal patterns: the effects of social structure on population genetics*. Edited by B.D. Chepko-Sade and Z.T. Halpin. University of Chicago Press, Chicago. pp. 287–321.
- Chesser, R.K. 1983. Genetic variability within and among populations of the black-tailed prairie dog. *Evolution*, **37**: 320–331.
- Chesser, R.K. 1991. Gene diversity and female philopatry. *Genetics* **127**: 437–447.
- Crow, J.F., and Denniston, C. 1988. Inbreeding and variance effective population numbers. *Evolution*, **42**: 482–495.
- Dobson, F.S. 1994. Measures of gene flow in the Columbian ground squirrel. *Oecologia*, **100**: 190–195.
- Dobson, F.S., Chesser, R.K., Hoogland, J.L., Sugg, D.W., and Foltz, D.W. 1998. Breeding groups and gene dynamics in a socially structured population of prairie dogs. *J. Mammal.* **79**: 671–680.
- Gaines, M.S., Diffendorfer, J.E., Tamarin, R.H., and Whittam, T.S. 1997. The effects of fragmentation on the genetic structure of small populations. *J. Hered.* **88**: 294–304.
- Gavin, T.A., Sherman, P.W., Yensen, E., and May, B. 1999. Population genetic structure of the northern Idaho ground squirrel (*Spermophilus brunneus brunneus*). *J. Mammal.* **80**: 156–168.
- Grant, P.R., and Grant, B.R. 1992. Demography and the genetically effective sizes of two populations of Darwin’s finches. *Ecology*, **73**: 766–784.
- Halpin, Z.T. 1987. Natal dispersal and the formation of new social groups in a newly established town of black-tailed prairie dogs. *In* *Mammalian dispersal patterns: the effects of social structure on population genetics*. Edited by B.D. Chepko-Sade and Z.T. Halpin. University of Chicago Press, Chicago. pp 104–118.
- Hastings, A., and Harrison, S. 1994. Metapopulation dynamics and genetics. *Annu. Rev. Ecol. Syst.* **25**: 167–188.
- Hedrick, P.E., and Gilpin, M.E. 1997. Genetic effective size of a metapopulation. *In* *Metapopulation biology: ecology, genetics and evolution*. Edited by I. Hanski and M.E. Gilpin. Academic Press, San Diego. pp. 165–181.

- Hoelkamp, K.E. 1984. Dispersal in ground-dwelling sciurids. *In* The biology of ground-dwelling squirrels. *Edited by* J.O. Murie and G.R. Michener. University of Nebraska Press, Lincoln. pp. 297–320.
- Hoffman, R.S., Anderson, C.G., Thorington, R.W., Jr., and Heaney, L.R. 1993. Family Sciuridae. *In* Mammal species of the world: a taxonomic and geographic reference. 2nd ed. *Edited by* D.E. Wilson and D.M. Reeder. Smithsonian Institution Press, Washington, D.C. pp. 419–465.
- Holsinger, K.E. 1996. The scope and limits of conservation genetics. *Evolution*, **50**: 2558–2561.
- Kochert, M.N., and Pellant, M. 1986. Multiple use in the Snake River Birds of Prey Area. *Rangelands*, **8**: 217–220.
- Koenig, W.D., Van Vuren, D., and Hooge, P.N. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends Ecol. Evol.* **11**: 514–517.
- Knick, S.T., and Rotenberry, J.T. 2000. Ghosts of habitats past: contribution of landscape change to current habitats used by shrubland birds. *Ecology*, **81**: 220–227.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science (Washington, D.C.)*, **241**: 1455–1460.
- Lidicker, W.Z., and Patton, J.L. 1987. Patterns of dispersal and genetic structure in populations of small rodents. *In* Mammalian dispersal patterns: the effects of social structure on population genetics. *Edited by* B.D. Chepko-Sade and Z.T. Halpin. University of Chicago Press, Chicago. pp. 144–161.
- Lynch, M. 1996. A quantitative-genetic perspective on conservation issues. *In* Conservation genetics: case histories from nature. *Edited by* J.C. Avise and J.L. Hamrick. Chapman and Hall, New York. pp. 417–501.
- Lynch, M., and Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. *Mol. Ecol.* **3**: 91–99.
- May, B.A., Gavin, T.A., Sherman, P.W., and Korves, T.M. 1997. Characterization of microsatellite loci in the northern Idaho ground squirrel *Spermophilus brunneus brunneus*. *Mol. Ecol.* **6**: 399–400.
- McCauley, D.E. 1991. Genetic consequences of local population extinction and recolonization. *Trends Ecol. Evol.* **6**: 5–8.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583–590.
- Nunney, L. 1993. The influence of mating system and overlapping generations on effective population size. *Evolution*, **47**: 1329–1341.
- Nunney, L., and Campbell, K.A. 1993. Assessing minimum viable population size: demography meets population genetics. *Trends Ecol. Evol.* **8**: 234–239.
- Nunney, L., and Elam, D.R. 1994. Estimating the effective population size of conserved populations. *Conserv. Biol.* **8**: 175–184.
- Olson, G.S., and Van Horne, B. 1998. Dispersal patterns of juvenile Townsend's ground squirrels in southwestern Idaho. *Can. J. Zool.* **76**: 2084–2089.
- Roach, J.L. 1999. Genetic analysis of a black-tailed prairie dog (*Cynomys ludovicianus*) metapopulation in shortgrass steppe. M.S. thesis, Colorado State University, Fort Collins.
- Schaffer, H.E., and Sederoff, R.R. 1981. Improved estimation of DNA fragment lengths from agarose gels. *Anal. Biochem.* **115**: 113–122.
- Smith, G.W., and Johnson, D.R. 1985. Demography of a Townsend ground squirrel population in southwestern Idaho. *Ecology*, **66**: 171–178.
- Smith, H.R., and Sloan, R.J. 1988. Estimated effective population size in a wild population of *Peromyscus leucopus*. *J. Mammal.* **69**: 176–177.
- Templeton, A.R. 1987. Inferences on natural population structure from genetic studies on captive mammalian populations. *In* Mammalian dispersal patterns: the effects of social structure on population genetics. *Edited by* B.D. Chepko-Sade and Z.T. Halpin. University of Chicago Press, Chicago. pp. 257–272.
- U.S. Department of the Interior. 1996. Effects of military training and fire in the Snake River Birds of Prey National Conservation Area. BLM/IDARNG Research Project Final Rep., U.S. Geological Survey, Biological Resources Division, Snake River Field Station, Boise, Idaho.
- Van Horne, B., Olson, G.S., Schooley, R.L., Corn, J.G., and Burnham, K.P. 1997. The effects of drought and prolonged winter on Townsend's ground squirrels in shrubsteppe habitats. *Ecol. Monogr.* **67**: 295–315.
- van Staaden, M.J. 1995. Breeding tactics, social structure, and genetic variation in mammals: problems and prospects. *Acta Theriol. Suppl. No. 3*. pp. 165–182.
- van Staaden M.J., Chesser, R.K., and Michener, G.R. 1994. Genetic correlations and matrilineal structure in a population of *Spermophilus richardsonii*. *J. Mammal.* **75**: 573–582.
- Vaughn, T.T., and Antolin, M.F. 1998. Population genetics of an opportunistic parasitoid in an agricultural landscape. *Heredity*, **80**: 152–162.
- Waser, P.M., and Elliot, L.F. 1991. Dispersal and genetic structure in kangaroo rats. *Evolution*, **45**: 935–943.
- Weddell, B.J. 1991. Distribution and movements of Columbian ground squirrels (*Spermophilus columbianus* (Ord)): are habitat patches like islands? *J. Biogeogr.* **18**: 385–394.
- Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**: 1358–1370.
- Williams, J.G., Kubric, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful genetic markers. *Nucleic Acids Res.* **18**: 6531–6535.
- Wright, S. 1978. *Evolution and genetics of populations*. Vol. 4. University of Chicago Press, Chicago.