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# Insights into recently fragmented vole populations from combined genetic and demographic data

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**Abstract**

**We combined demographic and genetic data to evaluate the effects of habitat fragmentation on the population structure of the California red-backed vole (*Clethrionomys californicus*). We analysed variation in the mitochondrial DNA (mtDNA) control region and five nuclear microsatellite loci in small samples collected from two forest fragments and an unfragmented control site in 1990–91. We intensively sampled the same forest fragments and two different control sites in 1998 and 1999. Vole abundances fluctuated greatly at sizes below 50 individuals per fragment. Fragment populations had significantly lower mtDNA allelic diversity than controls, but not nuclear heterozygosity or numbers of alleles. The use of only trapping and/or mtDNA marker data would imply that fragment populations are at least partially isolated and vulnerable to inbreeding depression. In contrast, the abundance estimates combined with microsatellite data show that small fragment populations must be linked to nearby forests by high rates of migration. These results provide evidence for the usefulness of combining genetic and demographic data to understand nonequilibrium population structure in recently fragmented habitats.**

*Keywords*: genetic variation, habitat fragmentation, population dynamics

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## Introduction

Habitat loss and fragmentation threaten species throughout the world. As a result of habitat fragmentation, many species are limited to a small number of habitat patches separated by a matrix of less suitable habitat. Species with limited dispersal capabilities are particularly vulnerable to the negative genetic and demographic impacts of habitat fragmentation. Adverse impacts can include a reduction in local population size, reduced migration, increased population size fluctuations and inbreeding depression. After roughly a decade of debates over the relative importance of demographic and genetic factors (e.g. Lande 1988), empirical evidence has shown that each can affect vulnerability to extinction (Newman & Pilson 1997; Groom 1998; Saccheri *et al*. 1998).

Despite the importance of demographic and genetic changes to fragmented populations, very few studies of recently fragmented populations have integrated rigorous demographic and genetic analyses. When rigorous demographic and genetic approaches are not combined, important questions about population structure are often left unanswered and understanding of how well theory meshes with real-world examples is not improved. For example, mark–recapture data may fail to detect important long-distance dispersal events because such events can be rare and difficult to observe (Koenig *et al*. 1996). Genetic analyses may be superior for detecting dispersal events, but are usually inadequate for estimating migration among populations because the distribution of genetic variation among populations can be the result of historic or current gene flow (Milligan *et al*. 1994). However, the combination of these two complementary approaches can be used to tease apart confounded interpretations of the effects of fragmentation on wild populations.

We combined genetic and demographic analyses in our study of the California red-backed vole (*Clethrionomys californicus*), a species that is thought to be very important to the fragmented forest communities where it is found. In the region of our study, California red-backed voles depend upon below-ground fruiting bodies, or truffles, of ectomycorrhizal fungi for 70–80% of their diet (Ure &

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Maser 1982). In turn, truffle specialists such as this vole and the northern flying squirrel (*Glaucomys sabrinus*) are thought to be the primary dispersers of the ectomycorrhizal fungi spores contained in truffles (Maser *et al*. 1978). Conifers require mycorrhizal fungi in order to obtain adequate water and soil nutrients for growth, so the decreased abundance and diversity of ectomycorrhizal fungi in clearcuts may explain why some forests are slow or unable to regenerate (Perry *et al*. 1989; Amaranthus *et al*. 1994; Hagerman *et al*. 1999). Given this interesting web of interactions among these forest ecosystem components, any negative effects of forest fragmentation upon voles may have indirect negative impacts upon the dispersal of mycorrhizal fungi and, ultimately, the regeneration of forests from clearcuts.

Trapping studies conducted over the past 50 years have shown that California red-backed voles disappear following clearcutting and burning of coniferous forests (Tevis 1956; Gashwiler 1970), and that they have much more limited movement patterns than other sympatric rodents (Gashwiler 1959). Mills (1995) extended these findings to show more detailed effects of forest fragmentation on California red-backed voles on forest fragments surrounded by recent clearcuts in south-western Oregon. He found that the relative abundance of voles decreased from the forest interior toward the forest edge and that voles were nearly absent from 5–35-year-old clearcuts. In addition, DNA fingerprints of a small sample of voles from forest fragments showed higher band-sharing than voles from unfragmented (control) forests (Mills 1993). These data imply possible isolation and inbreeding effects for vole populations on forest fragments.

During the summers of 1997–99, we revisited 12 isolated forest fragments studied by Mills (1995, 1996) in the summers of 1990 and 1991. However, it became apparent after the 1997 field season that adequate sample sizes for rigorous demographic and genetic analyses could not be obtained by trapping a large number of sites for only a few nights each field season. Therefore, we intensified our trapping efforts at two of the forest fragments and two controls throughout the summers of 1998 and 1999, which permitted us to use rigorous abundance estimates instead of abundance indices (Nichols & Pollock 1983).

Our primary objective was to investigate the effects of habitat fragmentation on vole population structure by integrating intensive mark–recapture studies with analyses of mitochondrial DNA (mtDNA) and nuclear microsatellite loci variation. First, we estimated vole abundances on forest fragment and control sites in 1998 and 1999 and asked whether fragment populations are small enough to lose variation due to genetic drift if they are isolated. Next, we analysed vole tissue samples collected in 1990–91 by Mills (1993) and samples collected in 1998 to determine whether genetic variation was lower on fragments compared with controls in both 1990–91 and 1998. Finally, we combined our demographic and genetic data to determine whether the observed distribution of genetic variation is consistent with that expected for populations isolated by fragmentation.

## Materials and methods

### *Site descriptions and trapping protocols*

The forest fragment and continuous forest control sites we studied are located in the Sucker Creek drainage of southwest Oregon, USA (Fig. 1). These forests are dominated by old-growth Douglas-fir (*Pseudostuga menzesii*) trees and

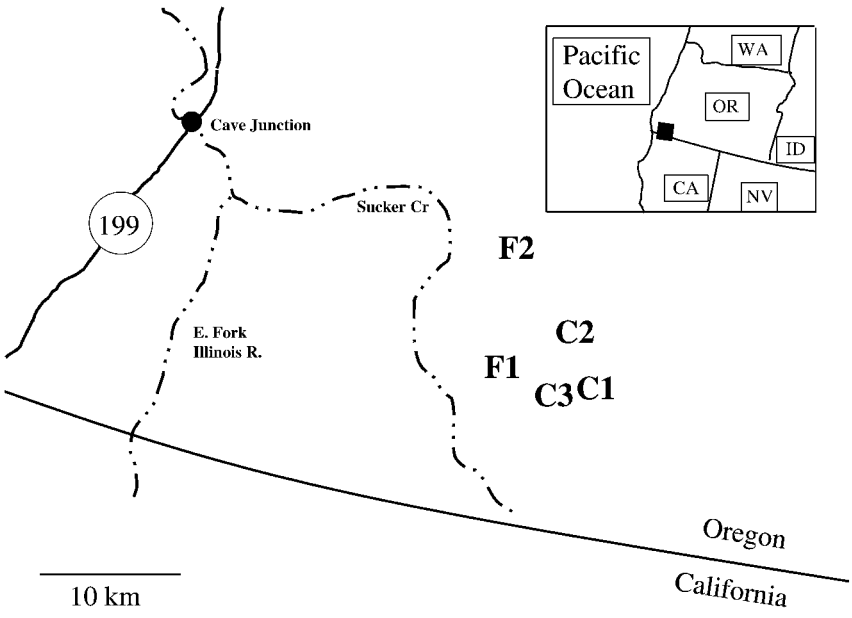


**Fig. 1** Map of the area used to study California red-backed vole genetics and demography in forest fragments (F1 and F2) and large, unfragmented forest sites (C1, C2 and C3).

have herbaceous understories. This habitat has been heavily fragmented by timber harvest, most of which occurred between the 1950s and 1980s (for detailed history see Jules *et al*. 1999). The fragment sites F1 and F2 (referred to as E and O in Mills 1995) are 3.0 and 3.7 ha in size and were created by clearcutting in 1987 and 1981, respectively. They are over 150 m from the nearest continuous forests. We completely covered each fragment with a grid of traps spaced at 15-m intervals. F1 was covered with 122 traps and F2 was covered with 154 traps. We also surrounded each fragment with four transects of six traps each, placed 50 m into the clearcut from the forest edge and parallel to the edge.

We used the same grid and peripheral transect configuration to trap voles in control sites located in continuous forest (> 1000 ha in size). Control sites C1 and C2 are located > 150 m from the nearest forest/road edge near the southern and northern borders of Oregon Caves National Monument, respectively. Site C3 (referred to as G in Mills 1995), is 0.8 km east of C1, and was not used in 1998 or 1999 because it abuts a forest edge. At C1 and C2, we set 102 traps in a $17 \times 6$ grid with 15-m spacing between traps. In addition, a transect of six traps with 15-m spacing was set parallel to each edge of the grid and 50 m from the grid, yielding 24 traps in peripheral transects that were analogous to the those in the clearcuts surrounding fragments.

We trapped all four sites (F1, F2, C1 and C2) from June to August of 1998 and 1999. Our first trapping session at each site was eight consecutive nights, which allowed us to capture many unique individuals. All seven subsequent trapping sessions were four consecutive nights. Each evening of the trapping sessions, we baited small Sherman live traps with oat groats, sunflower seeds and 1 $cm^3$ of fresh apple. We also placed polyester batting inside each trap, and placed each trap inside a pint milk container that was lined with batting to increase protection from inclement weather. Each morning we checked and closed all traps to minimize mortality. The four trapping sessions conducted on all sites in the summers of both 1998 and 1999 were separated by 16 days, except the first and second sessions of 1998, which were 20 days apart. This trapping approach, referred to as the robust design (Pollock *et al*. 1990), allowed us to estimate vole abundance each trapping session and to estimate survival between trapping sessions. Details of the population dynamics of these populations, including sex, site and time-specific survivorship estimates, are presented elsewhere (Tallmon 2001).

### *Demography*

We recorded individual capture histories for each vole during each trapping session. This allowed us to estimate vole abundance ($\hat{N}$) on F1, F2, C1 and C2 each session using closed capture models in the computer program MARK (White & Burnham 1999). Prior to estimating $\hat{N}$ values, we developed a set of simple models representing possible patterns in the capture and recapture probabilities. These candidate models were necessarily simple because of the small numbers of individuals captured, but they included different combinations of variation in capture and recapture probability among sites and through time (Appendix 1). $\hat{N}$ values presented in the results are from the candidate model that best described the data collected for each trapping session, as determined using Akaike's Information Criteria (AIC). AIC is a parsimony-based approach used in mark–recapture studies to quantitatively rank candidate models of population demography (White & Burnham 1999). We presented $\hat{N}$ s from the best model only ($\Delta AIC = 0.00$), because the other models that received some support ($\Delta AIC < 2.00$) provided identical $\hat{N}$ s in nearly all cases.

### *Genetic analysis*

All voles captured were toe-clipped for individual identification. In 1990–91, tissue was collected from 16 and 9 individuals on fragments F1 and F2, respectively, and 24 individuals on control C3. In 1998, ≈35 individuals captured in the first two trapping sessions at each site were used in genetic analyses, for a total of 139 voles. The genetic samples from each site were roughly equally composed of males and females, contained three or fewer juveniles, and were collected from the entire area covered by each trapping grid.

We extracted vole DNA using a Gentra Puregene kit and then analysed both mitochondrial and nuclear genetic variation. A random subset of ≈20 individuals was selected from the 1998 samples for mtDNA analysis. Modified Kocher primers (Kocher *et al*. 1989; Shields & Kocher 1991) L16007 (5′-CCCAAAGCTAAAATTCTAA-3′) and H16498 (5′-CCTGAAGTAGGAACCAGATC-3′) were used to amplify the mtDNA control region of individual voles in polymerase chain reactions (PCR; Appendix 2). The PCR profile included a 94 °C denaturing phase for 5 min followed by 35 repetitions of this cycle: 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min. We then incubated the amplified mtDNA from each individual with five restriction enzymes (*Nla*III, *Mbo*I, *Hin*dIII, *Rsa*I and *Hae*III) for 6 h at 37 °C. Restriction fragments were electrophoresed through 2.5% agarose gels, and visualized using ethidium bromide staining. Mitochondrial DNA fragments were sized by comparing them with a 100-bp standard and allelic diversity within forest fragments and control sites was estimated using REAP Version 4.0 (McElroy *et al*. 1992).

We amplified alleles at five microsatellite loci in PCR reactions with primer sequences developed for congenerics by Ishibashi *et al*. (1997) or Gockel *et al*. (1997; Appendix 2). Each 10-μL reaction contained 20–200 ng DNA amplified in a thermocycler for 30 cycles with the following

temperature regime: 2 min at 94 °C (first cycle only), 30 s at 94 °C, 30 s at an annealing temperature (Appendix 2), and 60 s at 72 °C. PCR products were run in 7% denaturing polyacrylamide gels in 1× TBE buffer, visualized using a Hitachi FM-BIO 100 scanner, and genotyped by comparing PCR product lengths with MapMarkerLOW size standards. We included two sample standards on all gels to ensure consistent genotyping.

We analysed the microsatellite data using the computer programs FSTAT (Goudet 1995) and GENEPOP (Raymond & Rousseau 1995). Specifically, we used FSTAT to calculate expected and observed heterozygosities, the mean number of alleles per population, and to test for departures from Hardy–Weinberg (HW) proportions. GENEPOP was used to test for gametic disequilibrium between each pair of loci in each population and to estimate gene flow.

We tested for lower levels of nuclear heterozygosity ($\hat{H}_o$), numbers of alleles ($\hat{A}$) and mtDNA allelic diversity ($\hat{h}$) in 1998 samples from fragment populations ($n = 2$) relative to controls ($n = 2$) using a one-tailed $t$-test. This approach was based on an *a priori* expectation of lower levels of genetic variation in fragment populations (Mills 1993, 1995). $\hat{H}_o$ and $\hat{h}$ were arcsine square-root transformed before statistical analysis.

In order to compare $\hat{A}$ among samples of unequal sizes collected in 1990–91, we developed a TURBOPASCAL (Borland 1991) bootstrapping program (available from DAT). In each of 100 replicates, the program drew alleles with replacement from the C3 sample at sample sizes equal to those collected from F1 ($n = 16$) and F2 ($n = 9$) in 1990–91. We did not use a statistical test to compare variation among populations sampled in 1990–91 because individuals were collected over two years, which could bias estimates of genetic variation.

### *Simulations*

We calculated expected losses of genetic variation for isolated, finite populations under conditions similar to those found on the forest fragments using the simulation model EASYPOP (Version 1.7.1, Balloux 1999). Specifically, we used the relationships between effective population size ($N_e$), the female effective population size ($N_f$), and our estimates of vole population sizes ($N$) on fragments to examine the expected rate of drift in vole populations if truly isolated on forest fragments. In an isolated, finite population, the initial heterozygosity ($H_i$) present at nuclear loci will decrease over $t$ generations to a lower level ($H_t$), following the equation

$$H_t = (1 - 1/(2N_e - 1))^t H_i,$$

where $N_e$ is the effective population size (Wright 1931). $N_e$ is an adjusted $N$ that accounts for 'nonideal' conditions that occur in natural populations, such as nonrandom variability in lifetime reproductive success, unequal sex-ratio, and fluctuating population size, that reduce $N_e$ below $N$. $N_e$ is usually between 30 and 50% of $N$ in wild vertebrate populations (Kalinowski & Waples 2001).

Mitochondrial DNA generally has an effective size that is one-fourth that of nuclear genes (Takahata & Palumbi 1985), and is maternally inherited in mammals. Therefore, the decay in initial mtDNA allelic diversity ($h_i$) over $t$ generations can be modelled using the equation

$$h_t = (1 - 1/N_f)^t h_i,$$

where $N_f$ is the female effective population size.

EASYPOP simulated genetic drift at five nuclear loci in a randomly mating population ($N_e = 10$ or 24) with equal numbers of males and females, $H_i = 1.00$, and a mutation rate of $10^{-2}$ per locus per generation. In addition, EASYPOP simulated genetic drift at a single, nonrecombining mtDNA locus in haploid population ($N_f = 5$ or 15) with $h_i = 1.00$. Because logging records indicate that these fragments have been surrounded by clearcuts (and therefore potentially isolated) for at least 10 years and voles have between 2 and 3 generations per year (Gashwiler 1977), $H_t$ or $h_t$ was recorded at the 10th, 20th and 30th generations in each of 50 replicate simulations.

We compared the empirical data to the model results, in order to determine whether the amounts of genetic variation found on the fragments are consistent with those expected for isolated populations. If the 1998 fragment samples show allelic diversity and heterozygosity values that are low relative to the samples from the controls and consistent with the reductions in genetic variation in the simulated populations relative to starting conditions, then the empirical data are consistent with the hypothesis that fragment populations are isolated. In contrast, if the allelic diversity and heterozygosity in fragment samples are not decreased relative to the controls and are higher than the simulated populations under isolation, then the empirical data are consistent with the hypothesis that fragment populations are not isolated.

## Results

### *Population dynamics*

We trapped and marked 368 voles at F1, F2, C1 and C2 in the summers of 1998 and 1999. The abundance estimates ($\hat{N}$s) were quite small and extremely precise (Fig. 2). The abundance estimates had standard errors of nearly zero because capture and recapture probabilities were high. The estimated capture probabilities for each trapping session ranged from 0.92 to 1.00 and recapture probabilities ranged from 0.87 to 0.98 (Appendix 1), suggesting that abundances

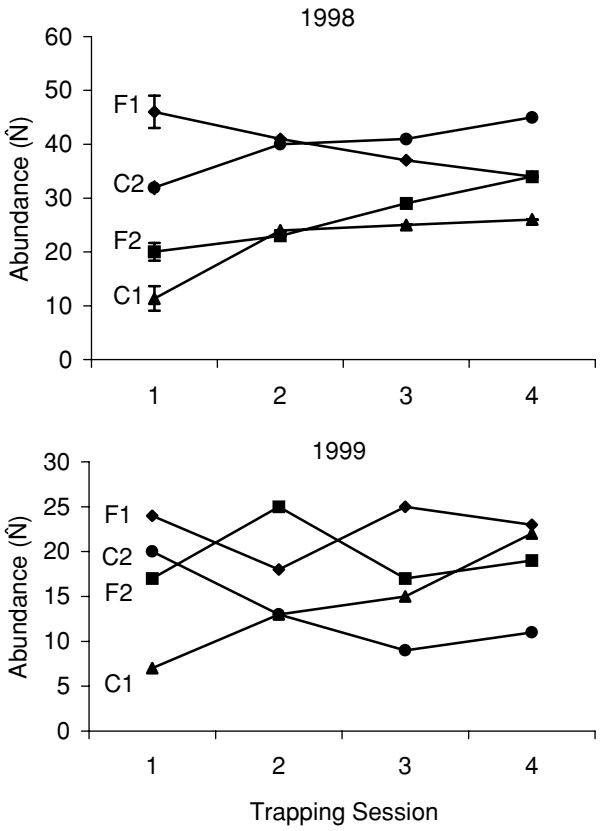


**Fig. 2** Vole abundances on forest fragment (F1, F2) and control sites (C1, C2) estimated each of four trapping sessions during the summers of 1998 and 1999. Standard errors (SE) are shown only for first trapping session of 1998 because high capture and recapture probabilities resulted in SE values of zero in all other sessions.

are known almost exactly. Because unweaned individuals could not be trapped, these abundances represent only the subadults and adults.

In each session, there were fewer than 50 voles on each fragment (range ≈17–46). All populations fluctuated greatly in size both among trapping sessions and between years, but population sizes were generally larger in 1998 than 1999. The turnover of individuals captured in successive trapping sessions was highly variable, with previously uncaptured voles comprising 10–60% of the total individuals trapped in the second through fourth trapping sessions at each site each summer. Three to six individuals survived at each site from trapping sessions in 1998–99. Despite our extensive trapping efforts, we detected only one immigration event, in which a vole captured in a clearcut was later found to have immigrated into a fragment.

### *Mitochondrial DNA analysis*

Mitochondrial DNA variation was lower in vole populations on forest fragments than in the control populations. In 1990–91, F1 and F2 samples had no mtDNA variation and were fixed for the same allele (Table 1). In contrast, allelic diversity ($\hat{h}$) = 0.34 (SE = 0.09) and three alleles were present at control site C3.

The pattern of lower mtDNA variation on the fragments relative to the controls was consistent in the larger 1998 samples (Table 1), even though $\hat{h}$ was generally greater in 1998 than 1990–91. In larger samples collected in 1998 at all four sites, the fragments showed lower $\hat{h}$ than the controls ($P < 0.05$, $t = 3.21$, df = 2). In fact, F1 and F2 showed ≈70 and 100% reductions in $\hat{h}$ relative to the controls, and there was no overlap of the standard errors of control and fragment estimates. F1 had three mtDNA alleles, but two alleles were represented by single individuals, and F2 was fixed for the same allele present at this site in the 1990–91 sample. In contrast, control sites C1 and C2 had four and three alleles each, together contained all five alleles observed in this study, and $\hat{h} \approx 0.60$ in both populations.

### *Microsatellite DNA analysis*

In contrast to the mtDNA results, we found extremely high levels of variation at five nuclear microsatellite loci in all populations (Table 2). Three microsatellite loci showed significant departures from HW proportions due to heterozygote deficits in 1990–91 and 1998 samples. There are many possible explanations for the departure seen at *CRB-5*, *Cgl-15* and *Cgl-19*, including natural selection, population subdivision and null alleles. The observed pattern is most consistent with null alleles as the cause. The primers we used were developed for other species, so mutations in primer locations could have caused allele amplifications to fail. In fact, there were 1–3 different individuals that failed to provide PCR products at each of these three loci in the 1998 samples, which suggests the presence of null allele homozygotes at fairly high frequencies. Whatever the cause of the heterozygote deficit, it does not appear to have affected the relative amount of genetic variation in fragment and control samples. However, it led us to compare observed heterozygosity ($\hat{H}_o$), rather than expected heterozygosity ($\hat{H}_e$), among populations. Only one of 40 pairwise tests showed evidence of gametic disequilibrium among loci, which is not greater than that expected by chance alone.

For 1990–91 samples uncorrected for sample size differences among populations, $\hat{H}_o$ was generally high, but it was somewhat lower on the fragments than the control (Table 2). Similarly, the estimated mean number of alleles per locus (Â) was lower in fragments than the control. The apparent reduction in Â on fragments disappeared after accounting for the larger sample size on the control. Bootstrapping of the C3 data at sample sizes equal to those collected from fragments F1 and F2 in 1990–91 indicated that Â is not lower on fragments. In bootstraps with $n = 16$ and $n = 9$, C3

**Table 1** Mitochondrial DNA allele frequencies, number of alleles (A), and allelic diversity ($h$) in voles samples from forest fragments (F1, F2) and control sites (C1, C2, C3)

| | | | mtDNA Alleles | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Site | $n$ | 1 | 2 | 3 | 4 | 5 | A | $\hat{h}$ (SE) |
| 1990–91 | F1 | 11 | 1.00 | — | — | — | — | 1 | 0.00 (0.00) |
| | F2 | 9 | 1.00 | — | — | — | — | 1 | 0.00 (0.00) |
| | C3 | 20 | 0.80 | 0.15 | — | — | 0.05 | 3 | 0.34 (0.09) |
| 1998 | F1 | 22 | 0.91 | 0.04 | 0.04 | — | — | | 0.17 (0.07) |
| | F2 | 21 | 1.00 | — | — | — | — | 1 | 0.00 (0.00) |
| | C1 | 23 | 0.56 | 0.30 | 0.04 | 0.09 | — | 4 | 0.59 (0.06) |
| | C2 | 22 | 0.59 | 0.22 | — | — | 0.18 | 3 | 0.58 (0.08) |

**Table 2** The mean number of alleles (Â), observed heterozygosities ($\hat{H}_o$), and expected heterozygosities ($\hat{H}_e$) at five microsatellite loci in samples (n) from vole populations on forest fragments (F1, F2) and control sites (C1, C2, C3). All statistically significant departures from Hardy–Weinberg proportions were due to heterozygote deficits and are noted by*

| | | | | Locus | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Site | $n$ | | *CRB-5* | *CRB-6* | *Cgl-4* | *Cgl-15* | *Cgl-19* | Mean (SE) |
| 1990–91 | F1 | 16 | Â | 12 | 11 | 12 | 6 | 9 | 10.0 (2.6) |
| | | | $\hat{H}_o$ | 0.67 | 0.80 | 0.94 | 0.46 | 0.44 | 0.66 (0.22) |
| | | | $\hat{H}_e$ | 0.94* | 0.92 | 0.92 | 0.76* | 0.86* | 0.88 (0.07) |
| | F2 | 9 | Â | 9 | 8 | 7 | 7 | 9 | 8.0 (1.0) |
| | | | $\hat{H}_o$ | 0.40 | 0.77 | 0.64 | 0.64 | 0.76 | 0.64 (0.15) |
| | | | $\hat{H}_e$ | 0.92* | 0.88 | 0.89 | 0.87 | 0.88 | 0.89 (0.02) |
| | C3 | 24 | Â | 13 | 12 | 13 | 7 | 10 | 11.0 (2.6) |
| | | | $\hat{H}_o$ | 0.87 | 0.78 | 0.91 | 0.65 | 0.67 | 0.78 (0.12) |
| | | | $\hat{H}_e$ | 0.91 | 0.84 | 0.91 | 0.83 | 0.88* | 0.87 (0.04) |
| 1998 | F1 | 34 | Â | 13 | 10 | 14 | 8 | 11 | 11.2 (2.4) |
| | | | $\hat{H}_o$ | 0.60 | 0.82 | 0.88 | 0.74 | 0.63 | 0.73 (0.20) |
| | | | $\hat{H}_e$ | 0.91* | 0.86 | 0.91 | 0.78 | 0.88* | 0.87 (0.05) |
| | F2 | 35 | Â | 13 | 11 | 11 | 9 | 10 | 10.8 (1.5) |
| | | | $\hat{H}_o$ | 0.81 | 0.85 | 0.98 | 0.48 | 0.73 | 0.77 (0.18) |
| | | | $\hat{H}_e$ | 0.91 | 0.87 | 0.91 | 0.71* | 0.86* | 0.85 (0.08) |
| | C1 | 36 | Â | 14 | 11 | 14 | 10 | 13 | 12.4 (1.8) |
| | | | $\hat{H}_o$ | 0.79 | 0.71 | 0.98 | 0.64 | 0.48 | 0.72 (0.18) |
| | | | $\hat{H}_e$ | 0.89 | 0.86 | 0.91 | 0.81* | 0.91* | 0.88 (0.04) |
| | C2 | 34 | Â | 11 | 11 | 15 | 10 | 11 | 11.6 (2.0) |
| | | | $\hat{H}_o$ | 0.81 | 0.91 | 0.77 | 0.89 | 0.83 | 0.78 (0.13) |
| | | | $\hat{H}_e$ | 0.89 | 0.88 | 0.86 | 0.57 | 0.88* | 0.87 (0.02) |

showed Â = 8.69 (var = 0.002) and Â = 7.33 (var = 0.002), respectively. These are not higher levels than those observed in F1 ($n$ = 16; Â = 10.0) and F2 ($n$ = 9; Â = 8.0) samples.

In 1998 samples, which included over 30 genotypes at all sites, neither $\hat{H}_o$ ($P > 0.05$, $t = 0.01$, df = 2) nor Â ($P > 0.05$, $t = 2.24$, df = 2) was greater in control than fragment populations. Values of $\hat{H}_o$ in fragment samples were intermediate to those from the controls, and there was considerable overlap among the standard errors of $\hat{H}_o$ and Â from controls and fragments (Table 2). In addition, $\hat{H}_o$ and Â did not decrease in F1 and F2 from 1990–91 to 1998, as would be expected for isolated populations. Rather, there was a trend toward increased Â in fragment samples from 1990–91 to 98.

### *Simulations*

The simulations of isolated, finite populations produced results consistent with the observed patterns of mtDNA allelic diversity, but not nuclear heterozygosity, in the fragment populations (Table 1). The range of values for $h$ loss after 10, 20 and 30 generations of isolation in the simulations were consistent with the 70 and 100% lower $h$ in F1 and F2 relative to the controls in 1998 (Table 3). In

**Table 3** Mean (and range) of mitochondrial DNA allelic diversity ($h$) and nuclear heterozygosity ($H$) lost in populations isolated for 10–30 generations in simulations of the computer program EASYPOP (Balloux 1999). Simulations that produced a range of values consistent with the observed reduction in genetic variation in forest fragment populations relative to control populations are shown in bold

| | Allelic diversity ($h$) | | Heterozygosity ($H$) | |
|---|---|---|---|---|
| Generation | $N_f = 5$ | $N_f = 12$ | $N_e = 10$ | $N_e = 24$ |
| 10 | **0.90 (0.40–1.00)** | **0.58 (0.16–1.00)** | 0.34 (0.20–0.51) | 0.16 (0.12–0.23) |
| 20 | **0.96 (0.40–1.00)** | **0.88 (0.46–1.00)** | 0.53 (0.35–0.75) | 0.27 (0.19–0.37) |
| 30 | 1.00 (1.00–1.00) | **0.93 (0.46–1.00)** | 0.61 (0.42–0.85) | 0.35 (0.23–0.69) |

contrast, all of the simulations of the decay of nuclear heterozygosity in isolated populations were greater (12–85%) than the maximum pairwise difference in $\hat{H}_o$ between fragment and control samples (6%).

## Discussion

### *Ecological and molecular evidence for isolation*

Previous studies suggest the California red-backed vole is susceptible to negative effects of habitat loss and fragmentation (Tevis 1956; Gashwiler 1970), including ecological isolation on forest fragments and possible inbreeding effects (Mills 1995, 1996). We found that vole populations on fragments are quite small and that very few voles are ever captured in clearcuts surrounding forest fragments, implying that the small fragment populations are isolated, at least during summer trapping sessions. In a larger geographical study, we have captured only 13 voles in the clearcuts surrounding 12 forest fragment sites in five summers of live-trapping, a capture rate significantly lower than in fragment or control sites (Mills *et al*. in press). The fragment populations also appear to be true populations, and not simply temporary residences, because some adults were present over several consecutive trapping sessions and most were in breeding condition throughout the summer.

The mtDNA data show that fragment populations have lower genetic variation. This pattern was consistent in 1990–91 and in 1998 samples, which implies the possibility of an inbreeding effect in fragment populations. If we had limited our inferences to trapping and mtDNA data, we would have inferred that fragment populations are at least somewhat isolated. This would have been cause for concern because fitness may be reduced in recently isolated, small populations (Newman & Tallmon 2001).

### *Ecological and molecular evidence for migration*

In contrast to the trapping and mtDNA data, the microsatellite data show that inbreeding effects are not currently a threat to the fragment populations. Although fragment populations appeared to have lower heterozygosity and numbers of alleles per locus than the control in 1990–91, this pattern could well have resulted from sample size artefacts and not underlying biological causes. In 1998, when sample sizes were larger and roughly equal across sites, heterozygosities in fragment populations were not different from the controls. In addition, there was no evidence for a loss of alleles in fragment populations through time, which is a more sensitive indicator of a population bottleneck than is the loss of heterozygosity (Allendorf 1986). Instead, the mean number of alleles per locus increased from 1990–91 to 1998 in conjunction with increased sample sizes. The nuclear genetic data refute the hypothesis that vole populations are losing genetic variation due to inbreeding effects on fragments and provide insights into the effects of habitat fragmentation on voles that could not be obtained with trapping and mtDNA data alone.

Nonetheless, these data are of limited usefulness if not combined with the abundance data. In most studies of molecular genetic variation, results showing little or no loss of variation within populations and little divergence among populations can be interpreted as the result of either large local effective population sizes or ongoing gene flow. That is, the failure to detect any loss of genetic variation in vole populations on forest fragments could be the result of one of two ecological mechanisms that are confounded in most molecular genetic studies. First, the vole populations may be relatively unaffected by genetic drift because, although isolated, large $N_e$s maintain prefragmentation levels of genetic variation. Alternatively, these fragment populations may be small enough to rapidly lose genetic variation due to genetic drift, but they are linked to other populations by sufficiently high levels of ongoing gene flow so that prefragmentation levels of genetic variation are maintained within fragment populations.

We used our vole abundance estimates in conduction with computer simulations to find out whether gene flow must be invoked to explain the observed patterns in the genetic data. Vole abundances on these fragments varied from 17 to 46 subadults and adults, which translate into extremely small $N_f$ and $N_e$ values based on previous findings from a wide variety of taxa (Frankham 1995). In EASYPOP simulations of isolated populations with $N_f = 5$ and 12, the range of mtDNA allelic diversity lost was not inconsistent with the 70–100% lower mtDNA allelic diversity on fragments relative to controls. Assuming the control sites represent prefragmentation levels of allelic diversity, these results suggest female-mediated gene flow may be low in these populations. These results are consistent with

live-trapping data collected over in the clearcuts surrounding these and 10 other forest fragments in south-western Oregon, which suggested male-biased dispersal (Mills *et al*. in press), and evidence of male-biased dispersal in many different rodents (e.g. Chepko-Sade & Halpin 1987). However, one of the two unique mtDNA alleles in the 1998 sample from F1 was from a female vole, implying at least some low level of female-based gene flow.

In simulations of isolated populations with $N_e$ = 10 and 24, the loss of nuclear heterozygosity was always greater than the observed relative difference in heterozygosity between fragments and controls. Therefore, we can safely conclude that the small fragment populations are not isolated and have maintained high levels of heterozygosity due to nuclear gene flow. Based on the differences in patterns of maternally inherited mtDNA and bi-parentally inherited microsatellite markers, it appears that gene flow is common and male-biased.

### *Conservation implications*

Vole abundances are mere tens of individuals on forest fragments and fluctuate greatly. Despite small, volatile sizes, the fragment populations do not show a loss of nuclear variation or evidence of decreased demographic rates (Tallmon 2001). Our combined genetic and demographic data show that the clearcut matrix surrounding forest fragments is not an impermeable barrier to vole dispersal and that immigration of voles into forest fragment populations must be high. Immigration is likely important not only to avoid inbreeding effects in the small fragment populations, but also to reduce extinction risk due to chance demographic and environmental events that can threaten small populations (Lande 1993). Details about the population structure of California red-backed voles described in this study could not have been obtained without both rigorous estimates of local population sizes and the use of appropriate genetic markers. This study provides an example of the need to combine the strengths of demographic and genetic data to understand the structure of populations that may be subject to nonequilibrium conditions in recently fragmented habitats.

The primary community-level implication of our data is that voles may well be capable of dispersing mycorrhizal fungi spores as they move through clearcuts between habitat patches. How effectively these voles disperse the spores of mycorrhizal fungi, which are necessary for forest growth and regeneration, remains an important, unanswered question for the health of forest ecosystems.

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This study represents some of David Tallmon's graduate research on the genetic, demographic, and ecological effects of habitat fragmentation. Hope Draheim recently received her BSc in Wildlife Biology at UM and is interested in bird conservation genetics. Scott Mills and Fred Allendorf are professors at UM and have long been interested in evolutionary and conservation issues.

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## Appendix 1

Descriptions, numbers of parameters, and ΔAIC values* of models used to estimate vole abundances at four sites in each of eight trapping sessions. Models included different combinations of variation in capture probabilites (*p*) and recapture probabilites (*c*). Values in bold are the number of parameters and ΔAIC values for the best-supported model in each session. Also shown are *p* and *c* estimates for each trapping session.

| | | 1998 | | | | 1999 | | | |
|---|---|---|---|---|---|---|---|---|---|
| Model | Description | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| *p*(.)*c*(.) | constant *p* and *c* | 6 | 6 | 6 | 6 | 6 | 6 | 6 | **6** |
| | | 19.24 | 15.94 | 5.57 | 4.57 | 3.55 | 5.92 | 1.41 | **0.00** |
| *p*(*T*)*c*(.) | linear time trend in *p*; constant *c* | 7 | 7 | 7 | 7 | 7 | **7** | **7** | 7 |
| | | 0.34 | 11.47 | 4.09 | 1.88 | 0.99 | **0.00** | **0.00** | 2.00 |
| *p*(*T*)*c*(*T*) | linear time trends in *p* and *c* | **8** | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | | **0.00** | 3.99 | 4.58 | 1.16 | 3.12 | 2.00 | 2.01 | 3.75 |
| *p*(*t*)*c*(.) | time dependent *p*; constant *c* | 13 | 9 | **9** | **9** | **9** | **9** | 9 | 9 |
| | | 0.29 | 2.49 | **0.00** | **0.00** | **0.00** | 0.17 | 1.41 | 3.45 |
| *p*(*t*)*c*(*t*) | time dependent *p* and *c* | 19 | **11** | 11 | 11 | 11 | 11 | 11 | 11 |
| | | 2.94 | **0.00** | 2.45 | 1.34 | 4.08 | 4.35 | 5.51 | 7.01 |
| *p*(*t*)*c*(*s***t*) | time dependent *p*; site by | 40 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | time dependent *c* | 27.22 | 10.03 | 15.07 | 7.30 | 11.15 | 12.40 | 20.39 | 11.03 |
| *p*(*s* + *T*)*c*(*s* + *T*) | site and time trend effects on *p* and *c* | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 |
| | | 1.95 | 9.64 | 12.62 | 7.23 | 4.77 | 8.17 | 6.85 | 9.91 |
| *p*(*s***T*)*c*(*s***t*) | site by time trend effects on *p*; | 40 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| | site by time effects on *c* | 25.01 | 28.27 | 27.41 | 16.17 | 23.37 | 22.77 | 25.66 | 20.99 |
| *p*(*s***t*)*c*(*s*) | site by time effects on *p* and | 40 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| | site effects on *c* | 21.82 | 22.03 | 22.39 | 10.84 | 18.08 | 23.16 | 18.89 | 26.61 |
| *p*(*s***t*)*c*(*s***t*) | site by time effects on *p* and *c* | 60 | 32 | 32 | 32 | 32 | 32 | 32 | 32 |
| | | 54.03 | 28.29 | 23.51 | 16.19 | 35.16 | 35.84 | 36.32 | 34.15 |
| *p* estimate for trapping session | | 0.92 | 1.00 | 1.00 | 1.00 | 1.00 | 0.98 | 0.99 | 0.99 |
| *c* estimate for trapping session | | 0.96 | 0.89 | 0.96 | 0.98 | 0.94 | 0.87 | 0.87 | 0.93 |

[a]ΔAIC is the difference in AIC values between a given model and the one that provides the best fit to the data. Therefore ΔAIC is always zero for the best model.

## Appendix 2

Polymerase chain reaction reactants and annealing temperature used to amplify the mtDNA control region and five microsatellite loci in California red-backed voles. All concentrations are millimoles except where noted. Also shown are the number of alleles detected

| | | Locus | | | | |
|---|---|---|---|---|---|---|
| | mtDNA | *CRB-5* | *CRB-6* | *Cgl-4* | *Cgl-15* | *Cgl-19* |
| Tris-HCl (pH 8.3) | 10 | 10 | 10 | 10 | 10 | 10 |
| KCl | 50 | 50 | 50 | 50 | 50 | 50 |
| $MgCl_2$ | 2.0 | 1.2 | 1.0 | 1.0 | 1.1 | 1.2 |
| Each dNTP | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Each primer | 10 | 10 | 10 | 10 | 10 | 15 |
| Perkin-Elmer Taq (units) | 2.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Flouresceine label | — | HEX | FAM | HEX | HEX | HEX |
| Annealing temp (°C) | 55 | 59 | 52 | 59 | 52 | 52 |
| Total no. alleles detected | 5 | 17 | 15 | 17 | 13 | 14 |