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Population genetic structure of the cyclic snowshoe hare (*Lepus americanus*) in southwestern Yukon, Canada

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Abstract

Spatial population structure has important ecological and evolutionary consequences. Little is known about the population structure of snowshoe hares (*Lepus americanus*), despite their ecological importance in North American boreal forests. We used seven variable microsatellite DNA loci to determine the spatial genetic structure of snowshoe hares near Kluane Lake, Yukon during a cyclic population peak. We sampled 317 hares at 12 sites separated by distances ranging from 3 to 140 km, and used 46 additional samples from Alaska and Montana. The level of genetic variation was high (13.4 alleles/locus, 0.67 expected heterozygosity) and the distribution of alleles and genotypes was not homogeneous across the sites. The degree of differentiation was low among Yukon sites (F_{ST} = 0.015) and between Yukon and Alaska (F_{ST} = 0.012), but the Montana site was highly differentiated (F_{ST} = 0.20). A weak pattern of isolation by distance was found over the Yukon study area, with an indication that local genetic drift may be important in shaping the regional genetic structure. Landscape barriers expected to influence gene flow did not consistently affect genetic structure, although there was evidence for a partial barrier effect of Kluane Lake. The high level of inferred gene flow confirms that snowshoe hare dispersal is widespread, successful and equal between the sexes. A stepping-stone model of gene flow, potentially influenced by the synchronous density cycle, appears to best explain the observed genetic structure. Our results suggest that despite their dramatic fluctuations in density, snowshoe hares in the northern boreal forest have a large evolutionary effective population size and are not strongly subdivided by either physical or social barriers to gene flow.

Keywords: dispersal, gene flow, genetic differentiation, genetic drift, isolation by distance, landscape barriers, snowshoe hare

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Introduction

The spatial structure of natural populations has important consequences for both contemporary and long-term ecological processes. Studies of spatial population structure in mammals have revealed that most mammalian populations are genetically subdivided, with relatively small effective population sizes and low dispersal rates that are consequences of the social and mating systems (Chepko-Sade & Halpin 1987). The scale of subdivision varies considerably, however, and suggests that genetic structure is influenced by complex interactions between social organization, dispersal tendencies and environmental factors (e.g. Lidicker & Patton 1987; Waser & Elliott 1991; Fuller *et al*. 1997; Petit *et al*. 1997; Surridge *et al*. 1999; Ehrich *et al*. 2001; Goossens *et al*. 2001; Kyle & Strobeck 2001; Mossman & Waser 2001).

Many studies have focused on fragmented populations or on species with complex social structures, but relatively few have investigated genetic structure in continuously distributed species with simple social systems. Snowshoe hares (*Lepus americanus*) provide an opportunity to do so in a species that also shows cyclic fluctuations in density. The population ecology of snowshoe hares has been extensively studied (Keith 1990; Krebs *et al*. 2001a), yet little is known about their geographical structure. They are distributed more or less continuously throughout the boreal forests of North America (Banfield 1974; Hodges 2000a), as well as in many of the montane and subboreal forests of the continental USA (Hodges 2000b), but it is unclear at what scale they

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are subdivided into smaller functioning units. Furthermore, the extent of hare movements within local regions has not been well documented (Hodges 2000a,b). As Lidicker *et al*. (2000) recently expressed, 'we can only guess at what might be the spatial dimensions of the demographic and genetic structure of hares living in these boreal forests.'

The goal of our study was to examine snowshoe hare population structure and inferred movement patterns at a regional scale through the use of neutral genetic markers. We addressed two main questions. First, at what scale are snowshoe hares genetically subdivided? Second, does the genetic structure reflect predictions of gene flow based on landscape features and estimates of hare dispersal? Previous studies have shown that: (i) snowshoe hares disperse frequently and over the spatial scale of a few kilometres (Windberg & Keith 1976; Boutin 1984; Gillis & Krebs 1999; Hodges 1999) (ii) dispersers survive as well as nondispersers (Boutin 1984; Gillis & Krebs 2000; but see Windberg & Keith 1976 and Keith *et al*. 1993), and (iii) hares do not live in family units or other obvious social clusters (Boutin 1979; Burton 2001). Given these findings and the fact that hares are distributed more or less continuously in forested habitat, we predicted that there should be high levels of gene flow leading to genetic homogeneity at a local scale (e.g. 10–20 km), and decreasing levels of gene flow leading to increasing differentiation with distance at a larger scale. We further predicted that areas of unsuitable habitat, such as lakes and alpine habitat, should represent barriers to dispersal and gene flow, thereby causing genetic differentiation and departures from the pattern of isolation by distance (e.g. Castella *et al*. 2000; Gerlach & Musolf 2000). We also tested an alternative hypothesis that local differentiation, and thus genetic structure, is increased through bottleneck effects related to the snowshoe hare density cycle.

Materials and methods

Sample collection

The study was conducted in the southwest Yukon, Canada, near Kluane Lake (61° N, 138° W) during a peak phase of the 10-year snowshoe hare population cycle. The forest in this area is dominated by white spruce (*Picea glauca*) with an understorey of grey willow (*Salix glauca*), bog birch (*Betula glandulosa*) and soapberry (*Sheperdia canadensis*) (see Douglas 1974 for a more detailed description). Snowshoe hares were live-trapped at 12 sites between 5 April and 27 August 1999. These sites were separated by a range of distances (3–140 km) and potential landscape barriers to dispersal (e.g. alpine habitat, lakes; see Fig. 1). At each site, between 30 and 100 Tomahawk traps (Tomahawk Live Trap Co.) were set in areas showing signs of hare activity (e.g. pellets, browse, runways). Traps were baited with alfalfa, apple and rabbit chow, set in the evening and checked in the morning. Trapping was carried out over two to four nights at each site and trapping sessions were repeated where necessary in an attempt to capture a minimum of 30 hares from each location. A small amount of ear tissue was collected from each captured hare using a 3-mm biopsy punch (Mader Instrument Corp.). Tissue samples were placed in 95% ethanol at the time of collection and stored in a freezer upon return from the field. Each hare was also weighed, sexed, aged and fitted with an identifying ear tag (Monel # 3, National Band and Tag Co.).

A total of 317 hares were sampled from the Kluane region, with sample sizes at each site ranging from 10 to 56 (mean = 26.3, Table 1). To gauge the degree of genetic differentiation among the Yukon sites, additional hare samples were obtained in August 2000 from two distant populations: 27 samples from the Tanana River floodplain in interior Alaska (~64° N, 148° W) and 19 samples from Seeley Lake, Montana (47° N, 113° W). No known relatives (e.g. parent–offspring) were sampled at any of the sites.

Microsatellite analysis

DNA was extracted from the ear tissue samples using the Puregene Animal Tissue Protocol (Gentra Systems) with proteinase K digestion. Polymerase chain reaction (PCR) amplification was attempted with 11 microsatellite primer pairs developed in the European rabbit, *Oryctolagus cuniculus* (Sol03 — Rico *et al*. 1994; Sol33 — Surridge *et al*. 1997; Sat2, Sat3, Sat4, Sat5, Sat7, Sat8, Sat12, Sat13, Sat16 — Mougel *et al*. 1997). Initial amplification for each primer pair was carried out in a 10-μL reaction volume containing the following: 100 ng template DNA, 0.5–0.8 μM each primer, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 0.5 units *Taq* polymerase (GibcoBRL) and 1× reaction buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl). Amplifications were carried out in a Robocycler Gradient 96 (Stratagene). PCR products were run on a 1% agarose gel, stained with ethidium bromide and visualized under ultraviolet light.

Eight of the 11 primer pairs (Sol03, Sol33, Sat2, Sat3, Sat5, Sat12, Sat13 and Sat16) gave a specific product in the snowshoe hare, and these were amplified and optimized using a radioactive label. The forward primer was first 5′ end-labelled in a 1-μL reaction volume containing: 0.25 units T4 polynucleotide kinase (PNK, New England BioLabs), 1× PNK buffer (70 mM Tris–HCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, pH 7.6), 0.5 μM forward primer and 9.25 kBq [γ^{32}P]dATP. The 10 μL PCR reaction volume contained: 100 ng DNA template, 0.1 mM each dNTP, 1.5 mM $MgCl_2$, 0.6 μM reverse primer, 0.25 μM unlabelled forward primer, 0.05 μM radiolabelled forward primer, 0.5 units *Taq* polymerase (GibcoBRL) and 1× reaction buffer. The Sol03 and Sol33 reactions also contained 0.5 μL dimethyl sulphoxide (DMSO). PCR amplifications were performed in a

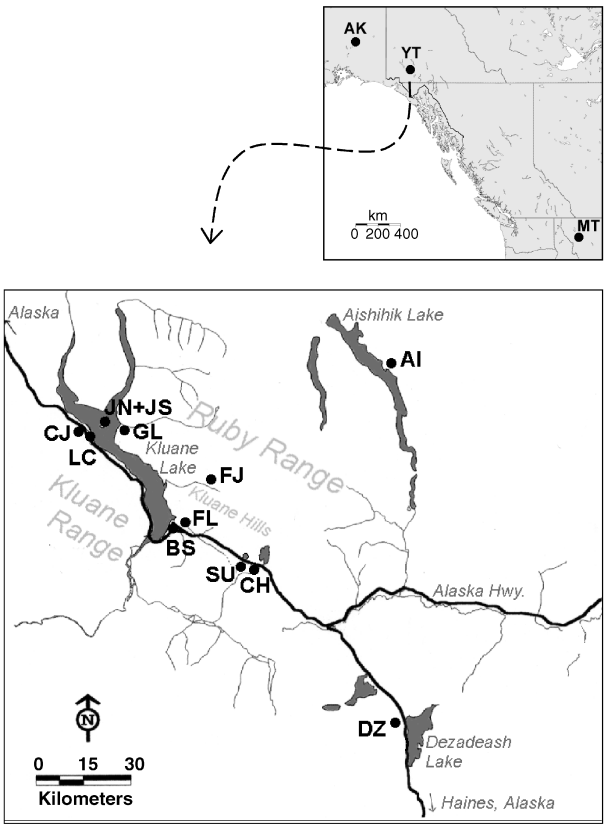

Fig. 1 Sampling locations. Upper box shows the Yukon (YT), Alaska (AK) and Montana (MT) sampling areas. Lower box is a detailed map of the 12 Yukon sampling sites (repres-ented by dark circles and name abbreviations — see Table 1). Also indicated is the general location of alpine habitats (Kluane Hills and Ruby Range) predicted to be barriers to gene flow.

PTC-100 (MJ Research) under optimal conditions for each locus (see Burton 2001 for details; optimal conditions did not differ significantly from those originally published for the European rabbit in Rico *et al*. 1994; Mougel *et al*. 1997; and Surridge *et al*. 1997). PCR products were mixed with 7 μL stop dye and denatured at 94 °C for 5–10 min before 4 μL each sample was loaded onto a 6% denaturing polyacrylamide gel (in 1.2× TBE buffer) for electrophoretic size determination. An M13mp18 control DNA sequencing ladder (T7 Sequenase v2.0, USB) was electrophoresed with the samples to allow accurate measurement of allele sizes. Dried gels were visualized by exposing to autoradiographic film for 24–48 h and were scored manually. Any individuals that failed to produce clear bands were reamplified under the same conditions.

Data analysis

Genetic variation. Microsatellite loci were tested for deviations from Hardy–Weinberg equilibrium and genotypic linkage equilibriums using the Markov chain methods in the computer program GENEPOP version 3.1d (Raymond & Rousset 1995b; see also Guo & Thompson 1992). Fisher's exact tests were used for loci with fewer than five alleles and default parameter settings of 1000 dememorizations, 100 batches and 1000 iterations per batch were used for Markov estimations. Significance levels were adjusted using the sequential Bonferroni correction for multiple comparisons (Rice 1989). The number of distinct alleles, their frequencies and the expected heterozygosity were calculated for each locus and each sampling site in GENEPOP.

Site	*N*	*A*	H_E
Flint (FL)	56	8.14 (3–20)	0.61 (0.18–0.93)
Sulphur (SU)	25	7.57 (3–22)	0.63 (0.41–0.96)
Chitty (CH)	12	6.57 (3–15)	0.72 (0.47–0.95)
Fourth of July (FJ) ,	15	7.00 (2–16)	0.65 (0.36–0.95)
Base (BS)	41	7.86 (3–21)	0.63 (0.35–0.93)
Aishihik (AI)	10	4.86 (2–10)	0.65 (0.39–0.86)
Copper Joe (CJ)	24	7.14 (3–16)	0.59 (0.36–0.92)
Dezadeash (DZ)	18	6.57 (2–13)	0.66 (0.44–0.89)
Gladstone (GL)	19	6.71 (3–14)	0.64 (0.41–0.93)
Lewis Creek (LC)	35	7.57 (4–19)	0.60 (0.35–0.93)
Jacquot Island North (JN)	35	8.29 (4–26)	0.60 (0.37–0.94)
Jacquot Island South (JS)	27	8.71 (4–25)	0.63 (0.42–0.96)
Yukon sites combined	317	12.14 (4–36)	0.64
Alaska (AK)	27	8.71 (4–22)	0.68 (0.40–0.94)
Montana (MT)	19	5.57 (1–13)	0.63 (0*–0.91)
All sites combined	363	13.43 (5–37)	0.67
Average per site	25.93	7.23 (2–26)	0.64

Table 1 The number of hares sampled (*N*), average number of alleles (*A*) and average expected heterozygosity (H_E) per locus for each of the 14 sites. Totals and averages over sites are also given. For the alleles and heterozygosities, the range of observed values across loci is indicated in parentheses.

*The Montana sample was monomorphic for the Sat3 locus.

Genetic differentiation. Population genetic structure was first examined by testing the null hypothesis that the distribution of alleles was identical across all sampling sites. An unbiased estimate of the probability was calculated for each locus using the Markov chain method in GENEPOP (Raymond & Rousset 1995a; parameter settings: 5000 dememorizations, 500 batches, 5000 iterations per batch), and Fisher's combined probability was calculated across all loci (Sokal & Rohlf 1995). Pairwise tests for allelic differentiation were also made between each of the sites and significance was evaluated after applying the sequential Bonferroni correction (Rice 1989). The degree of differentiation between and across all sites was quantified using Weir & Cockerham's (1984) estimator (θ) of Wright's F_{ST}, as calculated by the program FSTAT version 2.8 (Goudet 1999). Previous studies have indicated that measures of differentiation based on the variance in allele frequencies, such as θ, are more reliable than alternative measures based on the variance in microsatellite repeat numbers (e.g. R_{ST}) given the sample sizes, number of loci and population characteristics in our study (Paetkau *et al*. 1997; Gaggiotti *et al*. 1999). F_{ST} can theoretically range from 0 (no genetic divergence) to 1 (complete fixation of alternative alleles), but Wright (1978) suggested that values above ~0.15 indicate great genetic differentiation. Standard errors of θ were calculated by jackknifing over populations and loci, and a 95% confidence interval was generated by bootstrapping over loci (Goudet 1995). Significance of estimates (i.e. $\theta > 0$) was further evaluated with an exact *G*-test after 1000 randomizations of alleles among sites (Goudet *et al*. 1996).

To visualize the genetic relationships among sites, an unrooted neighbour-joining tree based on Cavalli–Sforza's chord distance (Cavalli-Sforza & Edwards 1967) was created using programs GENDIST, NEIGHBOUR and DRAWTREE in the PHYLIP software package (Felsenstein 1995). Reliability of tree nodes was evaluated by generating a consensus tree from 100 bootstrap replicates of the original allele frequencies (using programs SEQBOOT and CONSENSE).

As an alternative measure of the degree of genetic differences among sites, we used an assignment index to determine how unique individual hares' genotypes were to the site from which they were sampled. Unlike F_{ST} and other traditional divergence measures that compare allele frequencies using population models, the assignment index is based on individual multilocus genotypes (Waser & Strobeck 1998; Davies *et al*. 1999). It assigns an individual to the candidate source population in which its genotype has the highest likelihood of occurring. The software program GENECLASS (Cornuet *et al*. 1999) was used to assign individuals according to a Bayesian method developed by Rannala & Mountain (1997). This method was chosen for the following reasons: (i) it calculates the probability that an individual 'belongs' to a population based on a distribution of simulated genotypes (10 000 for each candidate population); (ii) it takes into account differences in diversity between candidate populations and the sampling error associated with estimating allele frequencies (Davies *et al*. 1999); (iii) it avoids the bias introduced by null frequencies in other assignment methods, and (iv) it has been found to be slightly more powerful than other methods (Luikart & England 1999). To avoid biasing likelihoods, we excluded the individual being tested from its sample population when estimating allele frequencies.

The assignment index was also used to test for sex-biased dispersal in hares, following the method of Favre *et al*. (1997; see also Mossman & Waser 1999). For each hare, the likelihood of its genotype at the site from which it was sampled was log-transformed and adjusted for site differences by subtracting the site mean. The resulting corrected assignment index (AI_c) indicates how likely an individual is to be an immigrant relative to the other individuals at its site. Differences in AI_c values between males and females were tested within each site and over all sites using the nonparametric Wilcoxon two-sample test (Sokal & Rohlf 1995).

Isolation by distance. We tested for a positive correlation between geographical and genetic distances to determine if the observed genetic structure was consistent with the isolation-by-distance model (Wright 1943; Slatkin 1993; Hutchison & Templeton 1999). The straight-line distance between all pairs of sites was plotted against pairwise F_{ST} (θ), and statistical significance was evaluated using a Mantel test (Mantel 1967). The Mantel *Z* statistic and correlation coefficient, *r*, were calculated using the R-PACKAGE version 4.0 software program (Casgrain & Legendre 2001), with 9999 matrix permutations used to determine significance. Following Hutchison & Templeton (1999), we further examined the relative influences of gene flow and genetic drift on regional structure by testing for a correlation between the variability in pairwise differentiation (using the residuals from the isolation-by-distance plot) and geographical distance. Under a condition of regional equilibrium, greater variability in population differences is expected at greater distances as drift becomes relatively more important (Hutchison & Templeton 1999).

We also examined the effect of geographical distance on genetic structure using the assignment index. The proportion of 'misassigned' genotypes at each site assigned to each other site was compared with the distance between sites. A Mantel test could not be used for this comparison because the proportion of cross-assignments was not symmetrical between sites (i.e. the number from site A assigned to site B differs from the number assigned from B to A, and the total number of 'misassignments' differs). Statistical significance was therefore tested using a regression test in the program RT 2.1 (Manly 1997) where the proportion of cross-assignments (the *y* variable) was randomized 5000 times.

Barrier effects. We identified three major landscape features in the Yukon study area that may act as barriers to gene flow for snowshoe hares (see Fig. 1): Kluane Lake, the Kluane Hills (~1200 m elevation, alpine tundra and rock) and the Ruby Range Mountains (similar alpine habitat and ~1800 m elevation). Sites were chosen to allow comparison of the degree of genetic differentiation across a barrier with the differentiation across an equal distance of relatively continuous forest. A paired *t*-test was used to directly compare θ-values and a partial Mantel test (after Smouse *et al*. 1986; calculated in R-PACKAGE) was used to test for a correlation between the presence of one of these barriers and pairwise θ while controlling for geographical distance. To perform this latter test, another pairwise matrix was constructed containing a value of 1 for sites separated by one of the identified barriers and a value of 0 for sites separated by more continuous forest habitat (cf Gerlach & Musolf 2000 and Kyle & Strobeck 2001).

Genetic bottlenecks. Snowshoe hare populations are characterized by cyclic fluctuations in density that typically have an amplitude of 10–25 fold (Keith 1990; Hodges 2000a), and periods of low density could possibly represent genetic bottlenecks. The effective population size (N_e) could be reduced to an extent that genetic drift results in significant genetic differentiation between hares in different areas. Such reductions in N_e are accompanied by correlated reductions in the number of alleles and expected heterozygosity (H_E), however, the alleles (especially those at low frequency) are expected to be lost more quickly (Cornuet & Luikart 1996; Luikart *et al*. 1998). A population showing greater H_E than predicted based on the observed number of alleles, and/or a distortion in allele frequency distribution, may have experienced a recent reduction in N_e. We used the computer program BOTTLENECK version 1.2. (Piry *et al*. 1999) to test for such genetic bottleneck signatures in each of the sample populations. A Wilcoxon sign-rank test was used to test for heterozygosity excess and a mode-shift test was used to test the allele frequency distribution (both tests were performed using the two-phased mutation model).

Results

Microsatellite variation

All pairs of loci were found to be in genotypic linkage equilibrium and all but one locus conformed to Hardy–Weinberg equilibrium. The Sat5 locus had a highly significant heterozygote deficiency ($P < 0.001$), presumably as a result of one or more high-frequency nonamplifying (null) alleles, and was thus excluded from all other analyses. The level of genetic variation in the other seven loci was high, with an average of 13.4 alleles per locus and an expected heterozygosity of 0.67 over all sites, and genetic diversity was similar across all sites (Table 1; see Burton 2001 for allele frequencies at each locus). None of the sampling sites had a significant heterozygosity excess or distorted allele frequency distribution (as tested in program BOTTLENECK), suggesting that they had not undergone significant genetic bottlenecks during the low phase of the cycle.

Table 2 Genetic differentiation among the sampling sites

	Yukon sites only		Among regions
Locus	Prob. of allelic homogeneity	F_{ST} (θ)	F_{ST} (θ)
Sol33	< 0.001	0.025 (0.013)*	0.088
Sol03	0.006	0.020 (0.011)	0.244
Sat2	< 0.001	0.014 (0.003)*	0.024
Sat3	0.012	0.010 (0.014)*	0.089
Sat12	0.004	0.016 (0.010)	0.136
Sat13	0.099	0.009 (0.024)	0.187
Sat16	< 0.001	0.012 (0.007)*	0.045
Overall	< 0.001	0.015 (0.002)*	0.104 (0.030)
95% CI	—	0.012–0.020	0.058–0.165

An estimate of the probability that the distribution of alleles was identical across all 12 Yukon sites was calculated in program GENEPOP. Fisher's combined probability was calculated across loci. Weir and Cockerham's (1984) estimator of Wright's F_{ST} (θ) was calculated in program FSTAT, both within Yukon and among Yukon, Alaska and Montana. For the among-region calculation all Yukon sites were combined. Jackknife standard errors are shown in parentheses (except for the among region estimates for individual loci, where jackknifing was not possible over only three populations) and the 95% bootstrap confidence intervals for the overall estimates are also given. Significance of θ at $P < 0.05$ (after sequential Bonferroni correction) is indicated with asterisks for the Yukon sites (all values were significant among regions).

Population genetic structure

There was highly significant allelic differentiation across the Yukon study area (Fisher's combined probability, $\chi^2 = 96.5$, d.f. = 14, $P < 0.0001$, Table 2). Twenty-three of the 66 pairwise comparisons between sites were significant ($P < 0.05$ after Bonferroni correction), indicating that some sites were genetically different but that many were genetically similar (Table 3). The overall level of differentiation, as estimated by θ, was relatively low at 0.015 ($P < 0.05$; Table 2), with pairwise estimates ranging from 0 to 0.062 (Table 3, negative estimates are equivalent to an F_{ST} of 0). When the Yukon sites were grouped and compared with the Alaska and Montana samples, the among-region differentiation was 0.104 (Table 2). This increase was primarily a result of the large divergence of the Montana sample. Pairwise θ was 0.201 between

Table 3 Pairwise matrix of genetic distance (θ, lower diagonal) and geographical distance (in km, upper diagonal) between all sampling sites (site codes correspond to the locations shown in Fig. 1)

Site	FL	SU	CH	FJ	BS	AI	CJ	DZ	GL	LC	JN	JS	AK	MT
FL		16.4	20.6	18.9	4.8	84.2	46.5	92.2	36.8	43.0	42.0	41.5	602.5	2208.4
SU	0.0103		4.3	19.6	20.9	77.6	62.3	76.7	51.6	58.8	57.0	56.8	618.4	2192.2
CH	0.026*	0.0138		22.7	25.0	77.4	66.6	72.5	55.9	63.0	61.2	61.0	622.7	2188.0
FJ	−0.0004	−0.0074	0.0078		22.5	65.4	51.7	92.3	39.2	48.1	44.3	44.9	606.0	2203.0
BS	0.0117*	0.0002	0.0179	−0.0035		88.0	42.9	96.0	34.1	39.6	39.2	38.4	598.6	2212.6
AI	0.0264	0.0071	0.007	−0.0038	0.0085		103.7	111.5	91.2	100.8	94.2	96.3	622.1	2185.5
CJ	0.0344*	0.0089	0.0399*	0.0122	0.0152*	0.0392*		138.7	13.1	3.6	9.6	7.5	556.4	2253.8
DZ	0.0288*	0.0117	0.0147	0.005	0.0174*	0.0057	0.0462*		128.3	135.3	133.7	133.4	693.1	2118.8
GL	0.0171*	−0.005	0.0173	−0.0063	−0.0003	0.0018	0.0206	0.0116		9.8	5.3	5.8	567.6	2241.9
LC	0.0356*	0.011	0.0367*	0.0142	0.0142*	0.038*	0.0029	0.0615*	0.0172		7.3	4.7	559.9	2250.2
JN	0.0144*	0.0079	0.0414*	−0.0034	0.0098*	0.0129	0.0239*	0.0229	0.0099	0.0278*		2.7	562.3	2247.1
JS	0.0172*	0.002	0.0213	−0.0002	0.0086*	0.0039	0.0077	0.0233*	0.0058	0.0153	−0.0009		562.1	2247.5
AK	0.0252*	0.0111	0.0216	−0.0036	0.0155*	0.0154	0.0322*	0.0218*	0.0073	0.0285*	0.0123	0.0081		2807.3
MT	0.2436*	0.1979*	0.1626*	0.1971*	0.1968*	0.1811*	0.2163	0.1859*	0.1941*	0.2073*	0.2191*	0.2058	0.1925*	

Sites for which the exact test for allelic differentiation was significant at $P < 0.05$ are marked with an asterisk (Fisher's combined probability over loci after sequential Bonferroni correction). When the Yukon sites were combined, the pairwise θ was 0.201 between Yukon and Montana and 0.012 between Yukon and Alaska.

Montana and Yukon and 0.193 between Montana and Alaska, whereas it was only 0.012 between Alaska and Yukon (Table 3, Fig. 2). There were also five novel alleles in the Montana sample, and eight other alleles that were present in all samples except Montana (see Burton 2001 for details). The measures of differentiation for all sites did not differ significantly when males were considered separately from females and adults from juveniles.

The assignment index also indicated little differentiation among Yukon sites (Table 4). Only 78 of the 317 hares (24.6%) were assigned to the site from which they were sampled (ranging from 6.7% to 37.5% of the hares at each site). All 19 of the Montana samples were assigned to the Montana site, whereas only six of 27 (22.2%) Alaska samples were assigned correctly, with the others assigned to various Yukon sites (Table 4). The comparison of individual assignment indices corrected for site differences (AI_c) suggested that there is no sex-bias in hare dispersal (Fig. 3). The mean AI_c for male hares (−0.065 ± 1.75 SD, $n = 148$) was marginally lower but not statistically different from the female mean (0.027 ± 1.85 SD, $n = 159$, Wilcoxon two-sample test, $z = -0.59$, $P = 0.56$). The result was similar when adults were considered separately from juveniles and when each site was tested separately.

There was a significant association between geographical and genetic distance (θ) among Yukon sites (Mantel $r = 0.38$, $P = 0.025$, Fig. 4), suggesting isolation by distance. There was, however, considerable scatter in this relationship. For example, samples from the Flint and Base sites were significantly differentiated (θ = 0.012, exact test $P < 0.0001$) despite being separated by less than 5 km, while on the other hand, the Aishihik and Dezadeash sites were separated by over 100 km but were not significantly differentiated (θ = 0.0057, exact test $P = 0.11$, Table 3). There was no correlation between the degree of scatter, as measured by the residuals of the isolation-by-distance plot, and the geographical distance (Mantel $r \approx 0$, $P = 0.5$). The assignment index suggested that nearby sites were somewhat more likely to share genotypes than distant sites, however, the positive correlation of cross-assignments with geographical distance was not statistically significant and explained little of the variation in cross-assignments ($r^2 = 0.06$, randomization test $P = 0.40$).

The potential landscape barriers to gene flow that we identified *a priori* did not consistently explain deviations from the isolation-by-distance model (Table 5). Genetic differentiation was not significantly greater between sites separated by these landscape features than between comparable sites without any obvious physical barriers (paired *t*-test, $t = 0.67$, d.f. = 3, $P = 0.28$). Kluane Lake does appear to act as a partial barrier to gene flow as θ-values between Jacquot Island and mainland sites, or between sites on opposite sides of the lake, were generally higher than between comparable sites not separated by the lake (Table 5). On the other hand, the potential alpine barriers (Kluane Hills and Ruby Range) do not appear to impede gene flow as differentiation was not higher between sites separated by these habitats. These results were supported by the Mantel tests across all sites in which the presence or absence of one of the potential barriers was tested against θ while controlling for geographical distance. When all three barriers were included, the correlation between presence of a barrier and increased θ was not significant (Mantel $r = 0.166$, $P = 0.17$); however, when only Kluane Lake was considered as a barrier the correlation was significant ($r = 0.44$, $P = 0.005$).

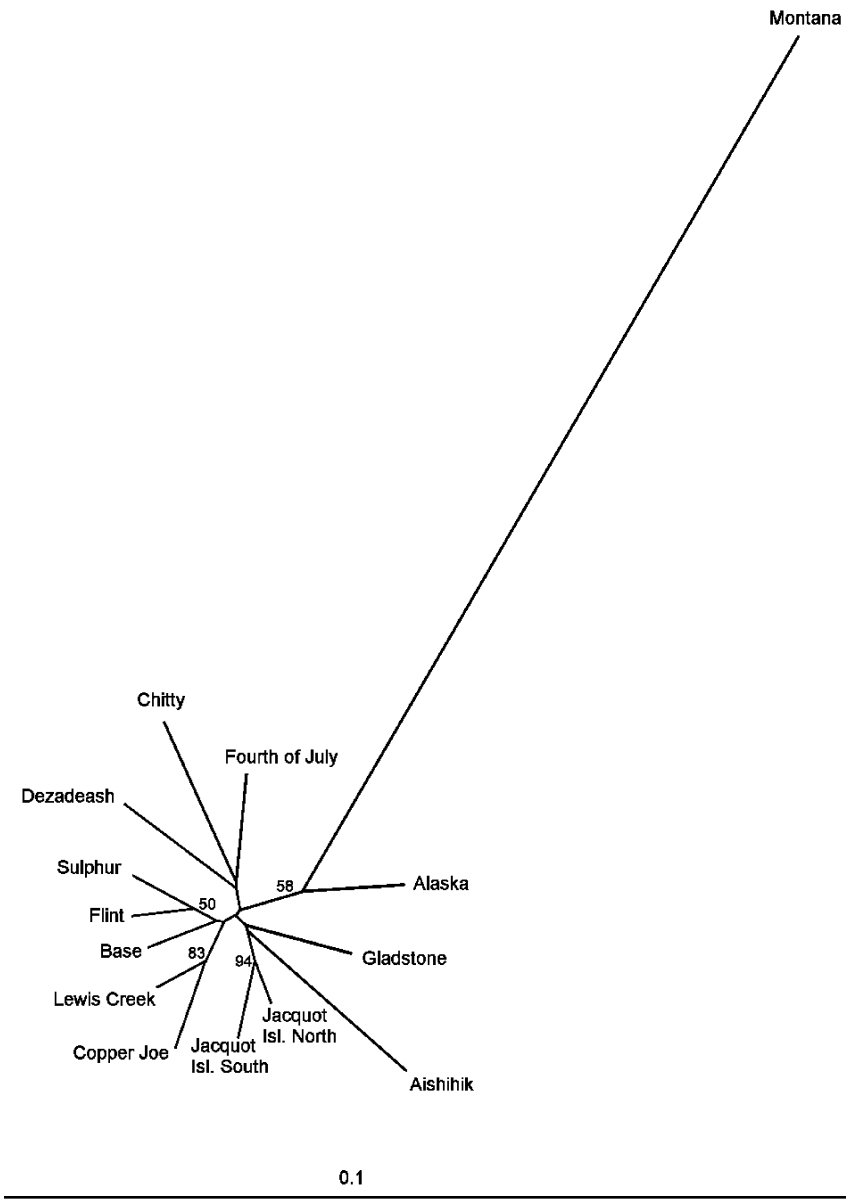

Fig. 2 Unrooted neighbour-joining tree based on Cavalli–Sforza chord distances among sampling locations. The length of tree branches is relative to the genetic distances (note scale). Bootstrap values are indicated for nodes with ≥ 50% support (100 replicates). Note that the Montana sample was highly differentiated but that the Alaska sample grouped closely with the Yukon sites. Some nearby Yukon sites were genetically very similar (e.g. Jacquot Island North and South), whereas other nearby sites were genetically more distant (e.g. Chitty and Sulphur). Lewis Creek and Copper Joe were the only two sites on the west side of Kluane Lake (see Fig. 1).

Discussion

Comparison with other mammals

Our results indicate that snowshoe hares have high genetic diversity but relatively little genetic differentiation over large areas in the southwest Yukon during a cyclic peak phase. The level of diversity, as measured by the number of alleles per locus and expected heterozygosity, is comparable to that reported for microsatellites in many other small mammal species [e.g. greater white-toothed shrew *Crocidura russula* (Favre *et al*. 1997; Balloux *et al*. 1998); European rabbit *Oryctolagus cuniculus* (Surridge *et al*. 1999); bank vole *Clethrionomys glareolus* (Gerlach & Musolf 2000); collared lemming *Dicrostonyx groenlandicus* (Ehrich *et al*. 2001)], although the reliability of such comparisons has been questioned because loci with low polymorphism are often unreported (Goossens *et al*. 2001). Lidicker *et al*. (2000) also reported that genetic (allozyme) variation in snowshoe hares was typical of other terrestrial mammals. It therefore appears that hares do not have reduced genetic diversity as a consequence of their cyclic density fluctuations.

The allelic and genotypic frequencies in hares were not homogeneous across the Yukon study area, suggesting significant genetic structure, and generally followed a pattern of decreasing similarity with increasing geographical distance. This pattern of isolation by distance has also been observed at comparable scales in other small and medium-sized mammals such as the white-toothed shrew (Favre *et al*. 1997), Alpine marmot (*Marmota marmota*, Goossens *et al*.

Table 4 Results of the assignment test

	n	FL	SU	CH	FJ	BS	AI	CJ	DZ	GL	LC	JN	JS	AK	MT
FL	56	**21**	5	2	2	6		4	2	5	3	5	1		
SU	25	6	**2**	1	5	1	3	1	1		2	1		2	
CH	12	2	2	**1**		1	1		2	2				1	
FJ	15	2	3		**1**	1		2	2	2		1		1	
BS	41	2	5	1	2	**14**	3	3	2	4	1	4			
AI	10				1	1	**1**	2		2		1	1	1	
CJ	24	2	2	2	1	1		**6**	1		3	1	4	1	
DZ	18	1	1	2	3	1	2		**6**					2	
GL	19	3	1			4		1		**3**	1	3	1	2	
LC	35	3	3	1	1		2	6	1	1	**11**	3	1	2	
JN	35	4	2		2		3	2	2	2		**7**	7	4	
JS	27	1	1			4	4	1		2	1	5	**5**	3	
AK	27		2	1	1	1	1	1	1	2	4	2	5	**6**	
MT	19														**19**

Each row contains the samples from one site and the columns indicate the sites to which these samples were assigned (i.e. in which their genotypes had the highest likelihood of occurring). Samples sizes (*n*) are indicated in the first column Site. Codes correspond to those shown in Fig. 1

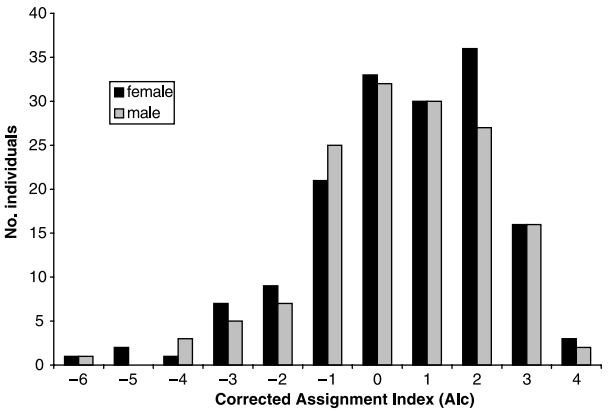

Fig. 3 Frequency distributions of assignment indices corrected for site differences (AI_c) for male and female hares from all 12 Yukon sites. There was not a significant difference between mean values for the sexes, implying that dispersal in these hares is not sex-biased.

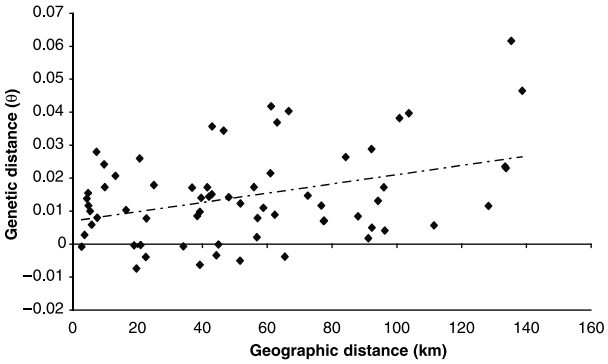

Fig. 4 Isolation by distance among Yukon sampling sites. Pairwise estimates of F_{ST} (θ) are plotted against the corresponding straight-line geographical distances (*d*) between sites (θ = 0.0070 + 0.00014*d*, Mantel *r* = 0.38, *P* = 0.025).

2001), wolverine (*Gulo gulo*, Kyle & Strobeck 2001), pine marten (*Martes americana*, Kyle *et al*. 2000), northern Idaho ground squirrel (*Spermophilus brunneus brunneus*, Gavin *et al*. 1999), and house mouse (*Mus musculus*, Dallas *et al*. 1995). By contrast, isolation by distance was not detected in the European rabbit (Fuller *et al*. 1996, 1997; Surridge *et al*. 1999), collared lemming (Ehrich *et al*. 2001) and white-footed mouse (*Peromyscus leucopus*, Mossman & Waser 2001). The degree of genetic structuring observed in snowshoe hares is less than reported for most of the small mammal species mentioned above (see Mossman & Waser 2001 for a review); however, it appears to be greater than in the larger carnivores (marten and wolverine). An interesting comparison is between the two leporid species, snowshoe hares and European rabbits: Surridge *et al*. (1999) reported F_{ST} values for rabbits in Britain that were an order of magnitude larger than for the Yukon hares over a comparable geographical scale. Conversely, Fuller *et al*. (1996, 1997) found very little genetic differentiation among rabbits over large areas of certain parts of eastern Australia. These genetic patterns were attributed to complex social structure in Britain and to dynamic extinction–recolonization processes related to environmental heterogeneity in Australia. Another comparison of note is with the collared lemming, a cyclic species that also showed low regional genetic differentiation in the Canadian north (Ehrich *et al*. 2001). The potential association between high gene flow and cyclic population dynamics is worthy of further investigation.

Table 5 Comparison of F_{ST} (θ) between four pairs of sites separated by similar distance but either a potential landscape barrier to gene flow or relatively continuous habitat

Pair	Site A	Site B	Distance (km)	Potential barrier	θ	Difference ($\theta_{barrier} - \theta_{no\ barrier}$)
i	LC	JS	4.7	Kluane Lake	0.0153	
	LC	CJ	3.6	none	0.0029	0.0124
ii	FL	LC	43.0	Kluane Lake	0.0356	
	FL	GL	36.8	none	0.0171	0.0185
iii	FL	FJ	18.9	Kluane Hills	−0.0004	
	FL	SU	16.4	none	0.0103	−0.0107
iv	FL	AI	84.2	Ruby Range	0.0264	
	FL	DZ	92.2	none	0.0288	−0.0024

Kluane Lake appeared to act as a partial barrier to gene flow, however the alpine habitats (Kluane Hills and Ruby Range) did not.

Gene flow and genetic drift

The low level of differentiation in hares suggests that there is considerable gene flow across the landscape. Regions separated by more than 100 km, and even up to 700 km for the Alaska site, appear to be connected by a substantial number of effective migrants. This result is surprising given indications from previous field studies that hare dispersal distances are typically in the range of a few kilometres (e.g. Gillis & Krebs 1999; Hodges 1999). An analysis of the straight-line distance between the location of first capture and the last known location for 1577 radiocollared snowshoe hares in the Kluane region also suggested that most hares move less than 2 km and that long-distance dispersal events do not exceed 25–30 km (C. J. Krebs, K. E. Hodges and C. Burton, unpublished data). Theoretical models have shown that few effective migrants are necessary to prevent strong differentiation between populations (Wright 1978; Slatkin 1985), thus it seems reasonable to conclude that the observed hare movements result in a large amount of gene flow and a low degree of differentiation at a local scale (e.g. tens of kilometres). Although long-distance dispersal events are often undetected in field studies (Koenig *et al*. 1996), it is highly unlikely that hares disperse over hundreds of kilometres. Rather, it is much more likely that the long-distance gene flow occurs through a series of smaller dispersal events, such as in the stepping-stone model (Kimura & Weiss 1964). The detection of significant isolation by distance, with nearby sites apparently exchanging more genes than distant sites, supports this view for hares. It is possible that the synchrony of the snowshoe hare cycle in northern boreal forests (Hodges 2000a) facilitates a large amount of stepping-stone gene flow between populations during the peak phase.

This model of gene flow is appealing for a continuously distributed species like the snowshoe hare, however, it does not explain the considerable deviation from isolation by distance exhibited by some of the Yukon sites (see Fig. 4 and Table 3). Some of the variation can be explained by the partial barrier effect of Kluane Lake, but other predicted alpine barriers were not associated with similar increases in genetic differentiation. Furthermore, the degree of differentiation on a local scale (e.g. over 5–20 km) was comparable to that on a much larger scale (e.g. 100–700 km), suggesting that a simple model of isolation by distance does not adequately explain genetic structure over the entire region. It is therefore important to consider other possible mechanisms that might underlie the observed structure.

One possibility is that the genetic structure, particularly on a regional scale, reflects historical rather than contemporary levels of dispersal and gene flow. For example, snowshoe hares may show relatively little differentiation over the northwestern boreal forest as a result of large-scale recolonization from a glacial refugium after the last glaciation period (e.g. Pielou 1991; Demboski & Cook 2001). The dynamic nature of snowshoe hare populations on a shorter time scale might also be important in shaping genetic structure. This study only represents a snapshot into hare genetic structure during a cyclic peak phase. The consequences of cyclic density fluctuations for genetic structure have previously attracted considerable attention from small mammal ecologists (Chitty 1967; Tamarin & Krebs 1969; Gaines & Krebs 1971; Gaines 1981; Bowen & Koford 1987; Lidicker *et al*. 2000). Snowshoe hare densities can change by over two orders of magnitude from the peak to low phases (Boutin *et al*. 1995), with associated changes in demographic parameters and behaviour (Keith 1990; Hodges 2000a; Hodges *et al*. 2001), and genetic structure is almost certainly affected to some extent. One hypothesis is that local differentiation because of genetic drift during the low phases could create significant structuring independently of distance. Some researchers have hypothesized that hares recede into patches of high-quality habitat during the low phase, and then expand back out into patches of lower quality habitat during the increase and peak phases (Keith 1966; Wolff 1980, 1981; Hik 1994). If hares in local patches

are relatively isolated and experience genetic bottlenecks during the low phase, local differentiation could result. Recolonization of lower quality habitat during the increase and peak would be expected to result in homogenizing gene flow, but if this recolonization process is not uniform it could create a 'mosaic' of genetic structure. For example, the direction and success of recolonizing movements might be influenced by environmental heterogeneity, such as differing food sources and predation pressures, rather than simply the distance between patches. This mechanism is speculative, however, and there has been limited evidence for consistent cyclic patterns in hare habitat use or dispersal rates (Hodges 2000a,b). Furthermore, local hare densities, and thus effective population sizes, may not get low enough to cause genetic bottlenecks. Our analysis did not show any genetic signatures of bottlenecks in hares. Power analyses on the heterozygosity excess and mode-shift tests, however, show that there is low power (e.g. < 0.4) to detect the short-term and relatively subtle reductions in N_e that would characterize the hare cycle given the sample sizes and number of loci in this study (Cornuet & Luikart 1996; Luikart *et al*. 1998). The observed isolation-by-distance effect is generally consistent with a regional equilibrium between gene flow and genetic drift, yet the considerable scatter around this relationship suggests that local drift may have a strong effect on regional structure (Slatkin 1993; Hutchison & Templeton 1999). The fact that the degree of scatter did not increase with geographical distance, as is predicted for regional equilibrium, is a further indication of the relative importance of drift (see case III in Hutchison & Templeton 1999). The potential for local extinction–recolonization or source–sink dynamics to shape genetic structure has been demonstrated in other systems (Wade & McCauley 1988; Whitlock 1992; Fuller *et al*. 1997; Giles & Goudet 1997, Newman & Squire 2001); however, more detailed data on cyclic changes in movement patterns and local effective population sizes are needed to address thoroughly such a mechanism in hares.

Implications for hare dispersal and population characteristics

Assuming that the observed genetic structure is largely a result of contemporary gene flow, more details of snowshoe hare dispersal can be inferred. A high degree of local gene flow is generally consistent with the findings of previous field studies on hare dispersal (Windberg & Keith 1976; Boutin 1984; Boutin *et al*. 1985; Hodges 1998; Gillis & Krebs 1999, 2000; see review in Hodges 2000a), although our results suggest that rates and distances of dispersal may be greater than previously reported. Philopatry does not appear to be common in hares and a significant proportion of individuals at any site are likely to be immigrants. Many dispersing hares must survive to pass on their genes, even when dispersing over long distances or across inhospitable habitat such as frozen lakes and alpine tundra. Our genetic data also confirm that there is no sex-bias in snowshoe hare dispersal. Most mammals have male-biased dispersal, potentially for reproductive enhancement and inbreeding avoidance, and equal dispersal by both sexes is rare (Greenwood 1980). There may be little risk of inbreeding in hares because of the lack of philopatry, the low level of relatedness in local populations, and multiple mating in both sexes (Burton 2001). Variance in reproductive success also appears to be relatively low for both sexes (Burton 2001), therefore selection for sex-biased dispersal should be weak.

The extensive amount of gene flow in snowshoe hares supports the idea that they do not exhibit any form of social organization that restricts dispersal and increases local differentiation (Sugg *et al*. 1996). The degree to which this high level of effective dispersal links different regions demographically is unclear. Boutin *et al*. (1985) showed that dispersal is not responsible for the cyclic density changes in hares; however, high levels of dispersal could certainly affect population dynamics over a large region and may synchronize hare cycles at a local to regional scale (see Ranta *et al*. 1995; Koenig 1999). Given the lack of obvious social or physical structure, defining the boundaries of a hare population is problematic. Over the approximately 7000 km^2 represented by our Yukon study area (perhaps greater than 70 000 km^2 when considering the Alaska samples), there was no indication of any strongly genetically isolated populations. Even the hares on Jacquot Island, which were previously thought to represent a demographically distinct, noncyclic population (Jardine 1995), showed little genetic differentiation from the mainland samples. While the number of sites sampled was not exhaustive, our results suggest that hares form very large, continuous populations in the northern boreal forest.

The Montana sample was by far the most geographically and genetically distant. Genetic differentiation between Yukon and Montana hares was more than an order of magnitude greater than within the Yukon (see Fig. 2). The presence of several novel alleles in the Montana sample also supports the idea that it represents a genetically differentiated population. While the level of differentiation does not suggest complete isolation between the regions, the indication is that there is very little gene flow. This divergence could simply be the result of distance, with the low amount of gene flow unable to balance the divergent forces of drift and mutation, or it could reflect deeper phylogeographic differences, such as distinct northern and southern lineages originating from different glacial refugia (e.g. Demboski & Cook 2001). Alternatively, the genetic differences might be a consequence of environmental differences between northern and southern hare populations. Southern

populations have been hypothesized to have different dynamics from those in the north, potentially as a result of greater habitat fragmentation and the presence of more facultative predators (see Hodges 2000b for a review). Habitat fragmentation can influence genetic structure in small mammals (Gaines *et al*. 1997) and may be linked to genetic differences between northern and southern carnivore populations in western North America (Paetkau *et al*. 1998; Kyle *et al*. 2000; Kyle & Strobeck 2001). Further investigation into the demographic and genetic differences among northern and southern hare populations is needed.

General implications and directions for future research

Our results represent a first look at the neutral genetic variation of snowshoe hares in the northern boreal forest. They reveal a complex pattern of genetic structure highlighted by a low degree of differentiation over both local and regional scales. The overall picture is that hares form large populations connected by high levels of effective dispersal. Further research is warranted to expand on these results both spatially and temporally. An assessment of genetic structure at different phases of the hare cycle is necessary to determine whether the patterns we observed are stable or unique to this particular peak phase. Detailed investigation of the low phase is especially critical for assessing the genetic consequences of the rapid decline in hare numbers. Comparative studies in different regions will be important for understanding large-scale geographical structure and historical gene flow patterns in hares. Of particular interest are the more southern populations, where the genetic effects of increased habitat fragmentation, reduced cyclic amplitude, and peripheral environmental conditions can be explored. Finally, potential demographic consequences of the considerable amount of long-distance dispersal require further investigation. The genetic results suggest that hares cannot be studied or managed at a local level without considering the influence of dispersal to and from surrounding areas. This may not only have implications for the interpretation and generality of previous studies (e.g. Lidicker *et al*. 2000; Hodges *et al*. 2001), but it could also extend to the management of many other boreal forest species that are strongly affected by the snowshoe hare cycle (Boutin *et al*. 1995; Krebs *et al*. 2001a,b).

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References

Balloux F, Goudet J, Perrin N (1998) Breeding system and genetic variance in the monogamous, semi-social shrew, *Crocidura russula*. *Evolution*, **52**, 1230–1235.

Banfield AWF (1974). *The Mammals of Canada*. University of Toronto Press, Toronto.

Boutin S (1979) Spacing behavior of snowshoe hares in relation to their population dynamics. MSc Thesis, University of British Columbia.

Boutin S (1984) The effect of conspecifics on juvenile survival and recruitment of snowshoe hares. *Journal of Animal Ecology*, **53**, 623–637.

Boutin S, Gilbert BS, Krebs CJ, Sinclair ARE, Smith JNM (1985) The role of dispersal in the population dynamics of snowshoe hares. *Canadian Journal of Zoology*, **63**, 106–115.

Boutin S, Krebs CJ, Boonstra R *et al*. (1995) Population changes of the vertebrate community during a snowshoe hare cycle in Canada's boreal forest. *Oikos*, **74**, 69–80.

Bowen BS, Koford RR (1987) Dispersal, population size, and genetic structure of *Microtus californicus*: empirical findings and computer simulation. In: *Mammalian Dispersal Patterns* (eds Chepko-Sade BD, Halpin ZT), pp. 180–189. University of Chicago Press, Chicago.

Burton C (2001) Genetic analysis of snowshoe hare population structure. MSc Thesis, University of British Columbia.

Casgrain P, Legendre P (2001) *The R package for multivariate and spatial analysis*, Version 4.03d — *User's Manual*. Department des sciences biologiques, Universite de Montreal. http://www.fas.umontreal.ca/BIOL/legendre.

Castella V, Ruedi M, Excoffier L, Ibanez C, Arlettaz R, Hausser J (2000) Is the Gibraltar Strait a barrier to gene flow for the bat *Myotis myotis* (Chiroptera: Vespertilionidae)? *Molecular Ecology*, **9**, 1761–1772.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution*, **32**, 550–570.

Chepko-Sade BD, Halpin ZT, eds (1987). *Mammalian Dispersal Patterns*. University of Chicago Press, Chicago.

Chitty D (1967) The natural selection of self-regulatory behaviour in animal populations. *Proceeding of the Ecological Society of Australia*, **2**, 51–78.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.

Dallas JF, Dod B, Boursot P, Prager EM, Bonhomme F (1995) Population subdivision and gene flow in Danish house mice. *Molecular Ecology*, **4**, 311–320.

Davies N, Villablance FX, Roderick GK (1999) Determining the source of individuals: multilocus genotyping in nonequilibrium population genetics. *Trends in Ecology and Evolution*, **14**, 17–21.

Demboski JR, Cook JA (2001) Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America. *Molecular Ecology*, **10**, 1227–1240.

Douglas GW (1974) Montane zone vegetation of the Alsek River region, south-western Yukon. *Canadian Journal of Botany*, **52**, 1016–1033.

Ehrich D, Jorde PE, Krebs CJ *et al*. (2001) Spatial structure of lemming populations (*Dicrostonyx groenlandicus*) fluctuating in density. *Molecular Ecology*, **10**, 481–495.

Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proceedings of the Royal Society of London Series B*, **264**, 127–132.

Felsenstein J (1995) PHYLIP *(Phylogeny Inference Package)*, Version 3.57*c*. Dept. of Genetics, University of Washington. http://evolution.genetics.washington.edu/phylip.html.

Fuller SJ, Mather PB, Wilson JC (1996) Limited genetic differentiation among wild *Oryctolagus cuniculus* L. (rabbit) populations in arid eastern Australia. *Heredity*, **77**, 138–145.

Fuller SJ, Wilson JC, Mather PB (1997) Patterns of differentiation among wild rabbit populations *Oryctolagus cuniculus* L. in arid and semiarid ecosystems of north-eastern Australia. *Molecular Ecology*, **6**, 145–153.

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology*, **8**, 1513–1520.

Gaines MS (1981) The importance of genetics to population dynamics. In: *Mammalian Population Genetics* (eds Smith MH, Joule J), pp. 1–27. University of Georgia Press, Athens, Georgia.

Gaines MS, Krebs CJ (1971) Genetic changes in fluctuating vole populations. *Evolution*, **25**, 702–723.

Gaines MS, Diffendorfer JE, Tamarin RH, Whittam TS (1997) The effects of habitat fragmentation on the genetic structure of small mammal populations. *Journal of Heredity*, **88**, 294–304.

Gavin TA, Sherman PW, Yensen E, May B (1999) Population genetic structure of the northern Idaho ground squirrel (*Spermophilus brunneus brunneus*). *Journal of Mammalogy*, **80**, 156–168.

Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology*, **14**, 1066–1074.

Giles BE, Goudet J (1997) A case study of genetic structure in a plant metapopulation. In: *Metapopulation Biology: Ecology, Genetics and Evolution* (eds Hanski IA, Gilpin ME), pp. 429–454. Academic Press, San Diego, CA.

Gillis EA, Krebs CJ (1999) Natal dispersal of snowshoe hares during a cyclic population increase. *Journal of Mammalogy*, **80**, 933–939.

Gillis EA, Krebs CJ (2000) Survival of dispersing versus philopatric juvenile snowshoe hares: do dispersers die? *Oikos*, **90**, 343–346.

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

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Goudet J (1999) FSTAT, *a program to estimate and test gene diversities and fixation indices*, Version 2.8. Institut d'Ecologie, Universite de Lausanne, Lausanne.

Goudet J, Raymond M, De Meeus T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1931–1938.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–372.

Hik DS (1994) Predation risk and the 10-year snowshoe hare cycle. PhD Thesis, University of British Columbia.

Hodges KE (1998) Snowshoe hare demography and behaviour during a cyclic population low phase. PhD Thesis, University of British Columbia.

Hodges KE (1999) Proximate factors affecting snowshoe hare movements during a cyclic population low phase. *Ecoscience*, **6**, 487–496.

Hodges KE (2000a) The ecology of snowshoe hares in northern boreal forests. In: *Ecology and Conservation of Lynx in the United States* (eds Ruggiero L, Aubry K, Buskirk S *et al*.), pp. 117–161. University Press of Colorado, Boulder, CO.

Hodges KE (2000b) Ecology of snowshoe hares in southern boreal and montane forests. In: *Ecology and Conservation of Lynx in the United States* (eds Ruggiero L, Aubry K, Buskirk S *et al*.), pp. 163–206. University Press of Colorado, Boulder, CO.

Hodges KE, Krebs CJ, Hik DS *et al*. (2001) Snowshoe hare demography. In: *Ecosystem Dynamics of the Boreal Forest: the Kluane Project* (eds Krebs CJ, Boutin S, Boonstra R), pp. 141–178. Oxford University Press, Oxford.

Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.

Jardine C (1995) A comparison of a cyclic and non-cyclic population of snowshoe hares in Kluane, Yukon. MSc Thesis, University of British Columbia.

Keith LB (1966) Habitat vacancy during a snowshoe hare decline. *Journal of Wildlife Management*, **30**, 828–832.

Keith LB (1990) Dynamics of snowshoe hare populations. In: *Current Mammalogy*, Vol. 2 (ed. Genoways HH), pp. 119–195. Plenum Press, New York.

Keith LB, Bloomer SEM, Willebrand T (1993) Dynamics of a snowshoe hare population in fragmented habitat. *Canadian Journal of Zoology*, **71**, 1385–1392.

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

Koenig WD (1999) Spatial autocorrelation of ecological phenomena. *Trends in Ecology and Evolution*, **14**, 22–26.

Koenig WD, Van Vuren D, Hooge PN (1996) Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology and Evolution*, **11**, 514–517.

Krebs CJ, Boonstra R, Boutin S, Sinclair ARE (2001a) What drives the 10-year cycle of snowshoe hares? *Bioscience*, **51**, 25–35.

Krebs CJ, Boutin S, Boonstra R, eds (2001b). *Ecosystem Dynamics of the Boreal Forest: the Kluane Project*. Oxford University Press, New York.

Kyle CJ, Strobeck C (2001) Genetic structure of North American wolverine (*Gulo gulo*) populations. *Molecular Ecology*, **10**, 337–347.

Kyle CJ, Davis CS, Strobeck C (2000) Microsatellite analysis of North American pine marten (*Martes americana*) populations from the Yukon and Northwest Territories. *Canadian Journal of Zoology*, **78**, 1150–1157.

Lidicker WZ Jr, Patton JL (1987) Patterns of dispersal and genetic structure in populations of small rodents. In: *Mammalian*

Dispersal Patterns (eds Chepko-Sade BD, Halpin ZT), pp. 144–161. University of Chicago Press, Chicago.

Lidicker WZ Jr, Wolff JO, Mowrey RA (2000) Genetic change in a cyclic population of snowshoe hares. *Ecoscience*, **7**, 247–255.

Luikart G, England P (1999) Statistical analysis of microsatellite DNA data. *Trends in Ecology and Evolution*, **14**, 253–256.

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238–247.

Manly BFJ (1997) *RT: a Program for Randomization Testing*, Version 2.1. Western Ecosystems Technologies Inc., Cheyenne WY.

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

Mossman CA, Waser PM (1999) Genetic detection of sex-biased dispersal. *Molecular Ecology*, **8**, 1063–1067.

Mossman CA, Waser PM (2001) Effects of habitat fragmentation on population genetic structure in the white-footed mouse (*Peromyscus leucopus*). *Canadian Journal of Zoology*, **79**, 285–295.

Mougel F, Mounolou JC, Monnerot M (1997) Nine polymorphic microsatellite loci in the rabbit, *Oryctolagus cuniculus*. *Animal Genetics*, **28**, 58–59.

Newman RA, Squire T (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology*, **10**, 1087–1100.

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

Paetkau D, Waits LP, Clarkson PL *et al.* (1998) Variation in genetic diversity across the range of North American brown bears. *Conservation Biology*, **12**, 418–429.

Petit E, Aulagnier S, Bon R, Dubois M, Crouau-Roy B (1997) Genetic structure of populations of the mediterranean mouflon (*Ovis gmelini*). *Journal of Mammalogy*, **78**, 459–467.

Pielou EC (1991) *After the Ice Age: the Return of Life to Glaciated North America*. University of Chicago Press, Chicago.

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.

Rannala B, Mountain JL (1997) Detecting immigration using multilocus genotypes. *Proceedings of the National Academy of Science USA*, **94**, 9197–9201.

Ranta E, Kaitala V, Lindstrom J, Linden H (1995) Synchrony in population dynamics. *Proceedings of the Royal Society of London Series B*, **262**, 113–118.

Raymond M, Rousset F (1995a) An exact test for population differentiation. *Evolution*, **49**, 1280–1283.

Raymond M, Rousset F (1995b) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rico C, Rico I, Webb N *et al.* (1994) Four polymorphic microsatellite loci for the European wild rabbit, *Oryctolagus cuniculus*. *Animal Genetics*, **25**, 367.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.

Sokal RR, Rohlf FJ (1995) *Biometry: the Principles and Practice of Statistics in Biological Research*. W.H. Freeman, New York.

Sugg DW, Chesser RK, Dobson FS, Hoogland JL (1996) Population genetics meets behavioral ecology. *Trends in Ecology and Evolution*, **11**, 338–342.

Surridge AK, Bell DJ, Rico C, Hewitt GM (1997) Polymorphic microsatellite loci in the European rabbit (*Oryctolagus cuniculus*) are also amplified in other lagomorph species. *Animal Genetics*, **28**, 302–305.

Surridge AK, Bell DJ, Ibrahim KM, Hewitt GM (1999) Population structure and genetic variation of European wild rabbits (*Oryctolagus cuniculus*) in East Anglia. *Heredity*, **82**, 479–487.

Tamarin RH, Krebs CJ (1969) *Microtus* population biology part 2: genetic changes at the transferrin locus in fluctuating populations of 2 voles species. *Evolution*, **23**, 183–211.

Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.

Waser PM, Elliott LF (1991) Dispersal and genetic structure in kangaroo rats. *Evolution*, **45**, 935–943.

Waser PM, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *Trends in Ecology and Evolution*, **13**, 43–44.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Whitlock MC (1992) Temporal fluctuations in demographic parameters and the genetic variance among populations. *Evolution*, **46**, 608–615.

Windberg LA, Keith LB (1976) Experimental analyses of dispersal in snowshoe hare populations. *Canadian Journal of Zoology*, **54**, 2061–2081.

Wolff JO (1980) The role of habitat patchiness in the population dynamics of snowshoe hares. *Ecological Monographs*, **50**, 111–130.

Wolff JO (1981) Refugia, dispersal, predation, and geographic variation in snowshoe hare cycles. In: *Proceedings of the World Lagomorph Conference* (eds Myers K, MacInnes CD), pp. 441–449. University of Guelph, Guelph.

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

Wright S (1978). *Evolution and the Genetics of Populations*, Vol. 4 Variability Within and Among Natural Populations. University of Chicago Press, Chicago.

This research was part of Cole Burton's MSc thesis work on population structure in snowshoe hares. Cole is interested in combining molecular and field techniques to investigate population structure and movement dynamics in terrestrial mammals. Charles Krebs has been studying the population ecology of snowshoe hares in the southwest Yukon for over 25 years. His main research interests centre around understanding population cycles in small mammals. Eric Taylor's research focuses on understanding patterns of genetic variation within and between natural populations. In particular, his laboratory develops and applies techniques in molecular biology to address questions in the evolution, ecology and conservation of natural fish populations.
