GENETIC ANALYSIS OF SNOWSHOE HARE POPULATION STRUCTURE

by

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ABSTRACT

Snowshoe hares are distributed throughout the boreal forests of North America and play a key role in the functioning of these ecosystems. Very little is known about the social and genetic structure of snowshoe hare populations. In this thesis, I used seven microsatellite DNA markers to investigate three levels of hare population structure: *mating structure*, *social structure* and *geographic structure*. I sampled 382 hares at 12 sites in southwestern Yukon (separated by 3-140 km) from April to August 1999, during a peak phase of the 10-year hare cycle. I also obtained samples from interior Alaska (n = 27) and western Montana (n = 19) for comparison. Genetic diversity was high, with 5 to 37 alleles per locus (mean = 13.4) and an overall expected heterozygosity of 0.67. At the level of mating structure, the genotypes of 65 newborn hares from 17 litters indicated that multiple paternity occurred at a low to moderate frequency (~25-30%), and that reproductive success was fairly widespread among male hares. In terms of social structure, the comparison of genetic relatedness with spacing behaviour among 68 hares on two 7.3 ha grids revealed that average group relatedness was low, and that related hares were not more likely to associate with each other than non-related hares. At a larger geographic scale I found significant genetic heterogeneity. The degree of genetic differentiation was low among Yukon sites (F_{ST} = 0.015) and between Yukon and Alaska (F_{ST} = 0.012); however the Montana site was highly differentiated (F_{ST} = 0.20). Geographic distance and landscape barriers explained some of the genetic differentiation between sites, but in general were poor predictors of geographic genetic structure. The indication of considerable long-distance gene flow implies that previous field observations may have underestimated dispersal, but confirms that hare dispersal is widespread, successful and equal between the sexes. The results of all three levels of investigation suggest that snowshoe hares have little population structure in southwestern Yukon during a cyclic peak phase. Hares do not form stable breeding groups, do not live in kin clusters, and do not experience significant social or physical barriers to gene flow across large areas. Future studies focusing on different phases of the hare cycle, and different regions, will be critical for understanding the mechanisms that shape the genetic structure of snowshoe hare populations.

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CHAPTER 1: GENERAL INTRODUCTION

Snowshoe hares are a keystone species in North American boreal forest ecosystems. They are distributed throughout the boreal forests of Canada and Alaska and into the sub-boreal and montane forests of the continental United States (Banfield 1974, Hodges 2000b). Their populations undergo synchronous cyclic fluctuations with a period of 8-11 years and a typical amplitude of 10 to 25 fold (Keith 1990, Hodges 2000a). During peak phases, snowshoe hares represent the dominant herbivore biomass in northern boreal forests, and the changes in hare density have important consequences for many predator, herbivore and plant species (Boutin *et al.* 1995). The snowshoe hare cycle has long interested population ecologists and experimental work has shown that it is primarily driven by an interaction between predation and food, potentially mediated by hare behaviour or stress effects (Krebs *et al.* 2001).

While the population dynamics of snowshoe hares have been well studied, little is known about the social and genetic structure of hare populations. Most species exhibit some degree of population substructure, be it through the formation of breeding or social groups, or the geographic subdivision of populations by environmental variation. Hares are thought to mate promiscuously (Flux 1979), but the difficulty of observing hares in the wild has left the mating system poorly described. The hare social system is similarly unclear. Hares do not maintain exclusive territories and are not known to form any social groups (Boutin 1979), yet they do exhibit social interactions and dominance hierarchies for which the mechanisms and consequences are unknown (Graf 1985). At a larger spatial scale, it is not known whether hares form continuous, genetically interconnected populations or smaller, partially isolated subpopulations. Many studies have focused on hare dynamics at local sites in various parts of their range, but few have expanded the spatial scale to consider regional movement patterns and their consequences to hare population structure and dynamics.

Genetic markers, such as microsatellite DNA, provide one means of examining population structure (Avise 1994, Hughes 1998, Ross 2001). These markers can be used to identify individuals, trace mating success and determine relationships among members of a social group. They can also be used to measure genetic differences among animals in different areas and infer patterns of movement. Furthermore, patterns of genetic diversity provide a window into historical population processes and the evolutionary mechanisms underlying population genetic

structure (Wright 1978). Microsatellite loci, consisting of tandem repeats of very short nucleotide sequences, are a useful mendelian marker for investigating population structure due to their codominance, selective neutrality, high degree of polymorphism and relative ease of amplification and scoring (Queller *et al.* 1993, Jarne & Lagoda 1996).

The objective of my thesis was to use microsatellite markers to investigate three levels of genetic variation in snowshoe hare populations corresponding to three interconnected aspects of population structure: *mating structure*, *social structure* and *geographic structure*. I studied snowshoe hares in the Kluane region of the southwestern Yukon from April to August 1999, during a peak phase of the density cycle. At the first level, I examined the genetic variation within complete hare litters and among their known mothers and potential fathers (Chapter 2). My goal was to assess male reproductive success by estimating the frequency of multiple paternity and the degree of reproductive skew. Multiple paternity has been well-documented in many species of birds and some monogamous mammals, however its frequency in promiscuous mammals is largely unknown. The number of reproductively successful males within and across litters has implications for female mate choice and male-male competition, as well as for the degree of local relatedness and the maintenance of genetic diversity (Reynolds 1996).

For the second level of population structure, I assessed the genetic similarity of hares interacting in a local group (Chapter 3). My goal was to document the degree of relatedness among residents and determine whether or not related hares associated preferentially. Kin selection can be an important force shaping animal behaviour (Hamilton 1964), and interactions among kin have been hypothesized to influence cyclic dynamics in small mammal populations (Charnov & Finerty 1980, Kawata 1990, Lambin & Krebs 1993). The detection of kin clusters in snowshoe hares could be important for understanding the basis and consequences of their social interactions.

Finally, for the third component of population structure, I examined the distribution of genetic variation at a larger spatial scale in the southwest Yukon (Chapter 4). My main objectives were to determine the scale and magnitude of genetic differentiation among hares in this region, and to test the hypothesis that such differentiation was correlated with observed and predicted patterns of hare dispersal. By comparing direct estimates of movement with estimates of gene flow

inferred from the distribution of genetic variation, greater insight can be gained into the ecological and evolutionary processes shaping geographic population structure (Slatkin 1987).

By using microsatellite markers to investigate these three hierarchical components of population structure, I have attempted to further our understanding of behavioural processes influencing snowshoe hare dynamics in the southwest Yukon. I have also made the first assessment of the amount and distribution of genetic variation in snowshoe hares during a cyclic peak phase, providing a starting point for further research into the genetic structure of hare populations.

Literature cited

Avise JC (1994) *Molecular markers, natural history and evolution.* Chapman and Hall, New York.

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, 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.

Charnov EL, Finerty JP (1980) Vole population cycles: A case for kin-selection. *Oecologia,* **45,** 1-2.

Flux JEC (1979) Field observations of behaviour in the Genus *Lepus*. In: *Proceedings of the World Lagomorph Conference* (eds. Myers K, MacInnes CD), pp.377-391. University of Guelph, Guelph.

Graf RP (1985) Social organization of snowshoe hares. *Canadian Journal of Zoology,* **63,** 468-474.

Hamilton WD (1964) The genetical evolution of social behaviour. *Journal of Theoretical Biology,* **7,** 1-52.

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.

Hughes C (1998) Integrating molecular techniques with field methods in studies of social behavior: a revolution results. *Ecology,* **79,** 383-399.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution,* **11,** 424-429.

Kawata M (1990) Fluctuating populations and kin interaction in mammals. *Trends in Ecology and Evolution,* **5,** 17-20.

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

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

Lambin X, Krebs CJ (1993) Influence of female relatedness on the demography of Townsend's vole populations in spring. *Journal of Animal Ecology,* **62,** 536-550.

Queller DC, Strassman JE, Hughes CR (1993) Microsatellites and kinship. *Trends in Ecology and Evolution,* **8,** 285-288.

Reynolds JD (1996) Animal breeding systems. *Trends in Ecology and Evolution,* **11,** 68-72.

Ross KG (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology,* **10,** 265-284.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science,* **236,** 787-792.

Wright S (1978) *Evolution and the genetics of populations,* vol. 4: *Variability within and among natural populations.* University of Chicago Press, Chicago.

CHAPTER 2: MULTIPLE PATERNITY AND MALE REPRODUCTIVE SUCCESS

Introduction

Multiple paternity occurs when offspring from a single litter or brood are fathered by more than one male. The frequency of multiple paternity has important implications for the intensity of sexual selection and sperm competition (Reynolds 1996, FitzSimmons 1998, Birkhead & Moller 1998, Kelly *et al.* 1999). Relative to single paternity, multiple paternity may also increase the genetic diversity of offspring, increase effective population size (Sugg & Chesser 1994), reduce inbreeding (Stockley *et al.* 1993), increase intrapopulation gene flow (Kelly *et al.* 1999), influence interactions among offspring (Ridley 1993) and decrease estimated genetic correlations in heritability studies (Rhen & Lang 1995).

Genetic studies of paternity have challenged our understanding of mating systems (Reynolds 1996, Hughes 1998). In mammals, genetic analysis has both revealed multiple paternity in seemingly monogamous species (Goosens *et al.* 1998) and confirmed strict monogamy (Ribble 1991, Brotherton *et al.* 1997). While many genetic studies have focussed on monogamous species, only 3% of mammalian mating systems are classified as monogamous (Kleiman 1977) and over 90% are considered polygynous or promiscuous (Clutton-Brock 1989). Few genetic studies have investigated patterns of paternity in the latter class of species, but results to date suggest significant variation in the frequency of multiple paternity: 80% or greater has been found in several promiscuous species (Stockley *et al.* 1993, Boellstorf *et al.* 1994, Baker *et al.* 1999) whereas less than 20% has been reported in others (Ribble & Millar 1996, Lacey *et al.* 1997). Such differences in the frequency of multiple paternity reflect considerable variation in mating behaviour and reproductive success, much of which had been previously undetected and which remains poorly understood.

The mating system of snowshoe hares is poorly defined. Most species of the genus *Lepus*, including the snowshoe hare, are thought to have promiscuous mating systems (Banfield 1974, Flux 1979). In previous studies snowshoe hares have displayed traits consistent with promiscuous mating (Clutton-Brock 1989) such as limited parental care (Graf & Sinclair 1987, O'Donoghue & Bergman 1992), overlapping home ranges (Boutin 1979) and multiple mating in captivity (Graf 1981). However, snowshoe hare behaviour is difficult to observe in the field

(Graf 1981) and the mating system has not been well studied. The goal of this chapter is to examine mating behaviour in a wild population of snowshoe hares by using genetic markers to determine the frequency of multiple paternity and assess male reproductive success. Given that snowshoe hares mate promiscuously in captivity, and that female home ranges are overlapped by those of several males (e.g., 3-7) at peak hare densities (Boutin 1979, see also Chapter 3), I predict that the majority of snowshoe hare litters will be fathered by more than one male and that reproductive success will be widespread among males.

Methods

Sample collection

The study was conducted over a 1 km^2 area near Kluane Lake, Yukon Territory (61^o N, 138^o W). The local forest is dominated by white spruce (*Picea glauca*) with an understory of grey willow (*Salix glauca*), bog birch (*Betula glandulosa*) and soapberry (*Sheperdia canadensis*) (for a more detailed description see Douglas 1974). I live-trapped hares beginning in May 1999 using Tomahawk traps (Tomahawk Live Trap Co., Tomahawk WI) distributed in areas of high hare activity (i.e., fresh pellets, heavy browsing, runways, etc.). Traps were baited with alfalfa cubes, apples and rabbit chow and were set late in the evening and checked early in the morning. The reproductive stage of captured females was assessed by weight, lactational tissue colour and gentle palpation of the abdomen. Females that were about to give birth were kept in 60 x 60 x 120 cm chicken wire cages (O'Donoghue and Krebs 1992) until the young were born. Cages were covered to provide shelter, lined with straw for nesting material, and included a partitioned refuge area to reduce stress. I checked the females each morning and gave them water, rabbit chow, apples and natural forage. When they gave birth I removed the females from the cage and sexed, weighed and measured the right hind foot length of each newborn. A small amount of ear tissue was collected from the mother and each newborn using a 3mm biopsy punch (Mader Instrument Corp., Stamford CT). Tissue samples were placed in 95% ethanol at the time of collection and put in the freezer within 1-2 hours. Immediately after collecting the samples, I returned the mother and offspring to the site of her original capture in the field. The leverets were placed in a suitable "nest" area in thick cover and the mother was released at the nest. All adult hares from which tissue was sampled were identified with a Monel # 3 eartag (National Band and Tag Co., Newport KY).

Eleven females were taken into captivity during the time of the first litter (May 24 - June 2, 1999). Eight of these produced litters between May 31 and June 11, and the other 3 were released without having given birth. Seven females were taken into captivity between July 5 and July 11, 1999, the time of the second litter (5 of the 7 had produced first litters in captivity). All seven produced litters between July 9 and July 22, 1999. I also obtained samples for two additional litters by collecting tissue from two pregnant females (and their fetuses) killed on the highway. The average number of offspring per litter was 3.8 (range 1-5, see Appendix 1). Tissue samples were also collected from 24 adult males trapped near the females to identify potential fathers (see Table 2.1).

One of the second litters was excluded from the multiple paternity analysis because it contained only one leveret. For the paternity assignment and reproductive success analyses, the two roadkill females and their litters (10 leverets in total) were excluded since they were most likely not resident in the same area as the males (Table 2.1).

Genetic analysis

I extracted DNA from the ear tissue samples using the Puregene Animal Tissue Protocol (Gentra Systems) with Proteinase K digestion. Aliquots of nine microsatellite primer pairs for the European rabbit, *Oryctolagus cuniculus* (Mougel *et al.* 1997, Table 2.2) were kindly provided by Monique Monnerot (CNRS, France). Two other microsatellite primer pairs were prepared using sequences from a research team at the University of East Anglia, England (Rico *et al.* 1994, Surridge *et al.* 1997, Table 2.2).

Initial PCR (Polymerase Chain Reaction) amplification for each primer pair was carried out in a 10 µl reaction volume containing the following: 100 ng of template DNA, 0.5-0.8 µM of each primer, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 0.5 units of *Taq* polymerase (GibcoBRL) and 1 x 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.

Table 2.1 Number of individuals sampled and included in the different analyses. One litter had to be excluded from multiple paternity analyses as it contained only one leveret. The two roadkill litters (2 mothers, 10 offspring) were excluded from analyses involving the sampled males since they were likely resident in a different area.

	Number of Mothers	**Number of Litters**	**Number of Offspring**
Total	12	17	65
Multiple Paternity Analysis (CERVUS analysis in parentheses)	12 (10)	16 (14)	64 (54)
Reproductive Success Analysis	10	15	55
Number of males sampled = 24			

Table 2.2 Microsatellite loci descriptions and PCR amplification conditions. The notation 30 s @ 94°/54° means 30 seconds at 94°C followed by 30 seconds at 54°C. All reactions ended with 5 minutes at 72°C and were held at 6°C.

Locus	**Reference**	**Repetition**	**Primer sequence** (5'-3')	**Amplification conditions**
Sol03	Rico *et al* 1994	$(TC)_{14}(T)_4(TC)_6$	**F:**TACCGAGCACCAGATATTAGTTAC **R:**GTTGCCTGTGTTTTGGAGTTCTTA	3 min @ 94°C 7 x (30s @ 94°/54°/72°) 23 x (30s @ 89°/54°/72°)
Sol33	Surridge *et al* 1997	$(TG)_3CG(TG)_{18}$	**F:**GAAGGCTCTGAGATCTAGAT **R:**GGGCCAATAGGTACTGATCCATGT	same as Sol03 *
Sat02	Mougel *et al* 1997	$(TC)_{15}(TG)_{10}$	**F:**GCTCTCCTTTGGCATACTCC **R:**GCTTTGGATAGGCCCAGATC	5 min @ 94° 25 x (30s @ 94°/58°/72°)
Sat03	Mougel *et al* 1997	$(TC)_{22}$	**F:**GGAGAGTGAATCAGTGGGTG **R:**GAGGGAAAGAGAGAGACAGG	5 min @ 94° 25 x (30s @ 94°/62°/72°)
Sat05	Mougel *et al* 1997	$(TC)_{23}TTT(CT)_5$	**F:**GCTTCTGGCTTCAACCTGAC **R:**CTTAGGGTGCAGAATTATAAGAG	5 min @ 94° 30 x (30s @ 94°/62°/72°)
Sat12	Mougel *et al* 1997	$(CTAT)_{10}$	**F:**CTTGAGTTTTAAATTCGGGC **R:**GTTTGGATGCTATCTCAGTCC	2 min @ 94° 3 min @ 55° 2 min @ 72° 30 x (30s @ 94°/55°/72°)
Sat13	Mougel *et al* 1997	$(GT)_{13}$	**F:**CAGTTTTGAAGGACACCTGC **R:**GCCTCTACCTTTGTGGGG	5 min @ 94° 5 x (30s @ 94°/53°/72°) 25 x (30s @ 94°/55°/72°)
Sat16	Mougel *et al* 1997	$(TG)_{15}$	**F:**AATCAGCCTCTATGAATTCCC **R:**AATGCTACATGGTAACCAGGC	2 min @ 94° 3 min @ 57° 2 min @ 72° 4 x (30s @ 94°/57°/72°) 25 x (30s @ 94°/55°/72°)

***** Sol03 and Sol33 were successfully diplexed

Primer pairs that gave a specific product were amplified and optimized using a radioactive label. The forward primer was first 5' endlabelled in a 1µl reaction volume containing: 0.25 units of T4 polynucleotide kinase (PNK, New England BioLabs), 1 x PNK buffer (70 mM Tris-HCl, 10 mM $MgCl_2$, 5mM DTT, 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 x reaction buffer. The Sol03 and Sol33 reactions also contained 0.5 µl of dimethyl sulphoxide (DMSO). PCR amplifications were performed in a PTC-100 (MJ Research) under optimal conditions for each locus (Table 2.2). PCR products were mixed with 7 µl of stop dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylenecyanol FF) and denatured at 94^{o}C for 5-10 minutes before 4 µl of each sample was loaded onto a 6% denaturing polyacrylamide gel (in 1.2x 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 hours and scored manually. Any individuals that failed to produce clear bands were reamplified under the same conditions.

Of the 11 European rabbit primer pairs tested, I successfully amplified eight in the snowshoe hare (Table 2.3 and Figure 2.1). I calculated the allele frequencies and heterozygosity for each locus over all of the sampled individuals using the program GENEPOP version 3.1 (Raymond and Rousset 1995). Each locus was also tested for adherence to Hardy Weinberg Equilibrium (HWE) and genotypic linkage equilibrium in GENEPOP using the genotypic data for the adults only (offspring were excluded from these tests to maintain the assumption of independent sampling). None of the loci were in linkage disequilibrium and all except Sat 5 conformed to HWE. The Sat 5 locus showed a significant heterozygote deficiency ($p<0.001$), presumably due to one or more high frequency nonamplifying (null) alleles, and was thus excluded from further analysis.

Data analysis - multiple paternity

The genotype of each offspring in a litter was compared with the mother's genotype to identify the maternal and paternal alleles for each locus. I identified paternal alleles as: (i) an allele

Table 2.3 Number of alleles, allele size range, observed (H_o) and expected (H_e) heterozygosities, and the probability of detection (*d*) for each locus. The power to detect multiple paternity over all loci combined (*D*) is also given. The total number of individuals sampled was 101.

Locus	**No. alleles**	**Allele size range (bp)**	**H_o**	**H_e**	***d***
Sol03	5	268 - 279	0.396	0.380	0.239
Sol33	8	212 - 221	0.733	0.774	0.527
Sat02	22	218 - 255	0.911	0.918	0.839
Sat03	5	138 - 160	0.485	0.488	0.221
Sat05 *	10	197 - 233	0.426 *	0.710 *	-
Sat12	7	112 - 136	0.693	0.706	0.505
Sat13	3	119 - 123	0.297	0.299	0.163
Sat16	7	95 - 115	0.733	0.835	0.659
Average **	8.38 (8.14)		0.584 (0.607)	0.639 (0.629)	***D** = 0.994*

* Sat05 had a highly significant heterozygote deficiency ($p<0.0001$) and was thus excluded from further analysis
** Number in parentheses is the average value excluding Sat05

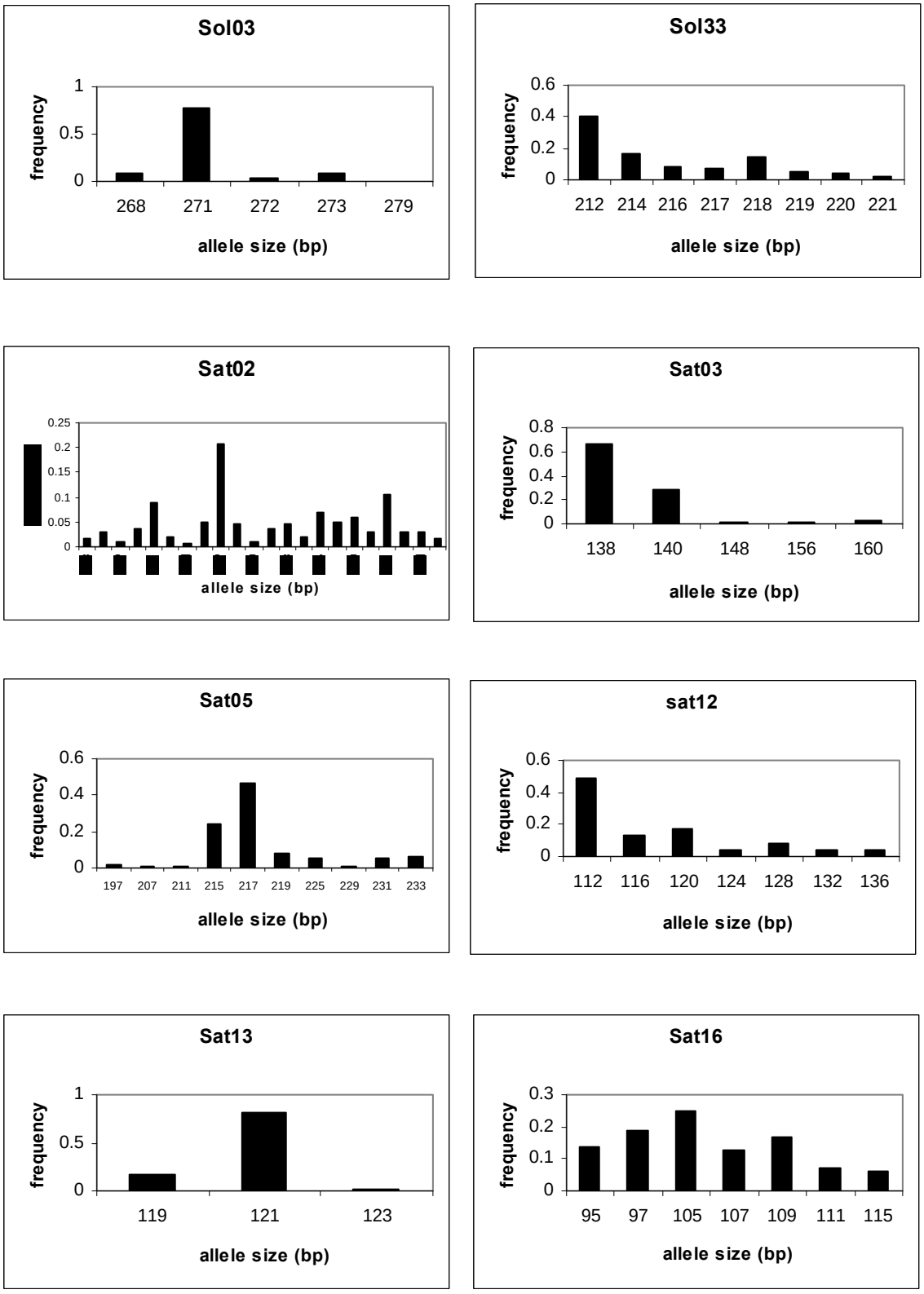


Figure 2.1 Allele frequencies at each locus for all individuals sampled (n = 101).

present in the offspring that is not present in the mother; (ii) an allele present in homozygous condition in the offspring; or (iii) one of the two alleles of a heterozygous offspring with a genotype identical to the mother's (which allele is paternal cannot be determined in this case). If the minimum number of paternal alleles required to explain the observed genetic variation in a litter is greater than two, multiple paternity can be assumed for that locus. The robustness of this assumption increases with the number of different loci that meet the criterion (FitzSimmons 1998).

The presence of only one or two paternal alleles in a litter does not necessarily preclude multiple paternity as several different males could have contributed the same allele. In order to determine the power of this analysis I calculated a detection index (*d*), defined as the probability of detecting alleles from more than one father given the population allele frequencies and calculated as:

$$d = 1 - 2a_2 + a_3 + 3(a_2a_3 - a_5) - 2(a_2^2 - a_4)$$

where $a_x = \sum_{i=1}^{n} p_i^x$

and p_i is the frequency of the *i*th allele for *n* alleles (Westneat *et al.* 1987, FitzSimmons 1998). The probability of detecting multiple paternity across all loci (*D*) was calculated as:

$$D = 1 - \prod_{i=1}^{m} (1 - d_i)$$

for *m* loci (Westneat *et al.* 1987, FitzSimmons 1998).

In addition to detection power, the estimate of multiple paternity may be affected by an inability to distinguish paternal alleles (see situation (iii) above) and by genotyping errors and mismatches due to mutation or null alleles. Furthermore, the method is locus-specific and thus does not take into account multi-locus genotypes. For these reasons, I also used two likelihood-based paternity inference methods to estimate multiple paternity. The computer program CERVUS, version 1.0, (Marshall *et al.* 1998) uses the observed multi-locus genotypes to determine the most-likely

father for each offspring from a pool of candidate males. It calculates statistical significance for these assignments based on simulations using population allele frequencies and estimates of genotyping error and sampling bias. Multiple paternity can be inferred from this program if offspring from the same litter are assigned to different fathers at a high confidence level. Since there is a trade-off between the number of paternity assignments and their accuracy, I included results for strict (95%) and relaxed (80%) levels of confidence. Marshall *et al.* (1998) suggest that paternities assigned with 80% confidence are more accurate than those determined by direct observation for most species, as well as being better than results obtained using a purely exclusionary approach.

Program KINSHIP, version 1.3 (Goodnight & Queller 1999), also performs likelihood calculations and determines statistical significance for hypotheses about pedigree relationships between individuals. The two programs differ in their sensitivity to deviations from Hardy Weinberg Equilibrium and their treatment of typing errors (Clinchy 1999). Since the paternity assignments in both programs depend on the pool of potential fathers sampled, I also used KINSHIP to generate pairwise relatedness values and test the likelihood that offspring in a litter were full-siblings against the null hypothesis that they were only maternal half-siblings.

Data analysis - male reproductive success

I used three methods to assess male reproductive success. I first examined the distribution of success by comparing the frequencies of paternal alleles across all offspring with the allele frequencies in the adult males sampled. If all males were equally successful, the observed paternal allele frequencies should follow the distribution found in the adult males. I performed a likelihood ratio chi-square goodness-of-fit test for each locus in program JMP IN (version 3.2.1, SAS Institute Inc.) and calculated Fisher's combined probability across all loci (Sokal and Rohlf 1995). Within a locus, alleles with very low expected frequencies were grouped together. When considering alleles for which the precise frequency in the offspring was uncertain (see situation (iii) above), I used a conservative estimate that maximized similarity with the adult male frequencies.

Paternity assignments from programs CERVUS and KINSHIP were used to determine how many offspring were assigned to each male. The simulation parameters used in CERVUS were:

10,000 cycles, 100% of loci typed, an error rate of 0.001 and a pool of 50 candidate males of which 48% were sampled (see Appendix 2). I included CERVUS results significant at a relaxed confidence level of 50% to provide a "maximum" estimate of reproductive success. Such a low level of statistical confidence can still provide useful biological information for species in which copulations are very difficult to observe in nature (Coltman *et al.* 1998).

The paternity results of both programs may be biased by the selection of adult males sampled and by assignment errors (see Appendix 2), therefore I also used KINSHIP to test the relatedness of the offspring independently of the candidate fathers. The number of offspring in a litter that had paternal half-siblings in other litters was calculated, and the total proportion of half-sibling relationships determined. If a few males were responsible for most of the paternity, it would be expected that a large proportion of offspring from different litters would be half-siblings. In order to visualize the relationships among all the offspring I constructed a UPGMA tree with the pairwise relatedness values calculated in KINSHIP (UPGMA is a clustering method using unweighted arithmetic averages).

Results

Multiple paternity

Four of sixteen litters (25%) showed evidence of multiple paternity from the minimum number of paternal alleles (Table 2.4, see Appendix 3 for complete genotype information). One litter had three different paternal alleles at both the Sat03 and Sat02 loci, two other litters had three paternal alleles at Sat02, and another litter had a third paternal allele at Sat12. The genotypes at the other loci in these litters and at all loci in the remaining 12 litters could be explained with only one or two different paternal alleles. The direct evidence for multiple paternity was thus seen in five of 112 possible cases (7 loci x 16 litters) and mainly at the most variable locus (three of five cases at Sat02). There were 20 other cases where a third paternal allele was possible, but because the maternal and paternal alleles could not be distinguished (the offspring and mother had the same genotype) the minimum possible number of paternal alleles was two. The probability of detecting multiple paternity across all seven loci was extremely high ($D = 0.994$). Three of the loci - Sol03, Sat03 and Sat13 - had one or two common alleles in the population (see Figure 2.1) and thus had low power to detect different paternal alleles (Table 2.3).

Table 2.4 Results of the multiple paternity analyses. The minimum number of paternal alleles detected for each litter at each locus is shown along with the corresponding minimum number of fathers. The results based on the paternity assignments in programs CERVUS and KINSHIP are also shown. CERVUS results include assignments under both strict (95%) and relaxed (80%) levels of confidence. Instances of multiple paternity are highlighted. The number of offspring in each litter is given in parentheses.

Litter (size)	Minimum number of paternal alleles at locus							Minimum no. of fathers	CERVUS		KINSHIP
	Sol03	Sol33	Sat02	Sat03	Sat12	Sat13	Sat16		95	80	
7950L1 (3)	1	2	**3**	1	2	2	2	**2**	1	1	1
7950L2 (4)	2	2	2	2	2	2	2	1	1	**2**	**2**
5925L1 (4)	1	2	2	2	1	2	1	1	1	1	1
5925L2 (4)	1	1	2	1	1	2	1	1	1	1	1
418L1 (4)	2	1	2	2	2	1	2	1	0	0	0
418L2 (4)	1	2	2	1	1	2	2	1	1	1	1
8220L1 (3)	2	2	1	2	1	1	1	1	1	1	1
474L1 (5)	1	2	**3**	2	2	2	2	**2**	0	0	1
8010L1 (3)	1	2	2	2	2	1	2	1	0	0	0
7973L2 (4)	1	2	2	1	2	1	2	1	0	0	0
8270L2 (5)	2	2	**3**	**3**	2	1	2	**2**	0	1	0
9412L1 (3)	2	2	1	1	2	1	2	1	1	1	0
9412L2 (5)	1	2	2	1	2	1	2	1	0	**2**	**2**
7901L1 (3)	2	2	2	2	**3**	1	2	**2**	1	1	1
RK1 (5)	2	2	2	2	2	1	2	1	-		-
RK2 (5)	2	1	2	1	2	1	2	1	-		-

Nevertheless, Sat03 did show a third allele for one litter (Table 2.4). There were three cases at Sat13 where a mismatch occurred between mother and offspring. In all three cases the mother and offspring appeared homozygous for different alleles (Appendix 3), indicating the likely presence of a low frequency null allele.

In the multi-locus paternity assignment programs, estimates of multiple paternity for litters in which more than one offspring were assigned fathers were 0% (CERVUS - 95% C.L.) and 28.6% (CERVUS - 80% C.L. and KINSHIP, see Tables 2.4 and 2.5). In CERVUS, only two litters had all offspring assigned paternity at the 95% level, and in both cases all were to the same father. At the 80% level, five litters had all offspring assigned, four of them to one father and the other to two fathers. Results were similar for KINSHIP, with three litters having all offspring assigned to one father and one complete litter with offspring assigned to two fathers. In CERVUS, five litters had more than one male assigned as most-likely father but without statistical significance, suggesting that an unsampled male (or several) fathered the offspring.

Only four of the sixteen litters had statistically significant full-sib relationships for all offspring in KINSHIP ($p<0.05$). In all the other litters, the maternal half-sib relationship could not be ruled out. This analysis did, however, report high probabilities of Type II errors (e.g., 0.503 for $p<0.05$ and 0.727 for $p<0.01$), and is thus conservative. A less conservative estimate of sibling relationships can be made using the pairwise relatedness (r) values calculated in KINSHIP. Full siblings are expected to have $r = 0.5$ and half-siblings to have $r = 0.25$, although in practice there is considerable variation around those values (Queller & Goodnight 1989, Blouin 1996, see Chapter 3). Five litters had low mean r-values (< 0.4) with high standard deviations, indicating that at least some of the littermates were half-siblings and thus suggesting multiple paternity (Figure 2.2). Two additional litters had intermediate r-values (0.35-0.4), making paternity unclear. A UPGMA tree constructed using the r-values (Figure 2.3) illustrates the fact that not all littermates grouped together despite sharing the same mother. Although r-values can be influenced by other factors, such as the relatedness of different parents, it seems likely that the large discrepancies between observed and expected values are due to multiple paternity.

Table 2.5 Summary results of paternity tests in programs CERVUS and KINSHIP. Results using both strict (95%) and relaxed (80%) confidence levels are reported for CERVUS. KINSHIP results are based on the highest likelihood value.

Program (C. L.)	no. litters tested	no. litters with > 1 offspring assigned	no. litters with offspring assigned to >1 father
CERVUS (95%)	14	6	0
CERVUS (80%)	14	7	2
KINSHIP	14	7	2

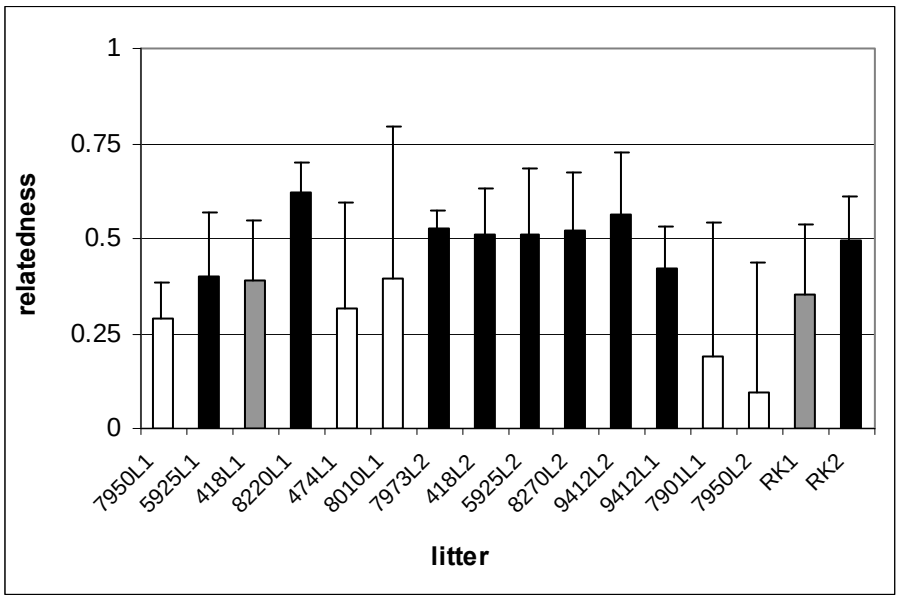


Figure 2.2 Mean pairwise relatedness (and standard deviation) among offspring within litters. Relatedness values, calculated in KINSHIP, have a theoretical value of 0.5 between full-sibs and 0.25 between half-sibs. Litters with values strongly suggestive of multiple paternity have white bars and those with marginal results have light shading.

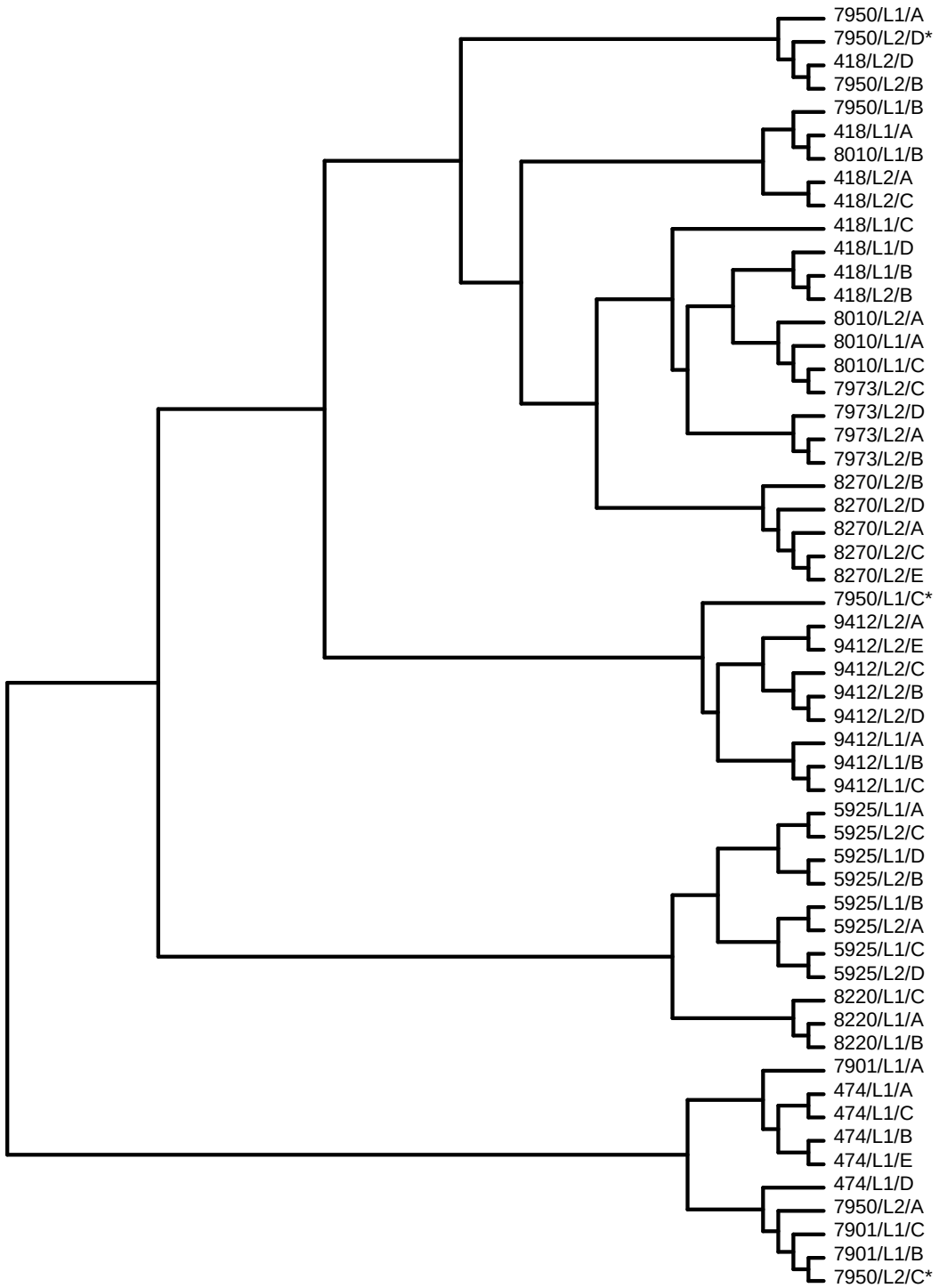


Figure 2.3 UPGMA cladogram showing degree of relatedness among the 55 offspring. Pairwise relatedness values were calculated in KINSHIP. Offspring are named according to mother (e.g., 7950), seasonal litter number (e.g., L1) and individual identifier (e.g., C). Some offspring did not group closely with their littermates (three examples from two litters are highlighted with asterisks), suggesting multiple paternity.

Male reproductive success

The paternal allele frequencies in the offspring differed significantly from those in the adult males when all loci were combined ($p<0.001$). This was largely due to discrepancies at Sol33 and Sat02 ($p<0.01$). While some alleles were significantly over- or under-represented among the offspring (Figure 2.4), the majority of the paternal allele frequencies were similar to those in the candidate males (mean deviation = 0.065 ± 0.041 S.D.). Most of the alleles at each locus that were detected in the entire population were accounted for in the paternal alleles (between 66.7% and 100% per locus, mean of 85%). There were 16 different paternal alleles at the most variable locus, Sat02, suggesting that a minimum of eight different males fathered the offspring from the fifteen litters. When the number of different paternal alleles was compared with the number of different maternal alleles (across all offspring), there were more paternal alleles at every locus except Sat16, with an average of 1.3 times more alleles across all loci.

The estimated distribution of paternities differed with the program and significance level used (Figure 2.5, Table 2.6). A large proportion of the sampled males were not assigned paternities by either program, however two factors must be considered when interpreting these results. Firstly, many of the offspring were not assigned fathers in the analyses (14-37 unassigned offspring), and secondly, some males probably fathered offspring from unsampled females in the area. Four males that were not assigned paternities and were known to have died before the second oestrus period were excluded from the assessment of reproductive success, however it is also possible that more of the sampled males did not survive through the breeding period.

The proportion of relationships between offspring from different litters that KINSHIP classified as paternal half-sibs was 5.14% (± 2.3% S.D.). Offspring from a single litter had paternal half-sibs in 25.7% (± 15.0% S.D.) of the other litters. The relatedness tree (Figure 2.3) shows that some offspring from different litters clustered together despite having different mothers, suggesting that they shared the same father.

Two of the five females for which both first and second litters were sampled appear to have mated with the same male, whereas the successive litters of the other three females were probably sired by different males (Table 2.7).

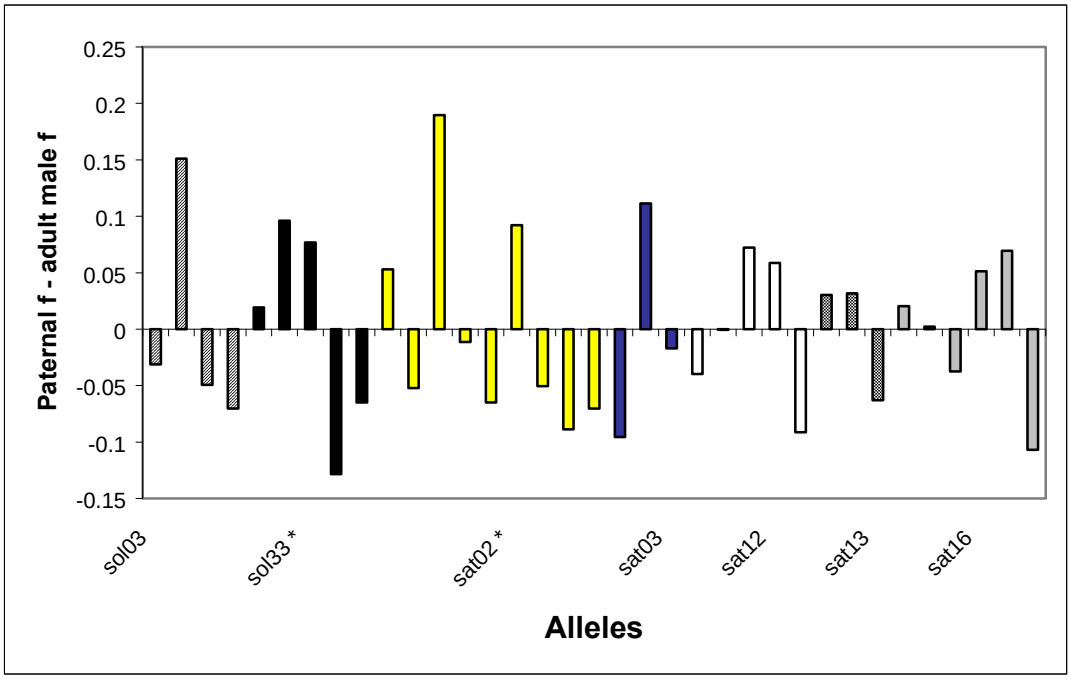


Figure 2.4 Difference in allele frequencies (f) between the paternal alleles in the offspring and the alleles in the adult male sample (alleles are shaded differently for the different loci). There were significant differences at Sol33 and Sat02 (chi-square test, $p<0.01$).

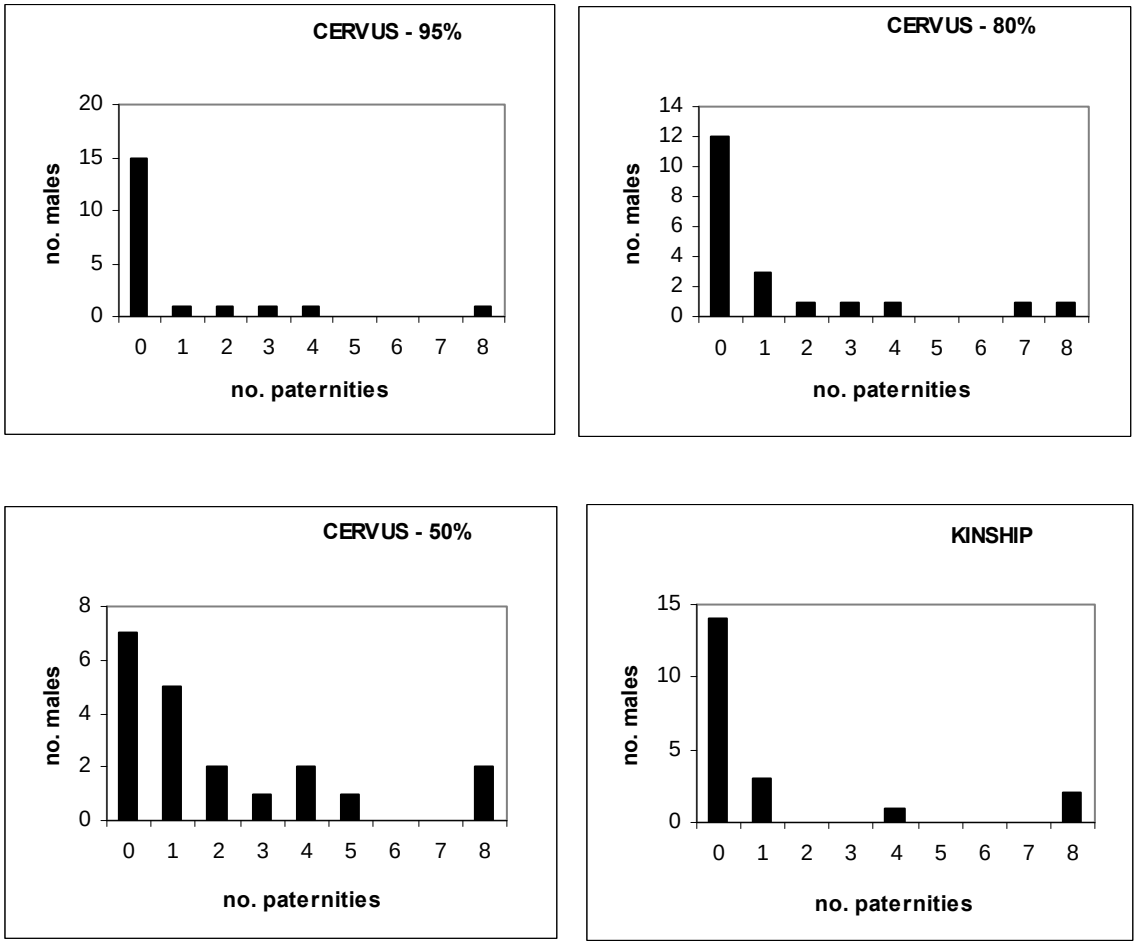


Figure 2.5 Distribution of paternities among adult males according to program CERVUS at confidence levels of 95%, 80%, 50% and program KINSHIP. Four males not assigned paternities and known to have died before the second oestrus period were excluded.

Table 2.6 Summary statistics for the estimates of male reproductive success using different paternity assignment criteria. Offspring were assigned fathers using program CERVUS at 95%, 80% and 50% confidence levels and using program KINSHIP. The standardized variance is the variance divided by the mean (Boness *et al.* 1993). Four males that were not assigned paternities and were known to have died before the second oestrus period were excluded from calculations.

Analysis	No. paternities assigned	Maximum per male	Mean per male	Variance	Standardized variance
CERVUS - 95%	18	8	0.90	4.09	4.55
CERVUS - 80%	27	8	1.35	5.71	4.23
CERVUS - 50%	41	8	2.05	6.47	3.16
KINSHIP	23	8	1.15	6.34	5.52

Table 2.7 The most-likely fathers in program CERVUS for females with both first (L1) and second (L2) litters sampled.

Mother	Litter	Most-likely father
7950	L1	420
	L2	420 and 2645
5925	L1	8047
	L2	8047
418	L1	unassigned
	L2	420
8010	L1	unassigned
	L2	8207
9412	L1	7910
	L2	unassigned

Discussion

Limitations of analyses

My results illustrate the difficulties in assessing paternity and reproductive success using microsatellite markers. In general, genetic studies of mating systems are affected by the number and variability of markers used. Failure to detect multiple paternity or assign paternities with high confidence may simply be due to a lack of informative loci. For example, in one study of multiple paternity in Columbian ground squirrels (*Spermophilus columbianus*), Murie (1995) found a low occurrence (16%) of multiple paternity using five loci with low levels of polymorphism. In contrast, a later study on the same species (S. Stevens unpublished) used nine highly polymorphic loci and detected a much higher frequency (65%) of multiple paternity. The seven loci that I used in this study showed a relatively high level of polymorphism and thus had a high combined probability of detecting multiple paternity. Higher levels of multiple paternity and variance in reproductive success have been reported for other species using comparable or fewer polymorphic markers (e.g., Boellstorf *et al.* 1994, Goosens *et al.* 1998, Valenzuela 2000). Nevertheless, the power to detect different fathers increases with the number of alleles observed (see Table 2.3) and the results here would be strengthened by the addition of more polymorphic loci.

Two other factors that can confound paternity analysis are null alleles and mutation (Pemberton *et al.* 1995, FitzSimmons 1998, Marshall *et al.* 1998). I found three mismatches between mother and offspring at Sat13 that were most likely due to a null allele. The occurrence of such nonamplifying alleles could have "hidden" true paternal alleles and resulted in false paternity exclusions. However, I reran some of the paternity analyses excluding this locus and the results did not significantly change (data not shown). Mutation processes in microsatellites are poorly known (Jarne & Lagoda 1996), making it difficult to determine whether "extra" paternal alleles are due to mutation or multiple paternity. Some authors consider that an extra paternal allele at only one locus is likely due to mutation and that extra paternal alleles at several loci are the result of multiple paternity (Fitzsimmons 1998, Valenzuela 2000). In my study, an extra paternal allele was detected at only one locus for three of four litters, and at two loci for the fourth. Furthermore, three of the five extra alleles were found at Sat02, the most variable locus and one likely subject to high mutation rates. A mutation rate of 5.6×10^{-3} would be required to explain

the extra alleles by mutation alone, which is within the range reported in the literature (10^{-2} to 10^{-5}, Jarne & Lagoda 1996). These results thus fall in a region of uncertainty in terms of making conclusions about multiple paternity. Nevertheless, I found no mother-offspring mismatches for any locus except Sat13 (see above), suggesting that mutations were uncommon and unlikely to explain the observed extra paternal alleles. Paternity assignments based on multi-locus genotypes can also be affected by mutations (Marshall *et al.* 1998). Most parentage programs, such as KINSHIP, do not account for genotyping errors due to mutation or otherwise. This can lead to false exclusion of true parents, as seen in this study when I tested known mother-offspring pairs (Appendix 2). Program CERVUS does allow for mutation and other forms of genotyping error, however determination of the error rate is uncertain (SanCristobal & Chevalet 1997, Marshall *et al.* 1998). Even with various error rates, program CERVUS excluded some known mothers from being the most likely mother in this study (Appendix 2).

Another important consideration for paternity studies involves sample sizes and the determination of the proportion of adults sampled. The detection of multiple paternity is obviously limited by the size of the litters sampled in that a minimum of three littermates is required and the power increases with litter size. The litter sizes in this study allowed for the detection of multiple paternity in all but one litter, however the average size (3.8 ± 1.1 S.D.) was small. The paternity assignments and assessments of male reproductive success are sensitive to estimates of the proportion of males and pregnant females in the study area that were sampled. With a complete census of the population, stronger conclusions could be made on paternity assignments and variance in reproductive success.

Multiple paternity

The direct paternal allele counts suggest that multiple paternity is infrequent in snowshoe hares. Given that hares are thought to be promiscuous (Banfield 1974, Flux 1979), and that females have multiple mates in captivity (Graf 1981), I expected multiple paternity to be frequent. However, only 25% of the litters had extra paternal alleles. A conservative assessment, assuming that extra alleles at only one locus in a litter are due to mutation (Fitzsimmons 1998, Valenzuela 2000), would only consider one litter (6.25%) to have shown multiple paternity. These estimates, however, are based on the minimum number of paternal alleles per litter and thus represent a minimum level.

The paternity analyses in programs CERVUS and KINSHIP support the idea that multiple paternity is infrequent in the hares (0% - 29%). However, a greater frequency of multiple paternity cannot be ruled out from these results as many offspring were not assigned fathers. In several litters, CERVUS assigned paternity for at least one offspring with a high degree of confidence but left the others unassigned. The fact that not all littermates were assigned to the same male raises the possibility that there may have been more than one father. The low proportion of litters that had a high likelihood of containing only full-sibs (in KINSHIP) also suggests of more multiple paternity, although the test most likely excluded some true full-sibs. The pairwise relatedness values are consistent with ~ 30% multiple paternity, but they do not rule out a frequency closer to 44%. It is interesting to note that none of the four litters for which multiple paternity was indicated from the single-locus paternal allele counts gave a statistically significant multiple paternity result in the multi-locus CERVUS or KINSHIP analyses. This lack of concordance may reflect problems with both types of analyses (see above) and highlights the need to carefully consider such biases in paternity studies. Although the relatedness values do not give a direct test of paternity, they provide a useful means of confirming results from the other methods.

I conclude from these results that multiple paternity does occur in snowshoe hares. While up to 56% of the litters (9 of 16) may have had multiple fathers, the data suggest a frequency of 25%-35%. This level of multiple paternity confirms that at least some wild female hares mate with multiple males during one oestrus period. The potential fitness benefits to females from multiple mating include: fertility assurance, procurement of good genes, increased offspring viability, increased genetic diversity of offspring and reduced harassment from courting males (Reynolds 1996, FitzSimmons 1998). However, the observed frequency of successful multiple mating in snowshoe hares is lower than expected considering that female hares likely encounter several different males during an oestrus period (e.g., 3-7 males, Boutin 1979, Chapter 3). The observed frequency is also lower than the 80-90% reported in several other promiscuous small mammals (e.g., Hanken & Sherman 1981, Stockley *et al.* 1993, Boellstorf *et al.* 1994, Baker *et al.* 1999). In fact it is close to the range reported for some socially monogamous species (e.g., 34% in Alpine marmots, Goosens *et al.* 1998).

There are several possible explanations for the low level of multiple paternity in snowshoe hares. Firstly, female hares may not frequently engage in multiple mating. Graf (1981) observed multiple mating only in captive hares. The male dominance hierarchies and female breeding dominance that Graf observed may restrict multiple mating in wild hares. Boutin (1979, 1980) suggested that females use their home ranges so as to avoid interactions with neighbouring females, and they may do the same to reduce encounter rates with males. Furthermore, Boutin's observation that both males and females have stable home ranges raises the possibility of stable mating associations. Post-copulatory sperm competition could also limit the number of males that fertilize one female. Sperm competition may influence fertilization in many promiscuous mammal species (Moller & Birkhead 1989, Gomendio *et al.* 1998). Testis size correlates with sperm competition in mammals (Kenagy & Tombulak 1986) and snowshoe hares have relatively large testes (~ 0.92% of body weight, R. Boonstra personal communication). According to Kenagy and Trombulak's (1986) allometric relationship between mammalian testes mass and body mass, this corresponds to a relative testes size (observed/predicted) of 1.96, which is consistent with a high degree of sperm competition. The high synchrony of oestrus in female snowshoe hares (Cary & Keith 1979) also suggests an important role for sperm competition in male mating success.

The social and dispersal behaviour of snowshoe hares may also not favour multiple paternity. For example, multiple mating may be advantageous for some female mammals in that it confuses paternity and prevents infanticide by adult males (Agrell *et al.* 1998), but infanticide has never been reported in hares. Multiple paternity can also reduce inbreeding (Stockley *et al.* 1993), yet there may be little risk of inbreeding in hares due to frequent dispersal and low local relatedness (see Chapter 3). Similarly, multiple paternity may not be needed to maintain genetic diversity if hares have other effective mechanisms, such as high gene flow between subpopulations (Chapter 4).

The phase of the snowshoe hare population cycle (Keith 1963, 1990, Krebs *et al.* 1995) could affect the level of multiple paternity. Other studies have found considerable variation in multiple paternity associated with changes in population density, habitat structure (Say *et al.* 1999) and predation pressure (Kelly *et al.* 1999). All of these factors change markedly during the hare cycle (Krebs *et al* 2001). This study took place during peak or early-decrease conditions and it would be interesting to know how the results might change under different conditions. I expect that the

frequency of multiple paternity would be greatest during this high-density phase since increased competition, greater home range overlap, and elevated predation risk may all promote multiple mating in females (Kelly *et al.* 1999, Say *et al.* 1999).

Male reproductive success

The difference between the frequencies of paternal alleles in the offspring and those in the adult males suggests that reproductive success was not evenly distributed among these males. However, given that 15 litters and 24 males were sampled and that multiple paternity was infrequent, it is not surprising that some of the males did not achieve paternity. It is also likely that unsampled males contributed some of the observed paternal alleles. Since most paternal allele frequencies were similar to those in the adult males and most of the alleles detected in the population were present among the paternal alleles, reproductive success was not limited to a few dominant males. The observed variation in paternal alleles suggests that a minimum of eight males fathered the 55 offspring. Given that ten different females contributed the observed maternal alleles, the results suggest that more than ten males were responsible for the greater number of paternal alleles.

It is difficult to make conclusions about reproductive success based on the analyses using CERVUS and KINSHIP. The low proportion of offspring for which paternity was assigned limits the generality of the results. Furthermore, the fact that not all males, females or leverets in the study area were sampled makes it impossible to confirm that the males not assigned as fathers of sampled offspring were actually reproductively unsuccessful. The results may thus overestimate the number of males who did not achieve paternity while underestimating those who fathered several offspring, potentially biasing the estimated variance in reproductive success. Nevertheless, if I assume the program results are representative of the male population, the indication is that a few males do obtain considerably more paternities than the average. Most of the above-average paternities were the result of fathering many offspring from one or two litters rather than achieving paternities in many different litters. The low proportion of paternal half-sibs in the KINSHIP analysis also suggests that individual males did not mate successfully with many different females. This implies that males do not mate successfully with all the females that they encounter (up to seven females per male with an average of three, Boutin 1979). The detection of some paternal half-sibs between litters, as well as the clustering pattern

in the relatedness tree, does however indicate that certain males did achieve paternities in more than one litter. Estimates of the standardized variance in reproductive success are consistent with those expected from low to moderate levels of polygyny (Boness *et al.* 1993, Coltman *et al.* 1998, Coltman *et al.* 1999) and are comparable to levels found in the socially monogamous white-toothed shrew (*Crocidura russula*, Bouteiller & Perrin 2000). The implication is that the intensity of sexual selection in snowshoe hares is limited.

When the trapping locations of males are considered relative to those of the mothers, there is some evidence suggestive of competition for mates with unequal reproductive success. For instance, eight different males were trapped at the same trap locations as four females (7950, 5925, 7901 and 418), however only three of these males (8047, 420 and 2645) were assigned paternity for the females' offspring. None of the other five males were assigned any paternities with high confidence, implying that they may have been outcompeted by the successful males.

The five females for which first and second litters were sampled provide evidence of both mate fidelity and "sequential" multiple paternity. Male 8047 fathered all of the offspring in both of female 5925's litters, despite the presence of several other males in the same area. Either 8047 outcompeted the other males and prevented them from successfully mating with 5925, or 5925 mated preferentially with 8047. Some offspring from both of female 7950's litters were fathered by male 420, but some in her second litter were fathered by 2645. The other females had unassigned offspring in one of the two litters, suggesting that the same male was not responsible for paternity of both. It is unclear whether 5925 and 7950 demonstrated mate fidelity by choosing the same mate over time, or if the fathers were simply stronger competitors. Such questions warrant further investigation for a species not known to form any pair bonds.

The low proportion of offspring assigned paternity with high statistical confidence in CERVUS and KINSHIP suggests that I did not sample many of the true fathers (see Appendix 2). I consider it unlikely that enough unsampled resident males were present near the mothers to account for the unassigned offspring, suggesting that the females were mating with transient males that were less likely to be caught in my traps. Chu (1996) has shown that male hares may increase their home ranges five-fold during the breeding season, therefore the "resident" males I trapped may have been outcompeted by males ranging over larger distances.

Conclusion

Offspring from one snowshoe hare litter may be fathered by more than one male. The level of multiple paternity in hare litters (~ 25-35%) is lower than expected in an unstructured promiscuous mating system. A low variance in male reproductive success indicates that no males dominated paternity, however there was an indication that a few males were significantly more successful than average. The observed multiple mating in both sexes confirms that snowshoe hares are promiscuous, yet successful multiple mating is limited. An important role for pre- and/or post-copulatory competition is implied.

Literature Cited

Agrell J, Wolff JO, Ylonen H (1998) Counter-strategies to infanticide in mammals: costs and consequences. *Oikos,* **83,** 507-517.

Baker RJ, Makova KD, Chesser RK (1999) Microsatellites indicate a high frequency of multiple paternity in *Apodemus* (Rodentia). *Molecular Ecology*, **8,** 107-111.

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

Birkhead TR, Moller AP (eds.) (1998) *Sperm competition and sexual selection.* Academic Press.

Blouin MS, Parsons M, Lacaille V, Lotz S (1996) Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology,* **5,** 393-401.

Boellstorf DE, Owings DH, Penedo MCT, Hersek MJ (1994) Reproductive behaviour and multiple paternity of California ground squirrels. *Animal Behaviour,* **47,** 1057-1064.

Boness DJ, Bowen WD, Francis JM (1993) Implications of DNA fingerprinting for mating systems and reproductive strategies of pinnipeds. *Symposium of the Zoological Society of London,* **66,** 61-93.

Boulanger J (1993) *Evaluation of capture-recapture estimators using a cyclic snowshoe hare population.* MSc thesis, University of British Columbia.

Bouteiller C, Perrin N (2000) Individual reproductive success and effective population size in the greater white-toothed shrew *Crocidura russula*. *Proceeding of the Royal Society of London Series B,* **267,** 701-705.

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

Boutin S (1980) Effect of spring removal experiments on the spacing behaviour of female snowshoe hares. *Canadian Journal of Zoology,* **58,** 2167-2174.

Brotherton PNM, Pemberton JM, Komers PE, Malarky G (1997) Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (*Madoqua kirkii*). *Proceeding of the Royal Society of London Series B,* **264,** 675-681.

Cary JR, Keith LB (1979) Reproductive change in the 10-year cycle of snowshoe hares. *Canadian Journal of Zoology,* **57,** 375-390.

Chu TKY (1996) *Fluctuations in home range size of male snowshoe hares during summer breeding season.* BSc thesis, University of British Columbia.

Clinchy M (1999) *Does immigration "rescue" populations from extinction?* PhD thesis, University of British Columbia.

Clutton-Brock TH (1989) Mammalian mating systems. *Proceedings of the Royal Society of London Series B,* **236,** 339-372.

Coltman DW, Bancroft DR, Robertson A*, et al.* (1999) Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. *Molecular Ecology,* **8,** 1199-1209.

Coltman DW, Bowen WD, Wright JM (1998) Male mating success in an aquatically mating pinniped, the harbour seal (*Phoca vitulina*), assessed by microsatellite DNA markers. *Molecular Ecology,* **7,** 627-638.

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

FitzSimmons NN (1998) Single paternity of clutches and sperm storage in the promiscuous green turtle (*Chelonia mydas*). *Molecular Ecology,* **7,** 575-584.

Flux JEC (1979) Field observations of behaviour in the Genus *Lepus*. In: *Proceedings of the World Lagomorph Conference* (eds. Myers K, MacInnes C), pp. 377-391. University of Guelph.

Gomendio M, Harcourt AH, Roldan ERS (1998) Sperm competition in mammals. In: *Sperm competition and sexual selection,* (eds. Birkhead TR, Moller AP), pp. 667-753. Academic Press.

Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology,* **8,** 1231-1234.

Goossens B, Graziani L, Waits LP, *et al.* (1998) Extra-pair paternity in the monogamous Alpine marmot revealed by nuclear DNA microsatellite analysis. *Behavioural Ecology and Sociobiology,* **43,** 281-288.

Graf RP (1981) *Some aspects of snowshoe hare behavioural ecology.* MSc thesis, University of British Columbia.

Graf RP (1985) Social organization of snowshoe hares. *Canadian Journal of Zoology,* **63,** 468-474.

Graf RP, Sinclair ARE (1987) Parental care and adult aggression toward juvenile snowshoe hares. *Arctic,* **40,** 175-178.

Hanken J, Sherman PW (1981) Multiple paternity in Belding's ground squirrels. *Science,* **212,** 351-353.

Hughes C (1998) Integrating molecular techniques with field methods in studies of social behavior: a revolution results. *Ecology,* **79,** 383-399.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution,* **11,** 424-429.

Keith LB (1963) *Wildlife's ten-year cycle.* University of Wisconsin Press, Madison.

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

Kelly CD, Godin J-GJ, Wright JM (1999) Geographical variation in multiple paternity within natural populations of the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London Series B,* **266,** 2403-2408.

Kenagy GJ, Trombulak SC (1986) Size and function of mammalian testes in relation to body size. *Journal of Mammalogy,* **67,** 1-22.

Kleiman DG (1977) Monogamy in mammals. *Quarterly review of biology,* **52,** 39-69.

Krebs CJ, Boutin S, Boonstra R*, et al.* (1995) Impact of food and predation on the snowshoe hare cycle. *Science,* **269,** 1112-1115.

Krebs CJ, Boutin S, Boonstra R (eds.) (2001) *Ecosystem dynamics of the boreal forest: the Kluane Project.* Oxford University Press, New York.

Lacey EA, Wieczorek JR, Tucker PK (1997) Male mating behaviour and patterns of sperm precedence in Arctic ground squirrels. *Animal Behaviour,* **53,** 767-779.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology,* **7,** 639-655.

Moller AP, Birkhead TR (1989) Copulation behaviour in mammals: evidence that sperm competition is widespread. *Biological Journal of the Linnean Society,* **38,** 119-131.

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

Murie JO (1995) Mating behavior of Columbian ground squirrels. I. Multiple mating by females and multiple paternity. *Canadian Journal of Zoology,* **73,** 1819-1826.

O'Donoghue M, Bergman CM (1992) Early movements and dispersal of juvenile snowshoe hares. *Canadian Journal of Zoology,* **70,** 1787-1791.

O'Donoghue M, Krebs CJ (1992) Effects of supplemental food on snowshoe hare reproduction and juvenile growth at a cyclic population peak. *Journal of Animal Ecology,* **61,** 631-641.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology,* **4,** 249-252.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution,* **43,** 258-275.

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

Reynolds JD (1996) Animal breeding systems. *Trends in Ecology and Evolution,* **11,** 68-72.

Rhen T, Lang JW (1995) Phenotypic plasticity for growth in the common snapping turtle: effects of incubation temperature, clutch, and their interaction. *American Naturalist,* **146,** 726-747.

Ribble DO (1991) The monogamous mating system of *Peromyscus californicus* as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology,* **29,** 161-166.

Ribble DO, Millar JS (1996) The mating system of northern populations of *Peromyscus maniculatus* as revealed by radiotelemetry and DNA fingerprinting. *Ecoscience,* **3,** 423-428.

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

Ridley M (1993) Clutch size and mating frequency in parasitic Hymenoptera. *American Naturalist,* **142,** 893-910.

SanCristobal M, Chevalet C (1997) Error tolerant parent identification from a finite set of individuals. *Genetical Research,* **70,** 53-62.

Say L, Pontier D, Natoli E (1999) High variation in multiple paternity of domestic cats (*Felis catus* L.) in relation to environmental conditions. *Proceedings of the Royal Society of London Series B,* **266,** 2071-2074.

Sokal RR, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research.* W.H. Freeman and Company, New York.

Stockley P, Searle JB, MacDonald DW, Jones CS (1993) Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proceedings of the Royal Society of London Series B,* **254,** 173-179.

Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. *Genetics,* **137,** 1147-1155.

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.

Valenzuela N (2000) Multiple paternity in side-neck turtles *Podocnemis expansa*: evidence from microsatellite DNA data. *Molecular Ecology,* **9,** 99-105.

Westneat DF, Fredrick PC, Haven WR (1987) The use of genetic markers to estimate the frequency of successful alternative reproductive tactics. *Behavioral Ecology and Sociobiology,* **21,** 35-45.

CHAPTER 3: THE INFLUENCE OF RELATEDNESS ON SPACING BEHAVIOUR

Introduction

Studies of population regulation in snowshoe hares have focused primarily on extrinsic rather than intrinsic factors (Keith 1990, Krebs 1996). Predation and food are the dominant forces influencing hare dynamics, but their direct effects are insufficient to explain all aspects of the 8-11 year density cycle (Krebs *et al*. 1995, Hodges *et al*. 1999, Hodges 2000). Recent models have focused on the interaction between predation and food, and the role of hare behaviour in linking the two, such as through predator-sensitive foraging (Hik 1995, Boonstra *et al*. 1998a). Experimental manipulations of predation and food, however, have not always resulted in consistent or predicted changes in hare behaviour (Hik 1994, Hodges 1998, 1999). The role of social interactions in these behavioural patterns remains unclear. Contrary to many other cyclic small mammals, interactions among hares seem unlikely to influence population dynamics due to a lack of obvious spacing behaviour or direct social mortality (Boutin 1980, Krebs 1996). Nevertheless, the potential for significant behavioural interference has been demonstrated in hares (Boutin 1984, Graf 1985, Sinclair 1986, Graf & Sinclair 1987, Ferron 1993, Quenette *et al*. 1997), and the consequences of interference for individual fitness (e.g., reduced foraging efficiency, greater predation risk, increased stress, lower reproductive success) require further investigation.

Identifying the mechanisms influencing social interactions is an important step in understanding their consequences. Such mechanisms remain poorly understood in snowshoe hares due to the difficulty of studying their behaviour in the field (Graf 1985). In many species of small mammals, the degree of relatedness between individuals has been hypothesized to influence social interactions (Charnov & Finerty 1980, Sherman 1981, Lambin & Krebs 1991, Surridge *et al*. 1999). Individuals may react less aggressively towards kin than non-kin, and the proportion of related individuals in a group could thus affect the frequency and severity of aggressive encounters experienced by a group member. This could directly or indirectly affect the survival, reproduction or dispersal of individuals and therefore influence population dynamics (e.g., Lambin & Krebs 1993). Relatedness may also be linked to dispersal through inbreeding avoidance and competition among kin (Ferriere *et al*. 2000).

Patterns of relatedness in natural populations of small mammals are poorly known (Krebs 1996, Boonstra et al. 1998b), but recent studies using genetic markers have detected clusters of related individuals in some species (e.g., *Clethrionomys rufocanus* - Ishibashi *et al*. 1997, *Crocidura russula* - Balloux *et al*. 1998, *Oryctolagus cuniculus* - Surridge *et al*. 1999). No studies have investigated spatial population structure in snowshoe hares and it is unknown whether or not relatives live near each other. The goal of this chapter is to examine the influence of kin interactions on snowshoe hare behaviour by: (i) determining the degree of relatedness among individuals in a wild population, and (ii) testing for an association between relatedness and spacing behaviour. If interactions among kin are less costly than among non-kin, I predict that hares should associate more closely with related individuals. Alternatively, if costs associated with inbreeding or kin-competition outweigh benefits of kin-association, related individuals would be expected to avoid each other. If kin interactions do not play a role in hare social behaviour I expect related and unrelated individuals to associate randomly.

Methods

Trapping and telemetry

This study took place on two 7.3 ha grids ("Flint" and "Chitty") separated by approximately 20 km near Kluane Lake, Yukon (see Chapter 2 for general information). Traps were set 30 m apart in a 10 x 10 pattern and trapping sessions were conducted weekly from early June to mid-August, 1999. Adult hares captured more than twice were considered to be resident on a grid. The majority of these residents (79% on Flint, 100% on Chitty) were fitted with 40 g radiocollars (Lotek, Newmarket ON). Radiotelemetry locations were collected daily by approaching hares to within ~ 5-10 m (without disturbing them) using handheld receivers and antennas (TR2, Telonics, Mesa AZ, see Hodges 1998 for more details on this methodology). Locations were recorded at different times of the day in order to obtain observations when hares were both active and resting. I quantified each radio-location by using the grid stakes as reference points. Locations for hares found away from the grids were estimated by measuring the direction and distance to a nearest reference point. Universal Transverse Mercator (UTM) coordinates for grid stakes and off-grid reference points were collected using a handheld GPS unit (GPS II plus, Garmin International Inc., Olathe KS).

On the Flint grid, 56 hares were caught over the trapping period. Forty of these were adults (16 males, 24 females) and 16 were juveniles (6 males, 10 females; "juvenile" is defined here as young of the year). An average of 3.4 trapping locations was obtained for each hare (range = 1-10; many juveniles were only caught once). Twenty-two of the adults (7 males, 15 females) were radiocollared and an average of 18.7 radio-locations per hare was obtained for 21 of them (range = 12-24). One female died after only five locations and was excluded from the telemetry analyses described below.

The Chitty grid had a lower density of hares. Eight adults (5 males, 3 females) and four female juveniles were trapped and an average of 2.7 trapping locations were obtained per hare (range = 1-9). Six of the adults (4 males, 2 females) were radiocollared and an average of 20.5 locations were obtained for these hares (range = 18-25, trapping + telemetry). Because of the low sample size on this grid, informative statistical analyses between the sexes and age classes were not possible.

Microsatellite analysis

DNA was extracted from tissue samples and seven microsatellite loci were amplified and scored as described in Chapter 2. Tests performed using the computer program GENEPOP version 3.1 (Raymond & Rousset 1995) indicated that all of the loci conformed to Hardy Weinberg Equilibrium and were in genotypic linkage equilibrium (data not shown). The number of distinct alleles and expected heterozygosity for each locus was calculated for the two grids using GENEPOP (Table 3.1).

Relatedness

I estimated genetic relatedness among individuals on the grids using the computer program KINSHIP version 1.2 (Goodnight & Queller 1999). The coefficient of pairwise relatedness (r) is calculated by comparing the proportion of shared alleles between two individuals with the allele frequencies in the whole population (details in Queller & Goodnight 1989). Resulting r-values can range from -1 to 1, with negative values indicating that individuals share fewer alleles than average. Theoretical r-values in a randomly mating population are 0.5 for full-siblings and 0.25 for half-sibs, but in reality there is considerable variation around those values

Table 3.1 Number of alleles and expected heterozygosity at each locus for the hares sampled from the Flint (n = 56) and Chitty (n = 12) grids.

Locus	**No. alleles**		**Expected heterozygosity**	
	Flint	Chitty	Flint	Chitty
Sol03	6	4	0.397	0.716
Sol33	6	8	0.760	0.773
Sat02	20	15	0.930	0.947
Sat03	7	3	0.612	0.564
Sat12	7	7	0.590	0.799
Sat13	3	3	0.182	0.466
Sat16	8	6	0.821	0.765

(Queller & Goodnight 1989, Blouin *et al*. 1996). I calculated mean pairwise relatedness for all hares on a grid and separately for each sex and age class. Pseudoreplication in the multiple pairwise comparisons precluded customary statistical tests of the means, therefore I determined significance using randomization tests performed in program RT version 2.1 (Manly 1997b). Data were randomized 5000 times and the probability of obtaining the observed means or differences between means by chance was calculated (see Manly 1997a for more details). As an alternative method of assessing the general level of relatedness among adults on each grid, I compared the distribution of *r*-values for the sampled hares to a distribution generated from the random simulation of 1000 unrelated pairs (i.e., having mean $r \approx 0$) using the same allele frequencies (Blouin *et al*. 1996). The significance of pairwise relatedness estimates was also further evaluated by using program KINSHIP to test the likelihood that pairs of individuals were either full-sibs or half-sibs against the null hypothesis that they were not related (Goodnight & Queller 1999).

Spacing behaviour

(i) Activity centres

I converted all hare locations to UTM coordinates based on their distance and direction from the reference UTM locations. An "activity centre" was determined for each hare by calculating the arithmetic mean of its locations (using both trapping and telemetry locations). For animals with more than five locations, I calculated the mean using only the 80% core locations. The distance between activity centres for each pair of hares was calculated and grouped by sex and age categories, and group means were tested by randomization in program RT.

I compared the distance between activity centres for each pair of hares to their degree of relatedness using a nonparametric Mantel test (Mantel 1967). For this test, relatedness values were transformed into dissimilarity measures by subtracting from 1. Significance was determined by comparing the observed Mantel Z statistic with the distribution of Z statistics obtained in 1000 random permutations of the distance and relatedness matrices. I also considered males, females, adults and juveniles in separate tests to look for effects of sex and age on spacing.

(ii) Home range overlap

Activity centres provide only a coarse estimate of hare home ranges and may not give a reliable representation of spacing behaviour (K. Hodges personal communication). I performed a more detailed analysis of spacing for the radiocollared hares using the computer program RANGES V (Kenward & Hodder 1996). The 95% fixed kernel estimates of home range (Worton 1989) were determined and the degree to which each hare's home range was overlapped by every other hare was calculated to provide a measure of space sharing. Because the degree of overlap between two hares depended on the size of each individual's home range, each hare had a unique overlap value and a matrix of pairwise overlap could not be made. Consequently, I used two alternative methods of testing for an association between overlap and relatedness (as in Knight *et al*. 1999): (a) separate regressions of overlap vs. *r* were made for each individual hare and the slopes compared across all hares; and (b) *r*-values and corresponding degrees of overlap were ranked for each hare, the means across all hares were calculated for every level in the ranking, and the regression of mean *r* on mean overlap was tested. Nonparametric Wilcoxon tests were used to compare slopes and test for differences between males and females (Sokal & Rohlf 1995). Significance levels were adjusted using the sequential Bonferroni correction (Rice 1989) when assessing multiple tests.

(iii) Dynamic interactions

The amount of home range overlap may not reveal the full extent of interaction between snowshoe hares. Boutin (1980) suggested that female hares use their home ranges in such a manner as to avoid neighbouring females. It is thus possible that two animals could seldom encounter each other despite sharing a large proportion of their ranges. For this reason I further examined the spacing behaviour of overlapping hares by testing for "dynamic interaction" (Macdonald *et al*. 1980) using RANGES V. An index of cohesion (Jacob's index, Jacobs 1974, see Kenward & Hodder 1996 and Kenward *et al*. 1993) was calculated by determining the tendency of pairs of individuals to be close together at the same time. I arbitrarily defined "same time" as radio-locations obtained within 60 minutes. The index is a measure of the difference between the observed and possible distances separating two individuals and can range from -1 to 1, with negative values indicating avoidance, positive values indicating cohesion and a value of 0 suggesting no interaction. Possible distances were calculated assuming that one of the hares

being compared could be at any of its observed locations when the other hare is at each of its locations. The interaction values were plotted against pairwise *r*-values and the significance of the relationship tested in program RT using 5000 randomized regressions.

Results

Flint grid

(a) Relatedness

The degree of relatedness (*r*) between any pair of adult hares on the Flint grid ranged from -0.66 to 0.87 with an overall mean of -0.027 (Figure 3.1, Table 3.2). This mean was significantly less than zero (randomization test, $p < 0.001$) and fell below the lower 95% confidence limit (-0.010) for the *r*-values of 1000 randomly generated pairs (Figure 3.1). This suggests that, in general, adults on the grid were slightly less related than expected by chance. Only 4.6% of the adult relationships were classified as either full- or half-sibling in program KINSHIP (36 of 780 pairwise comparisons, $p < 0.05$). There was no significant difference between the mean pairwise relatedness among adult males and adult females ($p = 0.95$), however juveniles on the grid were significantly more related than adults ($p = 0.023$).

When only the radiocollared adults were considered, the mean *r* increased to 0.025 (Table 3.2). This was not statistically greater than zero ($p = 0.059$), however it was above the upper confidence limit for the random distribution (0.019, Figure 3.1). The difference can be attributed to higher relatedness among females due to the exclusion of several non-collared females who had very low *r*-values. These latter females were only trapped 1-3 times each and it is possible that they were transients on the grid. Although the radiocollared female mean *r* was not statistically greater than zero ($p = 0.058$) or different from the male mean ($p = 0.11$), it suggests that there might have been slightly higher relatedness among resident females.

(b) Spacing behaviour

The average distance between activity centres for adults was 185.2 m (range = 6.5 - 496.9 m, Figure 3.2, Table 3.3). Males were located significantly farther apart than females

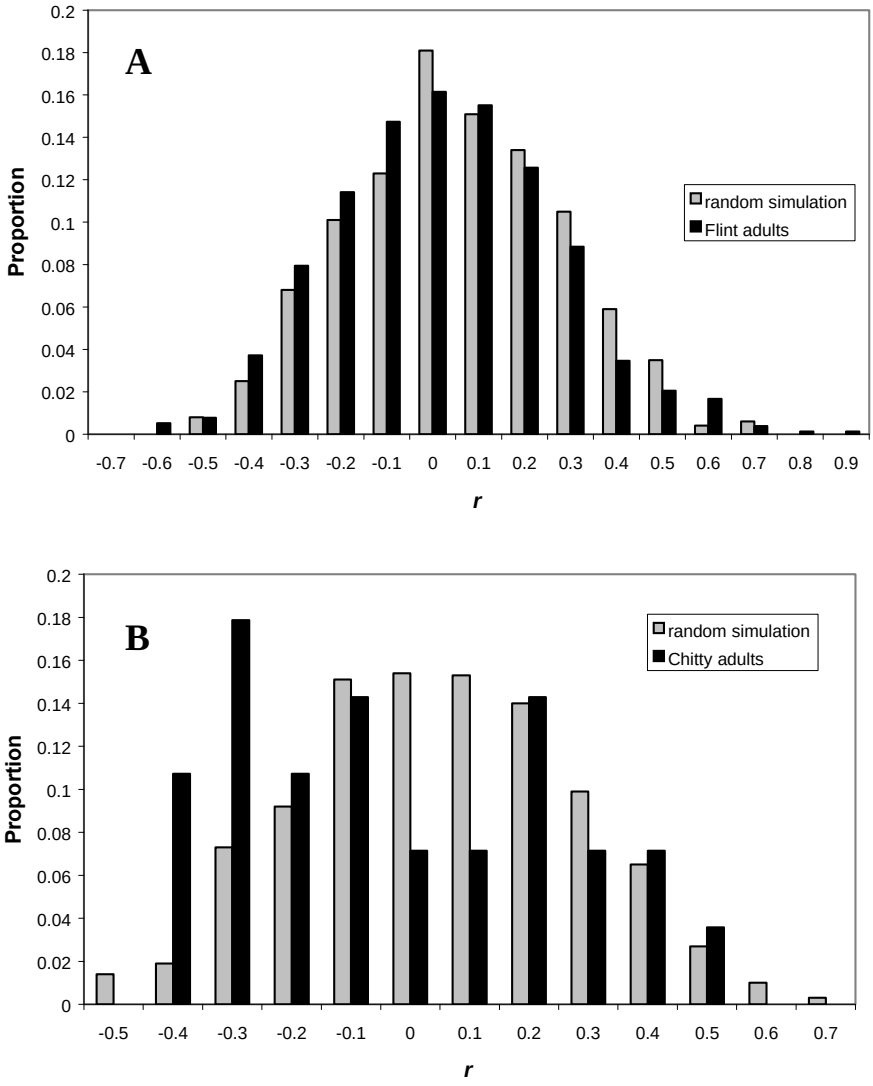
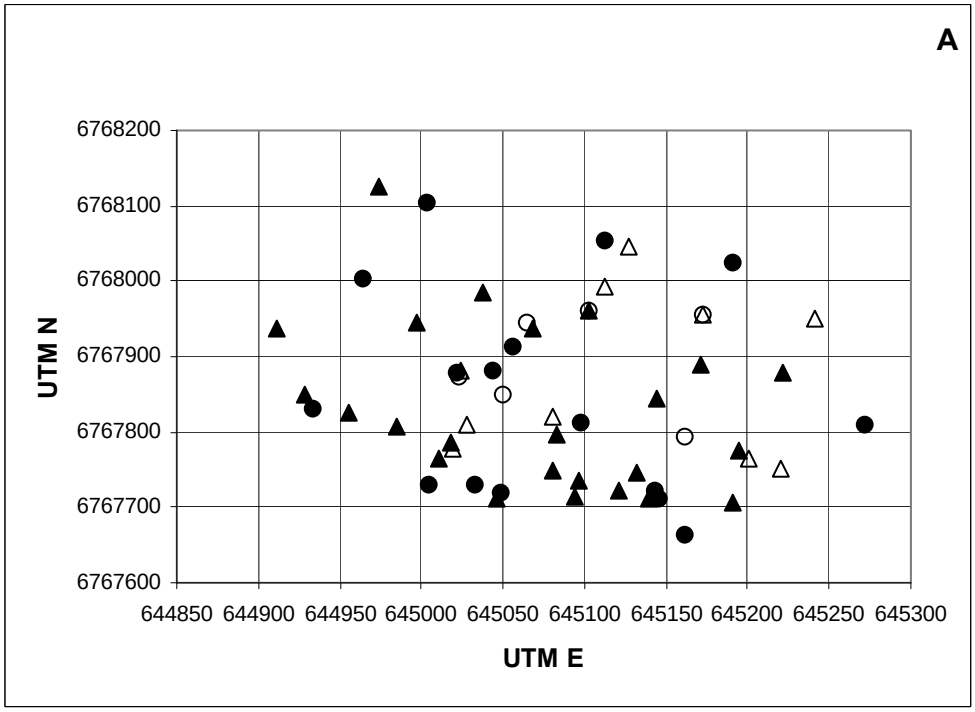


Figure 3.1 A. Distribution of pairwise relatedness values (*r*) for the 40 Flint grid adults (780 pairs) and 1000 pairs randomly generated in program KINSHIP using the Flint allele frequencies. The Flint adults had a few more closely related pairs ($r > 0.5$) but several more unrelated pairs (negative values) than the random group (test for goodness of fit, Pearson $\chi^2 = 74.3$, df = 15, $p < 0.0001$). The mean and standard deviation was -0.027 ± 0.24 for the Flint distribution and 0.004 ± 0.23 for the simulated distribution (95% C.I. = -0.010 to 0.019).
B. Distribution for the eight Chitty adults (28 pairs) and 1000 random pairs generated with the Chitty allele frequencies. There appears to be more unrelated individuals on the Chitty grid than expected by chance, however the Chitty distribution was not statistically different from the random distribution due to the low sample size (Pearson $\chi^2 = 19.33$, df = 12, $p = 0.081$). The mean and standard deviation was -0.080 ± 0.27 for the Chitty distribution and -0.001 ± 0.23 for the simulated distribution (95% C.I. = -0.015 to 0.013).

Table 3.2 Summary of sample sizes (*n*) and mean pairwise relatedness (*r*) with standard deviation (in parentheses) for the Flint and Chitty grids. The percentage of pairwise relationships classified as full-sib or half-sib by program KINSHIP ($p < 0.05$) are also shown. Values are reported over all adults and juveniles as well as separately for adult males and females. Results considering only the radiocollared hares are also given since it is possible that some of the non-collared hares were not residents on the grids. Standard deviations were calculated over all pairwise comparisons, where $N = [n \times (n-1)]/2$.

Group		*n* total	*r*	% full/half-sib relationships	*n* radiocollared	*r*	% full/half-sib relationships
Flint	All adults	40	-0.027 (0.24)	4.6	21	0.025 (0.24)	4.8
	Males	16	-0.032 (0.26)	3.3	7	-0.032 (0.25)	9.5
		24	-0.034 (0.23)	5.1	14	0.037 (0.23)	6.6
	Females						
	Juveniles	16	0.019 (0.25)	8.3	-	-	-
Chitty	All adults	8	-0.080 (0.27)	0	6	-0.073 (0.27)	0
	Males	5	-0.094 (0.28)	0	4	0.050 (0.25)	0
		3	0.091 (0.27)	0	2	-0.19	0
	Females						
	Juveniles	4	0.074 (0.15)	0	-	-	-



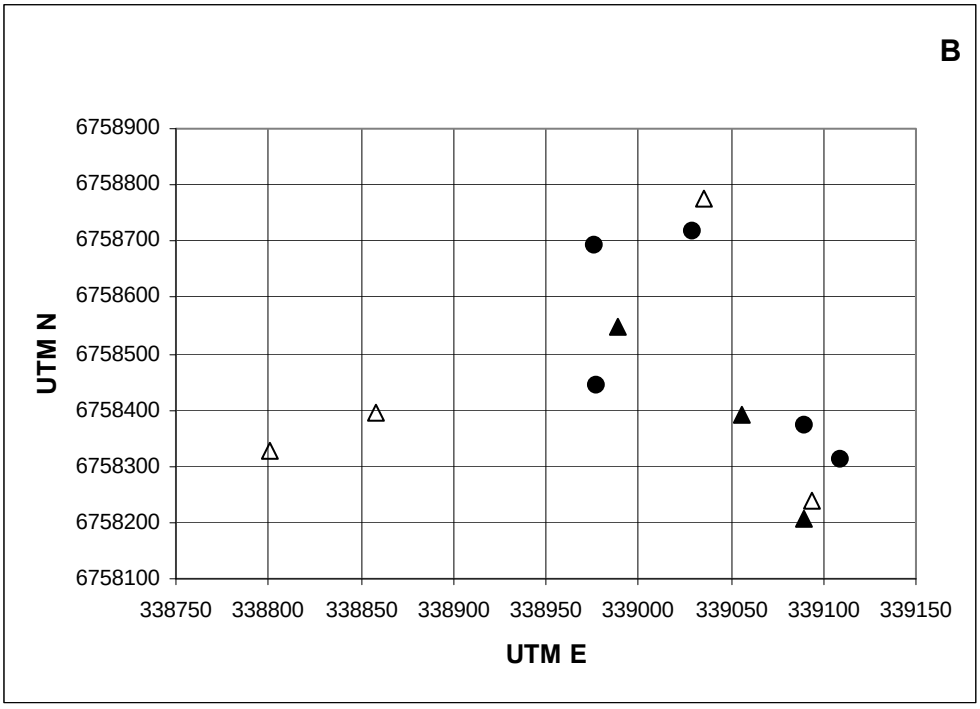


Figure 3.2 Activity centres for all hares trapped on the Flint (**A**) and Chitty (**B**) grids: adult males (●), juvenile males (○), adult females (▲) and juvenile females (△). UTM coordinates are in metres.

Table 3.3 Summary of spacing behaviour among hares on the Flint and Chitty grids. Means (and standard deviation) are given for the distance between activity centres, home range size, home range overlap and dynamic interaction (*J*). The distance between hares was calculated over all trapped individuals whereas the other measures included only radiocollared hares (sample sizes are given in parentheses as total/radiocollared) Standard deviations were calculated over all pairwise comparisons, equal to [*n* x (*n*-1)]/2, except for the dynamic interactions where the number of comparisons for all adults, males and females on the Flint and Chitty grids were 168, 17, 74 and 15, 6, 1 respectively.

Group		Distance (m)	Home range size (ha)	% overlap	*J*
Flint	All adults (40/21)	185.2 (97.2) *	3.4 (3.1)	24.2 (25.9)	0.041 (0.11) **
	Males (16/7)	209.3 (102.0) *	3.4 (1.5)	27.0 (24.1)	0.013 (0.09)
	Females (21/14)	173.8 (91.1) *	3.5 (3.8)	20.7 (25.1)	0.058 (0.12) **
	Juveniles (16)	151.7 (70.4) *	-	-	-
Chitty	All adults (12/8)	231.5 (131.9)	5.7 (3.3)	25.3 (23.4)	0.031 (0.12)
	Males (5/4)	246.9 (131.7)	6.3 (4.1)	22.8 (27.9)	-0.022 (0.05)
	Females (3/2)	236.8 (102.0)	4.6 (0.8)	21.0 (3.3)	-0.070
	Juveniles (4)	356.4 (167.1)	-	-	-

* On the Flint grid, adults were significantly further apart than juveniles and males further than females ($p < 0.001$)
** Mean *J* for Flint adults and adult females were significantly greater than zero ($p < 0.001$)

(randomization test, $p < 0.001$) and adults were significantly farther apart than juveniles ($p < 0.001$). There was no significant association between relatedness and the distance between activity centres for all adult hares (Mantel $Z = 299029.4$, $p = 0.16$, Figure 3.3). Similarly, there were no significant correlations for adult males ($Z = 52307.9$, $p = 0.26$), adult females ($Z = 99782.1$, $p = 0.29$) or juveniles ($Z = 35864.6$, $p = 0.33$).

Hares showed considerable variation in home range size and overlap (Figure 3.4, Table 3.3). The average home range size was 3.43 ha (range = 1.19-15.83) and did not differ significantly between males and females (Wilcoxon two-sample test, $z = 0.93$, $p = 0.35$). Most hares overlapped to some extent (82.6% of 420 pairwise comparisons) and the mean degree to which one individual's home range was overlapped by each of the others was 24.2% (range = 0.09-99.8%). The overlap among females was slightly less than among males (Table 3.3, randomization test, $p = 0.071$), and was also less than between males and females (mean = 27.6%, $p = 0.010$).

None of the slopes (b) of the individual regressions of relatedness on overlap were significantly different from zero ($p > 0.15$, mean $b = -0.00076$, Figure 3.5). Similarly, the regression of the mean ranked relatedness on the corresponding percent overlap over all individuals was non-significant ($r^2 = 0.052$, $p = 0.33$, Figure 3.6). When male radiocollared hares were considered separately, there was a trend towards decreasing relatedness with increasing home range overlap using the mean ranked values ($r^2 = 0.49$, $p = 0.12$, Figure 3.6). Similarly, six of the seven males had negative slopes for the individual regressions between males (Figure 3.5). None of these regressions were statistically significant after Bonferroni correction ($\alpha = 0.05$), however the mean of the individual slopes (-0.0041) was significantly less than zero (Wilcoxon signed-rank = -11.5, $p = 0.031$). Among the radiocollared females none of the individual regressions were significant nor was the mean slope (-0.0009) over all regressions significantly different from zero (Wilcoxon signed-rank = -21.5, $p = 0.19$, Figure 3.5). Regressions of the mean ranked relatedness and overlap values were not significant among the females ($r^2 = 0.019$, $p = 0.65$, Figure 3.6) or between males and females ($r^2 = 0.0057$, $p = 0.74$). There were also no significant differences between males and females in the slopes of either the individual regression lines (Wilcoxon $z = 1.34$, $p = 0.18$) or the regressions of mean ranked relatedness on overlap ($p > 0.05$).

The mean level of interaction between all pairs of overlapping hares was small but significantly greater than zero (mean Jacob's Index = 0.041, randomization test, p = 0.0002). Females appeared to interact more than males (Table 3.3) although the difference was not significant (p = 0.066). The mean level of interaction between the sexes (0.031 ± 0.11 SD, n = 77 pairs) was not significantly different than within the sexes (p > 0.07). As with the other measures of spacing, there was no correlation between the degree of interaction among hares and their relatedness (r^2 = 0.008, p = 0.26, Figure 3.7).

Chitty grid

(a) Relatedness

Results for the Chitty grid were similar to those for the Flint grid. Pairwise relatedness values ranged from -0.59 to 0.50 (Figure 3.1, Table 3.2) and the mean pairwise *r* over all individuals was significantly less than zero (-0.088 ± 0.26 SD, randomization test, p = 0.0034). The adult mean was not significantly less than zero (p = 0.065) however it fell below the lower 95% confidence limit of the random simulation (-0.015, Figure 3.1). None of the 66 pairwise comparisons among Chitty individuals were classified as statistically significant (p < 0.05) full-sib or half-sib relationships in program KINSHIP. There were trends toward lower mean relatedness between adult males than adult females (p = 0.17) and among all adults compared to juveniles (p = 0.094), however the differences were not significant due to the small sample sizes. Exclusion of the two non-collared adults whose resident status was unclear (they were each trapped only once) did not affect the adult mean, however it did change the male and female means (Table 3.2). Because of the small sample sizes on the Chitty grid these means should be interpreted with caution.

(b) Spacing behaviour

The distance between activity centres of Chitty hares varied from 30.4 m to 568.6 m (Figure 3.2, Table 3.3). The Mantel test indicated that the distance between individuals' activity centres was not correlated with their relatedness (Z = 14060.6, p = 0.43, Figure 3.3).

The average home range size for the six radiocollared hares was 5.73 ha (range = 3.05-12.11, Table 3.3) and the mean degree of overlap was 25.3% (range = 0.3-74.7%). The regression of

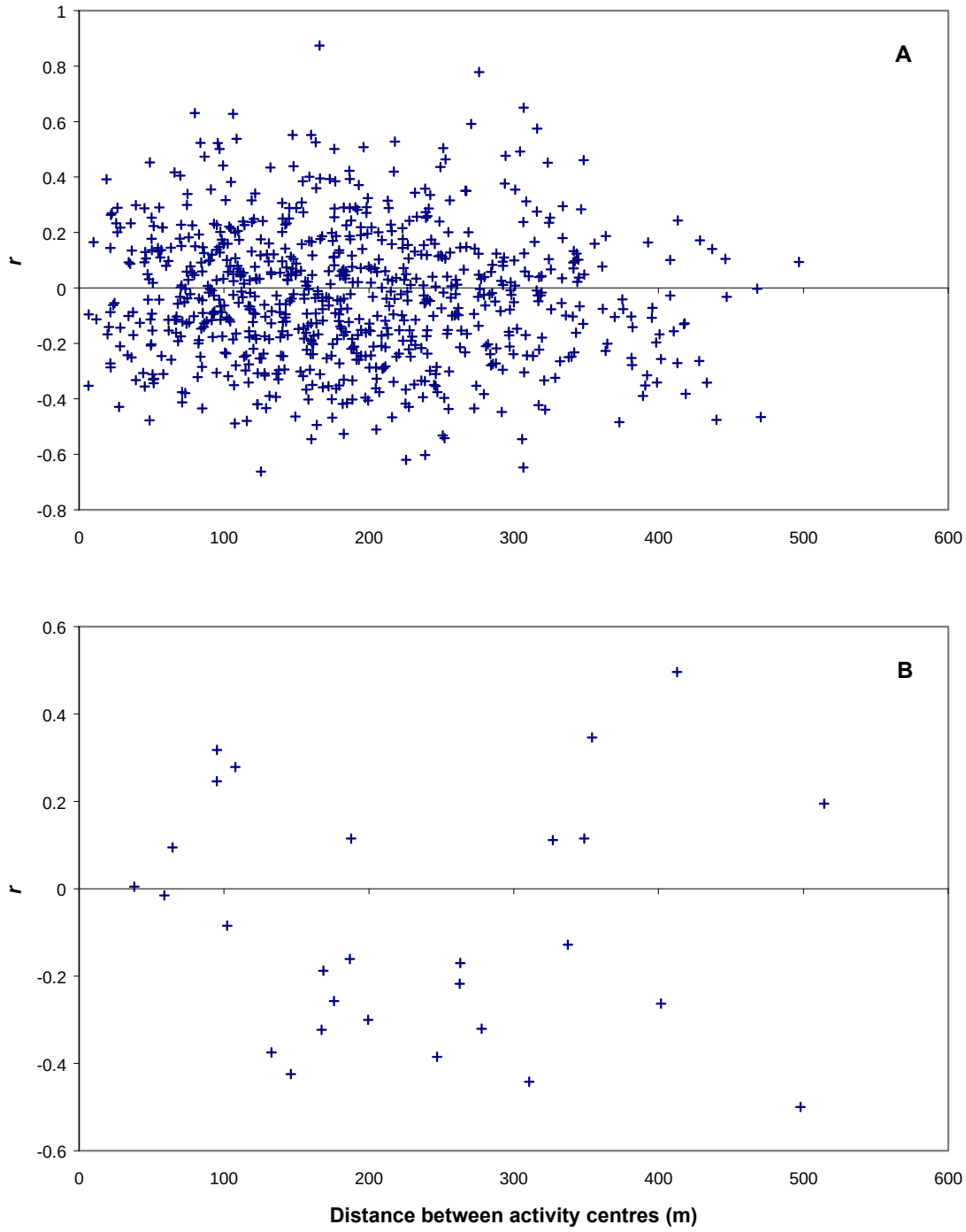


Figure 3.3 Relatedness (*r*) plotted against the distance between activity centres for all pairs of adults trapped on the Flint (**A**, 40 adults) and Chitty (**B**, 8 adults) grids. Mantel tests confirmed that there was no correlation between the two variables for either grid (Flint: Z = 299029.4, r = 0.067, p = 0.16, 780 pairwise comparisons; Chitty: Z = 14060.6, r = 0.031, p = 0.43, 28 pairwise comparisons).

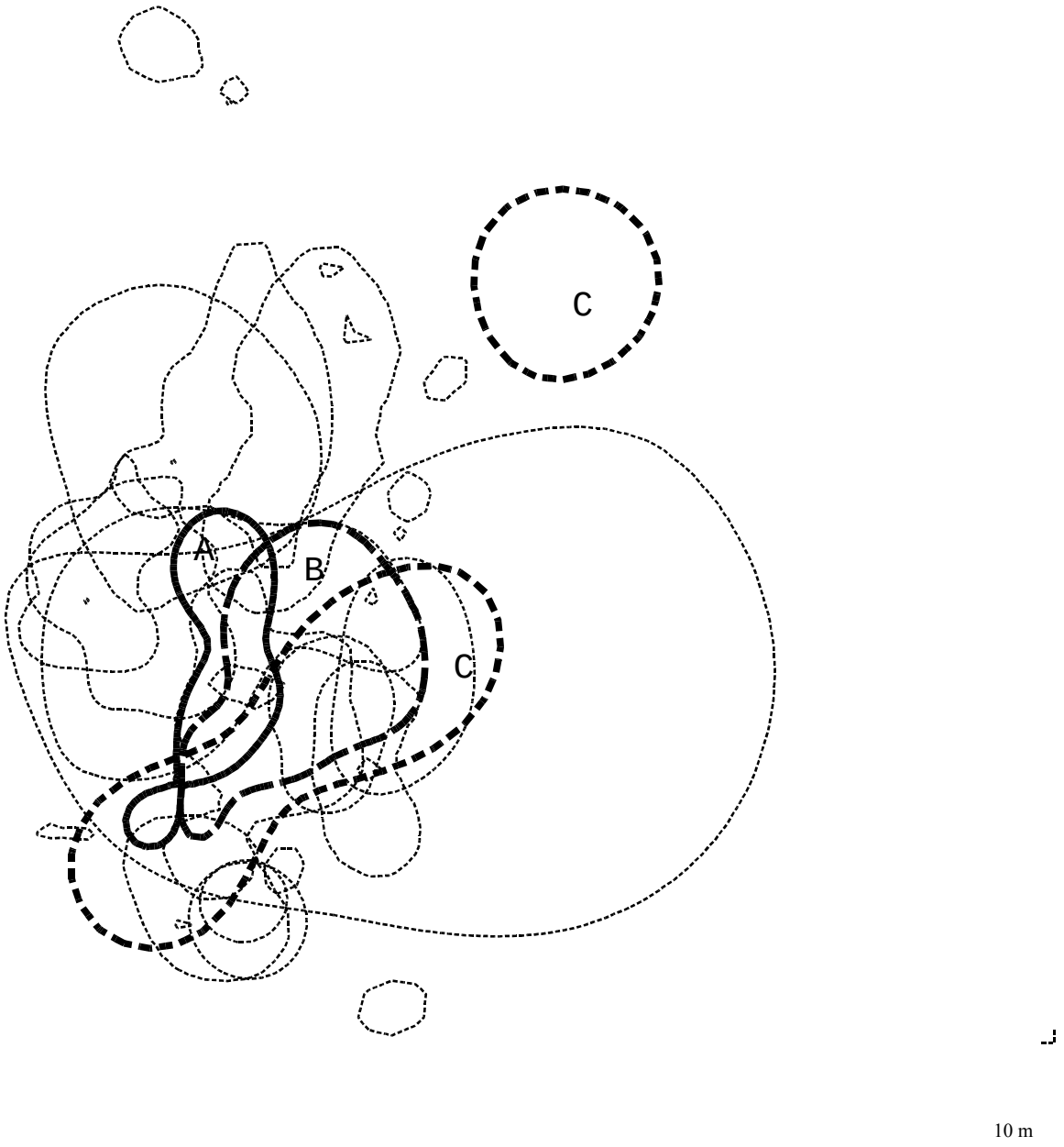


Figure 3.4 The 95% fixed kernel home ranges of the radiocollared females (n = 14) on the Flint grid. The extensive overlap was typical of both sexes and both grids. Three individual ranges are highlighted as an illustration of spacing between putative related and unrelated hares. Females **A** (—) and **B** (---) had a high relatedness value (r = 0.42) but did not overlap more with each other than with the less related female **C** (··· , r_{AC} = -0.30, r_{BC} = -0.02). Although single pairwise estimates of r must be treated with caution (see text), a comparable lack of predictable spacing among kin was found for the majority of individuals of both sexes and on both grids.

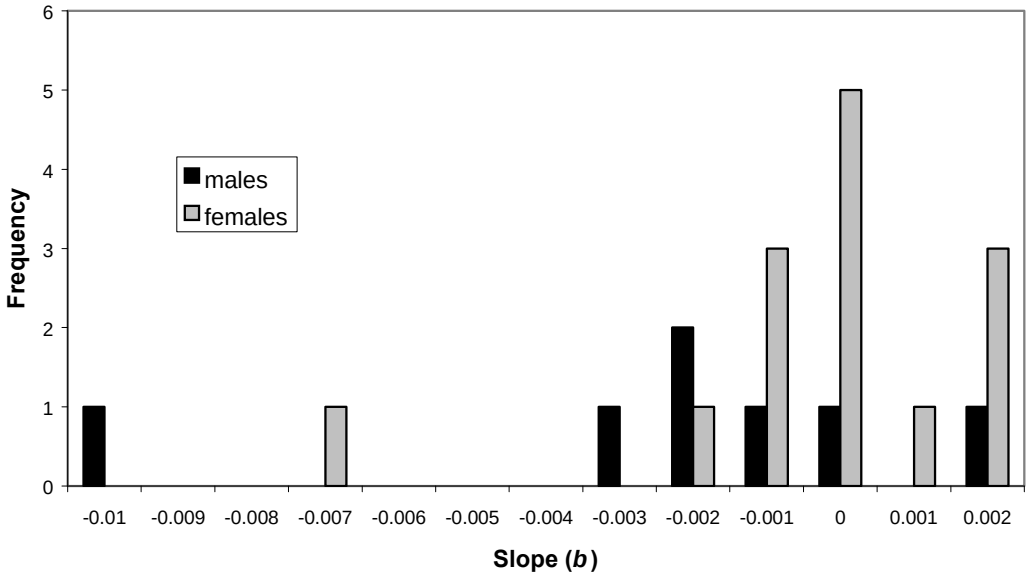


Figure 3.5 Frequency distribution of the slopes for individual regressions of pairwise relatedness on percent home range overlap for the Flint radiocollared hares. The mean slopes were: all individuals = -0.00076 ± 0.00047 SE (n = 21); males = -0.0041 ± 0.0027 SE (n = 7); females = -0.0009 ± 0.0006 SE (n = 14). Only the male mean was significantly less than zero ($p = 0.031$) and none of the individual slopes were significant after Bonferroni correction ($\alpha = 0.05$). There was not a significant difference between the individual male and female slopes ($p = 0.18$).

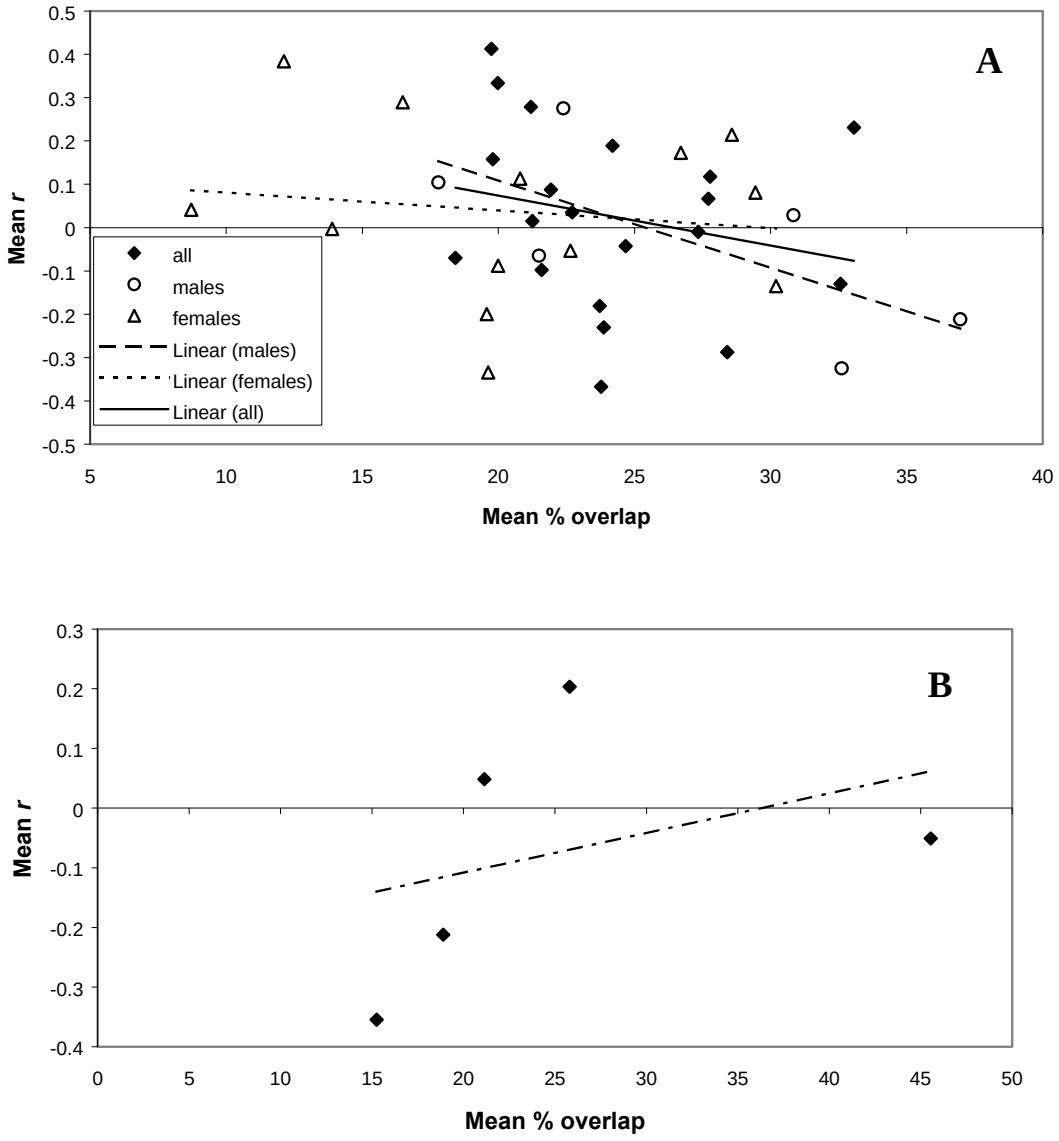


Figure 3.6 Relationship between the mean of the ranked pairwise relatedness values (*r*) and the corresponding mean percent home range overlap for the Flint (**A**) and Chitty (**B**) radiocollared hares. On Flint, relatedness showed a negative but non-significant trend with increasing overlap (overall $r^2 = 0.052$, $p = 0.33$; males $r^2 = 0.49$, $p = 0.12$; females $r^2 = 0.019$, $p = 0.65$). On Chitty, the trend of increasing relatedness with increasing overlap was not statistically significant ($r^2 = 0.13$, $p = 0.54$).

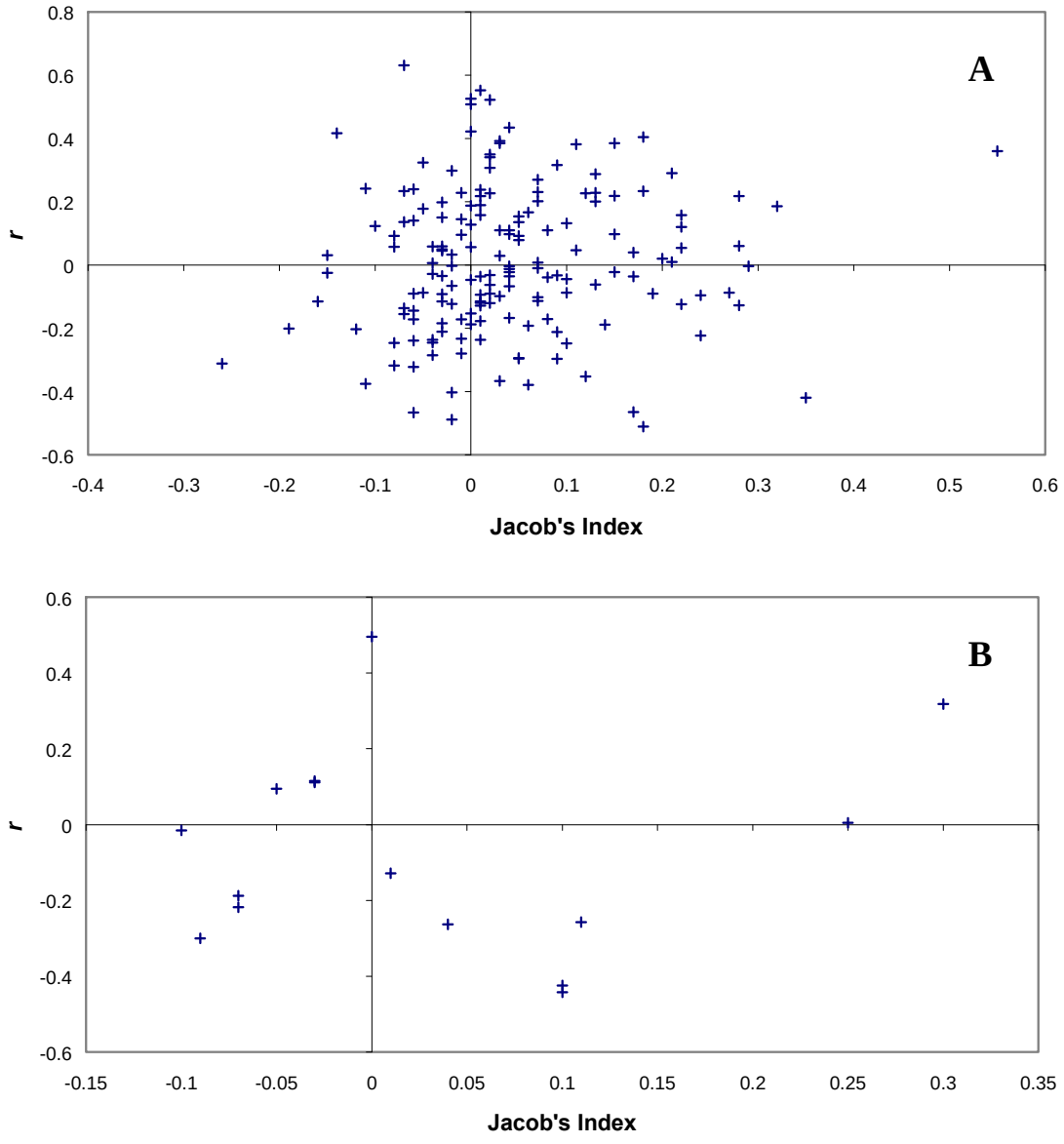


Figure 3.7 Relationship between pairwise relatedness (*r*) and the measure of dynamic interaction (represented as Jacob's Index of cohesion) for hares with overlapping home ranges on Flint, **A** (168 pairwise comparisons among 21 individuals), and Chitty, **B** (15 pairwise comparisons among 6 individuals). Hares that were more closely related were not more likely to associate together (Flint $r^2 = 0.008$, $p > 0.25$; Chitty $r^2 = 0.012$, $p > 0.7$).

Table 3.4 Mean, standard error and range of pairwise *r*-values for individuals of known relatedness from the paternity study in Chapter 2. The number of pairwise comparisons (*n*) and the theoretically expected values for each relationship are also given (Queller & Goodnight 1989, Blouin 1996). Note that there is a considerable amount of variation associated with the individual *r*-values but that the observed means closely match the expected values.

Relationship	*n*	Expected *r*	Mean *r* (SE)	Range
Full-sib	39	0.5	0.52 (0.027)	0.16 - 0.89
Half-sib	35	0.25	0.22 (0.038)	-0.26 - 0.60
Mother-Offspring	65	0.5	0.50 (0.019)	0.18 - 0.79
Unrelated	65	0	-0.02 (0.028)	-0.55 - 0.49

mean ranked relatedness on the corresponding percent home range overlap was not significant ($r^2 = 0.13$, $p = 0.54$, Figure 3.6). None of the individual regressions of relatedness on overlap were significant after Bonferroni correction ($\alpha = 0.05$), and overall the slopes were not significantly different from zero (mean $b = -0.0019 \pm 0.0047$ SE, Wilcoxon signed-rank = 3.50, $p = 0.56$). There were no apparent differences between males and females.

Coefficients of interaction (Jacob's Index) between overlapping hares ranged from -0.1 to 0.3 with a mean value of 0.031 (Table 3.3). There was no relationship between this measure of interaction and the degree of relatedness ($r^2 = 0.012$, $p = 0.70$, Figure 3.7).

Discussion

My results suggest that interactions between kin do not play an important role in snowshoe hare behaviour. There were few closely related hares on either grid and the overall degree of pairwise relatedness among individuals was equal to or less than that expected by chance. The location of an individual hare on a grid did not seem to be affected by the relatedness of neighbouring hares. Furthermore, these results were generally not different between males and females or adults and juveniles. There was some indication that resident adult females and juveniles may be more related than adult males; however the observed differences were slight.

A few factors should be noted with regard to the interpretation of the degree of relatedness among hares in this study. Firstly, it is possible that the spatial scale at which I sampled (7.3 ha grids) was too small to detect clusters of related individuals. Hares do show some genetic structuring at larger spatial scales (see Chapter 4), however the degree of structuring is low and appears unlikely to be caused by local kin clusters. Secondly, I made the assumption that the individual and mean *r*-values are good estimates of the true relatedness between hares. Queller and Goodnight (1989) cautioned that individual pairwise relatedness estimates may not always reliably represent true pedigree relationships and are best used in aggregate. On the other hand, the considerable variation in relatedness values that I observed on each grid calls into question the usefulness of the group means. In order to test my assumption, I examined *r*-values between individuals of known relatedness from the paternity study in Chapter 2. This analysis illustrated the potential variation in *r*-values but confirmed that the means accurately represented the true relationship among group members (Table 3.4). The conclusions I make in the current study are based on multiple pairwise comparisons of relatedness and should therefore be robust to

variation in *r*-values. Data from the paternity analysis also confirmed that program KINSHIP was generally reliable at identifying full- or half-sibs, although occasionally both Type I and Type II errors were made. It is thus possible that the number of siblings is slightly over- or underestimated in this study, however the general conclusions based on these estimates are valid.

The finding of low group relatedness in this study is consistent with previous observations of high juvenile dispersal (Gillis & Krebs 1999) coupled with low survival in snowshoe hares of both sexes (O'Donoghue 1994, Krebs *et al*. 1995, Gillis 1998). It is also consistent with the patterns of mating structure reported in Chapter 2: multiple paternity and widespread reproductive success among males likely decrease the chances that neighbouring juveniles will be closely related. Previous observations of limited parental care and short-term associations among littermates (Graf & Sinclair 1987, O'Donoghue & Bergman 1992) corroborate the suggestion that social relationships among kin are not important in hares.

Although my results suggest that some individuals on the grids were related, the degree of relatedness did not explain the considerable variation in spacing behaviour between hares. The fact that most of the radiocollared hares on both grids had overlapping home ranges confirms previous results (e.g., Boutin 1979) and is consistent with the idea that there is considerable potential for social interactions among hares (Graf 1985). Nevertheless, the average amount of overlap was low, as was the mean level of dynamic interaction. Measures of the latter (as Jacob's Index) indicated that most interactions were weak, but that in general hares of both sexes were more likely to associate (positive interaction) than to avoid each other (negative interaction). This pattern contrasts with Boutin's (1980) observation that during an increase phase females used their home ranges in a manner to reduce interaction. Further investigation is needed to test for other possible mechanisms underlying the observed variation in snowshoe hare social interactions and spacing behaviour.

The lack of strong differences in kin structure between males and females confirms that snowshoe hares are unusual among mammals in not exhibiting sex-biased dispersal (typically male) or philopatry (typically female, Greenwood 1980). I did find weak evidence that male hares may avoid other related males. This is not consistent with inbreeding avoidance since there was not a similar negative correlation of relatedness and spacing between individuals of the opposite sex. It is, however, consistent with the idea of kin-competition. Males on a grid would be competing for access to neighbouring females and less competition among related males

should be favoured. The effect may be weak since the overall relatedness among males was low. Furthermore, the results from Chapter 2 suggest that there is a relatively low variance in male reproductive success, thus limiting the severity of competition.

The failure to detect kin structuring for either sex in snowshoe hares contrasts with recent findings in other small mammal species. Clusters of related females have been reported in voles (Ishibashi *et al*. 1997), rabbits (Surridge *et al*. 1999) and ground squirrels (van Staaden *et al*. 1996) and male kin clusters have been detected in shrews (Balloux *et al*. 1998). It is possible that in hares, selective benefits of kin-association are outweighed by the costs of competition among kin, or overwhelmed by selective pressures on individuals to avoid predation. Low survival and the lack of territoriality in hares may weaken the potential benefits of strategies that increase kin-association or decrease the risk of inbreeding (such as female philopatry and male-biased dispersal). Regardless of the underlying reasons, the lack of kin clustering is consistent with the belief that spacing behaviour is relatively unimportant in snowshoe hare population regulation (Krebs 1996).

It is important to consider how this result might be affected by changes in density, predation and food associated with the population cycle. For example, in their hypothesis to explain the dynamics of vole cycles, Charnov and Finerty (1980) predict low relatedness during the high-density peak phase of the cycle and high relatedness during the low phase. The low relatedness observed among snowshoe hares during this peak phase is consistent with this prediction, however the lack of any significant correlation between relatedness and spacing suggests it is unlikely that kin-interactions play a role during any phase of the hare cycle. Furthermore, the density on the Flint grid was approximately five times that on the Chitty grid and yet there were no significant differences in the kin-spacing results for the two grids. There was some indication that the mean relatedness and proportion of putative siblings was lower on the Chitty grid, implying that relatedness might increase with density. This is opposite to the Charnov and Finerty prediction but consistent with those made by Lambin and Krebs (1991) for cycles in microtines. The average home range size and distance between hares were significantly larger on the Chitty grid, suggesting that density may have an effect on hare spacing behaviour. Temporal and manipulative investigation into the effects of changes in density on relatedness and spacing in hares would be useful to expand on these results.

Sinclair (1986) and Ferron (1993) suggested that spacing behaviour may be important in hare regulation only when food is limiting. It is possible that food was not limiting on either grid during this study and that social interactions were therefore not important. However, if food shortage is ever an important factor in snowshoe hare cycles it is most likely to be during the peak phase (Keith 1990, Hodges 2000), thus any patterns in spacing behaviour should have been most pronounced during this study. On the other hand, predation risk should be high during this phase (Hik 1995) and associated predator-sensitive behaviours may be much more important than social spacing behaviour. The latter may be important only when predation risk is reduced, such as during the low phase (Hodges *et al*. 1999). I do not have any quantitative data on food availability or predation risk for the sampling grids during this study, however my qualitative observations suggest that food and cover were more plentiful on Flint than on Chitty, which is consistent with the earlier decline in hare numbers on Chitty. If these observations correspond to actual differences in food and predation experienced by the hares, then my results suggest that these factors did not affect patterns of kin-interaction. It would, of course, be desirable to repeat this study during different phases of the hare cycle and under different manipulations of food and predation in order to confirm these speculations. Similarly, a direct experimental test of behaviour between related and unrelated hares would be important to confirm the suggestion that degree of relatedness does not affect hare interactions.

Conclusion

Relatedness is not a good predictor of spacing behaviour for either male or female snowshoe hares during the peak phase of the population cycle. In general there is low relatedness among hares in a group and related individuals associate more or less randomly. This is in contrast to other small mammals in which clusters of related individuals have been detected, such as voles and rabbits, and lends further support to the belief that spacing behaviour is relatively unimportant in snowshoe hare regulation.

Literature cited

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

Blouin M, Parsons M, Lacaille V, Lotz S (1996) Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology,* **5,** 393-401.

Boonstra R, Hik D, Singleton GR, Tinnikov A (1998a) The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs,* **68,** 371-394.

Boonstra R, Krebs CJ, Stenseth NC (1998b) Population cycles in small mammals: the problem of explaining the low phase. *Ecology,* **79,** 1479-1488.

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

Boutin S (1980) Effect of spring removal experiments on the spacing behaviour of female snowshoe hares. *Canadian Journal of Zoology*, **58,** 2167-2174.

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

Charnov E, Finerty J (1980) Vole population cycles: A case for kin-selection. *Oecologia,* **45,** 1-2.

Ferriere R, Belthoff J, Olivieri I, Krackow S (2000) Evolving dispersal: where to go next? *Trends in Ecology and Evolution,* **15,** 5-7.

Ferron J (1993) How do population density and food supply influence social behaviour in the snowshoe hare (*Lepus americanus*)? *Canadian Journal of Zoology,* **71,** 1084-1089.

Gillis EA (1998) Survival of juvenile snowshoe hares during a cyclic population increase. *Canadian Journal of Zoology,* **76,** 1949-1956.

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

Goodnight K, Queller D (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology,* **8,** 1231-1234.

Graf R (1985) Social organization of snowshoe hares. *Canadian Journal of Zoology,* **63,** 468-474.

Graf R, Sinclair ARE (1987) Parental care and adult aggression toward juvenile snowshoe hares. *Arctic,* **40,** 175-178.

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

Hik DS (1995) Does risk of predation influence population dynamics? Evidence from the cyclic decline of snowshoe hares. *Wildlife Research,* **22,** 115-129.

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 (2000) 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, Krebs CJ, Sinclair ARE (1999) Snowshoe hare demography during a cyclic population low. *Journal of Animal Ecology,* **68,** 581-594.

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

Ishibashi Y, Saitoh T, Abe S, Yoshida C (1997) Sex-related spatial kin structure in a spring population of grey-sided voles *Clethrionomys rufocanus* as revealed by mitochondrial and microsatellite DNA analyses. *Molecular Ecology,* **6,** 63-71.

Jacobs J (1974) Quantitative measurements of food selection. *Oecologia,* **14,** 413-417.

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

Kenward RE, Hodder KH (1996) *Ranges V. An analysis system for biological location data*. Institute of Terrestrial Ecology, Wareham UK.

Kenward R, Marcstrom V, Karlbom M (1993) Post-nestling behaviour in goshawks, *Accipiter gentilis*.II. Sex differences in sociality and nest switching. *Animal Behaviour,* **46,** 371-378.

Knight M, van Oppen M, Smith H, *et al.* (1999) Evidence for male-biased dispersal in Lake Malawi cichlids from microsatellites. *Molecular Ecology,* **8,** 1521-1527.

Krebs CJ (1996) Population cycles revisited. *Journal of Mammalogy,* **77,** 8-24.

Krebs CJ, Boutin S, Boonstra R, *et al.* (1995) Impact of food and predation on the snowshoe hare cycle. *Science,* **269,** 1112-1115.

Lambin X, Krebs CJ (1991) Can changes in female relatedness influence microtine population dynamics? *Oikos,* **61,** 126-132.

Lambin X, Krebs CJ (1993) Influence of female relatedness on the demography of Townsend's vole populations in spring. *Journal of Animal Ecology,* **62,** 536-550.

Macdonald D, Ball F, Hough N (1980) The evaluation of home range size and configuration using radio tracking data. In: *A handbook on biotelemetry and radio tracking,* (eds. Amlaner C, Macdonald D), pp. 405-424. Pergamon Press, Oxford.

Manly B (1997a) *Randomization, bootstrap and monte carlo methods in biology.* Chapman and Hall, New York.

Manly B (1997b) *RT: A program for randomization testing*.

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

O'Donoghue M (1994) Early survival of juvenile snowshoe hares. *Ecology,* **75,** 1582-1592.

O'Donoghue M, Bergman CM (1992) Early movements and dispersal of juvenile snowshoe hares. *Canadian Journal of Zoology,* **70,** 1787-1791.

Queller D, Goodnight K (1989) Estimating relatedness using genetic markers. *Evolution,* **43,** 258-275.

Quenette P, Ferron J, Sirois L (1997) Group foraging in snowshoe hares (*Lepus americanus*): Aggregation or social group? *Behavioural Processes,* **41,** 29-37.

Raymond M, Rousset F (1995) 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.

Sherman P (1981) Kinship, demography, and Belding's ground squirrel nepotism. *Behavioral Ecology and Sociobiology,* **8,** 251-259.

Sinclair ARE (1986) Testing multi-factor causes of population limitation: an illustration using snowshoe hares. *Oikos,* **47,** 360-364.

Sokal RR, Rohlf FJ (1995) *Biometry: the principles and practice of statistics in biological research.* W.H. Freeman and Company, New York.

Surridge A, Ibrahim K, Bell D*, et al.* (1999) Fine-scale genetic structuring in a natural population of European wild rabbits (*Oryctolagus cuniculus*). *Molecular Ecology,* **8,** 299-307.

van Staaden MJ, Michener GR, Chesser RK (1996) Spatial analysis of microgeographic genetic structure in Richardson's ground squirrels. *Canadian Journal of Zoology,* **74,** 1187-1195.

Worton B (1989) Kernel methods for estimating the utilisation distribution in home range studies. *Ecology,* **70,** 164-168.

CHAPTER 4: REGIONAL GENETIC STRUCTURE, GENE FLOW AND DISPERSAL

Introduction

The spatial structure of natural populations has important consequences for both contemporary and long-term ecological processes. The movement of individuals within and between populations influences social and demographic processes and determines the extent to which local populations function independently (Stenseth & Lidicker 1992, Hanski & Gilpin 1997). The movement of genes between populations counteracts the divergent effects of genetic drift, local adaptation and mutation, thereby influencing the evolutionary potential of a species (Wright 1978, Slatkin 1987). The dynamics of dispersal and gene flow are integral to spatial population structure, yet they are difficult to study in natural populations (Koenig *et al.* 1996, Bossart & Prowell 1998). Recent technical, theoretical and statistical methods build on traditional approaches and are rapidly improving our understanding of large-scale population processes (Hanski & Gilpin 1997, Neigel 1997, Waser & Strobeck 1998, Koenig 1999, Luikart & England 1999).

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 structure, but relatively few have investigated genetic structure in continuously distributed species with simple social systems. Snowshoe hares 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 geographic 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

sub-boreal forests of the continental U.S. (Hodges 2000b), but it is unclear at what scale they 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 this study is to examine snowshoe hare population structure and movement patterns at a regional scale through the use of neutral genetic markers. The main questions I am addressing are: (i) At what scale are snowshoe hares genetically subdivided? (ii) Does the genetic structure reflect predictions of gene flow based on landscape features and estimates of hare dispersal? Previous studies have shown that: (a) snowshoe hares disperse frequently and over the spatial scale of a few kilometers (Windberg & Keith 1976, Boutin 1984, Gillis & Krebs 1999, Hodges 1999), (b) dispersers survive as well as non-dispersers (Boutin 1984, Gillis & Krebs 2000, but see Windberg & Keith 1976 and Keith *et al.* 1993), and (c) hares do not live in family units or other obvious social clusters (Boutin 1979, see also Chapter 3). Given these findings and the fact that hares are distributed more or less continuously in forested habitat, I predict 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. I further predict that areas of unsuitable habitat, such as lakes and alpine habitat, will represent barriers to dispersal and gene flow, thereby causing genetic differentiation and departures from the pattern of isolation by distance. I also test an alternative hypothesis that local differentiation, and thus genetic structure, is increased through bottleneck effects related to the snowshoe hare density cycle.

Methods

Sample collection

Snowshoe hares were live-trapped at 12 sites between April 5th and August 27th 1999 (Figure 4.1). These sites were separated by a range of distances (3-140 km) and potential landscape barriers to dispersal (e.g., alpine habitat, lakes). At each site, between 30 and 100 traps were set (see Chapter 2 for general information). I trapped over 2-4 nights at each site and repeated

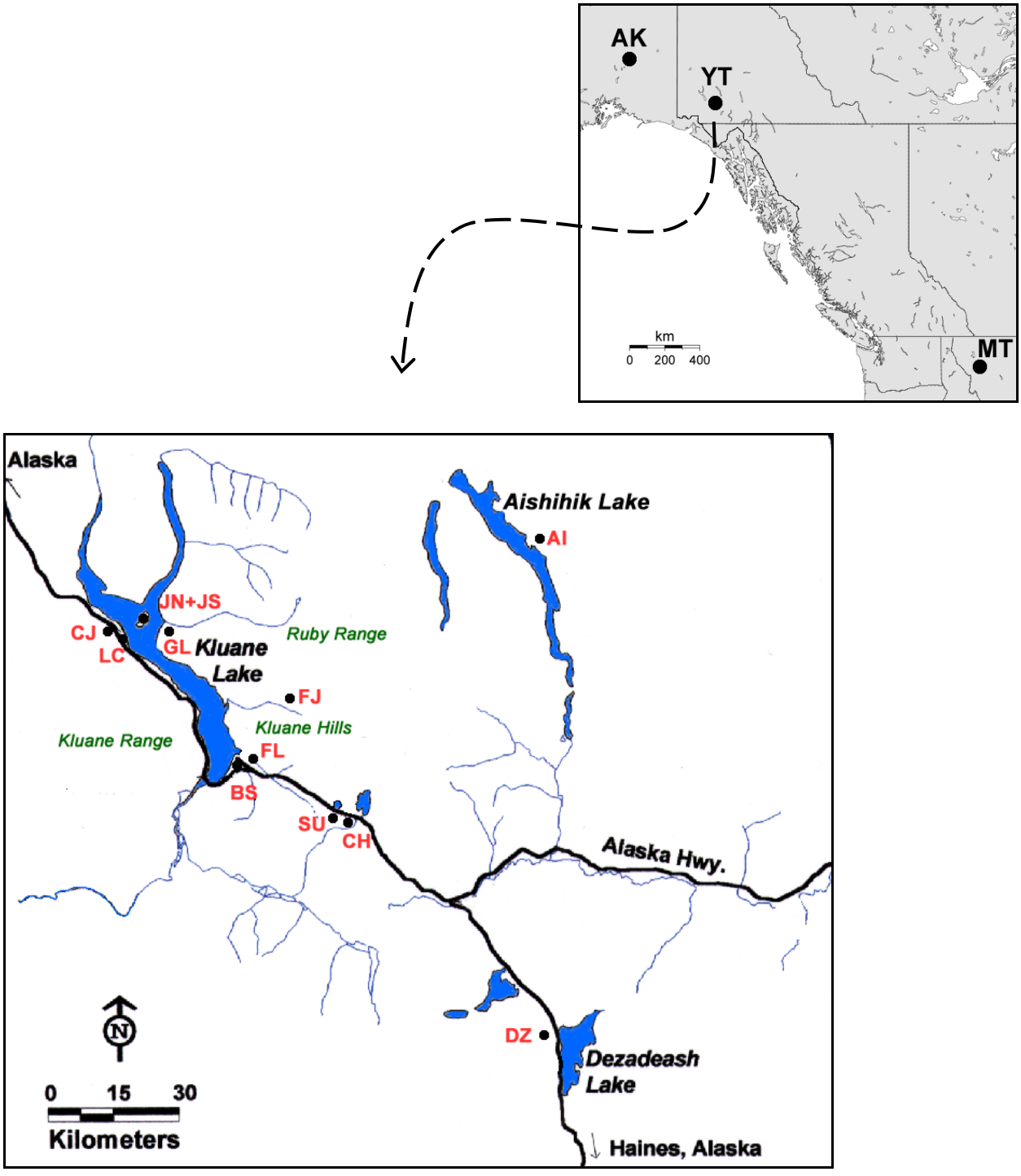


Figure 4.1 Sampling locations. Upper box shows location of Yukon (YT), Alaska (AK) and Montana (MT) sampling areas. Lower box is detailed map of the 12 Yukon sampling sites (see Table 1 for names). Also indicated are the general locations for the alpine habitats predicted to be barriers to gene flow (Kluane Hills and Ruby Range).

trapping sessions where necessary in an attempt to capture a minimum of 30 hares from each location.

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 4.1). In order to gauge the degree of genetic differentiation among the Yukon sites, additional hare samples were obtained in August 2000 from two distant populations (Figure 4.1): 27 samples from the Tanana River floodplain in interior Alaska (~ 64^{o} N, 148^{o} W) were provided by Eric Rexstad and Bjorn Flora (University of Alaska Fairbanks), and 19 samples from Seeley Lake, Montana (47^{o} N, 113^{o} W) were provided by Scott Mills and Paul Griffin (University of Montana). Locations of all sites were estimated from topographic maps using the Universal Transverse Mercator (UTM) system.

Microsatellite analysis

DNA was extracted from the tissue samples and eight microsatellite loci (Sol03, Sol33, Sat2, Sat3, Sat5, Sat12, Sat13, Sat16) were amplified and scored for each individual as described in Chapter 2.

Data analysis

(i) Genetic variation

Microsatellite loci were tested for deviations from Hardy Weinberg 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 less 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.

(ii) 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). I quantified the degree of differentiation between and across all sites using Weir and Cockerham's (1984) estimator (θ) of Wright's F_{ST}, as calculated by program FSTAT version 2.8 (Goudet 1999). F_{ST} can theoretically range from 0 (no genetic divergence) to 1 (complete fixation of alternative alleles). Values above ~ 0.15 indicate great genetic differentiation (Wright 1978). 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). Genetic relationships among sites were visualized using an unweighted arithmetic averages clustering method (UPGMA) based on the pairwise θ values.

As an alternative measure of the degree of genetic differences among sites, I 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 (Davies *et al.* 1999, Waser & Strobeck 1998). It assigns an individual to the candidate source population in which its genotype has the highest likelihood of occurring. I used the software program GENECLASS (Cornuet *et al.* 1999) to assign individuals according to a Bayesian method developed by Rannala and 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). In order to avoid biasing likelihoods, I excluded the individual being tested from its sample population when estimating allele frequencies.

(iii) Isolation by distance

I tested for a positive correlation between geographic and genetic distances to determine if the observed genetic structure could be explained by the isolation-by-distance model (Wright 1943, Slatkin 1993). The straight-line distance between all pairs of sites was compared with pairwise F_{ST} (θ) 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), and significance was determined by 9,999 matrix permutations. I also examined the effect of geographic 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 since 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" differ). I therefore tested statistical significance 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.

(iv) Barrier effects

Landscape features, such as mountains and lakes, may affect genetic structure by reducing the amount of gene flow between areas. I identified three major landscape features in the Yukon study area that may act as barriers to gene flow for snowshoe hares (see Figure 4.1): 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. I used a paired t-test to directly compare θ values and also used a partial Mantel test (after Smouse *et al.* 1986, calculated in R-package) 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, I constructed another pairwise matrix that contained 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).

(v) Genetic bottlenecks

Apart from geographic distance and landscape barriers, a third factor that might have an important effect on hare population genetic structure is genetic drift. Snowshoe hare populations are characterized by cyclic fluctuations in density that typically have an amplitude of 10 to 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. I 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).

(vi) Gene flow and dispersal

The spatial distribution of genetic variation provides an indication of the movement of genes (and hence individuals) within and among populations, yet it remains difficult to accurately quantify such movement using genetic data. Wright (1931) proposed estimating the number of effective migrants per generation (Nm) based on the island model relationship $Nm = (1-F_{ST})/4F_{ST}$, and Slatkin (1985a) developed an alternative estimate of Nm using the frequency of rare ("private") alleles in different populations. Both of these estimators are problematic, however, since most natural populations likely violate the model assumptions upon which they are based (Whitlock & McCauley 1999). Snowshoe hare populations are especially likely to violate model assumptions as they are distributed continuously rather than in discrete "island" subpopulations. More recently, Rousset (1997) developed an estimator of Nm for continuous populations based on the isolation by distance model. The slope (b) of the regression of $F_{ST}/(1-F_{ST})$ between pairs of populations against the natural log of the geographic distance separating them can be used to estimate "neighbourhood size" ($4\pi\sigma^2 d$) from the equation $b = 1/(4\pi\sigma^2 d)$,

where d is the population density and σ^2 is the variance in dispersal distance (see Appendix 5). The number of migrants per subpopulation can then be estimated from the relationship $2\sigma^2 d = Nm$ (Rousset 1997). I calculated all three estimates of Nm (using GENEPOP to calculate Slatkin's corrected Nm) as qualitative indices of gene flow among snowshoe hares in my study area.

I also used an "assignment index" to make qualitative estimates of hare dispersal. The assignment index can be a powerful method of quantifying the number of individuals dispersing between different populations (Waser & Strobeck 1998), however it requires a reasonable amount of reproductive isolation among candidate populations in order to effectively assign individuals (Davies *et al.* 1999). Furthermore, when sampling from sites in a continuously distributed population, such as in this study, it is difficult to assign individuals to specific sites with certainty since the allelic distribution in other nearby sites is unknown. In order to avoid false estimates of dispersal between sites, I defined dispersers as any individuals whose genotypes met the following criteria (as determined by the GENECLASS calculations): (i) the greatest likelihood of occurring at a site other than the one from which they were sampled, (ii) a high probability (> 0.90) of belonging to the population into which they were assigned, and (iii) a relatively low probability (< 0.10) of belonging to any of the other sampling populations. I classified individuals as potential immigrants from an unknown source if the probability of their genotype in the population from which they were sampled was less than 0.01.

I also used the assignment index 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).

In order to allow qualitative comparison of these indirect genetic estimates of hare movement with direct estimates from field observations, I analyzed unpublished data collected from radiocollared hares during the Kluane Boreal Forest Ecosystem Project (Krebs *et al.* 2001b). As an index of the extent of movement among hares in the Kluane region, I calculated the straight-

line distance between the location of first capture and the location of death (or radio-collar removal) for 1577 hares that were radio-tracked between 1986 and 1996. I analyzed the distribution of these distances and also tested for differences between male and female distances using the Wilcoxon test.

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 due to 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 4.1, see Appendix 4 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.

Population genetic structure

There was highly significant allelic differentiation across the Yukon study area (Fisher's combined probability, $\chi^2 = 96.5$, df = 14, $p << 0.0001$, Table 4.2). Twenty-three of 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 4.3). The overall level of differentiation, as estimated by θ, was relatively low at 0.015 (Table 4.2), with pairwise estimates ranging from 0 to 0.062 (Table 4.3, 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 4.2). This increase was primarily due to the large divergence of the Montana sample. Pairwise θ was 0.201 between Montana and Yukon and 0.193 between Montana and Alaska, whereas it was only 0.012 between Alaska and Yukon (Table 4.3, Figure 4.2). Measures of differentiation for all sites did not differ

Table 4.1 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.

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

* The Montana sample was monomorphic for the sat3 locus

Table 4.2 Genetic differentiation among the sampling sites. 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).

	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% C.I.	-	0.012 - 0.020	0.058 - 0.165

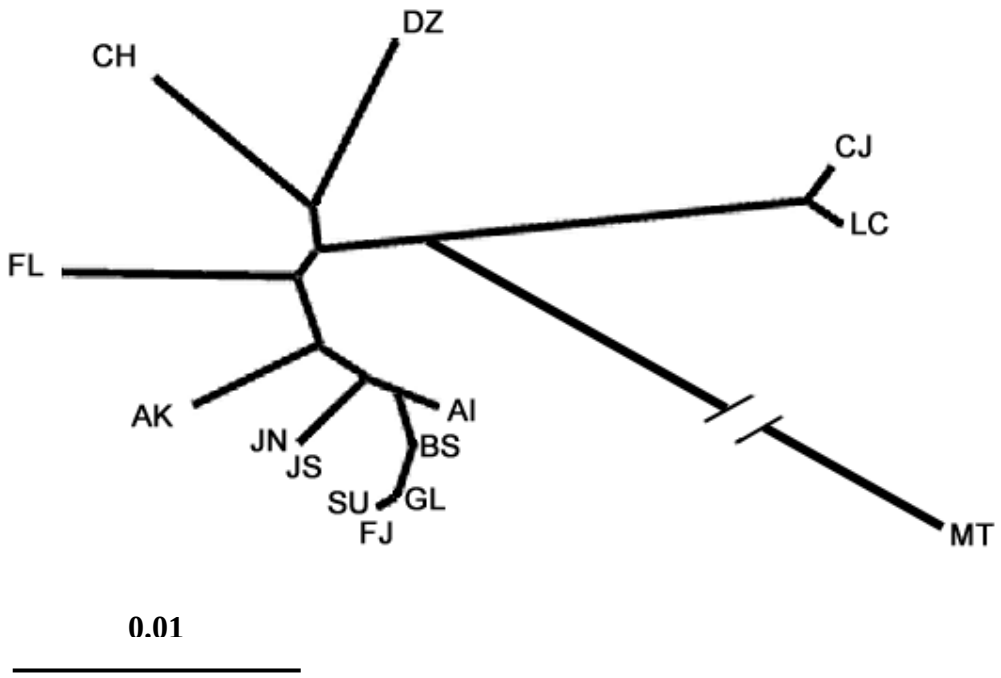


Figure 4.2 UPGMA tree showing the genetic relationships among sampling locations based on pairwise F_{ST} (θ) values. The Montana sample (MT) is highly differentiated from all other sites (a significant amount of the branch length has been omitted), however the Alaska sample (AK) clusters among the Yukon sites. Some nearby sites were genetically very similar, such as Jacquot Island North (JN) and South (JS), whereas others like Chitty (CH) and Sulphur (SU) were not. Copper Joe (CJ) and Lewis Creek (LC) were the only two Yukon sites on the west side of Kluane Lake (see Figure 1).

Table 4.3 Pairwise matrix of genetic distance (θ, lower diagonal) and geographical distance (in km, upper diagonal) between all sampling sites. 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.

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 *	

Table 4.4 Results of the assignment test. 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). Sample sizes (*n*) are indicated in the first column.

	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**

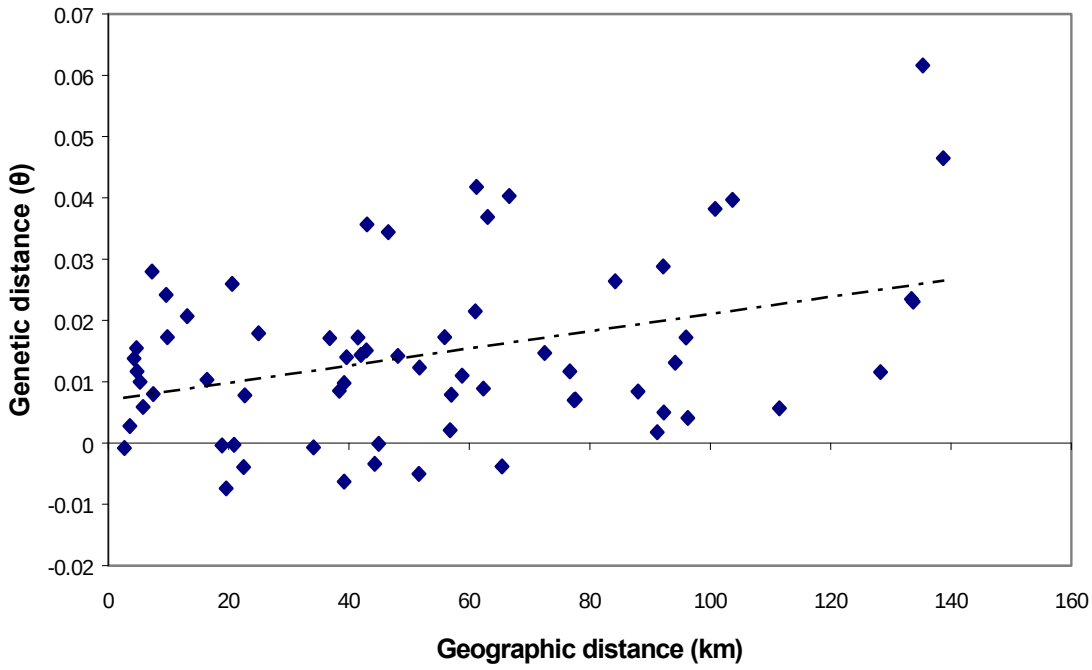


Figure 4.3 Isolation-by-distance among Yukon sampling sites. Pairwise estimates of F_{ST} (θ) are plotted against the corresponding straight-line geographic distances (*d*) between sites ($\theta = 0.0070 + 0.00014d$, Mantel r = 0.38, p = 0.025).

Table 4.5 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. Kluane Lake appeared to act as a partial barrier to gene flow, however the alpine habitats (Kluane Hills and Ruby Range) did not.

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

significantly when males were considered separately from females and adults from juveniles.

The assignment index also indicated little differentiation among Yukon sites (Table 4.4). 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 6 of 27 (22.2%) Alaska samples were assigned correctly, with the others assigned to various Yukon sites (Table 4.4).

There was a significant association between geographic and genetic distance (θ) among Yukon sites (Mantel $r = 0.38$, $p = 0.025$, Figure 4.3), suggesting isolation by distance. However, the pairwise comparisons show that this relationship was not consistent across all sites. Samples from the Flint and Base sites, for example, were significantly differentiated ($\theta = 0.012$, exact test $p < 0.0001$) despite being separated by less than 5 km. On the other hand, the Aishihik and Dezadeash sites were separated by over 100 km but were not significantly differentiated ($\theta = 0.0057$, exact test $p = 0.11$, Table 4.3). The assignment test suggested that nearby sites were somewhat more likely to share genotypes than distant sites, however the positive correlation of cross-assignments with geographic distance was not statistically significant and did not explain much of the variation in cross-assignments ($r^2 = 0.06$, randomization test $p = 0.40$).

The potential landscape barriers to gene flow that I identified *a priori* did not consistently explain deviations from the isolation by distance model (Table 4.5). 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, $t = 0.67$, $df = 3$, $p = 0.28$). 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 4.5). On the other hand, the alpine barriers that I identified (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 geographic 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).

Gene flow and dispersal

The low F_{ST} (θ) values among sites in the Yukon suggest a large amount of gene flow among hares in this region. Wright's island model of population structure suggests that approximately 16 effective migrants enter each "subpopulation" in the Yukon study area every generation. A lower Nm of 5.6 was obtained using Slatkin's private allele method. These theoretical estimates do not translate well into actual estimates of effective dispersal since real snowshoe hare populations are not structured like those in the model, making it difficult to determine what grouping of hares represent one "subpopulation" (Whitlock & McCauley 1999, see also Appendix 5). A higher estimate of 39.8 effective migrants moving between subpopulations was obtained using Rousset's (1997) method based on the stepping stone, isolation by distance model. The latter represents a more realistic model for snowshoe hare populations, but it remains difficult to directly compare model results with observations of dispersal from the field.

There was not sufficient genetic differentiation among Yukon sites to allow identification of dispersers using the assignment index. Although many hares were assigned to sites other than the one from which they were sampled, their genotypes either had low probabilities of belonging to that site (as determined by the simulated distribution in GENECLASS), or else they also had comparatively high probabilities of belonging to at least one other site. Using a critical probability of 0.01, 32 of the 363 hares (8.8%) across all sites were classified as potential immigrants into the "population" from which they were sampled (ranging from 0 to 26.7% at each site with a mean of 10.6%).

The comparison of individual assignment indices corrected for site differences indicated that there is no sex-bias in hare dispersal (Figure 4.4). 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 ±

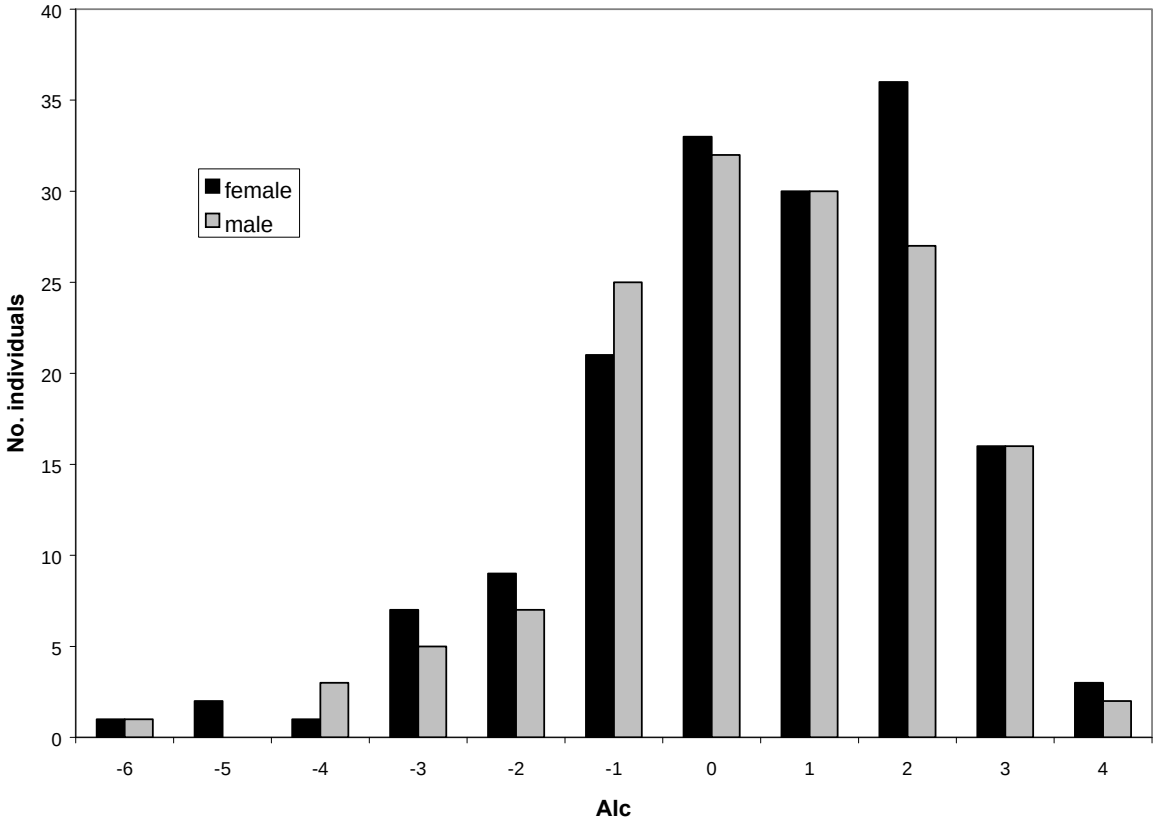


Figure 4.4 Frequency distributions of corrected assignment indices (AI_c) for male and female hares from all 12 Yukon sites. There was not a significant difference between mean values for the sexes, implying that dispersal in these hares is not sex-biased.

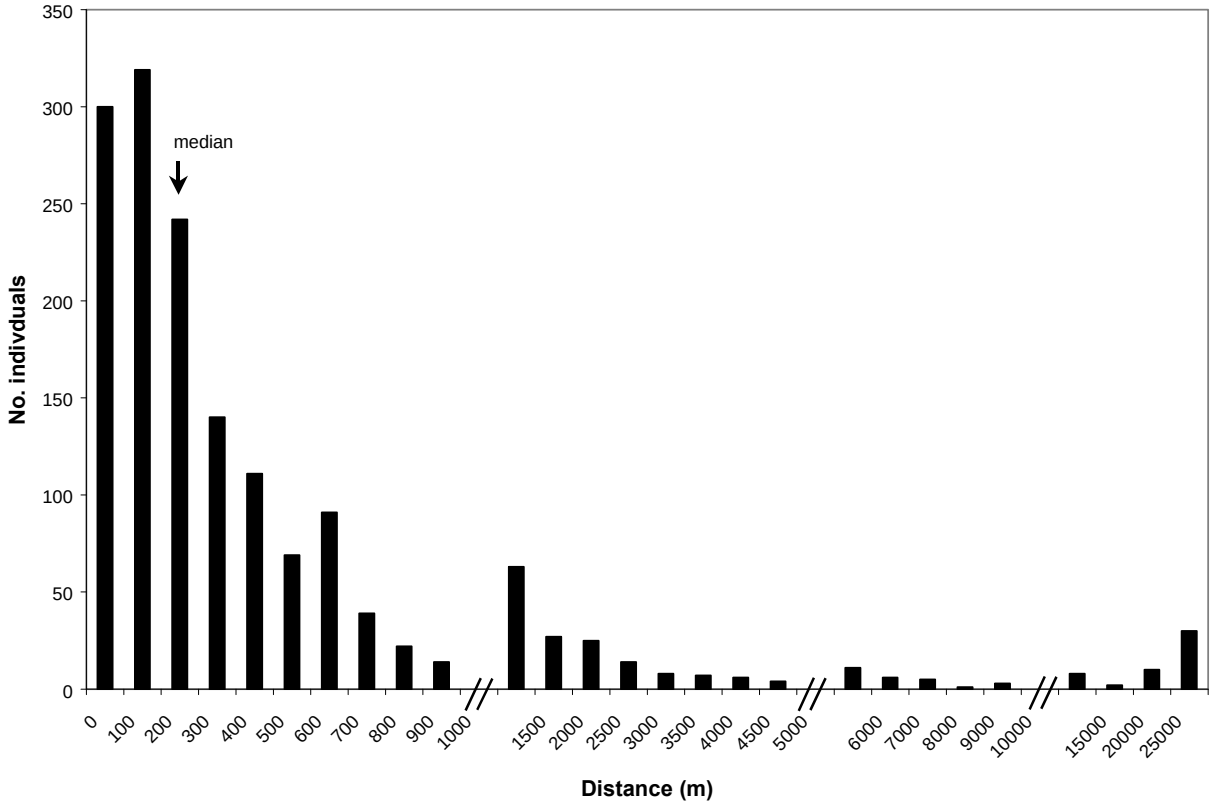


Figure 4.5 Frequency distribution of distances moved by radiocollared hares (note the three breaks in the x-axis scale). Straight-line distances were calculated between the first and last locations for 1577 hares collared from 1986-1996 during the Kluane Boreal Forest Ecosystem Project. The median distance (264 m) is indicated (range = 30 m - 29,833 m).

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.

The distance between first and last locations for the Kluane Project radiocollared hares ranged from 30 m to 29.8 km (Figure 4.5). Although most hares did not move very far (25th percentile = 129 m, median = 264 m, 75th percentile = 603 m), a significant proportion did move large distances (4.8% moved over 5 km and 1.9% over 25 km). There was not a significant difference in the distances moved by males (n = 755, mean = 1.3 km ± 4.3 SD) and females (n = 820, mean = 1.2 km ± 4.0 SD, Wilcoxon z = 0.16, p = 0.87).

Discussion

Comparison with other mammals

My 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 and 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 since 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 geographic 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.* 2001),

wolverine (*Gulo gulo*, Kyle and 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 I 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 geographic scale. Conversely, Fuller *et al*. (1996,1997) found very little genetic differentiation among rabbits over large areas in 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.

Gene flow and genetic drift

The low level of differentiation in hares suggests that there is considerable gene flow across the landscape. Nm estimates imply that a substantial number of effective migrants are connecting regions separated by more than 100 km, and even up to 700 km for the Alaska samples. This result is surprising given indications from the radiotelemetry data and previous field studies (e.g., Gillis & Krebs 1999, Hodges 1999) that most hares move less than 2 km and that long-distance dispersal events are not likely to exceed 25-30 km. Theoretical models have shown that few effective migrants are necessary to prevent strong differentiation between populations (Wright 1978, Slatkin 1985b). It therefore seems reasonable to conclude that the observed hare movements result in a large amount of gene flow and hence a low degree of differentiation at a local scale (e.g., tens of kilometers). Although long-distance dispersal events are often undetected in field studies (Koenig *et al*. 1996), I consider it highly unlikely that hares disperse over hundreds of kilometers. 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. The implication is that the rate of gene flow through smaller steps must be rapid enough to prevent the accumulation of significant genetic differences between distant sites.

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 Figure 4.3 and Table 4.3). 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 (see Pielou 1991). It seems unlikely, however, that strong local differences would not have developed in the rapidly mutating microsatellite loci over this time frame (> 10,000 years), therefore I consider this explanation less plausible.

Alternatively, the dynamic nature of snowshoe hare populations on a shorter time scale may be important in shaping genetic structure. My 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, 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 due to 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 isn't uniform it could conceivably 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 (see Appendix 5). My analysis did not show any genetic signatures of bottlenecks in hares. However, power analyses on the heterozygosity excess and mode-shift tests 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 my sample sizes and number of loci (Cornuet & Luikart 1996, Luikart *et al* 1998). The isolation by distance effect that I observed is 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 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 thoroughly address such a mechanism in hares.

Selection, mutation and sampling effects

Gene flow and genetic drift are not the only forces that can shape genetic structure. Natural selection is unlikely to have influenced my results since microsatellites are considered to be

selectively neutral (Jarne & Lagoda 1996). This is supported by the fact that none of the loci in this study were found to be out of Hardy Weinberg Equilibrium (except for Sat5, which was not included in the analyses) and that results were relatively consistent across loci (see Table 4.2). Nevertheless, I cannot completely dismiss the possibility that some loci could be linked to adaptive genes (e.g., "hitch-hiking", Charlesworth *et al.* 1997, Barton 1998, Slatkin & Wiehe 1998), or under selective pressure themselves (e.g., within exons, Jarne & Lagoda 1996). This might be important in light of the suggestion by Lidicker *et al.* (2000) that adaptive polymorphisms and temporal shifts of allozyme variation in snowshoe hares are a result of differing selective pressures over the course of the cycle.

Mutation is more likely to have played an important role in shaping the observed genetic structure. Microsatellites are characterized by high mutation rates (Jarne & Lagoda 1996), and the high level of polymorphism in this study shows that the loci I used are no exception. Mutation might explain some of the unexpected pattern of differentiation if the rate at which new alleles are generated is high relative to the rate of gene flow (Whitlock & McCauley 1999). Conversely, mutation processes could explain some of the homogeneity of genetic structure and cause an overestimation of gene flow if homoplasy is frequent (i.e., the presence of identical alleles due to mutation but not common ancestry, see Jarne & Lagoda 1996).

It is also possible that the observed genetic differences are not true differences, but rather the result of sampling artifacts. Declining densities and capture probabilities with the season resulted in small sample sizes for some of the sites (e.g., Aishihik, Chitty, Fourth of July), increasing the chances that the individuals sampled were not representative of the area from which they were captured. Indeed, both the Aishihik and Fourth of July samples were less differentiated than I expected, and the Chitty site was significantly different from the nearby Sulphur site. However, Flint and Base, the two largest samples, also had significantly different allele frequencies despite being separated by less than five kilometers. I therefore consider it unlikely that small sample sizes influenced the observed genetic patterns. The estimators of genetic differences that I used are also designed to account for differences in sample sizes (θ, Weir & Cockerham 1984, and Bayesian assignment index, Rannala & Mountain 1997, Cornuet *et al*. 1999). There were differences in the proportion of males or females and adults or juveniles caught among sample

sites, but these were also not likely to have caused the observed genetic differences since there were no significant differences in measures of differentiation when the sexes and age classes were considered separately. High genetic relatedness among hares within a site could distort measures of genetic differentiation between sites, however my results in Chapter 3 suggest that nearby hares are unlikely to be closely related. A final possibility to consider is that the sample sizes reflected differences in density that were created by differing environmental pressures (such as food availability or predation rate). As discussed above, differences in such selective factors are not likely to increase differentiation at neutral markers, however the effect of hare density on genetic structure is worthy of further investigation.

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. I was unable to accurately quantify the rates and distances of dispersal using the genetic methods, however the qualitative estimates provide a general picture of movement patterns. This picture largely supports 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 it does suggest that rates and distances 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 survive to pass on their genes, even when dispersing over long distances or across inhospitable habitat such as frozen lakes and alpine tundra. The 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 due to the lack of philopatry, the low level of relatedness in local populations (Chapter 3), and multiple mating in both sexes (Chapter 2). Variance in reproductive success also appears to be relatively low for both sexes (Chapter 2), therefore selection for sex-biased dispersal should be weak.

The extensive amount of gene flow in 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 my 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, non-cyclic population (Jardine 1995), showed little genetic differentiation. While the number of sites that I sampled was by no means exhaustive, my 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. While the level of differentiation does not suggest complete isolation between the regions, the indication is that there is very little gene flow (a theoretical Nm of ~ 1). 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 different glacial refugia. 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 due to 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.

Effective population size

The effective population size (N_e) is an important descriptor of a population that has consequences for its viability and evolutionary potential (Frankham 1995). N_e represents the number of individuals in a theoretically ideal population experiencing the same magnitude of random genetic drift as the actual population (Hartl & Clark 1997). Many factors can affect N_e, such as variance in reproductive success and fluctuation in population size, and it is usually smaller than the census population size (Frankham 1995). The high genetic diversity and low differentiation in snowshoe hares suggest a large effective population size, although accurately estimating N_e in natural populations is very difficult (Chepko-Sade *et al.* 1987). I used different methods to estimate current and long-term N_e for hares based on the field observations of dispersal distances and on the measured genetic diversity (see Appendix 5 for details). These estimates proved highly variable and highlight the uncertainty involved in quantifying N_e for continuously distributed populations. They generally suggest an N_e of several hundred to several thousand hares, however they can range from as little as 70 to as many as 100,000 hares depending on the interpretation of dispersal distributions and mutation processes. Although they are not quantitatively reliable, these estimates qualitatively support the conclusion that hares form large populations in the northern boreal forest, especially considering that N_e is usually smaller than the actual population size (Frankham 1995).

General implications and directions for future research

My 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. Although estimating actual and effective population sizes remains problematic, 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 I 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 geographic 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).

Literature cited

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.

Barton NH (1998) The effect of hitch-hiking on neutral genealogies. *Genetical Research,* **72,** 123-133.

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution,* **13,** 202-206.

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

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.

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

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.

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. www.fas.umontreal.ca/BIOL/legendre.

Charlesworth B, Nordborg M, Charlesworth D (1997) The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genetical Research,* **70,** 155-174.

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

Chepko-Sade BD, Shields WM, Berger J, *et al.* (1987) The effects of dispersal and social structure on effective population size. In: *Mammalian dispersal patterns,* (eds. Chepko-Sade BD, Halpin ZT), pp. 287-321. 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.

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.

Frankham R (1995) Effective population size/adult population size ratios in wildlife: A review. *Genetical Research,* **66,** 95-107.

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.

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, 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.

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

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, .

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.

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.

Hanski IA, Gilpin ME (eds.) (1997) *Metapopulation biology: ecology, genetics and evolution.* Academic Press.

Hartl DL, Clark AG (1997) *Principles of population genetics*, 3rd edition. Sinauer Associates Inc., Sunderland, Massachusetts.

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.

Hutchinson 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.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution,* **11,** 424-429.

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, 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.

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

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, 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.

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

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.

Neigel JE (1997) A comparison of alternative strategies for estimating gene flow from genetic markers. *Annual Review of Ecology and Systematics,* **28,** 105-128.

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*, 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.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145,** 1219-1228.

Slatkin M (1985a) Rare alleles as indicators of gene flow. *Evolution*, **39,** 56-65.

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

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science,* **236,** 787-792.

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

Slatkin M, Wiehe T (1998) Genetic hitch-hiking in a subdivided population. *Genetical Research,* **71,** 155-160.

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 and Company, New York.

Stenseth NC, Lidicker WZJ (eds.) (1992) *Animal dispersal: small mammals as a model.* Chapman & Hall, 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, Ibrahim KM, Hewitt GM (1999) Population structure and genetic variation of European wild rabbits (*Oryctolagus cuniculus*) in East Anglia. *Heredity,* **82,** 479-487.

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.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity*, **82,** 117-125.

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 (1931) Evolution in Mendelian populations. *Genetics*, **16,** 97-159.

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.

CHAPTER 5: GENERAL DISCUSSION

The results of my study suggest that snowshoe hares have very little population genetic structure during a cyclic peak phase in the southwest Yukon. The genetic data support previous descriptions of hares as promiscuous animals lacking social structure and displaying widespread movement patterns, and are consistent with the idea that hare populations are unlikely to be self-regulated by mechanisms involving kinship effects. My data also reveal a high level of genetic diversity in hares and suggest that European rabbit microsatellite primers are useful markers for investigating snowshoe hare population genetic structure.

At the level of the mating system, the microsatellites revealed that female hares can successfully mate with more than one male per oestrus period and per breeding season. Although the frequency of multiple paternity was relatively low, and some females mated with the same male in successive oestrus periods, the indication is that hares do not form stable breeding units. Similarly, there was no indication of stable associations among related hares in a local population. This supports field observations that hares are not philopatric, and implies that interactions among kin are not an important component of hare social behaviour. Widespread dispersal by both sexes was also suggested by the genetic structure at a larger scale. A remarkably low level of genetic differentiation across large distances and potential barriers in southwestern Yukon (and into Alaska) implies that there is considerable gene flow among hares in the northern boreal forest. However, hares did not form a single panmictic population and the observed structure suggests that non-equilibrium interactions between gene flow and drift, potentially linked to the density cycle, may be important in shaping hare population genetic structure (see Hutchinson & Templeton 1999, Newman & Squire 2001).

There are some important links between the three components of population structure that I addressed in this study. The interconnection between mating system attributes, local group composition, and the genetic diversity and structure of populations is being increasingly recognized by ecologists and population geneticists (Chepko-Sade & Halpin 1987, Sugg *et al.* 1996, Balloux *et al.* 1998, Surridge *et al.* 1999a, Ross 2001). Multiple paternity in snowshoe hares may play an important role in maintaining the observed high level of genetic variation and

large effective population size (Sugg & Chesser 1994). Successful paternity for more than one male in a single litter, and for many males in a local group, can also be expected to reduce the relatedness among nearby juveniles. This would make the formation of kin clusters less likely, thus diminishing the strength of kin selection, however it could also be beneficial in that direct competition among closely related siblings would be infrequent (Ridley 1993). Kin clustering and other forms of social structure are expected to increase overall genetic structure and reduce N_e (Chepko-Sade *et al.* 1987, Hartl & Clark 1997), therefore the fact that I did not detect kin clusters in hares is consistent with the low level of genetic differentiation at larger scales and the suggestion of large N_e. A low level of relatedness could also result in a high frequency of antagonistic social interactions among hares (see Graf 1985), thereby promoting widespread dispersal as suggested by the high estimates of gene flow.

An interesting evolutionary question that follows from these results is: why are snowshoe hare populations not more structured? Many other small mammal species show social structure in the form of territoriality, female philopatry, and breeding groups (Lidicker & Patton 1987, Stenseth & Lidicker 1992). Even other leporids, such as the European rabbit (*Oryctolagus cuniculus*), display social behaviour that causes fine-scale genetic structuring (Surridge *et al.* 1999b). In rabbits, group living is thought to have evolved in response to high predation risk and the patchy distribution of resources such as food and nesting sites (Bell 1983). The primary fitness benefit of sociality in many species is hypothesized to be greater success in dealing with predation (Alcock 1993). Since most snowshoe hares die from predation (Keith 1990, Krebs *et al.* 1995), it seems likely that the potential benefits of social living for reducing predation risk in hares are exceeded by associated costs (e.g., competition among group members, reproductive interference). The fact that hares are precocial and make use of more continuously distributed resources has presumably favoured alternative strategies for coping with predation. For example, the widespread reproductive success and high movement rates suggested by my results may protect against localized incidences of high predation that might otherwise eliminate an entire social group. I am not aware of any studies of population genetic structure in other hare species (genus *Lepus*), but I suggest that it would be interesting to determine if the level of structuring is similar in *Lepus* species with comparable life histories to snowshoe hares.

Aside from such comparative studies, I propose several directions for future research on snowshoe hare population structure. More insight into hare mating strategies would be gained by combining the genetic methods I have used with behavioural observations and manipulations of male-female and male-male interactions (see Graf 1981, 1985). Observational and experimental work is also needed to test potential mechanisms other than relatedness that might underlie hare social interactions (e.g., resident status, age, body condition). Although I consider it a lower priority in snowshoe hares, any further work on kin interactions should focus on a larger scale (in order to include more non-overlapping individuals) and should also involve direct manipulations of kin composition, followed by monitoring of reproduction and survival of group members.

Many interesting questions remain to be addressed for regional genetic structuring of hare populations. The first priority should be to investigate genetic structure during different phases of the hare cycle. The low phase is of particular interest since it is during this time that local extinctions or bottlenecks may have the most pronounced effect on genetic structure. Analysis of large-scale habitat use and movement patterns by hares throughout the cycle would complement this genetic work. In addition to expanding the temporal scale of this study, increasing the spatial scale will shed more light on the geographic structuring of hare populations throughout their range. The structure of southern hare populations is of particular interest given their peripheral nature and the hypothesized effects of human habitat fragmentation on their demography (see Keith 1990 and Hodges 2000). Combining microsatellite markers with other genetic markers, such as mitochondrial DNA, will be useful for better understanding historical gene flow patterns in hares. Finally, field analysis of the frequency, direction and fate of long-distance dispersers is necessary for assessing demographic consequences of the large amount of gene flow implied by the observed genetic structure.

Literature cited

Alcock J (1993) *Animal behavior: an evolutionary approach*, 5th edition. Sinauer Associates, Inc., Sunderland, MA.

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

Bell DJ (1983) Mate choice in the European rabbit. In: *Mate Choice,* (ed. Bateson P), pp. 211-223. Cambridge University Press, Cambridge.

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

Chepko-Sade BD, Shields WM, Berger J, *et al.* (1987) The effects of dispersal and social structure on effective population size. In: *Mammalian dispersal patterns,* (eds. Chepko-Sade BD, Halpin ZT), pp. 287-321. University of Chicago Press, Chicago.

Graf RP (1981) *Some aspects of snowshoe hare behavioural ecology.* MSc thesis, University of British Columbia.

Graf RP (1985) Social organization of snowshoe hares. *Canadian Journal of Zoology,* **63,** 468-474.

Hartl DL, Clark AG (1997) *Principles of population genetics.* Sinauer Associates Inc., Sunderland, Massachusetts.

Hodges KE (2000) 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.

Hutchinson 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.

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

Krebs CJ, Boutin S, Boonstra R, *et al.* (1995) Impact of food and predation on the snowshoe hare cycle. *Science,* **269,** 1112-1115.

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.

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

Ridley M (1993) Clutch size and mating frequency in parasitic Hymenoptera. *American Naturalist,* **142,** 893-910.

Ross KG (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology,* **10,** 265-284.

Stenseth NC, Lidicker WZJ (eds.) (1992) *Animal dispersal: small mammals as a model.* Chapman & Hall, New York.

Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. *Genetics,* **137,** 1147-1155.

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, Hewitt GM (1999a) From population structure to individual behaviour: genetic analysis of social structure in the European wild rabbit (*Oryctolagus cuniculus*). *Biological Journal of the Linnean Society,* **68,** 57-71.

Surridge AK, Ibrahim KM, Bell DJ, *et al.* (1999b) Fine-scale genetic structuring in a natural population of European wild rabbits (*Oryctolagus cuniculus*). *Molecular Ecology,* **8,** 299-307.

APPENDICES

Appendix 1 Details of litters sampled. All dates were in 1999. L1 corresponds to the first seasonal litter and L2 to the second. The total number of offspring is given with the number of those that were female indicated in parentheses.

Mother	**Date of Litter**	**Number of Offspring** (females)	**Mean weight of offspring** (grams)	**Mean right hind foot length of offspring** (mm)
8220	May 31 (L1)	3 (2)	67.8	36.0
418	June 2 (L1)	4 (3)	59.5	35.0
7950	June 5 (L1)	3 (1)	60.4	35.0
5925	June 6 (L1)	4 (1)	50.8	31.3
9412	June 7 (L1)	3 (2)	59.9	33.7
8010	June 9 (L1)	3 (2)	48.4	31.3
474	June 11 (L1)	5 (1)	54.3	34.2
7901	June 11 (L1)	3 (1)	55.0	38.3
418	July 7 (L2)	4 (1)	70.4	38.0
7950	July 12 (L2)	4 (0)	69.9	37.3
5925	July 13 (L2)	4 (3)	63.9	35.3
9412	July 14 (L2)	5 (3)	65.8	37.6
8270	July 15 (L2)	5 (0)	67.7	36.2
8010	July 16 (L2)	1 (0)	63.5	35.0
7973	July 22 (L2)	4 (3)	63.1	35.5
RK1*	(L2)	5	-	-
RK2*	(L2)	5	-	-
Overall means		3.8	61.6	35.4

*roadkill samples

Appendix 2 Details of simulation parameters and accuracy of parentage assignments in programs CERVUS and KINSHIP.

The results of the paternity assignments in program CERVUS are sensitive to the parameters used in the simulations, specifically the estimated error rate of the genetic data and the proportion of candidate males sampled (Marshall *et al.* 1998). Since both the error rate and the number of unsampled males were unknown, I ran preliminary tests to determine the parameter values that yielded the most accurate results.

The most accurate error rate was determined by running maternity tests for the offspring and known mothers using different simulated error rates. An error rate of 0.001 (0.1%) gave the most reliable results, assigning 89.1% of the offspring to the correct mother with 95% confidence and 92.7% with 80% confidence (3.6% were left unassigned while another 3.6% were assigned to the wrong female with 95% confidence). This low error rate is reasonable since it allows for some mismatch due to mutation or null alleles but reflects the care taken to avoid genotyping errors. It must be recognized, however, that the true error rate is unknown. I also tested the performance of KINSHIP with the known mother-offspring pairs: 87.7% were correctly assigned to their mother while the remaining 12.3% were assigned to the wrong female (the offspring assigned incorrectly in CERVUS were also mis-assigned in KINSHIP). These mother-offspring tests reveal the potential for incorrect paternity assignments in both programs, although the ability to make parentage assignments improves when the identity of one parent is known (Marshall *et al.* 1998). The probability of excluding a randomly chosen unrelated male from paternity in program CERVUS was high (99.19%), however false assignments due to genotyping errors or relatedness among candidate males might still be possible.

I did not know with certainty the total number of males present in the study area or the proportion that I sampled. Given the size of the study area and the relatively high density of hares, I consider it very unlikely that all candidate fathers were trapped and sampled. Nevertheless, I trapped repeatedly over a two-month period at the end of which there were very few untagged males being captured, suggesting the majority had been sampled. In order to best estimate the parameters, I ran three different CERVUS simulations that varied in the number of candidate males and proportion sampled (the other simulation parameters were held constant at

10,000 cycles, 100% of loci typed and an error rate of 0.001). The first simulation (A) assumed that I sampled all candidate males in the area, the second (B) assumed 80% were sampled, and the third (C) used a more conservative estimate of 48% sampled. The latter corresponds to an estimate of 50 candidate males, reflecting the potential for several different males to overlap the home range of each female (Boutin 1979, Chu 1996, see also Chapter 3) and the possible low capture probability of adult hares (Boulanger 1993).

The identity of the most-likely father was not affected by the different parameter values, however the significance of the paternity assignments was considerably affected (see Table below). Many more of the paternities were unresolved using simulation C as compared with simulations A and B. There were significant discrepancies, however, between the observed and expected number of resolved paternities for simulations A and B but not for simulation C. Such discrepancies likely reflect poorly estimated parameters (Marshall *et al.* 1998), suggesting that there was a large proportion of unsampled candidate fathers. A further test of the reliability of the different results was possible due to the fact that four of the sampled males were known to have died before females began their second period of oestrus. None of the second litter offspring should therefore have been assigned to these males (barring an ability of female hares to store sperm). The test using simulation A did assign paternity for three second litter offspring to two of the dead males with 95% confidence, and the test using simulation B also assigned one of these at the 95% level. It is therefore evident that the results from both of these tests include incorrect paternity assignments. Simulation C did not result in these faulty assignments at the 95% confidence level. Based on these results I rejected the parameter estimates from simulations A and B (100% and 80% sampled, respectively) and used the estimates from simulation C (50 candidate males of which 48% were sampled) for further analysis.

Appendix 2 Table Predicted and observed paternity assignments in program CERVUS using three different simulations varying in the estimated proportion of candidate males sampled. The actual number of offspring assigned paternity is given in parentheses.

Assignment Confidence Level	Simulation A (100% sampled)		Simulation B (80% sampled)		Simulation C (48% sampled)	
	pred.	obs.	pred.	obs.	pred.	obs.
95%	100%	80% (44)	72%	49% (27)	32%	33% (18)
80%	100%	80% (44)	92%	80% (44)	52%	51% (28)
Unresolved	0%	20% (11)	8%	20% (11)	48%	49% (27)

Appendix 3 Genotypic data for all mothers, offspring and candidate fathers sampled. Instances of extra paternal alleles are highlighted and underlined. Mother-offspring mismatches are highlighted. The four males that died before the second oestrus period are marked with an asterisk.

		Locus						
Mother	**Offspring**	**sol 33**	**sol 3**	**sat 3**	**sat 12**	**sat 13**	**sat 16**	**sat 2**
7950		214/217	271/271	138/140	112/120	121/121	097/107	243/250
	7950/L1/A	212/214	271/271	138/138	112/120	119/121	097/107	250/**251**
	7950/L1/B	212/217	271/271	138/140	112/112	121/121	095/097	**237**/250
	7950/L1/C	214/217	271/271	138/138	120/128	121/121	095/097	**239**/250
	7950/L2/A	217/218	271/273	140/160	112/112	121/121	097/109	243/250
	7950/L2/B	212/214	271/271	138/138	112/112	119/121	107/107	237/243
	7950/L2/C	212/217	271/273	140/160	120/132	121/121	097/109	243/243
	7950/L2/D	212/217	271/271	138/140	112/120	119/121	107/107	237/250
5925		212/219	271/273	138/140	112/112	121/121	109/109	219/237
	5925/L1/A	212/214	271/273	138/140	112/116	**119/119**	109/109	219/246
	5925/L1/B	214/219	271/271	138/140	112/116	121/121	109/109	219/246
	5925/L1/C	212/212	271/273	138/148	112/116	121/121	109/109	237/246
	5925/L1/D	212/214	271/273	138/138	112/116	**119/119**	109/109	235/237
	5925/L2/A	214/219	271/273	138/140	112/116	121/121	109/109	219/246
	5925/L2/B	212/214	271/271	138/138	112/116	119/121	109/109	237/246
	5925/L2/C	212/214	271/271	138/140	112/116	**119/119**	109/109	219/246
	5925/L2/D	212/214	271/273	138/138	112/116	121/121	109/109	235/237
418		212/216	271/271	138/140	112/112	121/121	105/111	229/237
	418/L1/A	212/216	271/271	140/140	112/112	121/121	097/111	229/237
	418/L1/B	212/212	271/271	138/138	112/120	121/121	097/105	229/229
	418/L1/C	212/212	268/271	138/138	112/120	121/121	111/111	233/237
	418/L1/D	212/216	271/271	138/140	112/120	121/121	105/111	229/233
	418/L2/A	212/220	271/271	138/140	112/112	121/121	095/105	229/237
	418/L2/B	212/216	271/271	138/138	112/112	121/121	095/105	229/251
	418/L2/C	216/220	271/271	138/140	112/112	119/121	095/105	237/237
	418/L2/D	212/212	271/271	138/138	112/112	119/121	105/107	237/237
8220		214/218	271/271	138/138	116/116	121/121	109/111	237/254
	8220/L1/A	214/218	271/272	138/138	112/116	121/121	107/109	237/239
	8220/L1/B	214/217	271/272	138/140	112/116	121/121	107/109	237/239
	8220/L1/C	214/218	271/271	138/140	112/116	121/121	107/111	237/239

Appendix 3 (cont'd)

474		212/219	268/268	138/140	112/128	121/121	095/097	237/247
	474/L1/A	212/220	268/271	138/140	128/136	119/121	095/097	247/255
	474/L1/B	212/212	268/271	138/138	112/136	119/121	097/097	237/**255**
	474/L1/C	212/220	268/271	140/140	128/136	121/121	095/097	**234**/247
	474/L1/D	212/219	268/271	138/140	112/120	121/121	095/105	**219**/247
	474/L1/E	212/212	268/271	138/140	112/136	121/121	097/097	247/255
8010		212/217	271/271	138/140	112/120	121/121	097/105	251/252
	8010/L1/A	212/212	271/271	138/140	112/120	121/121	097/105	237/252
	8010/L1/B	212/217	271/271	140/140	112/112	121/121	097/097	245/252
	8010/L1/C	212/212	271/271	138/138	120/120	121/121	105/105	237/252
	8010/L2/A	212/216	271/271	138/140	120/120	121/121	097/109	245/252
7973		212/212	271/271	138/138	112/128	121/121	097/105	218/251
	7973/L2/A	212/217	271/271	138/138	112/120	121/121	097/107	218/231
	7973/L2/B	212/217	271/271	138/138	112/128	121/121	097/097	237/251
	7973/L2/C	212/212	271/271	138/138	120/128	121/121	097/105	218/237
	7973/L2/D	212/212	271/271	138/138	112/128	121/121	105/107	231/251
8270		212/214	271/271	138/138	112/124	121/121	095/115	237/246
	8270/L2/A	212/212	271/272	**138/138**	112/112	121/121	105/115	231/246
	8270/L2/B	212/218	271/271	138/**156**	112/132	121/121	095/105	**231**/246
	8270/L2/C	212/212	271/271	138/**140**	112/112	121/121	105/115	**235**/246
	8270/L2/D	212/218	271/271	138/138	112/112	121/121	107/115	235/246
	8270/L2/E	212/212	271/271	138/140	112/124	121/121	105/115	**237/246**
9412		214/214	271/271	138/138	112/132	119/121	097/105	231/237
	9412/L2/A	214/214	271/271	138/138	112/120	121/121	105/105	231/237
	9412/L2/B	214/218	271/271	138/138	112/132	119/121	105/105	231/231
	9412/L2/C	214/218	271/271	138/138	112/132	119/121	097/107	231/237
	9412/L2/D	214/218	271/271	138/138	132/132	121/121	105/105	231/237
	9412/L2/E	214/214	271/271	138/138	112/120	121/121	105/107	237/237
	9412/L1/A	214/220	271/271	138/140	112/120	119/121	095/097	237/247
	9412/L1/B	214/220	268/271	138/140	112/128	119/121	105/105	237/247
	9412/L1/C	214/214	271/271	138/140	112/128	119/121	095/105	231/247
7901		212/217	271/271	138/140	120/136	121/121	095/115	237/239
	7901/L1/A	212/217	271/271	138/140	**136/136**	121/121	109/115	237/241
	7901/L1/B	212/212	268/271	140/160	120/**132**	121/121	109/115	239/243
	7901/L1/C	212/218	268/271	140/160	**112**/120	121/121	095/105	239/243

Appendix 3 (cont'd)

RK1		216/218	268/271	138/140	116/124	121/121	095/097	249/251
	RK1/A	216/218	271/273	138/140	116/120	121/121	095/105	241/251
	RK1/B	216/218	271/271	138/140	120/124	121/121	097/105	249/251
	RK1/C	216/216	268/271	140/140	120/124	121/121	095/105	249/251
	RK1/D	216/219	271/273	138/140	112/124	121/121	097/105	241/251
	RK1/E	218/219	268/273	138/138	116/120	121/121	097/111	249/251
RK2		218/221	271/271	138/140	112/112	119/121	107/115	249/251
	RK2/A	216/221	271/271	138/140	112/116	119/121	105/107	231/251
	RK2/B	216/221	271/273	138/140	112/116	119/121	095/107	231/249
	RK2/C	216/221	271/271	140/140	112/112	121/121	095/115	231/251
	RK2/D	216/218	271/271	138/140	112/116	121/121	095/115	231/249
	RK2/E	216/221	271/271	138/140	112/112	119/121	095/115	249/249

Males

409	212/218	271/271	138/138	112/124	121/121	107/109	237/254
6090	212/212	268/271	138/148	116/120	121/123	095/105	227/235
2645	212/218	268/273	160/160	112/132	121/121	105/109	243/251
9476*	217/219	271/273	138/140	112/120	121/121	105/111	251/251
6091	212/218	271/272	138/138	120/128	121/121	105/105	227/254
420	212/220	271/271	138/138	112/112	119/121	095/107	237/251
8244	212/212	271/271	138/138	112/116	121/121	097/111	235/251
9477*	212/217	271/272	138/138	128/128	119/121	097/111	237/254
7912	218/218	268/271	138/138	112/116	119/121	095/105	231/240
8206	212/218	271/271	138/138	112/112	121/121	105/109	249/254
8207	212/214	271/272	138/138	120/120	121/121	107/109	245/246
8209	218/219	271/273	138/138	112/112	121/121	097/107	241/254
7910	218/220	271/271	138/140	120/128	119/121	095/111	241/241
8049	214/219	271/273	138/138	112/116	121/123	095/109	243/251
9500*	218/218	271/273	138/148	124/136	119/121	105/109	247/249
8046	212/220	272/273	138/138	112/136	119/121	107/107	233/237
8205	218/219	271/271	138/138	112/112	121/123	097/097	247/249
8047	212/214	271/271	138/148	116/116	119/121	109/109	235/246
8044*	212/212	271/271	138/156	112/112	121/121	105/111	231/235
8045	212/214	271/271	138/138	120/128	121/121	105/105	241/245
8208	212/218	271/271	138/138	112/128	121/121	107/111	239/240
7902	212/212	268/271	140/140	112/116	121/121	095/105	233/252
7909	218/218	271/272	140/140	112/112	119/121	097/107	235/239
7923	212/214	271/279	138/138	112/128	119/121	105/105	231/235

Appendix 4 Allele sizes and frequencies at the seven microsatellite loci for each sampling site and over all sites (*n* is sample size).

Locus: sol 33

		Allele size										
Site	*n*	212	213	214	215	216	217	218	219	220	221	222
Flint	56	0.384		0.179		0.125		0.214	0.027	0.071		
Sulphur	25	0.52		0.16		0.16		0.12	0.02	0.02		
Chitty	12	0.417		0.042	0.042	0.042	0.042	0.25	0.042	0.125		
4th of July	15	0.433		0.133	0.033	0.067	0.067	0.233		0.033		
Base	41	0.439		0.134		0.012	0.073	0.195	0.098	0.049		
Aishihik	10	0.45		0.1				0.35		0.1		
Copper Joe	24	0.646		0.083		0.125	0.021	0.083	0.021	0.021		
Dezadeash	18	0.25		0.306	0.028	0.056		0.278		0.083		
Gladstone	19	0.474		0.211		0.026	0.079	0.132	0.053			0.026
Lewis Ck	35	0.7		0.043		0.114		0.114	0.014	0.014		
Jacquot Is. N	35	0.457		0.1		0.1		0.257		0.071		0.014
Jacquot Is. S	27	0.574		0.074		0.074	0.019	0.148		0.037		0.074
Alaska	27	0.5		0.074		0.056	0.037	0.185	0.019	0.056	0.056	0.019
Montana	19	0.184	0.289						0.026	0.368	0.105	0.026
All sites	363	0.47	0.015	0.12	0.004	0.079	0.022	0.178	0.026	0.066	0.01	0.011

Locus: sol 3

		Allele size										
Site	*n*	249	255	263	265	268	271	272	273	275	277	279
Flint	56				0.009	0.08	0.768	0.045	0.089		0.009	
Sulphur	25					0.06	0.72	0.02	0.2			
Chitty	12					0.167	0.458	0.125	0.25			
4th of July	15				0.033	0.033	0.8	0.033	0.1			
Base	41					0.073	0.756	0.073	0.085			0.012
Aishihik	10				0.05	0.15	0.55	0.1	0.15			
Copper Joe	24					0.063	0.792		0.146			
Dezadeash	18				0.028	0.056	0.583		0.333			
Gladstone	19				0.053	0.053	0.763		0.132			
Lewis Ck	35				0.029	0.086	0.8	0.014	0.057		0.014	
Jacquot Is. N	35				0.029	0.114	0.786		0.071			
Jacquot Is. S	27				0.019	0.185	0.63	0.037	0.111			0.019
Alaska	27					0.18	0.76		0.02	0.02	0.02	
Montana	19	0.167	0.028	0.5	0.111		0.139				0.056	
All sites	363	0.008	0.001	0.025	0.021	0.092	0.703	0.029	0.11	0.001	0.007	0.003

Appendix 4 (cont'd)

Locus: sat 3

Site	*n*	Allele size 136	138	140	144	148	150	156	158	160	162
Flint	56		0.554	0.277		0.089	0.018		0.018	0.009	0.036
Sulphur	25		0.7	0.2		0.04				0.06	
Chitty	12		0.583	0.333						0.083	
4th of July	15		0.633	0.267		0.033					0.067
Base	41		0.732	0.195		0.037		0.012		0.024	
Aishihik	10		0.75	0.2		0.05					
Copper Joe	24		0.813	0.063	0.021	0.063		0.021		0.021	
Dezadeash	18	0.028	0.667	0.25	0.028				0.028		
Gladstone	19		0.684	0.211		0.026		0.053			0.026
Lewis Ck	35		0.771	0.157		0.057					0.014
Jacquot Is. N	35		0.743	0.143		0.057		0.043		0.014	
Jacquot Is.S	27		0.722	0.167		0.019		0.093			
Alaska	27		0.5	0.346	0.058	0.058	0.019	0.019			
Montana	19		1								
All sites	363	0.001	0.695	0.2	0.007	0.046	0.004	0.018	0.004	0.014	0.011

Locus: sat 12

Site	n	Allele size 104	112	116	120	124	128	132	136	138	140
Flint	56		0.616	0.143	0.089	0.018	0.054	0.027	0.054		
Sulphur	25		0.56	0.12	0.14		0.06	0.08	0.04		
Chitty	12		0.333	0.292	0.083	0.042	0.042	0.125	0.083		
4th of July	15		0.567	0.033	0.1	0.1	0.067	0.1	0.033		
Base	41		0.476	0.134	0.146	0.037	0.134	0.024	0.049		
Aishihik	10		0.5	0.05	0.15			0.1	0.2		
Copper Joe	24		0.521	0.083	0.104	0.042	0.125	0.083	0.042		
Dezadeash	18	0.056	0.611	0.083	0.056	0.056	0.028	0.056	0.028		0.028
Gladstone	19		0.5	0.105	0.237	0.053	0.026	0.026	0.053		
Lewis Ck	35		0.357	0.143	0.171	0.1	0.143	0.029	0.057		
Jacquot Is. N	35		0.7	0.143	0.086	0.029		0.029	0.014		
Jacquot Is.S	27		0.648	0.093	0.13	0.019	0.019	0.037	0.056		
Alaska	27		0.577	0.096	0.058	0.038	0.077	0.077	0.058	0.019	
Montana	19			0.158		0.395	0.289	0.105			0.053
All sites	363	0.003	0.519	0.123	0.112	0.058	0.079	0.052	0.048	0.001	0.004

Appendix 4 (cont'd)

Locus: sat 13

Site	*n*	Allele size 117	119	121	123	125
Flint	56		0.071	0.902	0.027	
Sulphur	25		0.22	0.74	0.04	
Chitty	12		0.25	0.708	0.042	
4th of July	15		0.267	0.733		
Base	41		0.171	0.793	0.037	
Aishihik	10		0.25	0.75		
Copper Joe	24		0.208	0.688	0.063	0.042
Dezadeash	18		0.306	0.694		
Gladstone	19		0.263	0.711		0.026
Lewis Ck	35		0.214	0.714	0.057	0.014
Jacquot Is. N	35		0.229	0.729	0.029	0.014
Jacquot Is.S	27		0.185	0.741	0.056	0.019
Alaska	27		0.288	0.577	0.096	0.038
Montana	19	0.056	0.611	0.278	0.056	
All sites	363	0.003	0.223	0.724	0.039	0.011

Locus: sat 16

Site	*n*	Allele size 95	97	101	103	105	107	109	111	113	115
Flint	56	0.054	0.259	0	0.027	0.223	0.196	0.116	0.116	0	0.009
Sulphur	25	0.1	0.14	0	0.02	0.32	0.18	0.1	0.12	0.02	0
Chitty	12	0	0.333	0.042	0	0.333	0.167	0.042	0.083	0	0
4th of July	15	0.1	0.233	0	0.033	0.233	0.133	0.133	0.1	0	0.033
Base	41	0.146	0.159	0	0	0.244	0.159	0.159	0.11	0	0.024
Aishihik	10	0	0.15	0	0	0.4	0.15	0.25	0.05	0	0
Copper Joe	24	0.208	0.333	0	0	0.146	0.25	0.021	0.021	0	0.021
Dezadeash	18	0.083	0.167	0	0	0.306	0.083	0.222	0.083	0	0.056
Gladstone	19	0.079	0.105	0.026	0	0.421	0.211	0.132	0.026	0	0
Lewis Ck	35	0.143	0.271	0	0	0.171	0.171	0.057	0.171	0	0.014
Jacquot Is. N	35	0.1	0.171	0	0	0.171	0.157	0.271	0.043	0	0.086
Jacquot Is.S	27	0.13	0.278	0	0.019	0.222	0.111	0.167	0.019	0	0.056
Alaska	27	0.154	0.154	0	0.038	0.269	0.096	0.231	0.058	0	0
Montana	19	0	0	0	0.316	0.342	0.079	0.263	0	0	0
All sites	363	0.102	0.203	0.003	0.028	0.25	0.159	0.151	0.08	0.001	0.023

Appendix 4 (cont'd)

Locus: sat 2

Site	*n*	Allele size 216	217	218	219	220	221	223	225	226	227	228
Flint	56			0.009					0.027		0.027	0.009
Sulphur	25			0.02				0.02	0.06		0.02	0.04
Chitty	12				0.083				0.042		0.042	
4th of July	15			0.033					0.033		0.033	
Base	41			0.012	0.012						0.024	
Aishihik	10					0.05						
Copper Joe	24					0.021	0.021	0.021				0.021
Dezadeash	18				0.028				0.028			
Gladstone	19				0.079				0.026			
Lewis Ck	35				0.043	0.014		0.014	0.014			0.071
Jacquot Is. N	35			0.014	0.029		0.014		0.014	0.029	0.014	0.014
Jacquot Is. S	27		0.019	0.019	0.019	0.019	0.019	0.037		0.074	0.019	0.019
Alaska	27	0.019		0.019	0.058			0.019	0.019		0.019	
Montana	19								0.184		0.184	0.053
All sites	363	0.001	0.001	0.01	0.022	0.006	0.004	0.008	0.028	0.008	0.025	0.018

Locus: sat 2 (cont'd)

Site	n	Allele size 229	231	232	233	234	235	236	237	238	239	240
Flint	56	0.027	0.045		0.134	0.054	0.107		0.027		0.063	0.036
Sulphur	25	0.1	0.12		0.04	0.04	0.08		0.08	0.02	0.1	0.02
Chitty	12	0.042	0.042		0.042	0.042	0.042	0.042	0.042		0.042	
4th of July	15	0.133	0.033		0.133	0.033	0.033	0.033	0.033			
Base	41	0.012	0.073		0.037		0.085		0.171	0.012	0.049	0.024
Aishihik	10		0.05	0.05	0.1		0.1		0.15		0.05	
Copper Joe	24	0.042	0.063		0.042		0.021		0.104		0.104	
Dezadeash	18	0.056	0.028		0.028	0.028			0.25		0.139	
Gladstone	19		0.026		0.158		0.105	0.026	0.105		0.079	0.053
Lewis Ck	35	0.014	0.129		0.129		0.071		0.057		0.1	
Jacquot Is. N	35	0.029	0.057		0.186		0.057	0.014	0.057		0.057	0.057
Jacquot Is.S	27		0.056		0.13	0.019	0.037	0.019	0.093		0.056	0.056
Alaska	27	0.058	0.019	0.038	0.154	0.038	0.019		0.154	0.019	0.019	
Montana	19		0.053	0.026		0.026				0.079	0.053	0.079
All sites	363	0.033	0.061	0.006	0.101	0.021	0.061	0.007	0.09	0.008	0.066	0.026

Appendix 4 (cont'd)

Locus: sat 2 (cont'd)

Site	*n*	Allele size 241	242	243	244	245	246	247	248	249	250	251
Flint	56	0.107	0.009	0.054			0.036	0.054			0.009	0.125
Sulphur	25	0.04		0.02	0.02		0.02	0.02		0.06		0.04
Chitty	12	0.167				0.083	0.125					0.125
4th of July	15	0.067				0.033	0.067	0.133	0.067			0.1
Base	41	0.073		0.037		0.024	0.049	0.037		0.037	0.012	0.122
Aishihik	10			0.05				0.35		0.05		
Copper Joe	24	0.208		0.146				0.063				0.063
Dezadeash	18	0.139		0.028		0.083		0.056				0.111
Gladstone	19	0.053		0.079		0.105		0.026				0.079
Lewis Ck	35	0.029		0.143		0.043		0.014	0.029	0.014		0.057
Jacquot Is. N	35	0.014	0.029	0.043		0.014	0.029	0.057	0.014	0.029		0.086
Jacquot Is. S	27	0.056	0.037	0.074		0.019		0.056	0.019			
Alaska	27	0.058		0.077	0.038	0.077			0.019	0.038		
Montana	19	0.132	0.026	0.026						0.079		
All sites	363	0.079	0.008	0.061	0.004	0.029	0.022	0.048	0.01	0.021	0.003	0.072

Locus: sat 2 (cont'd)

Site	*n*	Allele size 252	253	254	255
Flint	56	0.045			
Sulphur	25	0.02			
Chitty	12				
4th of July	15				
Base	41	0.024		0.073	
Aishihik	10				
Copper Joe	24			0.021	0.042
Dezadeash	18				
Gladstone	19				
Lewis Ck	35			0.014	
Jacquot Is. N	35		0.014	0.029	
Jacquot Is. S	27			0.019	0.019
Alaska	27		0.019		
Montana	19				
All sites	363	0.011	0.003	0.015	0.004

Appendix 5 Estimating effective population size

Estimates of effective population size (N_e) can be made from data on dispersal distances or from estimates of genetic diversity and mutation. Wright (1943, 1978) estimated N_e based on the neighbourhood size (i.e., the area containing a randomly mating group, or deme) and the density of breeders (d) according to the equation $N_e = 4\pi\sigma^2 d$, where σ^2 is the one-way variance in dispersal distances. An alternative method that takes into account the skewed distribution of most dispersal distances is to multiply the 85th percentile dispersal distance (assumed to be the radius of a circular neighbourhood area) by the density of breeders (Chepko-Sade *et al.* 1987). I used the distribution of movement distances from the Kluane Project radiocollared hares to approximate dispersal distances, and 1999 spring hare densities from two control trapping grids in the Kluane area (C. J. Krebs unpublished data) as an estimate of the density of breeders (= 133 hares/km^2). Wright's equation yielded a neighbourhood size of approximately 107 km^2 and a corresponding N_e of about 14,000 hares. Because of the skewed distribution of distances (see Figure 5), the 85th percentile method gave a much smaller neighbourhood size of ~ 3 km^2 and an N_e of only ~ 400 hares. This large discrepancy points out the high degree of uncertainty surrounding these methods, however it also highlights the importance of long-distance dispersal in creating sufficient gene flow to account for the relatively small genetic divergence that I observed over the study area (Wright's neighbourhood size is biased upward by the rare long distances). It is possible that the distribution from the radiocollared hares underestimated the 85th percentile distance, since a second analysis using a smaller data set of 35 juvenile hares (from Gillis 1997) yielded intermediate neighbourhood size (15-55 km^2) and N_e (2000-7000 hares) using both methods.

Snowshoe hares appear to have even sex ratios and relatively low variance in reproductive success for both sexes (see Chapter 2), therefore such factors are unlikely to influence N_e. On the other hand, the low phase of the snowshoe hare density cycle would likely reduce the long-term N_e compared with estimates based on peak densities (Hartl & Clark 1997). Using spring densities from Kluane over an entire cycle (from 1990 peak to 1999 peak, C.J. Krebs unpublished data), Wright's neighbourhood equation indicated that N_e remained fairly high (~ 2700 hares), however the 85th percentile neighbourhood translated into a lower N_e (70 hares).

Although still relatively large, the latter estimate suggests that genetic drift could be important in creating differentiation among local areas during the low phase.

Rousset (1997) suggested a method for estimating neighbourhood size based on estimates of pairwise F_{ST} under isolation by distance. In this method, N_e ($= 4\pi\sigma^2 d$) is equal to the inverse slope of the regression of $F_{ST}/(1-F_{ST})$ on the logarithm of geographic distance between pairs of populations. For the Yukon hares, the slope of this regression was equal to 0.004, which corresponds to an N_e estimate of 250 hares.

Estimates of long-term N_e can also be derived from the average level of expected heterozygosity (H_E) and the mutation rate (v) under either the infinite alleles mutation model (IAM), where $N_e = H_E/4v(1-H_E)$ (Kimura & Crow 1963), or the stepwise mutation model (SMM), where $N_e = [(1/(1-H_E)^2)-1]/8v$ (Ohta & Kimura 1973). These estimates largely reflect the species-wide N_e, whereas the previous estimates based on neighbourhood size indicate the local potential for drift. As in the estimates based on dispersal distances, there is considerable uncertainty in these genetic estimates since mutation processes are poorly known for microsatellites (Jarne & Lagoda 1996). Assuming a mutation rate of 10^{-4}, which is in the middle of the range of observed values (Jarne & Lagoda 1996), I obtained an N_e of around 5000 hares under IAM and 10,000 under SMM. The estimate could be as low as 500 for a mutation rate of 10^{-3} (assuming IAM) or as high as 100,000 for a mutation rate of 10^{-5} (assuming SMM). With such a large range of potential values it is impossible to accurately quantify the effective size of snowshoe hare populations. More work is needed to bridge the gap between the theoretical concept of effective population size and the empirical data available from studies of dispersal and genetic diversity. Nevertheless, the general result from all of the above estimates is that N_e is relatively large in snowshoe hares.

Literature cited

Boulanger J (1993) *Evaluation of capture-recapture estimators using a cyclic snowshoe hare population.* MSc thesis, University of British Columbia.

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

Chepko-Sade BD, Shields WM, Berger J, *et al.* (1987) The effects of dispersal and social structure on effective population size. In: *Mammalian dispersal patterns,* (eds. Chepko-Sade BD, Halpin ZT), pp. 287-321. University of Chicago Press, Chicago.

Chu TKY (1996) *Fluctuations in home range size of male snowshoe hares during summer breeding season.* BSc thesis, University of British Columbia.

Gillis EA (1997) *Natal dispersal and post-weaning survival of juvenile snowshoe hares during a cyclic population increase.* MSc thesis, University of British Columbia.

Hartl DL, Clark AG (1997) *Principles of population genetics,* 3rd edition. Sinauer Associates Inc., Sunderland, Massachusetts.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution,* **11,** 424-429.

Kimura M, Crow JF (1963) The measurement of effective population number. *Evolution,* **17,** 279-288.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology,* **7,** 639-655.

Ohta T, Kimura M (1973) A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetic Research,* **22,** 201-204.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics,* **145,** 1219-1228.

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.