

Landscape location affects genetic variation of Canada lynx (*Lynx canadensis*)

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Abstract

The effect of a population's location on the landscape on genetic variation has been of interest to population genetics for more than half a century. However, most studies do not consider broadscale biogeography when interpreting genetic data. In this study, we propose an operational definition of a peripheral population, and then explore whether peripheral populations of Canada lynx (*Lynx canadensis*) have less genetic variation than core populations at nine microsatellite loci. We show that peripheral populations of lynx have fewer mean numbers of alleles per population and lower expected heterozygosity. This is surprising, given the lynx's capacity to move long distances, but can be explained by the fact that peripheral populations often have smaller population sizes, limited opportunities for genetic exchange and may be disproportionately affected by ebbs and flows of species' geographical range.

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Introduction

The distribution of genetic variation across the landscape is of interest to ecologists, taxonomists and conservation biologists. However, few systematic tests have been conducted to ascertain if populations located on the periphery of a species' genetic range have lower levels of genetic variation than core populations. Using empirical data and models, some studies have supported the premise that genetic variation is lower in the periphery of a species' range. For example, Anderson & Danielson (1997) modelled the effects of patch location on effective population size (N_e) and found that placing one patch in a peripheral location reduced the N_e of peripheral populations compared to core populations. These spatial models are consistent with the theories and observations of early *Drosophila* geneticists who found that the core of a species' range maintained greater levels of chromosomal polymorphisms than the periphery (Carson 1959; Dobzhansky 1970; Brussard 1984).

Lawton (1993) and Lesica & Allendorf (1995) proposed that geographical isolation and smaller N_e of most peripheral populations should significantly reduce multilocus heterozygosity and allelic variation. These predictions have been borne out in studies on lodgepole (*Pinus contorta*) and ponderosa pine (*P. ponderosa*; Cywnar & MacDonald 1987; Hamrick *et al.* 1989) and a variety of animals. For example, Gaines *et al.* (1997) found significantly less genetic variation within peripheral cotton rat populations (*Sigmodon hispidus*) compared to core populations, and Descimon & Napolitano (1993) found that both distance from the edge of a species' range towards the core, and N_e were correlated positively with genetic variation in butterfly populations (*Parnassius mnemosyne*).

On the other hand, there is nearly equal evidence against the idea that genetic variation is reduced on the periphery. In some cases, allelic diversity in *Drosophila* was not reduced in populations on the periphery of the geographical range (see Soule 1973; Brussard 1984; for review), nor was heterozygosity reduced on the periphery in firs (*Abies* spp.), *Silene nutans* and *Phlox* spp. (Levin 1970; Tigerstedt 1973; Lesica & Allendorf 1995; Van Rossum *et al.* 1997). In fact, some researchers have found greater genetic variation on the periphery of a species' range. Safriel *et al.* (1994)

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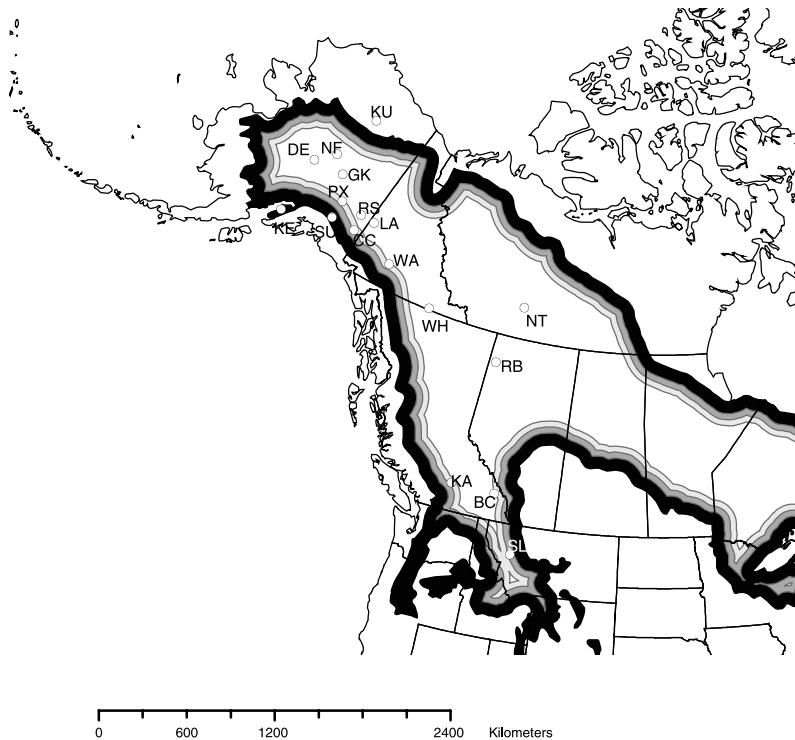


Fig. 1 Map of the lynx's geographical range. The shaded areas and internal white area represents the geographical range of Canada lynx. The populations sampled are noted with a solid circle and a letter code that corresponds to Table 2. The white area in the centre is the core of the lynx geographical range. The bands surrounding this core represent the periphery under each of our operational definitions of periphery (165 km, 123 km and 82 km). For example, the 165-km periphery is the area of all three shaded bands, while the 82-km periphery is the dark, outer black band.

and Volis *et al.* (1998) found higher neutral genetic diversity and phenotypic variability in peripheral chuckar partridge (*Alectoris chukar*) and wild barley (*Hordeum spontaneum*) populations.

Overall, little consensus exists regarding the pattern of genetic variation at the periphery of a species' range vs. the core. We may expect differences in the pattern of genetic variation at the periphery of a species' range because different species have different life histories. However, one other critical problem among all these studies is that periphery is not defined operationally, making comparisons between species difficult.

We compared genetic variation in core and peripheral populations of a wide-ranging species, the Canada lynx (*Lynx canadensis*), using an operational definition of core and periphery. The Canada lynx reaches the southern extent of its geographical range in the northern US Rockies and in the north Cascades (Fig. 1), where it was listed recently as 'Threatened' under the US Endangered Species Act (Federal Register 2000). Historically, lynx extended south into the mountains of Utah and Colorado, but currently no reproducing populations are thought to reside in these areas (McKelvey *et al.* 2000). The primary core habitat of the lynx is the boreal forest of Canada and Alaska, where their distribution today is roughly similar to historic times (McKelvey *et al.* 2000).

We predicted that little difference in genetic variation would be found in populations located in the core of the spe-

cies' geographical range vs. those located in the periphery because of the lynx's capability to move long distances (e.g. Ward & Krebs 1985; Slough & Mowat 1996; Mowat *et al.* 2000). Additionally, we previously reported low F_{ST} across 3100 km of the lynx geographical range (Schwartz *et al.* 2002). We interpreted this to indicate that high levels of gene flow may mediate any effect of the periphery on genetic variation.

Methods

Populations and samples

For this study a 'population' was considered any group of samples that was separated from other groups by more than 100 km or a human-perceived barrier such as a mountain range. We collected 599 samples from 17 populations (Fig. 1). In 16 populations, samples were either high quality tissue or blood collected during a state or province regulated trapping season or research efforts. We used hair samples from only one population, Kootnay-Banff; however, these samples were collected from individual lynx while they were being fitted with a radio collar (Apps 2000). The Kootnay-Banff samples thus consisted of a large number of hairs (> 20) with intact follicles, minimizing concerns about false polymerase chain reaction (PCR) products and allelic dropout (not measured in this study; Goossens *et al.* 1998; Taberlet *et al.* 1999).

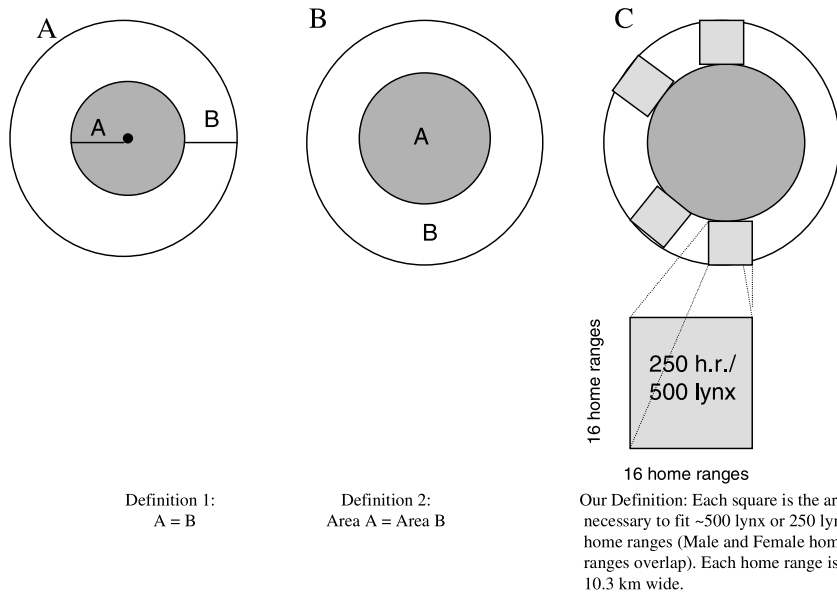


Fig. 2 Schematic of our definition of periphery compared to two other operational definitions of periphery (Channell & Lomolino 2000a,b).

Definition of periphery and core

The peripheral population concept has not been defined clearly in the literature. Most researchers approximate the periphery, or consider peripheral only those populations that are distinctly isolated at the geographical extent of a species' range. To our knowledge, the only operational definitions of periphery in the literature are those of Channell & Lomolino (2000a,b). They defined the periphery as the region that is within half the distance to the edge of a species' geographical range from a central point (see Fig. 2A). Subsequently, in a study considering the spatial dynamics of range contraction, Channell & Lomolino (2000b) defined the periphery by dividing the geographical range into two equal area bands, the inner band corresponding to the core and the outer band corresponding to the periphery (Fig. 2B).

We wanted a definition based on the basic biology of lynx and first principles of conservation biology. In particular, we believed that dimensions of an *isolated* peripheral population should scale with average home range size and be large enough to sustain a population in the short term. Therefore, we derived a coarse operational width of the periphery 'band' based on several small, isolated populations in a periphery each having an N_e of 50 (translating to approximately 500 individuals; Frankham 1995a), because this number ($N_e = 50$) is often used in conservation biology as a threshold population size for minimizing short-term effects of inbreeding depression (Franklin 1980; Soule 1986; Mace & Lande 1991). Five hundred individual lynx (or 250 pairs) fitted roughly into a $16 \times 16 (= 256)$ square matrix of home ranges. The average width of a home range (portrayed as a square) across several published lynx studies including

both males and females was approximately 10.3 km (Koehler 1990; Koehler & Aubry 1994). Thus, 16 home ranges extending 10.3 km each provides us with our periphery – the outer 165 km band of the lynx's geographical range (Fig. 2C). The strength of this operational approach is that it can be adapted to the biology of any organism and is grounded in both population genetics theory and natural history such that species with larger home ranges will have wider 'peripheries' than those with small home ranges.

Maps and geographic range

We used a digital version of Bailey's 'Ecosystems of North America' as our base map (Bailey 1998). Bailey subdivided North America into five ecodevelopments characterized by broad climatic similarities. The domains were each separated into divisions, characterized by the vegetational affinities of Köppen (1931) and Trewartha (1968). Lastly, the divisions were separated into province categories, identified by climatic zones, soil types and macro vegetation. We found evidence of either extant or recently extirpated (within the last 50 years) lynx populations in 12 province categories from six divisions and three domains (Table 1), encompassing 27 polygons from Bailey (1998). Using ARCTOOLS 7.1.2 we combined adjacent polygons that contained these province categories to produce our lynx geographical range map (Fig. 1; ESRI 1997). This map corresponds well to the high resolution map independently created by McKelvey *et al.* (2000) for the contiguous United States, but is extended to Canada and Alaska. Interestingly, one of our sampled lynx populations, Kuyuktuvuk Creek, Alaska, was outside our habitat association map and in the tundra–polar desert province of the polar domain. We

Table 1 A list of Bailey's eco-domains, divisions and provinces with extant lynx populations. The numbers and names of each domain, division and region correspond to Bailey (1998). ¹The only place where we subdivided a region is the deciduous or mixed forests – coniferous forest medium (M241). This region includes both the Cascade Mountains (WA and OR) and the Olympic Peninsula (WA) and Oregon Coastal Range (OR). Lynx have been reported only in the Cascades. After each province we provide a reference demonstrating evidence of lynx populations occurring in that area. ²Poole (1997), ³Stephenson *et al.* (1991), ⁴Ward & Krebs (1985), ⁵Erickson (1955), ⁶Mech (1980), ⁷Halter (1988), ⁸J. Vashon, Maine Department of Inland Fisheries and Wildlife, pers. comm., ⁹Koehler (1990), ¹⁰Apps (2000), ¹¹Koehler *et al.* (1979), ¹²J. Squires, USFS/Rocky Mountain Research Station pers. comm.

| Domain | Division | Province | Number |
|-----------------|-------------------------------|--|--------|
| Polar | SubArctic | | 100 |
| | | | 130 |
| | | Forest tundra, open woodland ² | 131 |
| | | Taiga (boreal forest) ² | 132 |
| | SubArctic mountains | | M130 |
| | | Open woodland-tundra ³ | M131 |
| | | Taiga/tundra/medium ⁴ | M132a |
| | | Taiga/tundra/high ⁴ | M132b |
| Humid temperate | Warm continental | | 200 |
| | | | 210 |
| | Warm continental mountains | Mixed deciduous/coniferous forest ^{5,6} | 211 |
| | | | M210 |
| | | Mixed forest, coniferous forest tundra medium ⁷ | M211a |
| | | Mixed forest, coniferous forest tundra high ⁸ | M211b |
| | Marine mountains ¹ | | M240 |
| | | Deciduous or mixed forest/coniferous forest medium ⁹ | M241 |
| Dry domain | Temperate steppe mountains | | 300 |
| | | | M330 |
| | | Forest steppe/coniferous forest/meadow/tundra ¹⁰ | M331 |
| | | Steppe/coniferous forest/tundra ¹¹ | M332 |
| | | Steppe/open woodland/coniferous forest/alpine meadow ¹² | M334 |

obtained the fewest samples for this population probably because Kuyuktuvuk Creek is at the extreme periphery of the lynx geographical range and may not represent a stable population.

We 'buffered' (ESRI 1997) the geographical range map towards the centre of the lynx geographical range by 165 km to define initially the periphery of the range (Fig. 1). This provided us with nine core and eight peripheral populations (Table 2). We also explored the influence of our definition of periphery by reducing the periphery by one-quarter and one-half and again comparing genetic variation measures between the core and the periphery.

Lastly, because of the novelty of our definition we also calculated the shortest distance between the approximate centre of each population and the edge of the geographical range and modelled the distance from the edge of a species' range with each measure of genetic variation.

Microsatellite loci

We isolated DNA from lynx tissue samples with the QIAmp DNA minikit using standard protocols (Qiagen, Hilden, Germany). The nine microsatellites (described originally in

the domestic cat), *FCA35*, *F41*, *FCA43*, *FCA45*, *FCA77*, *FCA78*, *FCA90*, *FCA96* and *FCA559* (Menotti-Raymond & O'Brien 1995; Menotti-Raymond *et al.* 1999), were in five different linkage groups (A1, A2, D2, B1 and C2) with the closest markers separated by 38 cM in domestic cats (*FCA35* and *FCA78*; Menotti-Raymond *et al.* 1999). All loci were dinucleotide repeats except *FCA559* and *F41*, which were tetranucleotide repeats. Each amplification was in a 10- μ L reaction volume comprised of 1 \times Perkin-Elmer *Taq* buffer; 1 unit of *Taq* polymerase; 0.8 mM MgCl₂; 200 μ M of each deoxynucleotide; and 1 μ M of each primer (labelled with a fluorescent dye – HEX or FAM). PCR were run in a thermal cycler (MJ Research PTC-200, Waltham, MA, USA) under the following conditions: 94 °C for 3 min; followed by 10 cycles of 94 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s; followed by 20 cycles of 89 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s, and completed with a step of 72 °C for 10 min. The subsequent products were electrophoresed using 7% polyacrylamide gels, and visualized on a florescent imager (Hitachi FMBIO-100, California). Allele sizes were estimated by comparing the allele to both lane size standards and samples with known allele sizes.

Table 2 Genetic diversity and sample size statistics for each population. H_O is observed heterozygosity, H_E is the mean expected heterozygosity, A is the mean number of alleles per locus. SE is one standard error from the mean. Populations are arranged from closest to the edge of the geographical range to furthest (i.e. in the order in which they are ranked on the x -axis in Fig. 3). In some analyses we re-sampled the Kenai and Fort Providence populations using only $n = 50$; statistics for the resampling are as follows: Kenai $A = 6.0$ (1.0), $H_O = 0.56$ (0.08), $H_E = 0.63$ (0.08); Fort Providence $A = 9.4$ (1.8), $H_O = 0.68$ (0.08), $H_E = 0.71$ (0.08)

| Location (165 km) | Population | Code | Sample size | A (SE) | H_O (SE) | H_E (SE) |
|-------------------|--------------------------|-----------|-------------|------------|-------------|-------------|
| Periphery | Kuyuktuvuk Creek, Alaska | KU | 7 | 4.8 (0.7) | 0.69 (0.10) | 0.66 (0.09) |
| | Susitna Lake, Alaska | SU | 35 | 6.7 (1.1) | 0.57 (0.08) | 0.66 (0.08) |
| | Kenai Peninsula, Alaska | KE | 115 | 6.7 (1.4) | 0.59 (0.08) | 0.65 (0.08) |
| | Copper Creek, Alaska | CC | 19 | 6.7 (1.2) | 0.72 (0.09) | 0.68 (0.09) |
| | Seeley Lake, Montana | SL | 32 | 7.6 (1.4) | 0.64 (0.07) | 0.66 (0.08) |
| | Kamloops, BC | KA | 25 | 7.1 (1.1) | 0.64 (0.09) | 0.66 (0.07) |
| | Paxson, Alaska | PX | 45 | 7.3 (1.1) | 0.72 (0.06) | 0.71 (0.06) |
| | Whitehorse, YU | WH | 52 | 8.2 (1.6) | 0.65 (0.08) | 0.69 (0.09) |
| | Mean (SE) | | | 6.9 (0.4) | 0.65 (0.02) | 0.67 (0.01) |
| Core | Kootnay-Banff, BC-AB | BC | 20 | 7.0 (1.1) | 0.62 (1.00) | 0.69 (1.00) |
| | Riverside, Alaska | RS | 43 | 8.3 (1.4) | 0.66 (0.09) | 0.71 (0.09) |
| | N. of Fairbanks, Alaska | NF | 19 | 7.3 (1.1) | 0.62 (0.07) | 0.71 (0.07) |
| | Ladue River, YU-AK | LA | 10 | 5.9 (1.0) | 0.72 (0.09) | 0.70 (0.08) |
| | Watson Lake, YU-BC | WA | 27 | 7.6 (1.2) | 0.67 (0.09) | 0.67 (0.08) |
| | Gold King Creek, Alaska | GK | 32 | 7.7 (1.3) | 0.66 (0.07) | 0.68 (0.09) |
| | W of Denali, Alaska | DE | 16 | 6.8 (1.2) | 0.69 (0.07) | 0.71 (0.06) |
| | Fort Providence, NT | NT | 84 | 10.1 (1.8) | 0.69 (0.07) | 0.71 (0.07) |
| | Rainbow Lake, BC | RB | 18 | 6.8 (1.2) | 0.67 (0.08) | 0.69 (0.08) |
| | | Mean (SE) | | | 7.5 (0.4) | 0.67 (0.01) |

Statistics

We tested for deviations from Hardy–Weinberg (HW) proportions with program GENEPOP (version 3.1d; Raymond & Rousset 1995). GENEPOP uses the Markov chain method of Guo & Thompson (1992) to calculate estimates of Fisher's exact test to assess the hypothesis of heterozygote deficiency in the sample. Because we had 17 populations and nine loci and tested across all loci for each population, we expected some significant deviations from HW proportions because of Type I errors. To minimize these Type I errors we used sequential Bonferroni tests to correct for multiple tests (Rice 1989). We also tested for genetic disequilibrium between marker pairs in each population using program GENEPOP and then used a Bonferroni correction.

We estimated genetic variability for each locus within a population by calculating the mean number of alleles (A), observed heterozygosity (H_O) and expected heterozygosity (H_E). Mean number of alleles per locus is expected to be more sensitive to sample size (n) and reductions in population size than heterozygosity (Allendorf 1986; Luikart *et al.* 1998). Therefore, we resampled the Kenai Peninsula and Fort Providence populations using only 50 samples to estimate A , H_O and H_E for our statistical analyses, because these populations were outliers in our sampling strategies

(Table 2). We tested A , H_O and H_E for differences between core and peripheral populations using general linear models (SAS 1999). In the basic model, we treated locus as a repeated measure within each population, and locus, location (i.e. core vs. periphery) and the interaction between locus and location as fixed factors.

Because of concerns that sample size affects mean number of alleles per locus (Leberg 2002), we constructed an additional model with sample size (n) as a covariate. We also evaluated a covariate interaction model adding interactions between n and locus, and n and location to the covariate model. We present results for models that are best supported on the basis of Akaike's information criteria (AIC); it is generally accepted that models within approximately four AIC values of the best approximating model are equally plausible (SAS 1999).

Results

Hardy–Weinberg (HW) proportions and gametic disequilibrium

After Bonferroni corrections nine tests (of 153) still deviated from HW proportions (Table 3). Loci *FCA35*, *FCA96* and *FCA45* diverged from HW proportions in two of 17 populations, while markers *FCA78*, *FCA90* and

Table 3 F_{IS} values at nine loci in 17 populations of lynx. Values in bold type indicate a significant ($P < 0.05$) deviation from Hardy–Weinberg proportions after Bonferroni corrections. Population codes are defined in Table 2

| | SL | KE | NT | SU | PX | CC | DE | RS |
|--------|--------|---------------|--------------|--------|--------|--------------|--------------|--------|
| FCA43 | -0.288 | 0.058 | -0.025 | 0.294 | 0.042 | -0.043 | -0.233 | -0.009 |
| FCA45 | 0.114 | 0.028 | 0.162 | 0.239 | 0.031 | -0.168 | 0.429 | 0.159 |
| FCA77 | -0.080 | -0.036 | -0.057 | -0.030 | 0.084 | -0.029 | 0.362 | -0.006 |
| FCA78 | -0.098 | -0.130 | 0.017 | 0.209 | -0.182 | 0.122 | -0.175 | 0.098 |
| FCA559 | -0.001 | 0.088 | -0.020 | -0.072 | -0.056 | 0.063 | 0.141 | 0.046 |
| FCA96 | 0.272 | 0.120 | 0.214 | 0.067 | 0.071 | -0.032 | -0.148 | 0.179 |
| FCA90 | -0.026 | 0.375 | -0.165 | 0.122 | 0.029 | -0.059 | -0.203 | 0.215 |
| F41 | 0.063 | 0.156 | -0.060 | 0.067 | 0.035 | -0.262 | -0.010 | -0.074 |
| FCA35 | 0.115 | 0.104 | 0.106 | 0.279 | 0.072 | -0.009 | -0.069 | -0.040 |
| All | 0.035 | 0.090 | 0.030 | 0.141 | 0.009 | -0.047 | 0.020 | 0.067 |

| | RB | WA | WH | BC | KA | GK | KU | LA | NF |
|--------|--------|--------------|--------------|--------------|--------|--------|--------|--------|--------|
| FCA43 | -0.321 | -0.231 | 0.147 | -0.209 | -0.037 | -0.180 | 0.273 | -0.145 | 0.173 |
| FCA45 | 0.161 | 0.288 | 0.039 | 0.014 | 0.017 | 0.211 | -0.042 | 0.176 | 0.262 |
| FCA77 | -0.090 | -0.045 | -0.032 | NA | -0.011 | -0.033 | 0.000 | 0.000 | 0.349 |
| FCA78 | 0.060 | -0.256 | 0.001 | 0.145 | 0.016 | 0.068 | -0.136 | 0.060 | 0.194 |
| FCA559 | 0.027 | 0.094 | 0.071 | 0.143 | -0.008 | 0.169 | -0.091 | -0.098 | 0.113 |
| FCA96 | 0.142 | 0.142 | 0.035 | 0.518 | 0.120 | -0.023 | -0.034 | -0.117 | 0.128 |
| FCA90 | 0.215 | 0.023 | -0.116 | 0.023 | -0.124 | -0.122 | -0.250 | 0.069 | -0.026 |
| F41 | 0.004 | -0.059 | 0.023 | 0.140 | -0.032 | -0.027 | 0.167 | -0.009 | 0.110 |
| FCA35 | 0.014 | -0.055 | 0.213 | 0.074 | 0.175 | 0.053 | -0.176 | -0.104 | 0.014 |
| All | 0.029 | 0.000 | 0.060 | 0.099 | 0.027 | 0.038 | -0.046 | -0.021 | 0.134 |

FCA559 deviated in one of 17 populations (Table 3). The only population that had greater than one of the nine markers depart from HW proportions was the Kenai Peninsula, where three markers (FCA78, FCA90 and FCA35) departed from HW proportions. Eight of nine significant deviations from HW proportions were associated with a positive F_{IS} . This is most likely because our samples from some populations unintentionally contained parent and offspring pairs, producing an excess of homozygotes relative to HW proportions.

Gametic disequilibrium was detected in five marker pairs (FCA45/FCA559, FCA45/FCA96, FCA78/FCA96, FCA90/FCA35 and FCA41/FCA35) of a possible 612 pairwise comparisons (testing for each locus pair within each population separately). As these five marker pairs were among five different pairs of loci and four different populations we continued with our analysis, assuming loci are, for the most part, independent (cf. Paetkau *et al.* 1999).

Genetic variation

Mean number of alleles per locus was highest in the Northwest Territories population (NT: 10.1 ± 1.8 ; core population) and lowest in Kuyuktuvuk Creek population (KU: 4.8 ± 0.7 ; peripheral population; Table 2). Mean H_O was highest in Ladue River, Yukon (LA: 0.72 ± 0.09 ; core

population) and lowest in the Susitna Lake population (SU: 0.57 ± 0.08 ; peripheral population; Table 2), and mean H_E was highest in the samples collected north of Fairbanks (NF: 0.71 ± 0.07 ; core population) and lowest in the samples collected from the Kenai Peninsula (KE: 0.65 ± 0.08 ; peripheral population).

Both mean number of alleles per population and expected heterozygosity tended to decrease in the periphery, with different models being the most parsimonious for different metrics of genetic variation. For each locus, core populations had a greater mean number of alleles per population than peripheral populations using the covariate model without interactions that controlled for n ($F_{1,15} = 7.48$, $P = 0.02$, Table 2; AIC for the covariate model was 4.5-values greater than the basic model). To further evaluate this result, we used a parallel approach to examine the relationship between mean number of alleles per population and distance from the edge of the lynx's geographical range. In a model that included both n and locus, mean A increased significantly with distance ($F_{1,15} = 6.64$, $P = 0.02$; Fig. 3). Mean n per population did not vary with distance of the population from the edge of the lynx's geographical range (Pearson's $r = -0.086$, $P = 0.74$).

The basic model, which included only locus, location and the interaction between locus and location, was most supported for testing differences between both H_O and H_E

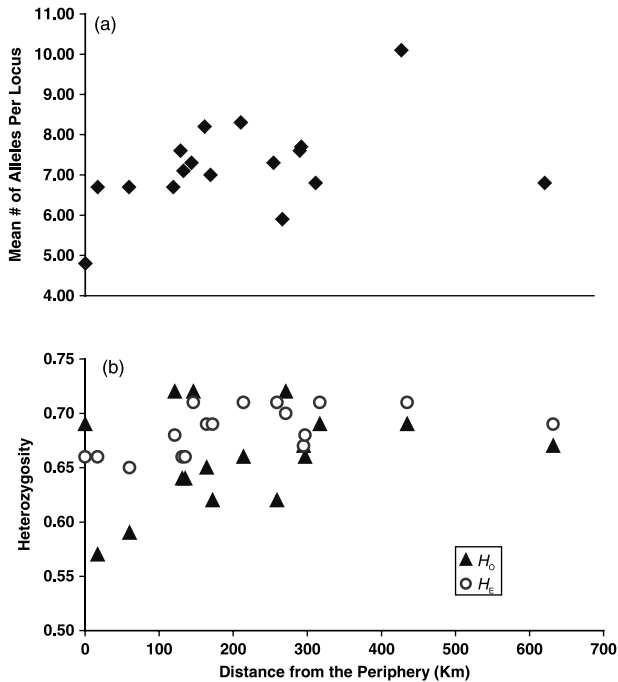


Fig. 3 Plot of three measures of genetic variation in lynx (averaged per population) vs. distance of the population from the edge of the geographical range. The top graph (A) is mean number of alleles per locus vs. distance from the periphery, and the bottom graph (B) is expected heterozygosity vs. distance from the periphery and observed heterozygosity vs. distance from the periphery. N is not accounted for in these graphs, but is accounted for in the statistical relationships presented in the text. Mean n per population did not vary with distance of the population from the edge of the lynx's geographical range (Pearson's $r = -0.086$).

in core and peripheral populations (> 10.5 AIC values better than the covariate and covariate–interaction model). This model showed no difference in H_O between core and peripheral populations ($F_{1,15} = 0.61$, $P = 0.45$). On the other hand, we found a difference in H_E between populations located in the core and periphery of the lynx's geographical range ($F_{1,15} = 7.02$, $P = 0.02$; > 13.7 AIC values better than the next competing model). Using parallel models to evaluate these variables as a function of distance from the edge of the lynx's geographical range yielded a nonsignificant relationship for H_O ($F_{1,15} = 1.61$, $P = 0.22$; Fig. 3), but a positive and significant correlation for H_E ($F_{1,15} = 4.80$, $P = 0.04$; Fig. 3).

Other definitions of periphery

We explored the impact of more restrictive definitions of periphery (123 km and 82 km periphery). Again, we found a higher mean number of alleles per population in core populations using the covariate model (123 km: $F_{1,15} = 7.00$, $P = 0.02$, AIC = 498.6; 82 km: $F_{1,15} = 12.97$, $P = 0.003$, AIC

= 486.9). The basic model (without n) was also well supported and showed a strong location effect (123 km: $F_{1,15} = 4.35$, $P = 0.05$, AIC = 501.3; 82 km: $F_{1,15} = 8.13$, $P = 0.01$, AIC = 490.7).

There were still no differences in H_O between the core and periphery under the basic model, which was the best supported model (123 km: $F_{1,15} = 0.1$, $P = 0.76$, AIC > 10.1 -values higher than the next model; 82 km: $F_{1,15} = 0.01$, $P = 0.94$, AIC > 10.0 -values higher than the next model). Furthermore, we still found differences in H_E , with the basic model being the most supported (123 km: $F_{1,15} = 8.47$, $P = 0.01$, AIC > 13.6 -values higher than the next model; 82 km, $F_{1,15} = 5.19$, $P = 0.04$, AIC 9.5-values higher than the next model which included n).

Discussion

Some locations on the landscape are expected to have low genetic variation. For example, island populations typically have small population size, thus decreased genetic variability and increased probabilities of extinction (Ashley & Willis 1987; Frankham 1998, 2001). Similarly, peninsulas have been implicated as places on the landscape where genetic variability is reduced, presumably because of small population size and isolation (Gaines *et al.* 1997). The extent to which the periphery of a mainland population acts as a landscape feature where genetic variation is reduced has been unclear.

In this study, we found evidence for decreased genetic variation at the periphery of the lynx's geographical range. Peripheral populations had fewer mean number of alleles per population, using our operational definition of periphery and a test based on relative distance from the edge of the species' range. Similarly, H_E was lower in populations located on the periphery of the lynx's geographical range; however, this pattern was not found with H_O . This apparent discrepancy between genetic variation measures is not surprising because as populations become small, rare alleles are rapidly lost while observed heterozygosity is diminished more slowly (Nei *et al.* 1975; Allendorf 1986; Luikart *et al.* 1998).

Genetic variation may be higher in the core of the geographical range for several reasons. First, peripheral populations tend to have smaller population sizes than core populations, which would lead to an expected reduction of heterozygosity and allelic diversity compared to a larger core population. Similarly, genetic variation may be reduced in the periphery due to a limited number of connections to other populations. For example, no populations of lynx exist to the west or south of the Seeley Lake, Montana population. Seeley Lake's only possible connections are to the north, whereas a central Alaskan population (e.g. Gold King Creek) can exchange migrants in all directions. Exchanging migrants in a metapopulation can boost N_e , and ultimately genetic

variation (Hedrick 1996). Thus, the simple geometry of being peripheral may lead to reductions in genetic variation.

Alternatively, core populations may have greater genetic diversity than peripheral populations because of large-scale, historic, landscape events. For example, a core population may be the result of mixing between two previously isolated peripheral populations. If lynx arrived in North America during an early glaciation, the last glaciation may have driven lynx and other carnivores into southern refugia. If several small, isolated lynx populations persisted in these refugia we may expect genetic drift to reduce genetic variation in each refugia. Subsequently, as glaciers retreated and lynx expanded their geographical range, genetic mixing between refugia stock may have occurred in the core of the range, thus boosting genetic variation in core populations.

Third, the pattern of genetic variation that we see today may be a result of historical microevolutionary or ecological forces, and not the result of current dynamics. For example, ebbs and flows in a species' geographical range may disproportionately change the size of peripheral populations over time, leading to drastic reductions in N_e , ultimately decreasing genetic variation. In addition, other forces such as historic migration or isolation may not be currently detected, but may have had large impacts on existing genetic variation.

The effect sizes we found in this study are not large (Table 2), but they are consistent and may be biologically meaningful. Importantly, our sampling scheme was biased towards having larger numbers of individuals sampled in the periphery (using our 165 km definition of periphery we had 330 samples collected in the periphery vs. 269 in the core). This would act to reduce differences between the core and peripheral populations, as mean number of alleles per population in the periphery could be inflated (Leberg 2002). The fact that we still found significantly less genetic variation in the periphery suggests that this effect may be larger given a more balanced sampling design. Therefore, we believe that this effect is real and not an artifact of our study design or sampling.

Slight differences in genetic variation may be the critical evolutionary potential needed for population persistence (Frankel 1974). In fact, populations with higher amounts of genetic variation have shown greater chances of surviving ecological or evolutionary changes (e.g. Quattro & Vrijenhoek 1989; Leberg 1993). Several researchers have shown that small changes in genetic variation can lead to large changes in population fitness (e.g. Frankham 1995b). On the other hand, the differences in heterozygosity shown in this study were small enough that Schwartz *et al.* (2002) estimated a very low global F_{ST} , suggesting a lack of significant population subdivision. Population subdivision is not supported by these data; movement was sufficient enough to keep pairwise estimates of F_{ST} low (Schwartz *et al.* 2002).

However, these data also do not support a panmictic system (nor should we expect one, given the biology of lynx). In this study we do not provide evidence against high levels of gene flow – clearly, lynx disperse and breed often – but instead show that gene flow is probably not strong enough to offset some loss of genetic variation caused by drift at the periphery of the lynx's geographical range.

We cannot determine whether the reductions in genetic variation for lynx at the periphery are due to human disturbance. If the reduction in genetic variation in peripheral populations was completely anthropogenic we would expect to see reductions only on the southern periphery where human impacts are greatest; this was not the case. Thus, the effect may be a result of biogeography.

In our analyses we examine genetic variation as a function of both a categorical variable (core vs. periphery) and a continuous variable (distance from the edge of the geographical range). Defining populations as core or peripheral is ubiquitous in the literature; thus we opted to provide, at minimum, an operational definition of core and periphery that can be generalized to other species. Knowing that some will object to our definition we wanted to show that our results were robust, and thus used the continuous variable as well.

We based our operational definition of core and periphery on home range instead of the maximum (or average) distance an animal travels in a given period of time for several reasons. The home range is defined as the area traversed by an individual in its normal activities of foraging, mating and parental care (Burt 1943), encompassing measures of average daily movements. Because this definition includes mating it also includes the normal spread of genes within and between populations. In addition, there is vastly more information on home range sizes of animals than on dispersal distances. For example, most data on lynx dispersal distances are anecdotal (Mowat *et al.* 2000). There is one documented case of a lynx moving 1100 km before being killed by a trapper (Mowat *et al.* 2000). This event may be anomalous compared to other lynx movements, such that derived definitions of periphery would be irrelevant. Information on dispersal may improve with the advent of satellite and global positioning system (GPS) technology; however, the number of animals for which long-distance dispersal is recorded will probably always be less than the number of animals for which home range can be estimated.

When data are plentiful for a species we would recommend using more complex operational definitions of periphery. For example, for some species incorporation of parameters such as differences in male vs. female home ranges with associated population sex ratios would make for a more precise estimation of the periphery. Alternatively, home range may vary by age or stage classes, and these data may be used to refine a definition of periphery.

The bottom line is that whatever definition is used for periphery it should be: (1) explicit, (2) rooted in the biology of the organism to the extent that the natural history data is available and (3) must be founded in evolutionary, conservation or population dynamic theory. We also recommend exploring the sensitivity of the results to any operational definition, such as we did by reducing the periphery by one-quarter and one-half.

Our basic definition of periphery can be adapted to other species with weaker dispersal capabilities that have known home ranges. There is a strong correlation between dispersal distances and home range for many species (Bowman *et al.* 2002); in cases where this correlation is known to be weak, other life-history information should be used to define periphery. Our definition does not work well for immobile species, such as plants. For plants dispersal of either pollen or seed may be a more pliable and pertinent measure. Again, our goal here was not to create a universal definition that works for all species, but rather to provide an explicit, flexible definition rooted in evolutionary theory. Overall, we encourage the wider use of operational definitions of core and periphery that scale to the biology of the organism under study, and greater examination of genetic data in a biogeographical and physiognomic context.

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