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RESEARCH ARTICLE

Patterns of within and between-colony microsatellite variation in the endangered Vancouver Island marmot (*Marmota vancouverensis*): implications for conservation

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Abstract Among the 14 extant species of the genus *Marmota* the Vancouver Island marmot (*Marmota vancouverensis*) is the most endangered. In 2007 as few as 85 individuals were left in the wild, with an additional 162 individuals maintained in captivity. To facilitate genetic monitoring of both wild and captive populations, polymorphic genetic markers were identified. Thirty-three different microsatellite loci were tested for amplification and variability in ≥30 wild-born individuals. Only 11 of these loci proved to be polymorphic and were subsequently analysed in 105 samples collected from wild Vancouver Island marmots. The average number of alleles (A) at those 11 loci was only 2.1, and the intraspecific variation (H_E between 8 and 23% within colonies) was low compared to other marmot species. Variation within the small and geographically isolated Mt. Washington colony was particularly low ($A = 1.3$, $H_E = 0.08$). Genetic distances between the Mt. Washington colony (11 individuals) and those of the Nanaimo Lakes region (94 individuals) on southern Vancouver Island were large (D values ranging from 0.42 to 0.50), while genetic distances among colonies within the latter area were much smaller (D values from 0.01 to 0.13). Given the low within-population genetic variation, and the resulting risk of inbreeding depression at Mt. Washington, we support the decision to maximize overall genetic variation of the species by crossbreeding marmots from the two different areas despite the possibility of local adaptation.

Keywords Genetic variability · Microsatellite analysis · Vancouver Island marmot · Endangered species · Conservation · Captive breeding program

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Introduction

The value of genetic research towards long-term conservation success has long been advocated (e.g., Frankel and Soulé 1981; Lande 1988). Given the large advances in modern molecular techniques and analytical tools, genetic analyses of endangered species have become increasingly important for conservation research and management (e.g., Hedrick 2001; Schwartz et al. 2007). In this paper we report results from microsatellite analyses designed to sample patterns of genetic variation in wild populations of a critically endangered mammal. Our intent was to provide managers with improved knowledge of population structure, and so enhance both captive-breeding and reintroduction programs.

The Vancouver Island marmot (*Marmota vancouverensis*) is a North American marmot species endemic to Vancouver Island, British Columbia, Canada (Nagorsen 1987). It is a member of the subgenus *Petromarmota*, together with the yellow-bellied marmot (*M. flaviventris*),

the hoary marmot (*M. caligata*), and the Olympic marmot (*M. olympus*). Genetic analyses suggest a close relationship to Olympic and hoary marmots (Kruckenhauser et al. 1999; Steppan et al. 1999). The Vancouver Island marmot originated fairly recently (Nagorsen 1987). Nevertheless, it is also the most divergent marmot with respect to cranial morphology (Cardini et al. 2005), and has unusual behavioural traits, characteristic vocalizations (Heard 1977; Blumstein 1999), and a unique, melanistic fur colour (Barash 1989). Thus, the Vancouver Island marmot might have undergone relatively fast differentiation after isolation from the mainland species.

The Vancouver Island marmot was among the first species to be listed as endangered in Canada (in 1978; Shank 1999). It is the only ground squirrel native to Vancouver Island (Banfield 1977). The natural habitat consists of small subalpine meadows 900–1,400 m in elevation (Milko and Bell 1986). Vancouver Island marmot colonies in historical times were generally small, with most containing only a single family group and fewer than five adults. The entire population has been described as a metapopulation, consisting of small colonies that occasionally suffer local extinction and recolonization (Bryant and Janz 1996). Dispersal is a fundamental aspect of the population ecology of this species, as is true for other marmot species (Ozgul et al. 2006; Griffin et al. 2008).

Although historic population sizes remain unknown, it is clear that Vancouver Island marmots experienced both rapid expansion and rapid declines during the last few decades of the 20th Century. Systematic population counts began in 1979. During the early 1980s some dispersing marmots colonized new habitats created by clear-cut logging of high elevation primary forests. As a consequence the population increased, with about half of the known population living in man-made clear-cuts by the mid 1980s (Bryant and Janz 1996). These new habitats proved to be unstable due to natural reforestation. Thus, populations did not survive over longer periods and most colonies declined sharply after 1994 (Bryant 2000). Annual population counts suggest that in the mid-1980s the Vancouver Island marmot population consisted of 300–350 individuals, declining to fewer than 100 in 1998 (Bryant 1998) and fewer than 35 individuals by 2003 (Bryant and Page 2005). In addition to losses of habitat due to natural forest regeneration, marmot populations in both natural and clear-cut habitats also suffered increasing levels of predation (Bryant and Page 2005) from wolves (*Canis lupus*), cougars (*Puma concolor*) and golden eagles (*Aquila chrysaetos*).

With the wild population nearing extinction by 1997, a captive breeding program was started at the Toronto Zoo. The program was later expanded, with marmots eventually breeding at Toronto, Calgary Zoo, Mountain View Farm near Vancouver, and a specially-designed facility on Vancouver Island that is situated in close proximity to natural marmot habitats (Bryant 2005). A total of 56 wild-born individuals were brought into captivity between 1997 and 2004 (Bryant 2007). This is a relatively large number compared to some other endangered species which are bred in captivity (e.g., Black-footed ferret: 18, European bison: 12, Red-ruffed lemur: 7; summarized by Frankham et al. 2004). As of 2007, 231 pups had been born in captivity, and 96 marmots had been returned to the wild. The population presently includes 162 captive and approximately 85 wild individuals, a remarkable increase brought about by intensive conservation efforts (unpublished minutes, Vancouver Island Marmot Recovery Team, 22nd October 2007).

In this paper we use microsatellite methods to describe the genetic structure of wild Vancouver Island marmots sampled from recently-occupied colonies. Our purpose was to establish whether Vancouver Island marmots were highly inbred compared to other marmot species, to identify patterns of genetic variation among colonies, and to determine whether the variation could be explained by standard isolation-by-distance models. All of these questions have direct relevance for future management of the captive-breeding program, particularly in terms of establishing breeding pairs that would retain maximum genetic variability from the wild genome (Bryant 2005). As such they are of critical importance towards eventual recovery of wild marmot populations.

Methods

Sampling

Vancouver Island marmots were live-trapped from 1993 through 2000 as part of a longer-term demographic study (Bryant 1998, 2005). Trapping and handling methods followed Bryant (1996). For DNA analyses hair samples were collected and stored dry in paper envelopes. All marmots were ear-tagged, thus preventing collection of duplicate samples. Although colony-specific sample sizes were variable, the cumulative sample of $n = 105$ individuals represents both the complete geographic range of recently-occupied colonies (Fig. 1), and a relatively large proportion (30–70%) of the wild population known to be alive during those years (Bryant 1998; COSEWIC 2008). Samples were obtained from six colonies in natural meadows (Green Mountain, Haley Lake, "P" Mountain, "Big Ugly", Heather Mountain, and Mt. Washington) and six colonies in clearcuts (Sherk Lake, K44a, Pat Lake, D13E, Butler Peak, and Mt. Franklin). All colonies except Mt. Washington were located in the Nanaimo Lakes region (Fig. 2).

The samples reflected a variety of age-classes (mean = 1.3 years, SD = 1.7 years, range = 1–9 years). Approximately half ($n = 52$) of the samples were from females.

DNA extraction

DNA extraction from hairs was done using ten hair roots per individual, which were rehydrated for 30 min at room temperature and subsequently incubated for 1.5 h at 60°C in a 200 µl Chelex suspension (10% Chelex 100 Resin in distilled water) with periodical mixing. After 8 min incubation at 95°C the mixture was vortexed and spun. From the supernatant 150 µl DNA solution were taken and stored in aliquots at −20°C.

Microsatellite analysis

PCR amplifications were conducted at two labs. Initial work was undertaken at University of Victoria, where amplifications were performed on a PTC-100^{TM} Programmable Thermal Controller (MJ Research Inc.) in a volume of 12.5 µl containing 10 mM Tris–HCl (pH 8.8), 1.5 mM $MgCl_2$, 150 mM KCl, 0.1% Triton X-100, 0.5 U DynaZyme DNA-polymerase (Finnzymes OY), 6 pM of each primer, 200 µM of each dNTP, and 2 µl template DNA, directly from the DNA extraction. The PCR reaction was covered with 30 µl mineral oil (ACP). The amplification protocols differed for the various primer pairs (Appendix 1, Table 3). A volume of 2 µl loading buffer was added to the PCR reaction and the PCR fragments separated on a 10% non-denaturising polyacrylamide gel (19:1 acrylamide to bis-acrylamide) in 2× TAE buffer at 80 V for 18–26 h. DNA fragments were stained with ethidium bromide (for 20 and 30 min destaining in distilled water) and visualized on a UV screen. Photographs were taken digitally (Stratagene Eagle Eye TM II). Alleles were scored by eye using a 1 kb length marker (Gibco) and a 20 bp superlow marker (Gensura) on the same gel as well as by comparisons with individuals of known genotypes. The accurate fragment sizes were calculated with the Bio Image Intelligent Quantifier 2.1.2.a software (B. I. Systems Corp.).

A second round of PCR amplifications were conducted on the same samples in the Montana Conservation Genetics Lab at the University of Montana. Additional loci were screened and samples were reamplified at all but three of the polymorphic loci identified in the previous analysis. Three multiplex PCRs and two single locus PCRs were optimized and 10 µl PCR reactions were performed on MJR PC200 thermocyclers using touch-down profiles (Appendix 1). The multiplex reactions contained: 2 µl of template DNA, 5 µl QIA multiplex mix (QIAGEN), and 1 µl 10× primer mix (primer concentrations in Appendix 1, Table 4). The single locus reactions contained: 2 µl of template DNA, 3.5 mM Invitrogen buffer, 2 pM forward and reverse primers, 400 µM dNTPs, $MgCl_2$ (3.5 µM

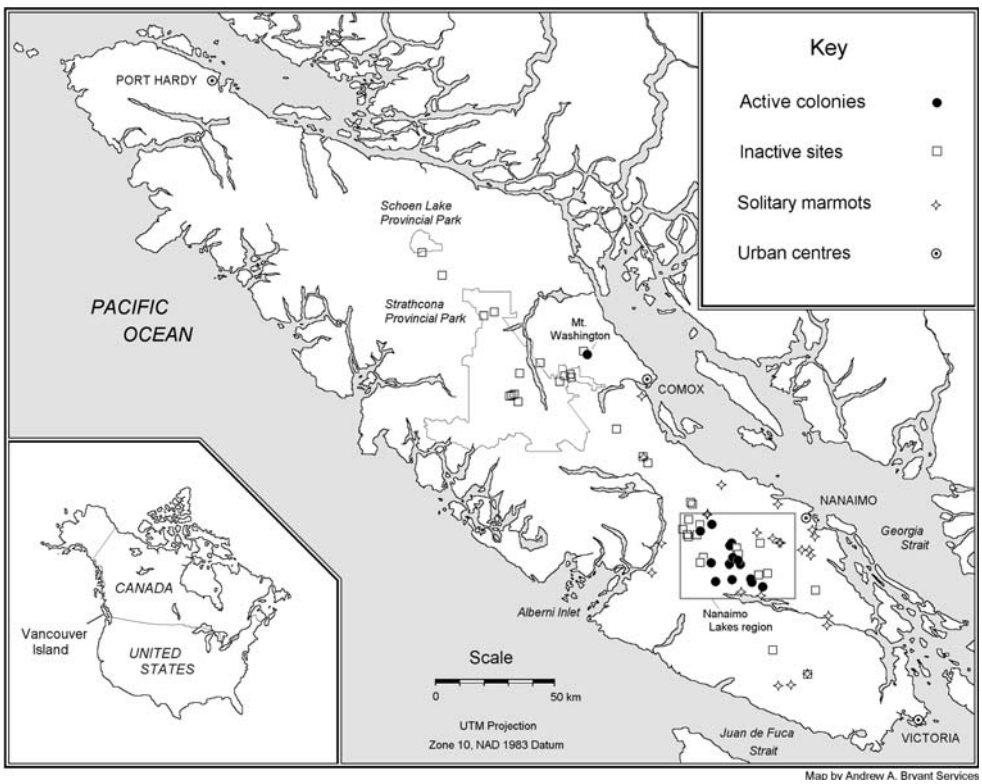

Fig. 1 Recently-active (1995–2000) and historical (1896–1994) location records for *M. vancouverensis*. The isolated Mt. Washington colony is located approximately 100 km from the cluster of recently-occupied sites in the Nanaimo Lakes region. The rectangle represents an area of about 1,000 km^2 and is shown in greater detail in Fig. 2. Adapted from Bryant and Janz (1996)

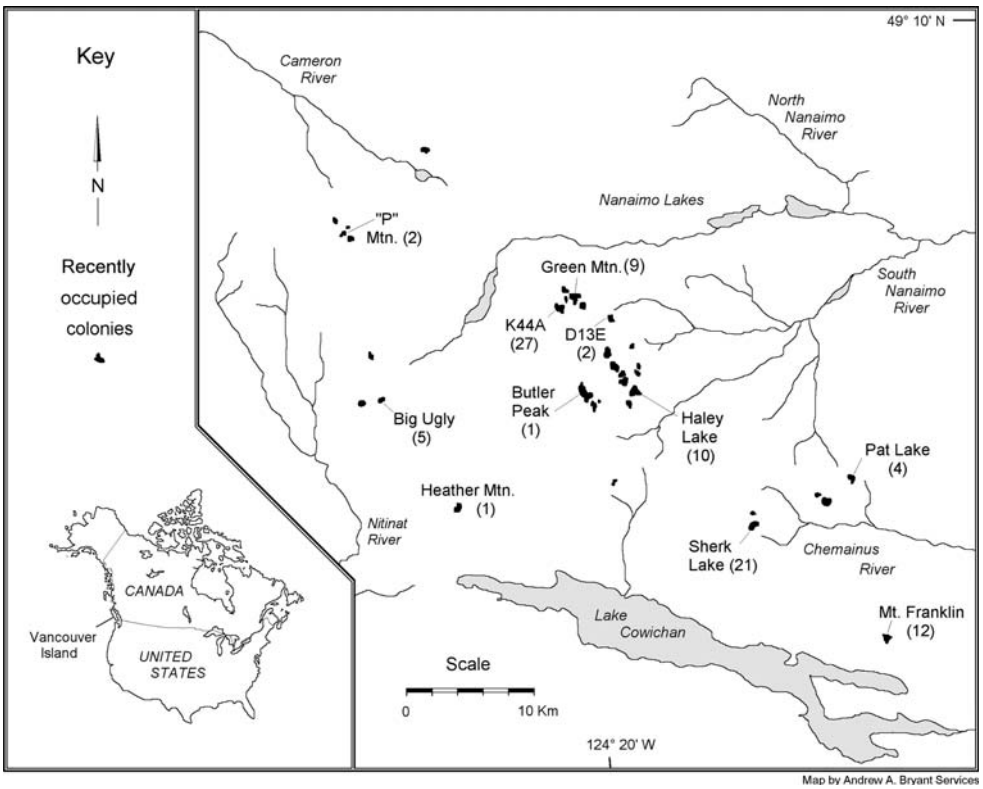

Fig. 2 Geographic distribution of marmot hair samples within the Nanaimo Lakes region. Active colonies during 1995–2000 are shown as black polygons, with names following Bryant and Janz (1996). Numbers of individuals sampled are shown in parentheses. The isolated Mt. Washington colony (from which $n = 11$ samples were obtained) does not appear on this map (see Fig. 1)

GS17, 2.5 μM MA018), and 0.8 U Invitrogen Platinum taq. The amplification conditions differed between the multiplex and single locus PCRs (Appendix 1, Table 4). Multiplex 2, GS17, and MA018 were combined at equal concentrations before fragment analysis. Fluorescently labelled DNA fragments were visualized on an ABI 3130xl automated capillary sequencer with the GS500 ladder (Applied Biosystems) and analyzed with GeneMapper software v3.7 (Applied Biosystems).

Data analysis

Deviations from Hardy–Weinberg equilibrium were calculated for each of the 11 polymorphic loci and a genotypic disequilibrium test was carried out with GENEPOP version 3.1d (Raymond and Rousset 1995).

We used the Bayesian methodology of STRUCTURE (Pritchard et al. 2000) to estimate the number of populations (K) within the Nanaimo Lakes colonies. This procedure has the advantage of not assuming a priori assignment of individuals to particular populations or colonies based on capture locations, allowing the use of all individuals irrespective of colony size. We assumed an admixture model with correlated allele frequencies (Falush et al. 2003). Ten independent runs of K = 1–12 were carried out each at 100,000 Markov Chain Monte Carlo

Table 1 Average number of alleles per locus (A), number of private alleles over all loci examined (pA), observed (H_O) and expected (H_E) heterozygosity, and departures from Hardy–Weinberg equilibrium (F_{IS})

	A	pA	H_O	H_E	F_{IS}	P	N
Populations							
Mt. Washington	1.27	5	0.07	0.08	0.02	NS	11
Nanaimo lakes region	1.40	6	0.21	0.20	−0.01	NS	94
Subpopulations							
“Northern” NL colonies	1.49	0	0.19	0.23	−0.09	0.02	61
“Southern” NL colonies	1.50	0	0.20	0.17	−0.04	NS	33
Nanaimo Lakes colonies							
Green Mtn.	1.55	0	0.29	0.23	−0.28	NS	9
K44A	1.55	0	0.29	0.23	−0.25	NS	27
Big Ugly	1.36	0	0.13	0.12	−0.11	NS	5
Haley Lake	1.64	0	0.18	0.18	−0.02	NS	10
Pat Lake	1.36	0	0.25	0.18	−0.50	NS	4
Sherk Lake	1.55	0	0.15	0.15	−0.01	NS	21
Mt. Franklin	1.45	0	0.24	0.19	−0.30	NS	12

N number of sampled individuals. Estimates are given for Nanaimo Lakes (NL) colonies separately as well as for population clusters suggested by later analyses (i.e., Mt. Washington, all NL colonies treated as one metapopulation = Nanaimo Lakes region; Sherk Lake + Mt. Franklin = “southern” NL colonies; remaining Nanaimo Lakes colonies = “northern” NL colonies). Colonies with fewer than four sampled individuals were excluded from these analyses

Table 2 Genetic differentiation of eight Vancouver Island marmot colonies based on 11 microsatellite loci

	Mt. Wash.	Green Mtn.	K44a	Big Ugly	Haley Lake	Pat Lake	Sherk Lake	Mt. Franklin
Mt. Washington	–	0.45	0.44	0.42	0.43	0.50	0.42	0.44
Green Mtn.	0.73*	–	0.02	0.03	0.04	0.04	0.09	0.13
K44a	0.66*	0.08	–	0.04	0.04	0.01	0.03	0.06
Big Ugly	0.80*	0.11	0.13*	–	0.04	0.10	0.08	0.13
Haley Lake	0.76*	0.15*	0.14*	0.19*	–	0.04	0.03	0.08
Pat Lake	0.81*	0.12	0.03	0.36*	0.17	–	0.04	0.08
Sherk Lake	0.75*	0.30*	0.10*	0.33*	0.16*	0.17	–	0.02
Mt. Franklin	0.74*	0.34*	0.19*	0.40*	0.26*	0.27*	0.11*	–

Data above the diagonal are Nei's (1978) genetic distance (D values), and those below the diagonal are Wright's fixation coefficients (F_{ST}). Significant ($P < 0.05$) differences among pair-wise F_{ST} values are indicated by an asterisk. The data illustrate significant genetic differences between marmots from the northern Mt. Washington colony and the seven colonies from the Nanaimo Lakes region. As in Table 1, colonies from which fewer than four individuals were sampled were excluded from the analyses

(MCMC) simulations following a burn-in of 10,000 repetitions. Following Pritchard et al. (2000), we used the value of K with the maximum posterior probability given by the data, Pr(X/K), to identify the most likely value for K. Outputs from STRUCTURE were graphed using DISTRUCT (Rosenberg 2004). In addition we used ΔK as described in Evanno et al. (2005), the maximum second order rate of change of Pr(X/K) standardized by the standard deviation of Pr(X/K) as calculated by STRUCTURE, as an estimator for the most likely number of populations.

GENEPOP version 3.1d was also used to assess the genetic structure within populations by calculation of allele frequencies, average observed (H_O) and expected (H_E) heterozygosity, average number of alleles per locus (A), probability of deviations from Hardy–Weinberg equilibrium and their significance (P), and the inbreeding coefficient (F_{IS}, Weir and Cockerham 1984). The genetic distances between populations were estimated by Nei's standard distance corrected for small sample size (D, Nei 1978) and the F_{ST} values between the populations (Weir and Cockerham 1984). All distance matrices were calculated with the software MICROSATELLITE ANALYZER (MSA 4.00; Dieringer and Schlötterer 2003). Significance levels for F_{ST} values were determined by permutating alleles 10,000 times among population pairs. The option assuming Hardy–Weinberg equilibrium was used, since no significant deviation was found for the Nanaimo Lakes colonies.

We used the software package PHYLIP version 3.65 (Felsenstein 1989) to construct unrooted neighbour-joining trees from the D distances. To determine support of the nodes, 1,000 replicates of the D distance matrices were produced by bootstrapping with MSA 4.00. This file was used as input for the neighbour option in PHYLIP, and a majority rule consensus tree was constructed from the resulting trees. The neighbour-joining tree was drawn with TREEVIEW version 1.6.5 (Page 1996) and manually adapted for the printed version.

We evaluated genetic variables (F_{ST} and D) and between-colony geographic distances from known colony locations (Bryant 1998). Significance of possible relationships were evaluated using Mantel tests (Mantel 1967) as implemented in the program IBD (Bohonak 2002), with 1,000 bootstrapped replicates for each pair-wise comparison. To minimize bias created by small sample sizes, input files in this case represented colony-specific F_{ST} and D variables using only those colonies from which at least four individuals were sampled. We also evaluated the significance of isolation by distance using several subsets of data suggested by previous analyses including (a) all colonies, (b) Nanaimo Lakes colonies with Mt. Washington removed, and (c) Nanaimo Lakes colonies with Mt. Washington and the Sherk Lake and Mt. Franklin colonies removed.

Results

Amplification of microsatellites

Thirty-three different primer pairs were tested for amplification of microsatellites in 30 individual Vancouver Island marmots. Five primer pairs did not produce unambiguous products and were therefore excluded from further analyses (SC4, SB10: Hanslik and Kruckenhauser 2000; IGS-BM1: May et al. 1997; GS20, GS26: Stevens et al. 1997). A total of 17 loci were monomorphic (MA091: da Silva et al. 2003; BIBL31: Klinkicht 1993; MS6, MS41, MS45, MS47, SC2, ST7, SX: Hanslik and Kruckenhauser 2000; IGS-1, IGS-6, IGS-110b, IGS-24d, IGS-BP1, IGS-CK1: May et al. 1997; GS12, GS14: Stevens et al. 1997). Only 11 of the tested microsatellite loci proved to be polymorphic and were analysed in all 105 samples (Appendix 1). Allele frequencies for the sampled colonies are given in Appendix 2.

Null alleles and linkage

To account for the possibility of null alleles, genotype distributions at the various loci were tested for deviations from Hardy–Weinberg equilibrium in those colonies with a sample number ≥10 (Mt. Franklin, Haley Lake, K44A, Sherk Lake, Mt. Washington). As no significant deficit of heterozygotes was observed, we concluded that the possibility of null alleles could be ignored for further analyses of the data set. To check for independence among the microsatellite loci, a test for genotypic disequilibrium was performed. There was no pair of loci with a significant *P*-value, suggesting that the loci are not physically linked, and thus all loci were retained for analysis.

Estimating the number of populations

We used STRUCTURE to estimate the probable number of "real" populations; the advantage of this method is that is uses only genetic data, ignoring geographic data, in order to assign individuals to particular population clusters. STRUCTURE analyses recorded highest posterior probability of the data for three [Pr(X/K: 3) = −693,81] clusters. However, posterior probabilities were similar for other K values and the variance of Pr(X/K) increased markedly as K increased. Therefore, it was not possible to determine unambiguously the most likely number of populations by the maximum Pr(X/K) criterion. The modal value of ΔK was at $K = 2$ with a high rate of change in ΔK, suggesting a strong signal in the data (Evanno et al. 2005). However, it seems possible that the clear separation of the Mt. Washington colony overrules the population subdivision signal within the Nanaimo Lakes colonies. Excluding this population from the dataset to distinguish between the probability of two or one populations is not possible since ΔK can not be calculated for $K = 1$. Assignment probabilities of individual genotypes placed all Mt. Washington individuals in one cluster. In addition, some structure within the Nanaimo Lakes region colonies becomes apparent (Fig. 3). All individuals from Mt. Franklin as well as most individuals from Sherk Lake were assigned to one cluster. Individuals from all other locations in the Nanaimo Lakes region formed a separate cluster but the individual assignment was seldom clear. For example, individuals of K44A were split almost evenly between the "southern" (Mt. Franklin and Sherk Lake) and "northern" (all other Nanaimo Lakes colonies) clusters. According to these results the Nanaimo Lakes colonies will be treated in the following as one metapopulation subdivided into a southern and a northern subpopulation.

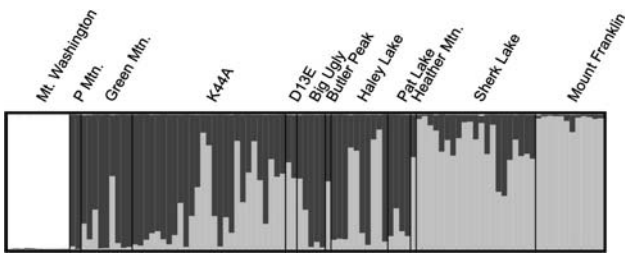

Fig. 3 Bayesian cluster analysis ($K = 3$) for *M. vancouverensis* (105 individuals, 11 loci) performed using STRUCTURE (Pritchard et al. 2000). Each column along the x axis represents one individual grouped by colony as in Fig. 2, plus Mt. Washington (Fig. 1). The y axis represents the assignment probability of each individual into the three clusters (*white* Mt. Washington cluster, *light grey* "southern" Nanaimo Lakes, *dark grey* "northern" Nanaimo Lakes)

Intraspecific variation in *Marmota vancouverensis*

The average number of alleles for the 28 successfully amplified loci (17 monomorphic + 11 polymorphic) was rather low (1.4). There was a single polymorphic locus with three alleles; all the others had only two alleles. Average expected heterozygosity (H_E) within colonies was calculated for the 11 polymorphic loci. The values varied between 0.08 and 0.23, which is low compared to other marmots (Kruckenhauser and Pinsker 2004; Kyle et al. 2004; Floyd 2003). To minimize problems arising from small sample sizes we calculated measures of genetic variation and substructuring only for those colonies with a sample size ≥4 individuals (Table 1). In addition we calculated these parameters treating Mt. Franklin and Sherk Lake as one subpopulation, with all other colonies from the Nanaimo Lakes area pooled as a second subpopulation. We also calculated the parameters treating all Nanaimo Lakes colonies as one population (see above for estimation of the real number of populations).

Genetic variability within colonies was lowest at Mt. Washington ($H_E = 0.08$), an isolated colony on central Vancouver Island. For none of the colonies did the data suggest inbreeding. Differences in average heterozygosity (H_E) did not reflect a geographic pattern and were not correlated with the number of individuals per colony. Nevertheless, the second lowest H_E value (0.12) was found in the rather separated Big Ugly colony consisting of five individuals only. Private alleles, i.e., alleles occurring in only one of the colonies, were only found in Mt. Washington, although the average number of alleles was relatively low in that isolated colony. When colonies in the Nanaimo Lakes region were pooled as suggested with STRUCTURE, only the subpopulation consisting of all the colonies but Mt. Franklin and Sherk Lake showed a significant deviation from Hardy–Weinberg equilibrium.

Genetic differentiation between colonies

As can be seen from the allele frequencies (Appendix 2), marmots from Mt. Washington are clearly distinguishable

from those occupying the "Nanaimo Lakes region" in the southern part of Vancouver Island (Bryant and Janz 1996). The distinctiveness of the Mt. Washington colony is already indicated by the presence of private alleles at the loci Bibl18 (139), Bibl4 (185), gs25 (136), 2g2 (118), and Ma018 (304). In the latter four cases, private alleles were fixed in the Mt. Washington colony, while the Nanaimo Lakes colonies proved monomorphic for different alleles.

To assess genetic differentiation among the eight colonies with a sample size ≥4, Nei's standard genetic distance corrected for small sample size (D) and the F_{ST} values between colonies (Table 2) were calculated. The distance measures underline the separated position of the Mt. Washington colony. For example, Nei's standard genetic distance values between Mt. Washington and the seven Nanaimo Lakes colonies (range of D values 0.42–0.50) are much higher than the highest value among the colonies within the Nanaimo Lakes area (range 0.01–0.13). Significant F_{ST} values among the Nanaimo Lakes colonies were found for Pat Lake and Big Ugly, which might be due to the low sample size, but also for Mt. Franklin and Sherk Lake (Table 2), which appeared differentiated from each other and from all other colonies.

We constructed unrooted neighbour-joining networks from the D values (Fig. 4) and F_{ST} values (data not shown). In both networks, the differentiation of the Mt. Washington colony from the rest was apparent. Relationships among colonies within the Nanaimo Lakes region were not clearly resolved and the groupings varied when different distance measures were applied. Only the grouping of the two colonies from Mt. Franklin and Sherk Lake appeared to be consistent. However, the geographically adjacent Pat Lake colony did not cluster with those two, a result that was also reflected by the significance of the F_{ST} values. For the other Nanaimo Lakes colonies no clear grouping and differentiation was observed. A consensus tree of 1,000 bootstrap replicates from the D values only gave values >50% for the node that separates Mt. Franklin and Sherk Lake from the other colonies (60%).

Using the same input data as described above (i.e., colony-specific values for cases in which ≥4 individuals were sampled), correlations between geographic and genetic distances were highly significant (for Nei's D: $r = -0.985$, $P < 0.001$, and for F_{ST}: $r = 0.954$, $P = 0.001$, $n = 28$ pairwise comparisons). These results were strongly influenced by the pronounced differentiation of the Mt. Washington colony. When results from Mt. Washington were removed, relationships between genetic similarity and distance were generally smaller but still significant (for D: $r = -0.641$, $P = 0.003$ and for F_{ST}: $r = 0.541$, $P = 0.009$, $n = 21$ pairwise comparisons). Removal of Sherk Lake, Mt. Franklin and Mt. Washington suggested no significant isolation by distance in the remaining Nanaimo Lakes colonies

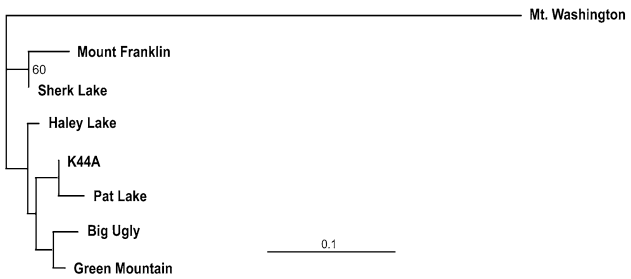

Fig. 4 Genetic relationships among recently-occupied marmot colonies from which more than four individuals were sampled. The nearest-neighbour-joining network was calculated from D values. A bootstrap value >50% (1,000 replicates) was obtained only for the node that separates Mt. Franklin and Sherk Lake from the other colonies

(for D: $r = -0.539$, $P = 0.102$ and for F_{ST}: $r = 0.274$, $P = 0.248$, $n = 10$ pair-wise comparisons). These results, like the neighbour-joining analyses, suggested that within the Nanaimo Lakes colonies only Sherk Lake and Mt. Franklin are slightly isolated.

Discussion

Loss of genetic variation is an important threat to small and endangered populations. Low genetic variation can lead to reduced fecundity and survival (Keller and Waller 2002; Reed and Frankham 2003), as well as loss of evolutionary potential (England et al. 2003; Reed et al. 2003). For these reasons, preservation and management of existing variation is an important goal of most captive management programs. Because the rate of loss of heterozygosity is inversely proportional to effective population size (N_e), the initial goal of most intensive management programs is to rapidly increase population size. However, unless the population is extremely small, this effort may be confounded by ancillary objectives such as assuring approximately equal representation of all founders or concerns about whether subpopulations should be managed as distinct units. The results of this study indicate that Vancouver Island marmots have extremely low genetic diversity and that there are at least two distinct populations, one of which consists of only a handful of animals.

Genetic diversity of Vancouver Island marmots

Although among-species comparisons of genetic variation can be problematic, our data indicate that Vancouver Island marmots have remarkably low variation at a large number of microsatellite loci compared to several congeners. Ascertainment bias often confounds such comparisons since loci can be more polymorphic in species for which

they were developed than in species from which they were borrowed (Ellegren et al. 1995; Forbes et al. 1995; Rubinsztein et al. 1995; Garner et al. 2005). However, recently published data for all four western North American species as well as the European Alpine marmot (*M. marmota*) allow us to compare variation among species without encountering this pitfall. The loci used in this study, with the exception of 2g2, were developed for use in *Spermophilus* spp. ground squirrels or *M. marmota*. Since the genus *Marmota* is monophyletic, none of the marmot species should have inherited greater diversity at the loci developed for another genus (e.g., ground squirrels). Similarly, the four North American species are believed to be evolutionarily equidistant from *M. marmota* (Kruckenhauser et al. 1999; Steppan et al. 1999) and so could be expected to have inherited similar variation at the loci developed for *M. marmota*. We examined all studies in which genetic variation at subsets of the *Spermophilus* or *M. marmota* loci was examined in *M. olympus*, *M. caligata*, *M. flaviventris*, and *M. marmota*; all authors (Goossens et al. 1998, 2001; da Silva et al. 2003; Floyd 2003; Kruckenhauser and Pinsker 2004; Kyle et al. 2004; Cohas et al. 2006; Griffin 2007) reported substantially greater diversity in terms of numbers of alleles per polymorphic locus and both observed and expected heterozygosities than we found across the entire population of Vancouver Island marmots. Thus, having eliminated the possibility of ascertainment bias, our results indicate that across its entire range, the gene pool of *M. vancouverensis* contains only a small fraction of the neutral genetic variation that other *Marmota* species carry.

The evolutionary history, population structure, and mating systems of different species can lead to different levels of expected and observed genetic diversity as well. All *Marmota* species discussed above are social and inhabit a relatively fragmented landscape. However, the Vancouver Island marmot and the Olympic marmot are unique in that they inhabit actual or effective islands that severely limit their population size. Allelic diversity in the Olympic marmot (Griffin 2007), while higher than in the Vancouver Island marmot, is lower than that of any other studied marmot population (see citations above).

Island populations often have lower genetic variation than mainland populations (Frankham 1997), and the low levels of both allelic diversity and heterozygosity in the Vancouver Island marmot are likely due in part to historic effects associated with the founding of the population, as well as any subsequent bottlenecks associated with climate fluctuations and changes in habitat availability. Even a few short-lasting bottlenecks could have led to the loss of rare alleles and any rare alleles that made it through historic bottlenecks would likely have been lost in the recent population decline. The extremely low levels of heterozygosity likely indicate that this population has been small for extended periods, consistent with the limited habitat available to this island species. However, we note that variability measured at neutral markers such as microsatellites may differ considerably from that measured at loci under selection, e.g., MHC genes, with the loss of immediately-useful variability tending to lag behind the loss of neutral variability (Amos and Balmford 2001; Aguilar et al. 2004). Indeed, allozyme studies performed before the most recent decline (in 1987–1989, Bryant 1990) revealed an H_E of 0.073 (44 individuals, 22 loci) similar to those of other North American marmots (*M. monax*: 0.053, Wright et al. 1987; *M. flaviventris*: 0.075, Schwartz and Armitage 1980). However, in contrast to the relatively high H_E value, the number of polymorphic loci detected by Bryant (1990) was considerably lower (18%) than in those two other marmot species (*M. monax*: 25%, Wright et al. 1987; *M. flaviventris*: 40%, Schwartz and Armitage 1980) suggesting that the variation at the four polymorphic enzyme loci recorded by Bryant (1990) might be maintained by selective forces.

Regardless of the root cause, the low measured genetic variability in the Vancouver Island marmot has to be considered as an additional threat to the continued survival of this endangered species. At low population size genetic drift will ultimately dominate selection and any remaining variation at loci under selection will be lost (Allendorf and Luikart 2006). Genetic depletion can harm a declining population in the short term due to fixation of deleterious alleles as a consequence of inbreeding (Amos and Balmford 2001; Frankham et al. 2004). This phenomenon may not be of immediate relevance for the Vancouver Island marmot, since no reduction of fitness in wild-living animals has been observed over the last 20 years (Bryant 2005), reproductive rates are comparable to those of related species (Bryant 2005), and captive breeding efforts have been successful, with at least 231 reported births and a positive overall population growth rate (lambda = 1.31, COSEWIC 2008). As recommended by Keller and Waller (2002), examination of the reproductive success of captive marmots descended from the Nanaimo Lake region, the Mt. Washington colony, and those of mixed ancestry would provide additional insight into whether reproductive rates are impacted by inbreeding levels. Over the long term, the limited gene pool of the Vancouver Island marmot may reduce its ability to evolve in response to environmental changes, e.g., pathogens or climatic changes (Lande 1988; Primack 1993; Hoffmann and Parsons 1997; Frankham et al. 1999).

Spatial genetic structure

Besides demonstrating low genetic variability, the second major result of this study was the clear genetic

differentiation of the marmots from two geographically isolated regions, which can be considered as separated populations. The high genetic distance between colonies in the Nanaimo Lakes region and Mt. Washington is not surprising since the geographic distance and the topography of Vancouver Island would restrict migration between the two populated habitats, especially in the previous several generations in which all intermediate colonies became extinct. Nevertheless the amount of differentiation remains striking. Eleven of the 23 detected alleles occur in only one of the populations. Although some of these may have been lost in one or the other population due to genetic drift, this finding might also suggest a longer lasting separation of these two populations.

We detected very little substructure within the Nanaimo Lakes region area, although marmots there lived in distinct colonies scattered across nearly 1,000 km^2 (Bryant 1998). In general the D values between these were very low, but two colonies (Mt. Franklin and Sherk Lake) had significant F_{ST} values, whereas the other five (Big Ugly, Green Mtn., Haley Lake, K44a, Pat Lake) were not clearly differentiated. These results were corroborated by the Bayesian cluster analysis. Thus we conclude that the Nanaimo Lakes colonies reflect a single metapopulation, as previously suggested by Bryant and Janz (1996), with two main subpopulations. Thus, for both genetic and empirical reasons, colonies in the Nanaimo Lakes region should not be considered to represent strongly isolated populations. Geographic distance did not completely explain the genetic patterns, suggesting that founder effects (i.e., a small number of colonizing marmots) and genetic drift may be important.

The genetic structure is likely driven by the dispersal patterns of the marmots in general and the occasional long-distance movement of a few marmots in particular. Based on ear-tagged animals, Bryant (1998) recorded dispersal movements of 1–11 km, but numerous records of solitary marmots in new habitats suggest dispersal movements >25 km (Bryant and Janz 1996). More recently, radio-tagged marmots have been observed to make movements of 10–30 km within periods of a few days or weeks (Bryant and Page 2005). Forests, clearcuts, rivers and roads do not appear to represent physical barriers to dispersal, although logging of high elevation forests may alter dispersal patterns through creation of new and nearby habitat (Bryant 1996). There is no evidence that dispersing marmots preferentially follow ridge lines or other topographic features. However, Vancouver Island does contain several apparent barriers created by large bodies of water, notably by Lake Cowichan in the south, Alberni Inlet in the west, and Buttle Lake, which separates the eastern and western portions of Strathcona Provincial Park. The empirical evidence that Vancouver Island marmots regularly move up to 30 km is consistent with the genetic population structure we detected. Occasional dispersal among natural habitat patches in the northern Nanaimo Lakes region would be expected to lead to genetic homogeneity within that group of colonies (Mills and Allendorf 1996), while the geographic isolation and small size of the Mt. Washington colony would be expected to lead to low heterozygosity and unique patterns of alleles. Finally, alleles found at the clear-cut colonies of Sherk Lake and Mt. Franklin are a subset of those in the northern Nanaimo Lakes colonies, as would be expected following a recent founding event.

Management implications

A major aim of this study was to provide information about the genetic make-up of the Vancouver Island marmot population to support the captive breeding program. Representatives from Mt. Washington and most Nanaimo Lakes colonies were taken into captivity. Through efforts to equalize the contribution of all founders and consideration of the geographic origin of individuals to be paired, studbook managers have attempted to maintain the remaining genetic variation of the species. Early on, a decision was made to risk the loss of local adaptation in the isolated Mt. Washington population by managing all animals as a single population.

Our results indicate that genetic variation in wild Vancouver Island marmots was primarily among the Mt. Washington populations and the "southern" (Sherk Lake and Mt. Franklin) and the "northern" (Green Mtn., K44 A, Big Ugly, Pat Lake, Haley Lake) Nanaimo Lakes subpopulations. Within (sub)populations diversity was extremely low, particularly at Mt. Washington (H_E = 0.08). As all three (sub)populations were represented, the founding captive population likely did contain the entire spectrum of genetic variation present in the wild population. And, given the extremely low within-population genetic variation, the under-representation of one or a few founders should not lead to substantial loss of diversity as long as all three (sub)populations are represented. Our results also indicate that the Mt. Washington population, in addition to being small and isolated, is genetically impoverished. Given that inbreeding depression is generally considered a more common problem than out-breeding depression, we support the decision to crossbreed marmots from different areas despite the possibility of local adaptations.

Early indications were that the captive Vancouver Island marmot population had reproductive fitness comparable to that of the wild population. Bryant (2005) found no significant differences between the two populations in the age

of first reproduction, litter size or probability of breeding. If, however, the low genetic variation within (sub)populations was negatively impacting reproductive success of wild animals, the captive population may eventually outperform the initial wild population as the Mt. Washington and Nanaimo Lakes animals are hybridized. The existing wild population (approximately 85 in late 2007) is not, of course, either stable or "viable" (Bryant and Page 2005; COSEWIC 2008). Bryant and Page (2005) stated that the long-term survival of the wild population of the Vancouver Island marmot will largely depend on the management of predator pressure and the availability of appropriate habitat.

However, it will be necessary to continue the careful management of the limited genetic resources of this population.

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Appendix 1

Table 3 PCR primer pairs used at University of Victoria

Locus	Reference	Primers	PCR conditions	Separation	Alleles
ms53	Hanslik and Kruckenhauser (2000)	fwd: attgaggagcagcatctagg rev: tcagggaaaggcagacctg	94°C 5 min (94°C 20 s, 59°C 20 s, 70°C 20 s) × 2; (94°C 20 s, 53°C 20 s, 70°C 20 s) × 32; 72°C 2 min	10%, 80 V-22 h	2 alleles 145/147
ms56	Hanslik and Kruckenhauser (2000)	fwd: cagactcccaccagtgac rev: cctgatctatgtaggttccat	94°C 5 min (94°C 20 s, 59°C 20 s, 70°C 20 s) × 2; (94°C 20 s, 53°C 20 s, 70°C 20 s) × 32; 72°C 2 min	10%, 80 V-18 h	2 alleles 101/103
bibl4	Klinkicht (1993)	fwd: cctaggttcagtcttcaac rev: tggtgttgccattgttctg	94°C 5 min (94°C 20 s, 56°C 20 s, 70°C 20 s) × 2; (94°C 20 s, 50°C 20 s, 70°C 20 s) × 33; 72°C 2 min	10%, 80 V-20 h	2 alleles 175/185
bibl18	Klinkicht (1993)	fwd: atggtcatggaagggaagg rev: gcatcttcacagttgatct	94°C 5 min (94°C 20 s, 59°C 20 s, 70°C 20 s) × 2; (94°C 20 s, 53°C 20 s, 70°C 20 s) × 31; 72°C 2 min	10%, 80 V-22 h	2 alleles 137/139
gs25	Stevens et al. (1997)	fwd: ccagcatgggggagagagag rev: cttgtcatttatccattcatag	94°C 5 min (94°C 20 s, 56°C 20 s, 70°C 20 s) × 2; (94°C 20 s, 50°C 20 s, 70°C 20 s) × 34; 72°C 2 min	10%, 80 V-18 h	2 alleles 126/128
St10	Hanslik and Kruckenhauser (2000)	fwd: ttgtgatcctccagggagtt rev: gtgatttccaaaccccattc	94°C 5 min (94°C 20 s, 52°C 20 s, 70°C 20 s) × 35; 72°C 2 min	10%, 80 V-22 h	2 alleles 136/138
igs-9d	May et al. (1997)	fwd: caaacatttattaaaccgtaaag rev: ggaatttgaaagaagtgacatc	94°C 5 min (94°C 20 s, 52°C 20 s, 70°C 20 s) × 35; 72°C 2 min	8%, 70 V-15 h	2 alleles 125/128
gs17	Stevens et al. (1997)	fwd: caattcgtggtggttatatc rev: ctgtcaacctatatgaacaca	94°C 5 min (94°C 20 s, 56°C 20 s, 70°C 20 s) × 2; (94°C 20 s, 50°C 20 s, 70°C 20 s) × 37; 72°C 2 min	10%, 80 V-22 h	2 alleles 147/151
gs22	Stevens et al. (1997)	fwd: tcccagagaacaacatcaacag rev: tccgcacaggtcttggactt	94°C 5 min (94°C 20 s, 56°C 20 s, 70°C 20 s) × 2; (94°C 20 s, 50°C 20 s, 70°C 20 s) × 36; 72°C 2 min	10%, 80 V-26 h	2 alleles 172/174

The table shows name, reference, primer sequences, amplification and separation protocols as well as allele sizes (in bp) of the microsatellite loci

Table 4 PCR primer pairs used at University of Montana

Locus	Reference	Primer sequence	Final primer concentration (μM)	Dye	PCR		Alleles	Alleles[b]
					Mix[a]	Conditions (TD)		
2g2	e	fwd: tgaactgggtcttgaggtct	0.15	NED	Multi1	60[c]	2 alleles	
		rev: gtctgctctgctctccatca					118/124	
ma018	f	fwd: atccgtccaataaagaaattc	0.1	FAM	Single2	65[d]	3 alleles	
		rev: gtttcttgtggctcagtggtcagatg					304/310/316	
ms53	a		0.15	HEX	Multi3	65[c]	2 alleles	
							145/147	
ms56	a		0.15	HEX	Multi3	65[c]	2 alleles	
							101/103	
bibl18	b		0.15	HEX	Multi1	60[c]	2 alleles	
							134/136	137/139
St10	a		0.1	FAM	Multi2	58[c]	2 alleles	
							134/136	136/138
gs17	c		0.1	FAM	Single1	50[d]	2 alleles	
							154/158	147/151
gs22	c		0.15	NED	Multi1	60[c]	2 alleles	
							179/181	172/174

The table shows name, reference, primer sequences (if not already included in Appendix 1a), amplification protocols and allele sizes (in bp) of the microsatellite loci

[a] Multiplexes were created for use in *M. olympus* (Griffin 2007) and thus contained primers for loci that were monomorphic in *M. vancouverensis*. In addition to loci identified above, multi 1 included primers for GS14 (reference c), MS47(a), and MA091 (f) and multi 2 included primers for BIBL31 (b), IGS-6 (d), and MS45 (a)

[b] At some loci allele size differed systematically between labs, the corresponding allele sizes at University of Victoria are given

[c] Multiplex PCRs had an initial denaturation at 95°C for 15 min, followed by 20 cycles with 30 s denature, 90 s annealing stepping down 0.5°C per cycle, and a 60 s extension. This is followed by an additional 18 cycles at 10°C below the starting annealing temperature with a final extension step at 60°C for 30 min

[d] Single locus cycling conditions differed by having an initial denaturation step of 5 min and 25 cycles (instead of 18) at 10°C below the starting annealing temperature

a–d see Appendix 1a

e Kyle et al. (2004)

f da Silva et al. (2003)

Appendix 2

Table 5 Allele frequencies for all 12 colonies

Locus	Allele	Mt. Washington	"P" Mtn.	Green Mtn.	K44A	D13E	Big Ugly	Butler Peak	Haley Lake	Pat Lake	Heather Mtn.	Sherk Lake	Mt. Franklin
2g2	118	0.09	–	–	–	–	–	–	–		–	–	–
	124	0.91	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	2*n*	22	4	18	54	4	10	2	20	6	2	38	24
ma018	304	1.00	–	–	–	–	–	–	–	–	–	–	–
	310	–	1.00	0.43	0.33	–	0.50	0.50	0.15	–	0.50	–	–
	316	–	–	0.57	0.67	1.00	0.50	0.50	0.85	1.00	0.50	1.00	1.00
	2*n*	18	4	14	54	2	8	2	20	6	2	40	22
ms53	145	1.00	0.50	0.72	0.44	1.00	1.00	1.00	0.95	0.25	1.00	0.60	0.54
	147	–	0.50	0.28	0.56	–	–	–	0.05	0.75	–	0.40	0.46
	2*n*	22	4	18	54	4	10	2	20	8	2	40	24

Table 5 continued

Locus	Allele	Mt. Washington	"P" Mtn.	Green Mtn.	K44A	D13E	Big Ugly	Butler Peak	Haley Lake	Pat Lake	Heather Mtn.	Sherk Lake	Mt. Franklin
ms56	101	–	–	–	0.15	–	–	0.50	0.05	–	–	0.21	0.27
	103	1.00	1.00	1.00	0.85	1.00	1.00	0.50	0.95	1.00	1.00	0.79	0.73
	2*n*	22	4	18	54	4	10	2	20	8	2	42	22
bibl4	175	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	185	1.00	–	–	–	–	–	–	–	–	–	–	–
	2*n*	22	4	18	54	4	10	2	20	8	2	42	24
bibl18	137	0.27	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	139	0.73	–	–	–	–	–	–	–	–	–	–	–
	2*n*	22	4	18	54	4	10	2	20	8	2	42	24
gs25	128	–	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	136	1.00	–	–	–	–	–	–	–	–	–	–	–
	2*n*	22	4	18	54	4	10	2	20	8	2	42	24
igs-9d	125	–	0.50	0.50	0.22	–	0.10	–	0.50	0.50	–	0.07	–
	128	1.00	0.50	0.50	0.78	1.00	0.90	1.00	0.50	0.50	1.00	0.93	1.00
	2*n*	22	4	16	54	4	10	2	20	8	2	42	24
st10	136	0.14	1.00	0.72	0.39	–	0.50	0.50	0.15	0.38	0.50	0.05	–
	138	0.86	–	0.28	0.61	1.00	0.50	0.50	0.85	0.63	0.50	0.95	1.00
	2*n*	22	4	18	54	4	10	2	20	8	2	42	24
gs17	147	–	0.75	0.56	0.63	1.00	0.90	0.50	0.75	0.75	0.50	0.62	0.46
	151	1.00	0.25	0.44	0.37	–	0.10	0.50	0.25	0.25	0.50	0.38	0.54
	2*n*	22	4	18	54	4	10	2	16	8	2	42	24
gs22	172	1.00	1.00	0.94	1.00	1.00	1.00	1.00	0.90	1.00	0.50	0.95	0.46
	174	–	–	0.06	–	–	–	–	0.10	–	0.50	0.05	0.54
	2*n*	22	4	18	54	4	10	2	20	6	2	42	24

2*n* Number of investigated genes

References

Aguilar A, Roemer G, Debenham S et al (2004) High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. Proc Natl Acad Sci USA 101:3490–3494. doi:10.1073/pnas.0306582101

Allendorf FW, Luikart G (2006) Conservation and genetics of populations. Blackwell, Cambridge

Amos W, Balmford A (2001) When does conservation genetics matter? Heredity 87:257–265. doi:10.1046/j.1365-2540.2001.00940.x

Banfield AWF (1977) The mammals of Canada. University of Toronto Press, Toronto

Barash DP (1989) Marmots: social behavior and ecology. Stanford University Press, Stanford

Blumstein DT (1999) Alarm calling in three species of marmots. Behaviour 136:731–757. doi:10.1163/156853999501540

Bohonak AJ (2002) IBD: a program for analyses of isolation by distance. (Version 1.52). J Hered 93:153–154. doi:10.1093/jhered/93.2.153

Bryant AA (1990) Genetic variability and minimum viable populations in the Vancouver Island marmot (*Marmota vancouverensis*). M.E.Des. Thesis, University of Calgary, Calgary

Bryant AA (1996) Reproduction and persistence of Vancouver Island marmots (*Marmota vancouverensis*) in natural and logged habitats. Can J Zool 74:678–687. doi:10.1139/z96-076

Bryant AA (1998) Metapopulation ecology of Vancouver Island marmots (*Marmota vancouverensis*). Ph.D. dissertation, University of Victoria, Victoria

Bryant AA (2000) Relative importance of episodic versus chronic mortality in the Vancouver Island marmot (*Marmota vancouverensis*). In: Darling L (ed) Proceedings of a conference on the biology and management of species and habitats at risk, Kamloops, B.C., 1999, vol. I. Queen's Printer, Victoria, pp 189–195

Bryant AA (2005) Reproductive rates of wild and captive Vancouver Island marmots (*Marmota vancouverensis*). Can J Zool 83:664–673. doi:10.1139/z05-056

Bryant AA (2007) Recovery efforts for Vancouver Island marmots, Canada. In: Soorae PS (ed) Re-introduction news, vol 26. IUCN/SSC Re-Introduction Specialist Group, Abu Dhabi, pp 30–32

Bryant AA, Janz DW (1996) Distribution and abundance of Vancouver Island marmots (*Marmota vancouverensis*). Can J Zool 74:667–677. doi:10.1139/z96-075

Bryant AA, Page RE (2005) Timing and causes of mortality in the endangered Vancouver Island marmot (*Marmota vancouverensis*). Can J Zool 83:674–682. doi:10.1139/z05-055

Cardini A, Hoffmann RS, Thorington RW Jr (2005) Morphological evolution in marmots (Rodentia, Sciuridae): size and shape of the dorsal and lateral surfaces of the cranium. J Zool Syst Evol Res 43:258–268. doi:10.1111/j.1439-0469.2005.00316.x

Cohas A, Yoccoz NG, da Silva A et al (2006) Extra-pair paternity in the monogamous Alpine marmot (*Marmota marmota*): the roles

of social setting and female mate choice. J Behav Ecol Sociobiol 59:597–605. doi:10.1007/s00265-005-0086-8
COSEWIC (2008) COSEWIC assessment and update status report on the Vancouver Island marmot (*Marmota vancouverensis*) in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa. Available at http//www.sarareregistry.gc.ca/status/status_e.cfm. Accessed 12 Sept 2008
da Silva A, Luikart G, Allaine D et al (2003) Isolation and characterization of microsatellites in European Alpine marmots (*Marmota marmota*). Mol Ecol Notes 3:189–190. doi:10.1046/j.1471-8286.2003.00392.x
Dieringer D, Schlötterer C (2003) MICROSATELLITE ANALYZER: a platform independent analysis tool for large microsatellite data sets. (Version 2.2). Mol Ecol Notes 3:167–169. doi:10.1046/j.1471-8286.2003.00351.x
Ellegren H, Primmer CR, Sheldon BC (1995) Microsatellite evolution: directionality or bias. Nat Genet 11:360–362. doi:10.1038/ng1295-360
England PR, Osler GHR, Woodworth LM et al (2003) Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. Conserv Genet 4:595–604. doi:10.1023/A:1025639811865
Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620. doi:10.1111/j.1365-294X.2005.02553.x
Falush D, Stephens M, Pritchard JK (2003) Inference of population structure: extensions to linked loci and correlated allele frequencies. Genetics 164:1567–1587
Felsenstein J (1989) PHYLIP: phylogeny inference package. (Version 3.65). Cladistics 5:164–166
Floyd CH (2003) Ecological genetics of dispersal and mating system in populations of yellow-bellied marmots (*Marmota flaviventris*). Ph.D. Dissertation, University of California, Davis
Forbes SH, Hogg JT, Buchanan FC et al (1995) Microsatellite evolution in congeneric mammals. Mol Biol Evol 12:1106–1113
Frankel OH, Soulé ME (1981) Conservation and evolution. Cambridge University Press, Cambridge
Frankham R (1997) Do island populations have less genetic variation than mainland populations? Heredity 78:311–327. doi:10.1038/hdy.1997.46
Frankham R, Lees K, Montgomery ME et al (1999) Do population size bottlenecks reduce evolutionary potential? Anim Conserv 2:255–260. doi:10.1111/j.1469-1795.1999.tb00071.x
Frankham R, Ballou JD, Briscoe DA (2004) Introduction to conservation genetics. Cambridge University Press, Cambridge
Garner A, Rachlow JL, Hicks JF (2005) Patterns of genetic diversity and its loss in mammalian populations. Conserv Biol 19:1215–1221. doi:10.1111/j.1523-1739.2005.00105.x
Goossens B, Graziani L, Waits LP et al (1998) Extra-pair paternity in the monogamous alpine marmot revealed by nuclear DNA microsatellite analysis. Behav Ecol Sociobiol 7:1237–1241
Goossens B, Chikhi L, Taberlet P et al (2001) Microsatellite analysis of genetic variation among and within alpine marmot populations in the French Alps. Mol Ecol 10:41–52. doi:10.1046/j.1365-294X.2001.01192.x
Griffin SC (2007) Demography and ecology of a declining endemic: the Olympic marmot. Ph.D. Dissertation, University of Montana, Missoula
Griffin SC, Taper ML, Hoffman R et al (2008) The case of the missing marmots: are metapopulation dynamics or range-wide declines responsible? Biol Conserv 141:1293–1309. doi:10.1016/j.biocon.2008.03.001
Hanslik S, Kruckenhauser L (2000) Microsatellite loci for two European sciurids. Mol Ecol 9:2163–2165. doi:10.1046/j.1365-294X.2000.10535.x
Heard DC (1977) The behavior of Vancouver Island marmots (*Marmota vancouverensis*). M.Sc. Thesis, University of British Columbia, Vancouver
Hedrick PW (2001) Conservation genetics: where are we now? Trends Ecol Evol 16:629–636. doi:10.1016/S0169-5347(01)02282-0
Hoffmann AA, Parsons PA (1997) Extreme environmental change and evolution. Cambridge University Press, Cambridge
Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241. doi:10.1016/S0169-5347(02)02489-8
Klinkicht M (1993) Untersuchungen zum Paarungssystem des Alpenmurmeltieres, *Marmota m. marmota* mittels DNA-fingerprinting. Ph.D. Dissertation, University of Munich, Munich
Kruckenhauser L, Pinsker W (2004) Microsatellite variation in autochthonous and introduced populations of the Alpine marmot (*Marmota marmota*) along a European west-east transect. J Zool Syst Evol Res 42:19–26
Kruckenhauser L, Pinsker W, Haring E et al (1999) Marmot phylogeny revisited: molecular evidence for a diphyletic origin of sociality. J Zool Syst Evol Res 37:49–56. doi:10.1046/j.1439-0469.1999.95100.x
Kyle CJ, Karels TJ, Clark B et al (2004) Isolation and characterization of microsatellite markers in hoary marmots (*Marmota caligata*). Mol Ecol Notes 4:749–751. doi:10.1111/j.1471-8286.2004.00810.x
Lande R (1988) Genetics and demography in biological conservation. Science 241:1455–1460. doi:10.1126/science.3420403
Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220
May B, Gavin TA, Sherman PW et al (1997) Characterization of microsatellite loci in the Northern Idaho ground squirrel (*Spermophilus brunneus brunneus*). Mol Ecol 6:399–400. doi:10.1046/j.1365-294X.1997.00203.x
Milko RJ, Bell AM (1986) Subalpine meadow vegetation of south central Vancouver Island. Can J Bot 64:815–821. doi:10.1139/b86-106
Mills LC, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. Conserv Biol 10:1509–1518. doi:10.1046/j.1523-1739.1996.10061509.x
Nagorsen DW (1987) Marmota vancouverensis. Mamm Species 270:1–5. doi:10.2307/3503862
Nei M (1978) Estimation of average heterozygosity and genetic distance from a number of individuals. Genetics 89:538–590
Ozgul A, Armitage KB, Blumstein DT et al (2006) Effects of patch quality and network structure on patch occupancy dynamics of a yellow-bellied marmot metapopulation. J Anim Ecol 75:191–202. doi:10.1111/j.1365-2656.2006.01038.x
Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. (Version 1.6.5). Comput Appl Biosci 12:357–358
Primack RB (1993) Essentials of conservation biology. Sinauer, Sunderland
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
Raymond M, Rousset F (1995) GENEPOP: population genetics software for exact tests and ecumenicism. (Version 1.2). J Hered 86:248–249
Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17:230–237. doi:10.1046/j.1523-1739.2003.01236.x
Reed DH, Lowe EH, Briscoe DA et al (2003) Fitness and adaptation in a novel environment: effect of inbreeding, prior environment, and lineage. Evol Int J Org Evol 57:1822–1828
Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. (Version 1.1). Mol Ecol Notes 4:137–138. doi:10.1046/j.1471-8286.2003.00566.x

Rubinsztein DC, Amos W, Leggo J et al (1995) Microsatellite evolution—evidence for directionality and variation in rate between species. Nat Genet 10:337–343. doi:10.1038/ng0795-337

Schwartz OA, Armitage KB (1980) Genetic variation in social mammals: the marmot model. Science 207:665–667. doi:10.1126/science.207.4431.665

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. Trends Ecol Evol 22:25–33. doi:10.1016/j.tree.2006.08.009

Shank CC (1999) The committee on the status of endangered wildlife in Canada (COSEWIC): a 21-year retrospective. Can Field Nat 113:318–341

Steppan SJ, Akhverdyan MR, Lyapunova EA et al (1999) Molecular phylogeny of the marmots (Rodentia, Sciuridae): tests of evolutionary and biogeographic hypotheses. Syst Biol 48:715–734. doi:10.1080/106351599259988

Stevens S, Coffin J, Strobeck C (1997) Microsatellite loci in Columbian ground squirrels *Spermophilus columbianus*. Mol Ecol 6:493–495. doi:10.1046/j.1365-294X.1997.t01-1-00192.x

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evol Int J Org Evol 38:1358–1370. doi:10.2307/2408641

Wright J, Tennant BC, May B (1987) Genetic variation between woodchuck populations with high and low prevalence rates of woodchuck hepatitis virus. J Wildl Dis 23:186–191