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RESEARCH ARTICLE

Past bottlenecks and current population fragmentation of endangered huemul deer (*Hippocamelus bisulcus*): implications for preservation of genetic diversity

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Abstract Small populations in fragmented habitats can lose genetic variation through drift and inbreeding. The huemul (*Hippocamelus bisulcus*) is an endangered deer endemic to the southern Andes of Chile and Argentina. Huemul numbers have declined by 99% and its distribution by 50% since European settlement. The total population is estimated at less than 2,000 individuals and is highly fragmented. At one isolated population in Chilean Patagonia we sampled 56 individuals between 2005 and 2007 and genotyped them at 14 microsatellite loci. Despite low genetic variability (average 2.071 alleles/locus and average H_O of 0.341), a low inbreeding coefficient (F_{IS}) of 0.009 suggests nearly random mating. Population genetic bottleneck tests suggest both historical and contemporary reductions in population size. Simulations indicated that the population must be maintained at 75% of the current size of 120 individuals to maintain 90% of its current genetic diversity over the next 100 years. Potential management strategies to maintain genetic variability and limit future inbreeding include the conservation and establishment of habitat corridors to facilitate gene flow and the enlargement of protected areas to increase effective population size.

Keywords Huemul deer · *Hippocamelus bisulcus* · Genetic variability · Inbreeding · Endangered · Ungulate

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Introduction

Reduced genetic variability caused by population bottlenecks is a major concern in conservation biology (Kirkpatrick and Jarne 2000). Population genetic theory predicts that bottlenecks will lead to diminished genetic variability, lower individual fitness, and decreased capacity to adapt to environment change (Keller and Waller 2002). In small populations, habitat fragmentation and limited gene flow can lead to reduced genetic variability and a rapid reduction in effective population size (N_e) within remaining habitat patches (Couvet 2002; Johansson et al. 2007).

Habitat fragmentation can increase mortality of dispersing animals that must traverse unsuitable areas (Hanski and Gilpin 1997). Fragmented populations may also face reduced population size and gene flow, potentially leading to lower genetic diversity, inbreeding depression, fixation of deleterious mutations, reduced population growth rate, and higher extinction risks (Couvet 2002; Dudash and Fenster 2000; Newman and Tallmon 2001). Small populations in fragmented habitats can also lose genetic variation through drift (Newman and Tallmon 2001; Nunney 1999). If mechanisms for purging deleterious alleles are inadequate, mating among close relatives can lead to

inbreeding depression, and in extreme cases to population extirpation (Keller and Waller 2002). Collectively, these effects of habitat fragmentation can accelerate extinction (Fahrig 2002) which implies that connectivity between patches is crucial for the long-term persistence of a population.

The role of genetic factors in species extinctions is controversial (Caro and Laurenson 1994; Frankham et al. 2002; Lande 1988). Although some have suggested that endangered species are typically driven to extinction by habitat loss and overexploitation, before genetic factors can impact them (Caro and Laurenson 1994; Caughley 1994; Lande 1988), increasing evidence highlights the importance of genetic information in the conservation and management of endangered species (reviewed in Sarre and Georges 2009). Low genetic variation can increase susceptibility to disease and reduces the ability to adapt to environmental changes (Keller and Waller 2002): threatened taxa often have lower genetic diversity than closely related taxa with healthy populations (Spielman et al. 2004).

The huemul (*Hippocamelus bisulcus*) is the most threatened deer in South America (IUCN 2009). It inhabits the Andes of southern Chile and Argentina, and has declined dramatically in numbers and distribution since the arrival of Europeans (Diaz and Smith-Flueck 2000; Flueck and Smith-Flueck 2006). This species was once abundant from central Chile (34°S) to the Strait of Magellan (54°S) (Cabrera and Yepes 1960). Currently, the total population is estimated at less than 2,000 individuals (Flueck and Smith-Flueck 2006; Vila et al. 2006), or probably less than 1% of its historical abundance (Redford and Eisenberg 1992). Such drastic reduction in numbers and distribution, plus the fragmentation of remaining populations through habitat loss, likely have reduced its genetic variability.

Numerous factors can lead to population declines associated with anthropogenic activities, such as overharvesting, introduced diseases, exotic species (Caughley and Gunn 1996), apparent competition (Sinclair et al. 2006), and habitat loss and fragmentation (Young and Clarke 2000). Many of these causes of decline have been proposed for huemul (Flueck and Smith-Flueck 2006; Frid 1994; Povilitis 1998), but thus far none have been investigated. Recent research on the huemul social organization (Corti 2008), population dynamics (Corti et al. in press), and ranging behaviour (Gill et al. 2008), allow stronger inferences about the possible genetic consequences of habitat fragmentation for this deer. Territorial males defend females in a specific area and sire most offspring, while non-territorial males rarely sire offspring (Corti 2008). Because of high site fidelity and small home ranges, isolated huemul populations may be more susceptible to the loss of genetic variability than other more wide-ranging species (Forbes and Hogg 1999).

To understand the impact of population reduction and current habitat fragmentation on genetic variation, we studied a population of huemul at the Lago Cochrane National Reserve, Chile, that appears isolated by unsuitable habitat (agricultural land, human settlements, and steppe). Habitat fragmentation and the isolation of this population began at least 90 years ago when settlers started cutting and burning the southern beech forest (*Nothofagus* spp.) to create cattle pastures (Donoso and Otero 2005; Velásquez et al. 2005). Here, we present the first evaluation of the genetic variability of a huemul population using microsatellite DNA markers. The territorial mating system of huemul, where one male can monopolize an area with a group of females for several years (Corti 2008), could lead to inbreeding. We measured the rate of inbreeding using F_{IS} (Weir and Cockerman 1984). We also tested for a recent bottleneck due to a possible population reduction and habitat fragmentation. Finally, we used simulations to provide management with conservation targets to maintain current genetic variability.

Materials and methods

Study area and the huemul population

The study was conducted in the Lago Cochrane National Reserve (LCNR) (47°12′S, 72°30′W; 69 km^2), Aysén District, Chilean Patagonia, northeast of the town of Cochrane (ca. 3,000 inhabitants) (Fig. 1). The LCNR was created in 1967 to protect lenga tree (*Nothofagus pumilio*) forest and the remaining huemul population. A large proportion of the area used by deer is dominated by steep terrain (23% with slopes $> 45°$) and flat rocky outcrops (Gill et al. 2008). The canopy of the deciduous forest is dominated by lenga at high altitude and coihue (*N. dombeyi*) at lower elevations; the shrub layer is dominated by ñirre (*N. antarctica*) and notro (*Embothrium coccineum*) trees, and the shrubs of chaura (*Pernettya mucronata*), calafate (*Berberis microphylla*), and zarzaparrilla (*Ribes* spp.); the main forb species is anemona (*Anemone multifida*). Evidence of old burned areas persists from human-caused fires in the 1940s. Mean annual temperature is 7.6°C and annual precipitation is nearly 750 mm, mostly falling between May and August. Snowfall occurs mostly between June and August.

The huemul population of the Cochrane Lake area, whose distribution extends beyond the limits of the LCNR, was bounded by private sheep and cattle ranches to the north and east until 2004, when additional lands became a wildlife protection area. To the west, there are

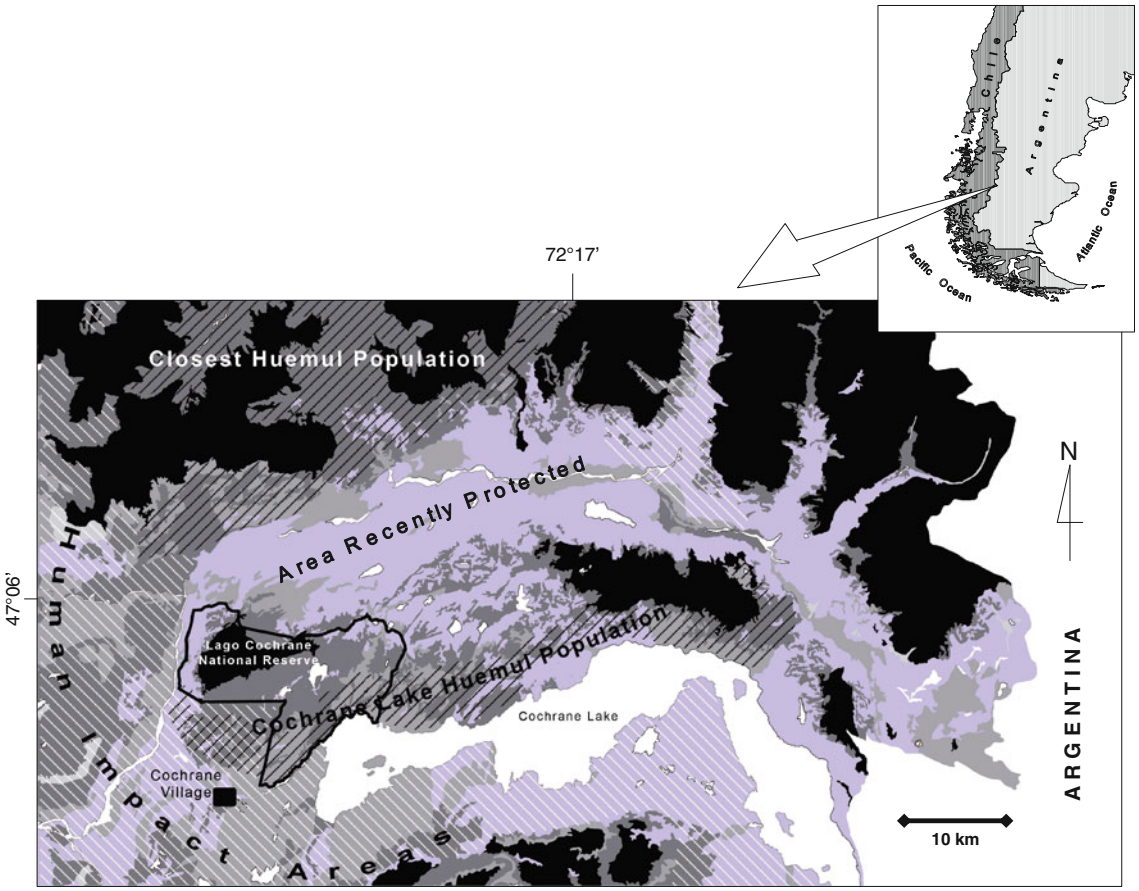

Fig. 1 The huemul study population at Cochrane Lake, Aysén District, Chilean Patagonia. Huemul used the area with *black right-slanted lines* north of the lake. Major human impacts (urban areas with dogs, livestock/agricultural areas, logging and exotic tree plantations) are indicated by *white left-slanted lines*. The approximate range of the closest huemul population is also shown in the northern range with *black right-slanted lines*. The valley separating the two huemul populations recently changed from cattle and sheep ranching to a private protected area. The black boundaries indicate the Lago Cochrane National Reserve. Habitat types are indicated with different tones: (■) areas above treeline, (■) forest areas, (■) shrub areas, (■) steppe and grassland areas

cattle ranches and the town of Cochrane, and further east, the Patagonian steppe begins where the mountains end, with sheep ranching in Argentina. The southern limit of this huemul population is Cochrane Lake and the Cochrane River (Fig. 1). The population appears isolated, surrounded by unsuitable habitats that prevented connection with other populations. The total population size is about 120 individuals (Corti 2008). This estimate combines the 43 individuals identified in the LCNR with 75 deer estimated outside the LCNR from field observations and non-systematic surveys (Corti 2008). The nearest population is approximately 10 km to the north, separated by a 5 km wide valley of steppe habitat, running east–west that is avoided by huemul (Gill et al. 2008) (Fig. 1). In addition, ca. 25,000 sheep and 2,500 cattle are held in the valley; however, since 2004 land has started to be converted into protected areas (P. Corti pers. obs.).

DNA extraction and genotyping

We collected 58 tissue samples from live-captured or dead huemul inside the LCNR between 2005 and 2007 (see Appendix I). Samples were stored in 70% ethanol until DNA extraction. DNA was extracted using the DNeasy™ Blood & Tissue Kit (Qiagen, Inc., Valencia, CA, USA). We used a previously optimized cervid multiplex PCR (Anderson et al. 2002), consisting of fourteen primers (Appendix II) that were evaluated in three separate multiplex reactions. The 10 µl multiplex reactions contained 0.5 µl of double-distilled water, 5 µl of 2X multiplex PCR Master Mix (Qiagen), 2.5 µl of DNA template, and 2 µl of a 10X primer mix in which one primer of each pair was fluorescently labelled (6-FAM, TET, PET or HEX). The multiplex PCR began with an initial 15 min denaturation at 95°C, followed by 33 cycles of 30 s at 94°C, 90 s at 60°C,

and 60 s at 72°C. The run ended after 30 min at 72°C. Twenty-four additional microsatellite loci (Appendix II) were screened individually in 10 µl reactions containing 4.94 µl of double-distilled water, 0.8 µl of $MgCl_2$ (20 mM), 1 µl of 10X PCR buffer, 2 µl of dNTPs (0.2 mM each), and 0.1 µl of *Taq*. Primer mixes were diluted to a final concentration of 0.16 µM, with one being fluorescently labelled. All PCR consisted of a 3 min denaturing period at 94°C, followed by 38 cycles of 30 s at 94°C, 60 s at 49°C, and 60 s at 72°C. The microsatellite amplicons were run (co-loaded when possible) on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a GS500LIZ size standard (Applied Biosystems). All bands were detected, scored, and manually verified using GENEMAPPER version 4.0 (Applied Biosystems) (Rinehart 2004).

Genetic analysis

We used GENEPOP version 3.4 (Raymond and Rousset 1995) to conduct exact tests for Hardy–Weinberg Equilibrium (HWE) and linkage disequilibrium for each locus across all individuals through a Markov chain process (Guo and Thompson 1992). *P* values for all loci were Bonferroni-corrected for multiple comparisons, where a new level of significance is established to test the different *P* values across loci ($\alpha = P/n$) (Rice 1989). We quantified genetic variability by assessing the number of alleles (*A*), together with expected (H_E) and observed heterozygosity (H_O). From these data, we calculated Wright's inbreeding coefficient (F_{IS}; Weir and Cockerman 1984), using GENEPOP and FSTAT version 2.9.3.2 (Goudet 2000). Null alleles were identified using CERVUS 3.0.3 (Kalinowski et al. 2007) and MICRO-CHECKER (Van Oosterhout et al. 2004). A subset of samples was genotyped two to three times to ensure reliability.

Bottleneck tests and simulation of loss of variability

Populations that experienced a bottleneck often exhibit a reduction of allele number and heterozygosity at polymorphic loci, with allele number being reduced faster than heterozygosity (Frankham et al. 2002; Luikart and Cornuet 1999). Thus, observed heterozygosity is larger than that expected based on allele numbers assuming mutation drift equilibrium (Cornuet and Luikart 1996). We tested for a population bottleneck using BOTTLENECK version 1.2.0.2 (Cornuet and Luikart 1996; Piry et al. 1999), which is based on the assumption that bottlenecked populations will show an excess of heterozygotes relative to allelic diversity. BOTTLENECK was run under three mutation models: the infinite alleles (IAM), two-phased (TPM) and stepwise mutation (SMM). The TPM was set at 95% stepwise mutation model and 5% multi-step mutations, as recommended by Piry et al. (1999). Wilcoxon signed-rank tests were used to identify heterozygosity excess (Piry et al. 1999).

We also calculated the *M*-ratio of Garza and Williamson (2001) for a sample of microsatellite loci to detect reductions in effective population size in relation to former huemul population sizes. The ratio is simply $M = k/r$, where *k* is equal to the number of alleles and *r* is the allelic size range, averaged across all loci (Garza and Williamson 2001). To assess for significance we calculated the critical ratio (M_C) using 10,000 simulations under one-step mutations (p_s) and the average size of multistep mutations (Δg) parameters respectively set to 90% and 3.5 as suggested by Garza and Williamson (2001). The θ parameter was estimated using a mutation rate of 5×10^{-4} (Estoup and Angers 1998) and a range of potentially historically biologically relevant N_e values expressing previous population sizes when this huemul population was more wide spread, connected with other populations, and higher in numbers (200, 500, 1000 and 2000).

To assess the chance of maintaining 90% of observed genetic variation over the next 100 years, as suggested by Frankham et al. (2002), we used BOTTLESIM version 2.6 (Kuo and Janzen 2003). Using present genetic diversity, BOTTLESIM simulates future population genetic parameters (observed number of alleles [OA] and H_O) based on different bottleneck scenarios. Genetic diversity estimates over 100 years were simulated when retaining 100, 90, 75, 50, and 25% of the current population of 120 deer. We performed 1,000 iterations with constant parameters (lifespan = 15 years, age at maturity = 3 years, completely overlapping generations, random mating, dioecious reproduction, and sex ratio of F:M: 1.5:1).

Results

Genetic diversity

Of 38 microsatellite loci screened (see Appendix II), six did not amplify, 16 were monomorphic and 16 were variable and amplified from 56 unique individuals (Table 1). Two samples taken from dead huemul did not amplify. Two loci, BL6 and N, deviated from HWE (both $P < 0.001$), with the latter containing a null allele according to MICRO-CHECKER (Van Oosterhout et al. 2004). Both loci were omitted from analysis. After correcting for multiple tests, no deviation from HWE was observed in the remaining 14 variable loci. Fisher's exact test for linkage disequilibrium showed no evidence of linkage ($P = 0.93$) among these 14 loci. The constructed genotype matrix was 98.72% complete with only 20 alleles

Table 1 Genetic diversity of huemul measured at 14 microsatellite loci

Locus	*N*	*A*	*A* size range (bp)	H_E	H_O	F_{IS}	Reference
RT27	55	2	171–173	0.071	0.073	−0.028	Wilson et al. (1997)
Q	56	2	271–275	0.419	0.339	0.149	Jones et al. (2000)
INRA011	56	2	203–205	0.473	0.500	−0.063	Vaiman et al. (1992)
ILSTS011	50	2	266–268	0.489	0.540	−0.077	Brezinsky et al. (1993)
BM203	56	2	218–220	0.404	0.411	−0.050	Bishop et al. (1994)
BM1225	55	2	231–235	0.467	0.509	−0.074	Bishop et al. (1994)
BBJ11	53	2	184–186	0.440	0.453	−0.038	Wilson and Strobeck (1999)
BBJ2	56	2	174–176	0.207	0.196	0.056	Wilson and Strobeck (1999)
BL25	56	2	180–184	0.164	0.179	−0.086	Bishop et al. (1994)
BM6438	56	2	271–273	0.018	0.018	0.000	Bishop et al. (1994)
BM6506	56	2	188–194	0.260	0.232	0.088	Bishop et al. (1994)
RT30	56	2	193–195	0.369	0.375	−0.027	Wilson et al. (1997)
RT5	56	3	151–157	0.589	0.500	0.177	Wilson et al. (1997)
RT7	56	2	207–209	0.446	0.446	0.017	Wilson et al. (1997)
Average		2.071		0.344	0.341	0.009	

Sample size (*N*), allele number (*A*), allele size range (bp), expected (H_E) and observed heterozygosity (H_O), and coefficient of inbreeding (F_{IS}) for each locus (calculated in GENEPOP and FSTAT) are shown for huemul from Lake Cochrane, Chile. References indicate in which species the primers for each locus were developed

missing from a total of 1,568 (calculated from 56 individuals, 14 microsatellite markers, and two alleles). The mean number of alleles per locus was 2.071 (SE = ±0.071), mean H_E was 0.344 (±0.046) and H_O was 0.341 (±0.046). F_{IS} across all loci was 0.009.

Bottleneck tests and simulations

Under the three mutation models in BOTTLENECK, 10 of 14 loci showed significant heterozygosity excess. Overall, Wilcoxon signed-rank tests showed evidence of a bottleneck ($P = 0.003$ for IAM; $P = 0.05$ for TPM; $P = 0.06$ for SMM). The *M*-ratio was 0.89 which exceeded M_C (0.83, 0.80, 0.77, and 0.74) at all four N_e values respectfully. For the estimated population size of 120 individuals BOTTLESIM projected a continued decrease in genetic diversity over the next 100 years (Fig. 2). H_O was expected to decrease faster than observed allele diversity (OA), with a minimum of 75% (90 individuals) of the population required to maintain 90% of its current genetic diversity. At 50% of the current population size, H_O was projected to drop below the 90% threshold in 80 years, but not OA (Fig. 2). Simulations were also run with a sex ratio of 1:1 (F:M), and the same trend was observed.

Discussion

The huemul population of LCNR showed essentially random mating ($F_{IS} = 0.009$) yet very low genetic diversity ($H_O = 0.341$, mean number of alleles = 2.071). Although interspecific comparisons of microsatellite variation suffer from ascertainment bias, they are useful in this context to compare huemul genetic diversity to that of other mountain ungulates and endangered species. Studies that used the same loci as reported here including mountain goats *Oreamnos americanus* (Mainguy et al. 2005; Poissant et al. 2009), chamois *Rupicapra* spp. (Pérez et al. 2002), and wild sheep *Ovis* spp. (Worley et al. 2004) all reported higher numbers of alleles per locus (range 2–22, average 5) than the huemul (Table 1). Two closely related Neotropical deer, the pampas deer (*Ozotoceros bezoarticus*) and the marsh deer (*Blastocerus dichotomus*), also have been screened for genetic variability through microsatellite markers (Cosse et al. 2007; Gilbert et al. 2006; Leite et al. 2007). Pampas deer showed much higher diversity (mean alleles per locus = 15 and $H_O = 0.703$) (Cosse et al. 2007) as did the marsh deer (mean alleles per polymorphic locus = 2.57 and $H_O = 0.413$; Leite et al. 2007). The screening of 38 microsatellite markers in the LCNR huemul population, of which only 14 were polymorphic, further supports our findings of extremely low levels of genetic diversity. Loss of genetic variability in the huemul is also supported by preliminary results using mitochondrial DNA which showed few haplotypes (range between 1 and 4) remaining in many huemul populations (J. Marín unpublished data).

Our simulation results showed a faster decline in H_O than OA and is likely a result of low allelic richness and/or the absence of rare alleles in this population. In a similar

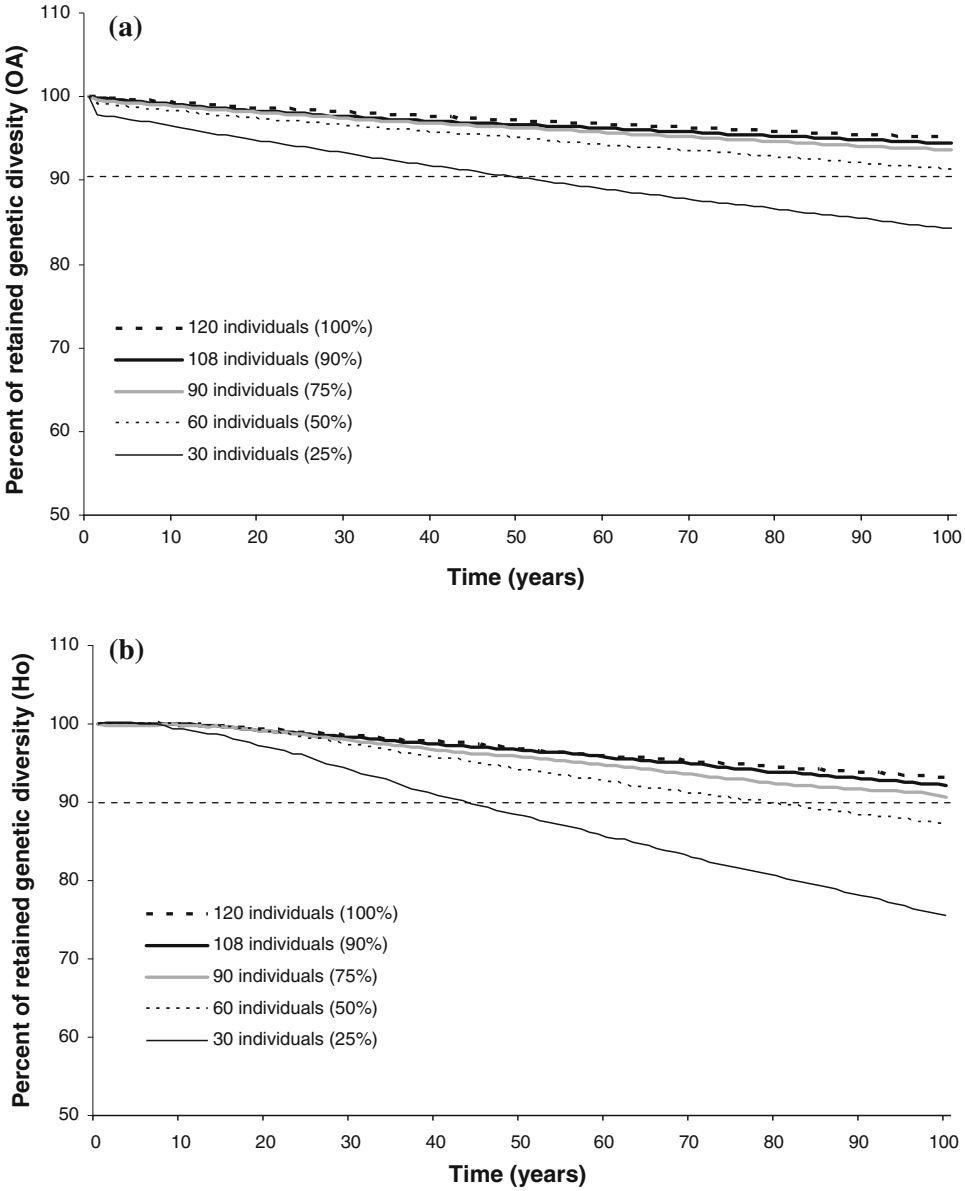

Fig. 2 Simulated genetic diversity of the huemul population in the Lago Cochrane National Reserve over 100 years using BOTTLESIM. At least 75% (90 individuals) of the current population is required to maintain 90% of current genetic diversity over the next 100 years. Both **a** the observed number of alleles (OA) and **b** the observed heterozygosity (H_O) were projected to decline (sex ratio: 1.5:1 F:M)

analysis of the endangered copper redhorse (*Moxostoma hubbsi*), an opposite trend was observed, where the OA declined faster than H_O (Lippé et al. 2006). The opposite trend is likely a result of higher retained diversity (both OA and H_O) due to a greater number of alleles (4 to 23) and larger population sizes in this fish (Lippé et al. 2006). Similarly, white-tailed eagle populations (*Haliaeetus albicilla*) in northern Europe retained significant levels of genetic diversity despite passing through demographic bottlenecks during the last century (Hailer et al. 2006).

Our simulations projected a future decline in genetic diversity. Because these simulations mimic genetic drift, they do not take into account gene flow between populations; this is an appropriate assumption because our observations suggest gene flow to this population is very small or non-existent. The LCNR population is surrounded by unsuitable habitat (see Methods and Fig. 1) and known maximum dispersal movements of adult huemul are limited to about 8 km (Gill et al. 2008). Known maximum dispersal distances for juveniles (1–3 years) do not go beyond 4 km (P. Corti unpublished data). The closest known population is 10 km to the north, and in 9 years of monitoring at the LCNR, no migration events have been documented. However, if migrants from a source population with novel alleles were to enter the LCNR and successfully reproduce, the loss of genetic diversity would be reduced; conversely, if current population levels are not maintained, the loss of genetic diversity will be exacerbated (Fig. 2).

Huemul have suffered a drastic decrease and many subpopulations have been extirpated (Redford and Eisenberg 1992). The last glaciation may have affected the genetic structure of huemul as populations became fragmented along the Andes during glacial advances in the Quaternary (Hewitt 2000). The arrival of European settlers some 500 years ago slowly accelerated the reduction of the huemul population, further exacerbated with increasing human settlement (Redford and Eisenberg 1992). The *M*-ratio analysis suggests that this huemul population has persisted in a small and isolated state for a long time (Garza and Williamson 2001); but BOTTLENECK indicates that a relatively recent reduction occurred in our study population. Approximately 90 years ago settlers arrived near the study area, which previously was sporadically used by native South Americans (Goñi et al. 2004; Velásquez et al. 2005). The entire area was used for cattle ranching and forest logging for firewood until 1967 when the LCNR was created. With the clearing of land for human settlement and cattle ranching starting 90 years ago, huemul populations became increasingly fragmented, and exposed to livestock-related diseases and poaching (Flueck and Smith-Flueck 2006). These cumulative effects are likely partly responsible for the reduced genetic diversity.

The reduction of genetic diversity in isolated populations can be accelerated due to the social and mating system (Banks et al. 2007). In a wood bison (*Bison bison athabascae*) population in northwestern Canada, two male founders monopolized the females and sired over 90% of offspring, reducing genetic diversity (Wilson et al. 2005). Huemul live in small mixed groups and territorial males defend a few females (Corti 2008). Both sexes are highly philopatric, and use a small home range of ca. 350 ha, defended by males all year for several years. Eight of 16 sexually mature males sampled (older than 2 years) and present during the rutting season of 2005–2007 sired no offspring, and 44% of fawns were sired by two males (Corti 2008). A few males appear to sire most fawns for several consecutive years with the same group of females (Corti 2008), possibly contributing to reductions in the genetic variation. More data on male lifetime reproductive success are required to properly evaluate the impact of huemul breeding system on genetic variability. Nevertheless, if mating opportunities are limited, inbreeding avoidance is often not detected in fragmented habitats, even when relatedness among potential mates is high (Banks et al. 2007).

The limited genetic variability of this huemul population suggests a need to expand genetic screening to other populations. A wider assessment will determine the global genetic diversity and connectivity of huemul populations, and establish whether the limited variability observed at LCNR is a general characteristic of this species. The historical small population size of huemul might have purged deleterious recessive alleles through natural selection (Hedrick 1994; Wang et al. 1999). In addition, because of their long history of isolation, huemul may have a low risk of inbreeding depression, as observed in some ungulates introduced to islands (e.g. Kerguelen mouflon *Ovis aries*) (Kaeuffer et al. 2007). To properly assess the risks posed by inbreeding and loss of genetic variability, a survey of several huemul populations to detect evidence of inbreeding depression (i.e. birth defects, low juvenile survival and fecundity) is required. Conservation efforts should seek to maintain current levels of genetic variability, using the projections presented here as a guideline. In the LCNR, a minimum of 90 individuals is required to maintain the current genetic diversity for the next 100 years. These simulations were based on the current low levels of diversity, highlighting the urgency to prevent any additional loss. Our finding of low genetic variability in the LCNR suggests that increasing connectivity and gene flow between populations should be a priority. Across the huemul's range, we recommend the enlargement of current protected areas, or the creation of buffer zones to facilitate migration and offset potential inbreeding depression (Brashares et al. 2001; Woodroffe and Ginsberg 1998). Conservation efforts should focus on identifying and preserving former (or contemporary) corridors used by huemul, so gene flow between populations can occur.

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Appendix I

Table 2 Huemul sampled at Lago Cochrane National Reserve, Chilean Patagonia

N°	Sex	Age	Year of sampling
1	F	A	2005
2[a]	F	A	2005
3	F	F	2005
4	F	F	2005
5	M	A	2005
6	M	A	2005
7	M	A	2005
8	M	A	2005
9	M	F	2005
10	M	F	2005
11	F	A	2006
12	F	A	2006
13	F	A	2006
14	F	A	2006
15	F	A	2006
16	F	A	2006
17	F	A	2006
18	F	F	2006
19	F	F	2006
20	F	F	2006
21	M	A	2006
22	M	A	2006
23	M	A	2006
24	M	A	2006
25	M	F	2006
26	M	F	2006
27	M	F	2006
28	M	F	2006
29	M	F	2006
30	M	F	2006
31[a]	M	F	2006
32[a]	U	F	2006
33	F	A	2007
34	F	A	2007
35	F	A	2007
36	F	A	2007
37	F	A	2007
38	F	A	2007
39	F	A	2007
40	F	F	2007
41	F	J	2007
42	M	A	2007
43	M	A	2007
44	M	A	2007
45	M	A	2007
46	M	A	2007
47	M	A	2007
48	M	F	2007
49	M	F	2007
50	M	F	2007
51	M	F	2007
52	M	F	2007
53	M	F	2007
54	M	F	2007
55	M	J	2007
56	M	J	2007
57	M	Y	2007
58	M	Y	2007

Number of individuals captured is indicated in the first column. Sex of the animal is indicated with F (female), M (male), and U (undetermined). Age is indicated with A (adults >3 years old), J (juveniles 1–3 years old), and F (fawns 0–1 year old). Also the year the animal was sampled is indicated

[a] Dead animals

Appendix II

Table 3 Loci names, number of alleles (*A*), chromosome number, and original reference for 38 microsatellite loci screened in huemul deer

Loci names	*A*	Chromosome number	Reference
ARO28	1	2	Crawford et al. (1995)
BBJ11	2	–	Wilson and Strobeck (1999)
BBJ2	2	–	Wilson and Strobeck (1999)
BL25	2	28	Bishop et al. (1994)
BL6	2	24	Grosz et al. (1997)
BM121	1	16	Bishop et al. (1994)
BM1225	2	20	Bishop et al. (1994)
BM203	2	27	Bishop et al. (1994)
BM4025	1	15	Bishop et al. (1994)
BM4107	1	20	Bishop et al. (1994)
BM415	No amplicon	6	Bishop et al. (1994)
BM4208	No amplicon	29	Bishop et al. (1994)
BM6438	2	1	Bishop et al. (1994)
BM6506	2	1	Bishop et al. (1994)
BM848	1	15	Bishop et al. (1994)
BovPRL	1	7	Moore et al. (1994)
Cervid1	No amplicon	–	DeWoody et al. (1995)
D	1	–	Jones et al. (2000)
ETH152	1	5	Steffen et al. (1993)

Table 3 continued

Loci names	*A*	Chromosome number	Reference
HUJ616	1	13	Barendse et al. (1994)
ILSTS011	2	14	Brezinsky et al. (1993)
INRA011	2	1	Vaiman et al. (1992)
K	1	–	Jones et al. (2000)
MAF64	No amplicon	1	Crawford et al. (1995)
McM527	1	5	Crawford et al. (1995)
N	2	–	Jones et al. (2000)
O	1	–	Jones et al. (2000)
OarFCB193	No amplicon		Buchanan and Crawford (1993)
OarHH62	1	16	Crawford et al. (1995)
OarJMP58	1	26	Crawford et al. (1995)
OCAM	No amplicon	29	Moore et al. (1994)
P	1	–	Jones et al. (2000)
Q	2	–	Jones et al. (2000)
Rt27	2	–	Wilson et al. (1997)
Rt30	2	–	Wilson et al. (1997)
Rt5	3	–	Wilson et al. (1997)
Rt7	2	–	Wilson et al. (1997)
Rt9	1	–	Wilson et al. (1997)

References

Anderson JD, Honeycutt RL, Gonzales RA, Gee KL, Skow LC, Gallagher RL, Honeycutt DA, DeYoung RW (2002) Development of microsatellite DNA markers for the automated genetic characterization of white-tailed deer populations. J Wildl Manag 66:67–74

Banks SC, Piggott MP, Stow AJ, Taylor AC (2007) Sex and sociality in a disconnected world: a review of the impacts of habitat fragmentation on animal social interactions. Can J Zool 85:1065–1079

Barendse W, Armitage SM, Kossarek LM et al (1994) A genetic-linkage map of the bovine genome. Nat Genet 6:227–235

Bishop MD, Kappes SM, Keele JW, Stone RT, Sunden SLF, Hawkins GA, Toldo SS, Fries R, Grosz MD, Yoo JY, Beattie CW (1994) A genetic-linkage map for cattle. Genetics 136:619–639

Brashares JS, Arcese P, Sam MK (2001) Human demography and reserve size predict wildlife extinction in West Africa. Proc R Soc Lond B Biol Sci 268:2473–2478

Brezinsky L, Kemp SJ, Teale AJ (1993) 5 Polymorphic bovine microsatellites (Ilsts010-014). Anim Genet 24:75–76

Buchanan FC, Crawford AM (1993) Ovine microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 loci. Anim Genet 24:145

Cabrera A, Yepes J (1960) Mamíferos sudamericanos, 2nd edn. Edian, Buenos Aires

Caro TM, Laurenson MK (1994) Ecological and genetic factors in conservation: a cautionary tale. Science 263:485–486

Caughley G (1994) Directions in conservation biology. J Anim Ecol 63:215–244

Caughley G, Gunn A (1996) Conservation biology in theory and practice. Blackwell Science, Cambridge

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

Corti P (2008) Organisation sociale, dynamique de population, et conservation du cerf huemul (*Hippocamelus bisulcus*) dans la Patagonie du Chili. Département de biologie, Université de Sherbrooke, Sherbrooke, p xv+143

Corti P, Wittmer HU, Festa-Bianchet M (in press) Dynamics of a small population of endangered huemul deer (*Hippocamelus bisulcus*) in Chilean Patagonia. J Mamm

Cosse M, Gonzalez S, Maldonado JE (2007) Cross amplification tests of ungulate primers in the endangered neotropical pampas deer (*Ozotoceros bezoarticus*). Genet Mol Res 6:1118–1122

Couvet D (2002) Deleterious effects of restricted gene flow in fragmented populations. Conserv Biol 16:276–369

Crawford AM, Dodds KG, Ede AJ et al (1995) An autosomal genetic-linkage map of the sheep genome. Genetics 140:703–724

Dewoody JA, Honeycutt RL, Skow LC (1995) Microsatellite markers in white-tailed deer. J Hered 86:317–319

Diaz NI, Smith-Flueck JAM (2000) El huemul patagónico: un misterioso cérvido al borde de la extinción. L.O.L.A. (Literature of Latin America), Buenos Aires

Donoso PJ, Otero LA (2005) Towards a definition of a forest country: where is Chile located? Bosque 26:5–18

Dudash M, Fenster C (2000) Inbreeding and outbreeding depression in fragmented populations. In: Young AG, Clarke GM (eds) Genetics, demography and viability of fragmented populations. Cambridge University Press, New York, pp 35–53

Estoup A, Angers B (1998) Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. In: Carvalho G (ed) Advances in molecular ecology. IOS Press, Amsterdam, pp 55–86

Fahrig L (2002) Effect of habitat fragmentation on the extinction threshold: a synthesis. Ecol Appl 12:346–353

Flueck WT, Smith-Flueck JM (2006) Predicaments of endangered huemul deer, *Hippocamelus bisulcus*, in Argentina: a review. Eur J Wildl Res 52:69–80

Forbes SH, Hogg JT (1999) Assessing population structure at high levels of differentiation: microsatellite comparisons of bighorn sheep and large carnivores. Anim Conserv 2:223–233

Frankham R, Briscoe DA, Ballou JD (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge

Frid A (1994) Observations on habitat use and social organization of a huemul *Hippocamelus bisulcus* coastal population in Chile. Biol Conserv 67:13–19

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. Mol Ecol 10:305–318

Gilbert C, Ropiquet A, Hassanin A (2006) Mitochondrial and nuclear phylogenies of Cervidae (Mammalia, Ruminantia): systematics, morphology, and biogeography. Mol Phylogenet Evol 40:101–117

Gill R, Saucedo C, Aldridge D, Morgan G (2008) Ranging behaviour of huemul in relation to habitat and landscape. J Zool 274:254–260

Goñi R, Barrientos G, Figuerero MJ, Mengoni GL, Mena F, Lucero V, Reyes O (2004) Distribución espacial de entierros en la cordillera de Patagonia centro-meridional (Lago salitroso-Paso Roballos Arg/Entrada Baker-Río Chacabuco Ch). Chungará 36:1101–1107

Goudet J (2000) FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.1. Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland. http://www.unil.ch/izea/softwares/fstat.html

Grosz MD, Solinas Toldo S, Stone RT et al (1997) Chromosomal localization of six bovine microsatellite markers. Anim Genet 28:39–40

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

Hailer F, Helander B, Folkestad AO, Ganusevich SA, Garstad S, Hauff P, Koren C, Nygård T, Volke V, Vilà C, Ellegren H (2006) Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline. Biol Lett 2:316–319

Hanski I, Gilpin ME (eds) (1997) Metapopulation biology: ecology, genetics, and evolution. Academic Press, San Diego

Hedrick PW (1994) Purging inbreeding depression and the probability of extinction: full-sib mating. Heredity 73:363–372

Hewitt G (2000) The genetic legacy of the quaternary ice ages. Nature 405:907–913

IUCN (2009) IUCN red list of threatened species. Version 2009.1. http://www.iucnredlist.org. Accessed 12 Oct 2009

Johansson M, Primmer CR, Merilä J (2007) Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (*Rana temporaria*). Mol Ecol 16:2693–2700

Jones KC, Levine KF, Banks JD (2000) DNA-based genetic markers in black-tailed and mule deer for forensic applications. Calif Fish Game 86:115–126

Kaeuffer R, Coltman DW, Chapuis JL, Pontier D, Réale D (2007) Unexpected heterozygosity in an island mouflon population founded by a single pair of individuals. Proc R Soc Lond B Biol Sci 274:527–533

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241

Kirkpatrick M, Jarne P (2000) The effects of a bottleneck on inbreeding depression and the genetic load. Am Nat 155:154–167

Kuo CH, Janzen FJ (2003) BOTTLESIM: a bottleneck simulation program for long-lived species with overlapping generations. Mol Ecol Notes 3:669–673

Lande R (1988) Genetics and demography in biological conservation. Science 241:1455–1460

Leite KCE, Collevatti RG, Menegasso TR, Tomas WM, Duarte JMB (2007) Transferability of microsatellite loci from Cervidae species to the endangered Brazilian marsh deer, *Blastocerus dichotomus*. Genet Mol Res 6:325–330

Lippé C, Dumont P, Bernatchez L (2006) High genetic diversity and no inbreeding in the endangered copper redhorse, *Moxostoma hubbsi* (Catostomidae, Pisces): the positive sides of a long generation time. Mol Ecol 15:1769–1780

Luikart G, Cornuet JM (1999) Estimating the effective number of breeders from heterozygote excess in progeny. Genetics 151:1211–1216

Mainguy J, Llewellyn AS, Worsley K, Côté SD, Coltman DW (2005) Characterization of 29 polymorphic artiodactyl microsatellite markers for the mountain goat (*Oreamnos americanus*). Mol Ecol Notes 5:809–811

Moore SS, Byrne K, Berger KT et al (1994) Characterization of 65 bovine microsatellites. Mamm Genome 5:84–90

Newman D, Tallmon DA (2001) Experimental evidence for beneficial fitness effects of gene flow in recently isolated populations. Conserv Biol 15:1054–1063

Nunney L (1999) The effective size of a hierarchically structured population. Evolution 53:1–10

Pérez T, Albornoz J, Domínguez A (2002) Phylogeography of chamois (*Rupicapra* spp.) inferred from microsatellites. Mol Phylogenet Evol 25:524–534

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

Poissant J, Shafer ABA, Mainguy J, Davis CS, Côté SD, Hogg JT, Coltman DW (2009) Genome-wide cross-amplification of domestic sheep microsatellite loci in bighorn sheep and mountain goats. Mol Ecol Resour 9:1121–1126

Povilitis A (1998) Characteristics and conservation of a fragmented population of huemul *Hippocamelus bisulcus* in central Chile. Biol Conserv 86:97–104

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Redford KH, Eisenberg JF (1992) Mammals of the neotropics. Vol. 2. The southern cone: Chile, Argentina, Uruguay, Paraguay. University of Chicago Press, Chicago

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

Rinehart TA (2004) AFLP analysis using GENEMAPPER® software and an EXCEL® macro that aligns and converts output to binary. Biotechniques 37:186–188

Sarre SD, Georges A (2009) Genetics in conservation and wildlife management: a revolution since Caughley. Wildl Res 36:70–80

Sinclair ARE, Fryxell JM, Caughley G (2006) Wildlife ecology, conservation, and management, 2nd edn. Blackwell Pub, Malden

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proc Natl Acad Sci USA 101:15261–15264

Steffen P, Eggen A, Dietz AB et al (1993) Isolation and mapping of polymorphic microsatellites in cattle. Anim Genet 24:121–124

Vaiman D, Osta R, Mercier D, Grohs C, Leveziel H (1992) Characterization of 5 new bovine dinucleotide repeats. Anim Genet 23:537–541

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Velásquez H, Mena F, Trejo V, Reyes O (2005) Historical and archaeological overview of XIX–XX centuries, transition at Aisen's mountain range. Werken 7:5–20

Vila AR, López R, Pastore H, Faúndez R, Serret A (2006) Current distribution and conservation of the huemul (*Hippocamelus bisulcus*) in Argentina and Chile. Mastozool Neotrop 13:263–269

Wang J, Hill WG, Charlesworth D, Charlesworth B (1999) Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. Genet Res 74:165–178

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

Wilson GA, Strobeck C (1999) The isolation and characterization of microsatellite loci in bison, and their usefulness in other artiodactyls. Anim Genet 30:226–227

Wilson GA, Strobeck C, Wu L, Coffin JW (1997) Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. Mol Ecol 6:697–699

Wilson GA, Nishi JS, Elkin BT, Strobeck C (2005) Effects of a recent founding event and intrinsic population dynamics on genetic diversity in an ungulate population. Conserv Genet 6:905–916

Woodroffe R, Ginsberg JR (1998) Edge effects and the extinction of populations inside protected areas. Science 280:2126–2128

Worley K, Strobeck C, Arthur S, Carey J, Schwantje H, Veitch A, Coltman DW (2004) Population genetic structure of North American thinhorn sheep (*Ovis dalli*). Mol Ecol 13:2545–2556

Young AG, Clarke GM (2000) Genetics, demography, and viability of fragmented populations. Cambridge University Press, New York