ORGANISATION SOCIALE, DYNAMIQUE DE POPULATION, ET CONSERVATION
DU CERF HUEMUL (*HIPPOCAMELUS BISULCUS*) DANS LA PATAGONIE DU
CHILI

par

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A mi hijo y su linda mamá

SOMMAIRE

Ce projet de recherche a examiné l'organisation sociale, le cycle biologique et la dynamique des populations de cerf huemul (*Hippocamelus bisculus*) par l'utilisation de techniques aussi bien moléculaires que de suivi des individus, et fournit des informations utiles pour la conservation de cette espèce en danger. La population de la Réserve Nationale Lago Cochrane, en Patagonie chilienne, a été suivie entre mars 2005 et juin 2008. Pour reconnaître et suivre les individus (n = 55), les cerfs ont été équipés de colliers émetteurs, d'étiquettes auriculaires émettrices, et d'étiquettes auriculaires conventionnelles. L'organisation sociale du huemul a été analysée par un index d'association (le "ratio simple"), pour établir les tendances d'associations spatiales et temporelles des individus identifiables de chaque sexe. Le huemul forme de petits groupes (moyennes ± ES: 1.68 ± 0.25 individus) mixtes, durant l'année. J'ai attribué cette association inhabituelle à leur faible dimorphisme sexuel en terme de taille, ce qui engendre des besoins nutritionnels et des budgets d'activité similaires pour les deux sexes. Les femelles adultes peuvent s'associer en fonction des relations de parenté. Les deux sexes sont nettement phylopatriques et non-migratoires. Les mâles adultes s'évitent, et peuvent bénéficier de l'association avec des femelles ce qui diminue leur propre risque de prédation. Les mâles adultes qui défendaient des territoires ont produit plus de faons que les mâles non-territoriaux. Les simulations du taux de croissance fini (λ) de cette population suggèrent qu'elle est soit stable soit en déclin. L'analyse d'élasticité a montré que les changements en terme de survie des femelles adultes auraient des effets sévères sur λ. Bien que la survie des femelles adultes est apparu élevée et stable, celles des faons femelles était basse, à cause de la prédation et de la stochasticité démographique. Les recommandations de gestion ont ainsi été axées sur la survie des jeunes. La population étudiée est isolée, entourée d'habitats non favorables, détruits par les activités humaines. Sa diversité génétique est parmi les plus basses de toutes les populations d'ongulés étudiées jusqu'à présent. D'après la diversité allélique et l'hétérozygotie, les simulations pour estimer l'impact de la fragmentation ont indiqué une perte continue de diversité. La gestion du huemul de cette zone d'étude devrait au moins maintenir la taille de la population actuelle, augmenter l'aire protégée pour permettre la connexion avec les populations voisines, réduire la prédation des jeunes, et maintenir la survie actuelle des femelles adultes. Les futures recherches devraient s'attacher à la relation qu'a le huemul avec son habitat et ses prédateurs naturels.

SUMMARY

This research project used individual-based monitoring and molecular techniques to examine the social organization, life-history and population dynamics of huemul deer (*Hippocamelus bisulcus*) and provide knowledge useful for the conservation of this endangered species. The population at Lago Cochrane National Reserve, Chilean Patagonia, was monitored between March 2005 and June 2008. Deer ($n = 55$) were radiocollared, radio ear-tagged, and conventionally ear tagged to recognize individuals. Huemul social organization was analyzed using an index of association (the 'simple ratio'), to establish spatial and temporal association patterns of recognizable individuals of both sexes. Huemul formed small (mean ± SD: 1.68 ± 0.25 deer) mixed-sex groups throughout the year. I attributed this unusual association pattern to the low sexual dimorphism in body size, which should generate similar nutritional requirements and time budget for both sexes. Adult females may associate with each other based on kin relationships. Both sexes were highly philopatric and non-migratory. Adult males avoid each other, and may benefit from associating with females by decreasing their risk of predation. Adult males that defended territories sired more fawns than non-territorial males. Simulations of the finite rate of growth (λ) of the study population suggest that it is either stable or declining. Elasticity analysis showed that changes in adult female survival would have drastic effects on λ. Adult female survival appeared high and stable but the survival of female fawns was low, because of predation and demographic stochasticity. Consequently, management recommendations were focused on young survival. The study population is isolated, surrounded by unsuitable habitats due to human-caused habitat destruction. Its genetic diversity is among the lowest of any ungulate population studied so far. Based on observed diversity of alleles and heterozygosity, simulations to estimate the impact of fragmentation indicated a continued loss in diversity. Management of huemul in the study area should at least maintain the current population size, increase the area protected to allow connection with other populations, reduce predation on fawns, and maintain current adult female survival. Future research should focus on the relationship of huemul with its natural predators and habitat.

RESUMEN

A través del monitoreo individual y el uso técnicas moleculares se investigó la organización social, la historia de vida, y la dinámica poblacional del huemul (*Hippocamelus bisulcus*), para así proporcionar conocimiento para la conservación de esta especie en peligro. Desde marzo del 2005 a junio del 2008 se siguió en forma sistemática a la población de huemules de la Reserva Nacional Lago Cochrane. Los huemules ($n = 55$) fueron marcados con radio-collares, radio-aretes, y aretes convencionales para reconocerlos individualmente. La organización social fue analizada usando un índice de asociación ('índice de asociación simple'), para establecer los patrones espaciales y temporales de agrupación de los huemules de ambos sexos. Los huemules formaron grupos pequeños (media ± SD: 1.68 ± 0.25 ciervos) y mixtos a través del año. Atribuí este patrón de asociación a la diferencia limitada en tamaño corporal entre hembras y machos resultando en requerimientos nutricionales y actividades diarias similares. Las hembras adultas se asocian entre ellas posiblemente por parentesco. Ambos géneros fueron muy filopátricos y no migratorios. Los machos adultos se evitan entre ellos y se beneficiarían al asociarse con las hembras al disminuir el riesgo de ser depredados. Los machos adultos que defendieron territorios engendraron más cervatillos que los machos sin territorios. Las simulaciones de la tasa finita de crecimiento poblacional (λ) sugieren que la población está estable o disminuyendo. El análisis de elasticidad demostró que los cambios en supervivencia de las hembras adultas tendrían efectos drásticos sobre λ. La sobrevivencia de las hembras adultas fue alta y estable pero la sobrevivencia de los cervatillos hembras fue baja debido a la depredación y a la estocasticidad demográfica. Por lo tanto, las recomendaciones de manejo se enfocaron en aumentar la sobrevivencia de los animales jóvenes. La población estudiada se encuentra aislada, rodeada de ambientes alterados por actividades humanas y su diversidad genética está entre las más bajas de los ungulados estudiados hasta ahora. De acuerdo con la diversidad observada en alelos y la heterocigocidad, las simulaciones para estimar el impacto de la fragmentación indicaron una pérdida continua en diversidad. El manejo del huemul en el área de estudio debe por lo menos tratar de mantener el tamaño actual de la población, aumentar el área protegida para permitir la conexión con otras poblaciones, reducir la depredación sobre los cervatillos, y mantener la tasa de sobrevivencia de las hembras adultas. La investigación futura debe centrarse en la relación del huemul con sus depredadores naturales y su hábitat.

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CHAPTER I

GENERAL INTRODUCTION – GENERAL MATERIAL AND METHODS

General introduction

Habitat fragmentation and the destruction of wild areas are causing the extinction of species at a rate exceeding past rates of extinction (Ceballos and Ehrlich, 2002; Gaston, 2005; Hughes et al., 1997; Primack, 2006). The extirpation of different forms of life that make up our planet's biodiversity, evolved over millions of years, has the potential for unprecedented alteration of natural ecosystem functions, with possibly dire consequences (Lande, 1988; Primack, 2006; Young and Clarke, 2000). Efforts to conserve biodiversity have been set in place in many parts of the world to stop or at least delay extinction processes; however, it is uncertain whether those efforts can preserve a sustainable natural diversity (Boyd et al., 2008; Lande, 1988).

Most endangered species are restricted to small and fragmented areas, often away from their former distribution cores. In the face of human-induced threats, remaining populations are not necessarily in their best habitats, but rather in areas where the threats are reduced (Ceballos and Ehrlich, 2002; Channell and Lomolino, 2000). For many species, the remoteness of these locations makes them difficult to study. Therefore, details in the natural history of most of these endangered species are practically unknown (Lomolino and Channell, 1995). Lack of ecological knowledge presents a challenge for managing endangered species and for identifying the causes threatening them (Caughley and Gunn, 1996). From this point of view, it is of vital importance to understand the ecology, evolutionary patterns, behaviour, and demography of small populations to ensure their persistence.

South America hosts a great diversity of environments and wildlife, but many of these could disappear in the near future (Olson and Dinerstein, 1998; Redford and Eisenberg, 1992). One of the groups that is most representative of this continent is the Cervidae, with six genera and 15 species (Geist, 1998; Weber and Gonzalez, 2003). Of 15 South American deer species, two are classified by the International Union for Conservation of Nature as data-deficient (red brocket deer *Mazama americana* and pigmy brocket deer *M. nana*), three as least concern (brown brocket deer *M. gouazoubira*, Amazonian brown brocket deer *M. nemorivaga*, and white-tailed deer *Odocoileus virginianus*), one is near threat of extinction (pampas deer *Ozotoceros bezoarticus*), eight as vulnerable to extinction (little red brocket deer *M. rufina*, dwarf grey brocket deer *M. bricenni*,

small red brocket deer *M. bororo*, dwarf brocket deer *M. chunyi*, marsh deer *Blastocerus dichotomus*, the lesser pudu *P. puda*, the greater pudu *Pudu mephistophiles*, and taruca or northern Andean deer *Hippocamelus antisensis*), and one species as in danger of extinction (huemul or southern Andean deer *H. bisulcus*) (IUCN, 2008). Weber and Gonzalez (Weber and Gonzalez, 2003) have proposed that marsh deer, pampas deer, taruca, huemul and lesser pudu, together with pigmy and small red brocket deer, should all be designated as endangered.

Huemul is currently the most threatened deer in South America (IUCN, 2008). It inhabits the Andes of southern Chile and Argentina and has probably declined dramatically in numbers and distribution since the arrival of Europeans (Diaz, 1993; Diaz and Smith-Flueck, 2000; Flueck and Smith-Flueck, 2006; Povilitis, 1983a; Vila et al., 2006). This species was originally abundant from central Chile (34° S) to the Strait of Magellan (54° S) (Cabrera and Yepes, 1960). The total huemul population is currently estimated at less than 2000 individuals (Flueck and Smith-Flueck, 2006; Vila et al., 2006), reduced to less than 1% of its historical abundance (Redford and Eisenberg, 1992). Its distribution has been reduced by more than 50% (Fig. 1.1) (Diaz, 1993; Smith-Flueck and Flueck, 2001b; Vila et al., 2006), with a relict isolated population in Central Chile (Povilitis, 1998) and the rest in the southern and less accessible part of the country. For these reasons, huemul has been included in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2008) and appendices of the Convention on the Conservation of Migratory Species of Wild Animals (CMS, 2006).
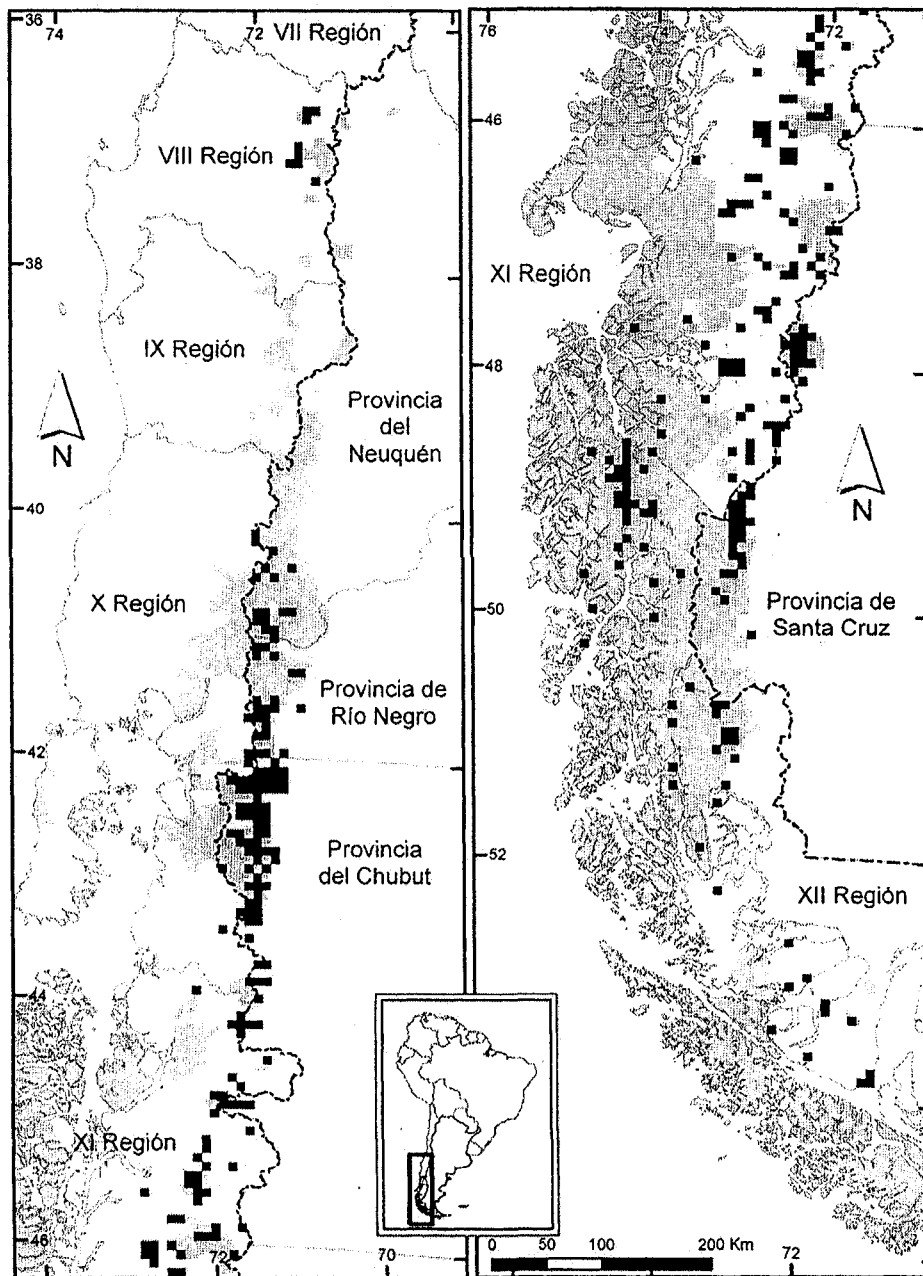
Figure 1.1. Current (2008) distribution range of huemul in Chile and Argentina. The black cells show records of occurrence of huemul and the light-gray areas represent protected areas (modified from Vila et al., 2006).

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The huemul is not only a flagship species for biodiversity and conservation in South America, it is also the national symbol of Chile (Frid, 1994; Povilitis, 1983a) and has promoted the protection of habitats and ecosystems which they inhabit. The temperate forest of Chile and Argentina, where the huemul is found, is classified by the World Wildlife Fund (WWF) as one of the top 200 areas where biological diversity is most distinctive, rich, and threatened (Armesto et al., 1998; Olson and Dinerstein, 1998). The huemul is protected in 13 Chilean and 12 Argentinean national parks and reserves. Protected areas in Chile are managed by CONAF (Chile National Forest Service) and in Argentina by APN (National Park Service). In addition, four private reserves have huemul in their properties (Corti et al., 2005).

Despite protection efforts, by all apparent indications huemul continue to decline. Declining populations of huemul and the reduction of its distributional range have been attributed to a variety of factors: habitat loss through conversion of native forest to farmland; poaching; attacks by domestic dogs; competition from domestic livestock and infection by the exotic diseases that they carry; introduction of exotic species, such as red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*); and cougar (*Puma concolor*) predation (Diaz and Smith-Flueck, 2000; Flueck and Smith-Flueck, 2006; Frid, 2001; Povilitis, 1983a, 1998; Saucedo and Gill, 2004; Serret, 2001; Smith-Flueck and Flueck, 1997, 2001a; Texera, 1974). Both the absolute and relative importance of these factors, however, remain unclear. No unbiased measurements of population parameters exist to establish tendencies or elucidate factors affecting huemul throughout its distribution.

Huemul exhibit several behaviours that distinguish them from other cervids. First, they form mixed-sex groups outside the rutting season (Frid, 1999; Povilitis, 1983b). Second, individuals have strong site fidelity (Gill et al., 2008). Third, the species is not particularly wary of humans (Diaz et al., 2007; P. Corti pers. obs.) The distinctive behaviours of the huemul could offer new insights for evolutionary theory and research, but they also make the management and conservation of this species more challenging.

To recover declining species it is crucial to accurately determine the causes of their decline (Sinclair et al., 2006). Sometimes, all it takes is legislative changes such as a ban on hunting or the establishment of high fines for poaching (e.g., commercial wildlife harvesting in Mongolia (Reading et al., 1998)). In other cases, active management to recover a declining species is required

(Sinclair et al., 2006), such as predator control (e.g., woodland caribou *Rangifer tarandus caribou* (Rettie and Messier, 1998)), translocations (e.g., bighorn sheep *Ovis canadensis* (Singer et al., 2000a)), or captive breeding and reintroductions (e.g., Arabian oryx *Oryx leucoryx* (Price, 1989)). However, to justify these intensive and expensive management actions, much knowledge needs to be acquired.

Previous research on declining ungulate species has recognized the importance of taking into account behaviour, such as social organization. One example is to not disrupt the social system of the animals when new individuals are used to establish a new population (Caro and Durant, 1995). Among reintroduced Przewalski horses (*Equus ferus przewalskii*) in Mongolia, some social groups of founder animals in their captive adaptation area were in bad physical condition due to malnutrition (Van Dierendonck and De Vries, 1996). Those animals did not belong to the dominant stallion group that monopolized the best grazing areas, and many of those animals died because they were forced to eat the only available vegetation, which were toxic, due to the social stress exerted by the dominant male (Van Dierendonck and De Vries, 1996). Following changes in the reintroduction protocol based in those observations of horse behaviour, the reintroductions proven successful (Van Dierendonck and De Vries, 1996).

It is also important to identify the relative importance of different population parameters. Demographic studies have identified potential causes of a species' decline and which demographic parameters have the greatest influence on changes in population growth. For woodland caribou (*Rangifer tarandus caribou*) in British Columbia, Canada, low survival rates of females mostly due to increased predation are driving the remaining population to extinction (Wittmer et al., 2007a). Habitat alterations had allowed an increase in alternative prey, leading to an increase in caribou predators (Wittmer et al., 2007a). In this case management actions were directed towards the increase in female and calf survival through predator and alternative prey control and habitat restoration (Wittmer et al., 2005a).

Additionally, the genetic status of declining species must be considered to investigate the impact of possible bottlenecks or 'founder effects' (Frankham et al., 2002). These factors are particularly relevant for populations that have been drastically reduced or that were established with few individuals (Frankham et al., 2002). Potential genetic isolation due to fragmentation of the

population plus a particular social system of a species can also decrease the long-term viability of endangered species. For example, in a recently established wood bison (*Bison bison athabascae*) population in northwestern Canada, two male founders sired over 90% of offspring, leading to a reduction in genetic diversity (Wilson et al., 2005). Monitoring of reproductive success and incorporation of selective breeding strategies were suggested to reduce the rate at which genetic diversity was being lost from this small and isolated population (Wilson et al., 2005).

The lack of scientific knowledge on huemul motivated me to initiate this research program aimed at understanding the causes and mechanisms of huemul decline and to contribute to the recovery of this endangered species. Hence, this study focused on applied research using current scientific theory, and methods to understand how huemul life history and behaviour may affect its conservation. I also wanted to explore the huemul's apparently unique social system, to contribute to knowledge about general theory on ungulate social organization and mating systems.

In one huemul population in Chilean Patagonia, I used radio collars, radio ear tags and conventional ear tags to individually recognize and follow huemul. Once individuals were recognized, they were monitored over 39 months from 2005 to 2008 to establish behavioural associations among individuals, and to establish their social organization and mating system. Through monthly systematic monitoring of the population, basic demographic parameters to determine population dynamics and causes of mortality were estimated. Molecular techniques, including paternity analyses were applied to supplement behavioural data on social structure and mating system and also to determine levels of genetic diversity, and inbreeding of this huemul population.

Research objectives

This research had 4 main objectives:

1) To understand the social organization and mating system of huemul. These behavioural characteristics are key factors associated with population viability and persistence, since they may affect recruitment and genetic variability when few males monopolize most of the reproduction.

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2) To determine huemul population dynamics. Variability in adult and yearling survival, fawn survival, production, and recruitment can have important effects on the persistence and growth of ungulate populations (Gaillard et al., 2000), but huemul vital rates are unknown. Causes of mortality in deer change with age (Gaillard et al., 2000), and strategies to increase or stabilize huemul populations require knowledge of age-specific causes and rates of mortality. Finally, knowledge about which age classes contribute most to changes in population growth (λ) is essential to guide management interventions.

3) To study the genetic variability of huemul. Preliminary mitochondrial DNA analyses suggested low variability (Jara, 2005), but no microsatellite markers have been used to establish allele diversity and heterozygosity. I sought to understand what potential factors produced this low variability and to relate it to mating behaviour and demography.

4) To establish a long-term population monitoring program. Monitoring of populations through time, together with accurate methods to determine population demography, will make it possible to estimate minimum viable population sizes and minimum reserve sizes for this endangered species (Van Vuren, 1998).

Dissertation outline

My dissertation includes three chapters and a general conclusion. Chapter II, on the social organization and mating system of this endangered deer, includes two subsections. Section II.a. presents findings regarding huemul social organization, obtained through an index of association, the 'simple ratio' (Cairns and Schwager, 1987; Ginsberg and Young, 1992), to establish association patterns of males and females; in subsection II.a., I present and discuss this association between sexes. Section II.b. describes the mating system of huemul and analyzes male reproductive success.

Chapter III examines the dynamics of the study population, its sex-age structure, and presents estimates of its sizes and trends. In this chapter I present estimates of vital rates and demographic parameters, such as the finite rate of population growth (λ), of a huemul population under three

different possible scenarios. Sensitivity and elasticity were calculated to establish which components of the population may contribute most to λ (Gaillard et al., 2000). Sensitivity estimates the impact on λ of a change in a vital rate. The level of change that is actually occurring was determined through the three different models named above. These population parameters were then used to propose management actions to target specific factors affecting this population of huemul. These analyses were conducted in collaboration with Dr. Heiko Wittmer of the University of California at Davis.

Chapter IV presents genetic analyses determining allele diversity and heterozygosity of the study population. This huemul population is isolated and surrounded by unsuitable environments because of human-caused habitat destruction. Predictions of genetic diversity loss are made with simulation models based on observed allele diversity and observed heterozygosity in relation to different population sizes (Kuo and Janzen, 2003). Management recommendations are presented to maintain the diversity of the huemul populations. The genetic analyses were performed in collaboration with Aaron Schafer at the University of Alberta.

Chapter V presents the general conclusions of my study, emphasizing its conservation implications and discussing management options for huemul deer. It also provides suggestions for future research.

General materials and methods

Study area

The study population is in the Lago Cochrane National Reserve (LCNR) (47°12'S, 72°30'W), Aysén District, Chilean Patagonia. The Reserve is 69.25 km^2 in size and it was created in 1967 to protect southern beech forests dominated by lenga (*Nothofagus pumilio*) and the remaining huemul population, which seems to have been relatively stable for at least 10 years (Conaf-Codeff, 2001). 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 town of Cochrane (about 3000 inhabitants) is located about 3 km to the southwest of the Reserve (Fig. 1.2).
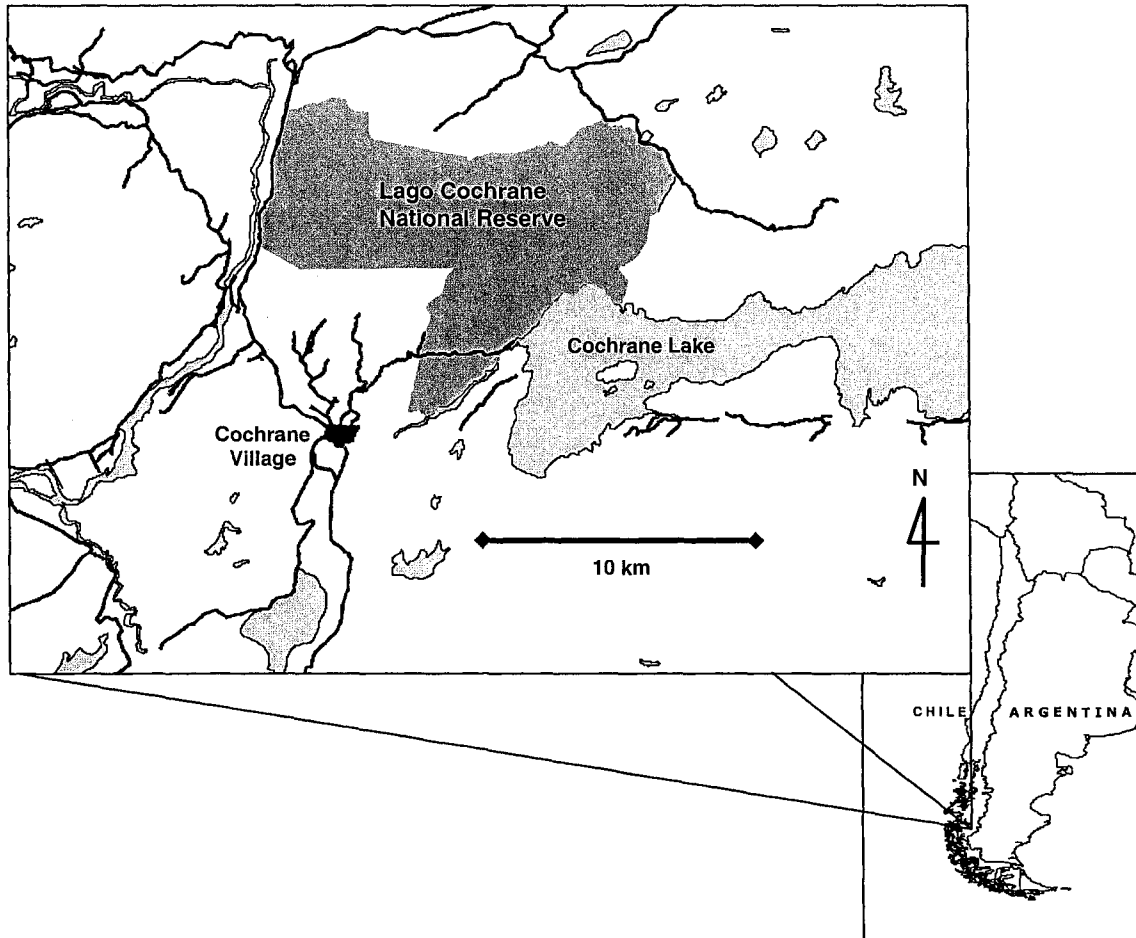
Figure 1.2. Huemul study area at Lago Cochrane National Reserve, Aysén District, Chilean Patagonia. The Reserve area is indicated in dark grey.

The vegetation of the Reserve includes at least 200 species of vascular plants, of which 81.5% are native and 18.5% are exotic (Teillier et al., unpublished report). 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*). Evidences of old burned areas persist from human-caused fires between 1942 and 1945 (Donoso and Otero, 2005). Mean annual temperature

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is 7.6°C and annual precipitation is nearly 730 mm, mostly falling between May and August. Snowfall occurs mostly between June and August.

Huemul capturing and sampling

Between 2005 and 2007, 55 huemul were captured and marked with ear tags, including 29 adults (15 males and 14 females), three two-year-olds (two males and one female), two male yearlings, and 21 fawns (15 males and six females). Deer were immobilized with a mixture of medetomidine (Zalopine, Orion Pharma, Espoo, Finland) and ketamine (Imalgene 1000, Merial SAS, Lyon, France) through teleinjection (Kreeger and Arnemo, 2007). The dosages used are approximately 0.09 ± 0.05 mg/kg medetomidine and 2.0 ± 0.7 mg/kg ketamine. All immobilized deer were revived with 0.40 ± 0.2 mg/kg atipamezole (Antisedan, Orion Pharma, Espoo, Finland) (P. Corti and C. Saucedo unpublished data). To deliver the drug mixtures, I fired darts from a DanInject compressed air rifle (Daninject Ltd, Børkop, Denmark). To capture and mark fawns, I monitored parturient or lactating females and hand-captured the newborns, that hide on the ground and can be captured until about 10 days of age. An additionally thirty deer (two adult males, five adult females, three young males, two young females, three yearlings, and 17 fawns) were identified by natural marks (scars in the face and body, body shape, coat coloration, colour distribution of the rump patch).

When darted individuals were recumbent and unresponsive (judged by observing ear movements), one person carefully approached the animal to cover its eyes with a dark cloth, while keeping noise to a minimum. Animals were positioned on sternal recumbency, with the head and neck held elevated. After the dart was removed and checked, the injection site was treated with antiseptics and antibiotics (local and general). Immobilized deer received a complete physical examination, with monitoring of rectal temperature, respiratory and heart rate using a pulse oximeter (Kreeger and Arnemo, 2007).

All captured animals were sexed and measured (total length, head length, chest girth, and hind foot length). Body condition was assessed through the palpation of fat deposits between the lumbar vertebras (Schröder and Staufenbiel, 2006). Incisor eruption allowed an estimate of age until four

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and half years old (Dimmick and Pelton, 1994). Older deer were classified as 'adult'. Hair, fecal, blood, and tissue samples were taken for genetic analyses. Blood was obtained through jugular puncturing with sterile needles and syringes. Tissues were obtained with a biopsy punch in the ear; the resulting hole was then used to place an ear tag. Feces were obtained directly from the rectum using latex gloves, and hair was pulled from areas where it is naturally short like the head. Blood was kept in vials, centrifuged and then the serum was frozen for antibody analyses. Tissue and feces were stored in 70% ethanol for later DNA extraction. Hair was stored in paper bags under dry conditions.

Animals younger than one year received VHF radio-ear tags (Sirtrack, Havelock North, New Zealand), while some animals older than one year old were fitted with conventional VHF radio-collars (Sirtrack). All animals older than one year of age also received conventional plastic numbered and coloured ear tags Allflex (Allflex Inc, Palmerston North, New Zealand), with a unique combination of colour and digits.

CHAPTER II

SOCIAL ORGANIZATION AND MATING SYSTEM OF HUEMUL DEER

SECTION II.a – SOCIAL ORGANIZATION

Introduction

The social organization of a species includes grouping, mating strategies and maternal attributes and is often highly plastic in response to ecological factors (Coakes and Whitehead, 2004; Jarman, 1974; Jarman and Jarman, 1979; Kie, 1999; Linklater, 2000). Habitat characteristics, nutrient requirements, body size and predation risk can modulate social systems (Jarman, 1974). The environment and demography of a population, particularly adult sex ratio and density, can affect intraspecific competition for resources and mates (Emlen and Oring, 1977). The presence of conspecifics and patterns of association can affect the persistence of a population, because individuals may cooperate to obtain or defend resources, protect themselves against predators, and acquire potential mating partners (Whitehead, 2008a). In addition, knowledge of the social structure of a population may help to understand dispersal, the spread of diseases, and genetic variability (Whitehead, 2008a).

The first theories to explain variation in mammalian sociality in relation to ecology were developed in the 1960's (Eisenberg, 1966; Hall and Devore, 1965). A seminal hypothesis for explaining variation in social organization of ungulates was developed by Jarman (1974) based on observations on the ecology, food habits, body size and social behaviour of African antelopes. The hypothesis states that sexual size dimorphism evolved as ancestral monomorphic and monogamous ungulate species occupying forested habitats moved into open grassland habitats, allowing for the aggregation of males and females and for the evolution of the consequently polygynous systems (Jarman, 1974). According to this hypothesis, ungulates with low sexual dimorphism, inhabiting visually dense environments, tend to form small mixed-sex groups and sometimes hold territories (e.g., oribi *Ourebia ourebi* (Brashares and Arcese, 2002; Brashares et al., 2000), Japanese serow *Capricornis crispus* (Kishimoto and Kawamichi, 1996; Ochiai et al., 1993), and bushbuck *Tragelaphus scriptus* (Apio et al., 2007)).

The hypothesis that body size and sexual dimorphism drive variation in social organization has been used to explain intra- and inter-specific variation in social organization, mostly in conspicuous and relatively abundant animals (Linklater, 2000). Endangered ungulate species have instead received little attention, mainly because most are rare or restricted to remote regions (Channell and Lomolino, 2000). This makes them difficult to study, but information on social organization and mating systems of endangered species can be crucial for their recovery and conservation (Caughley and Gunn, 1996; Caughley and Sinclair, 1994; Hogg, 2000; Sinclair et al., 2006; Stephens and Sutherland, 2000). The social organization of a population influences its distribution in the landscape, mating strategy, genetic variability, and sex- and age-specific survival, potentially affecting its extinction risk (Banks et al., 2007). Therefore, knowledge of factors that determine the associations between and within different sex-age classes is crucial for the conservation of species at risk (Banks et al., 2007; Sinclair et al., 2006).

Huemul (*Hippocamelus bisulcus*) or southern Andean deer, inhabits the southern Andes of Chile and Argentina and has a critical conservation status (Corti et al., 2005; Flueck and Smith-Flueck, 2006; Frid, 1994; Povilitis, 1983a, 1998; Vila et al., 2006). It is at risk of extinction, with no more than 2000 individuals remaining (IUCN, 2008). Information on the social organization of huemul is limited to brief descriptions from short-term studies (Frid, 1994, 1999) or studies based on few individuals (Povilitis, 1983b, 1985). All huemul populations are presently at low densities (Diaz and Smith-Flueck, 2000). They inhabit forested and rugged mountainous areas with stable food supply (Veblen et al., 1981; Veblen and Schlegel, 1982) and relatively mild winters with limited snow cover (Alaback, 1991).

Huemul social organization appears to be characterized by small groups (mean ± SD of 1.6 ± 0.7 deer (Povilitis, 1983b), and typical group size (*sensu* Jarman, 1974) of 2.2 (Frid, 1994)) with little or no sexual segregation (Frid, 1994, 1999; Povilitis, 1983b). This social behaviour is unusual for a middle-sized (about 60 kg for adult females) sexually dimorphic temperate ungulate (Bowyer, 2004; Main and Coblentz, 1990; Main et al., 1996; Ruckstuhl and Kokko, 2002; Ruckstuhl and Neuhaus, 2000, 2002) and the proposed explanations for this behaviour are speculative (see Frid, 1994; Povilitis, 1983b). Although huemul are sexually dimorphic because males have antlers, distinctive darker hair in the face, and they are apparently heavier (Diaz and Smith-Flueck, 2000), my data indicate very little dimorphism in mass. Chest girth, which was correlated with body mass

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($r_s = 0.59$, $n = 11$, $P = 0.027$), did not differ between adult males and females (mean ± SD: 105.56 ± 7.32 cm for 16 adult males and 102.38 ± 5.99 cm for 16 adult females; Mann-Whitney $U = 96.50$, $P = 0.233$).

The lack of quantitative knowledge on huemul social structure is an obstacle for the management and conservation of this species. Any recovery plan requires knowledge on social behaviour and grouping patterns, distribution and the consequences of social structure on population growth and genetic variability (Caro, 2007). The apparently unusual social organization of huemul deserves investigation. Recent studies suggest that huemul of both sexes have small, stable home ranges (ca. 400 ha) and high site fidelity, with no long-distance movements (Gill et al., 2008). The current and former distribution of huemul include a great variety of environments (Diaz and Smith-Flueck, 2000; Vila et al., 2006), yet variation in their sociality related to different ecological conditions is unknown and quantitative methods that would allow future comparisons among populations have not been employed.

Here I present a quantitative description of the social organization of huemul based on nearly three years of monitoring individuals in one population. I explored the social organization of this deer, and sought to identify the extrinsic and intrinsic factors that affect its social system. Based on the above, I formulated the following predictions:

1) Because of low sexual dimorphism, males and females should have similar nutritional requirements and foraging behaviour, therefore they should form mixed-sex groups.

2) Huemul of both sexes are philopatric on small home ranges; associations among females, and between females and males should therefore be stable in space and time.

3) Visually dense environments, like the ones huemul occupy, limit the formation of large groups. The high site fidelity of both sexes and the low population density should promote a social system where males avoid each other and associate with a stable group of females.

Using recently developed social network techniques (Whitehead, 2008a), I developed statistical models to quantify social interactions among adults. The models I used quantify individual

association patterns based on the proportion of time spent by dyads in the same group (Cairns and Schwager, 1987; Ginsberg and Young, 1992; Whitehead, 2008a). I also explored seasonal changes in group size and composition.

Material and methods

Study area

The study area was in the 69.25 km^2 Lago Cochrane National Reserve (LCNR) (47°12'S, 72°30'W), Aysén District, Chilean Patagonia. The Reserve was created in 1967 to protect southern beech forests, dominated by lenga (*Nothofagus pumilio*), together with a remaining huemul population. Details of the study area are given in Chapter I

Field observations

Observations were carried out on 41 individually marked huemul (33 ear tags, 14 radio-collars and eight deer recognized through natural marks), including 18 males (13 adults, older than three years and five juveniles aged one to three years) and 23 females (19 adults and four juveniles). Analyses were restricted to deer older than one year observed for at least six months, each with a minimum of 12 days of observations. Animals younger than one year were excluded because they were always seen with their mothers. Adult males were considered territorial if they appeared dominant to other males and held a specific area. Non-territorial adult and juvenile males were considered subordinates.

Huemul were monitored monthly from late March 2005 to December 2007. Deer could be readily located because 20 had radio collars and because they used small home ranges with high site fidelity (Gill et al., 2008). Deer without radio collars were located visually when found together with collared individuals or by searching within their known home ranges. Not all individuals were seen every month.

Wild huemul are easy to observe because they tolerate observers within 20-30m. When deer were encountered, I recorded the number, sex and age of all animals in the group. I started to record the behaviour of each animal when they were first seen using the instantaneous scan sampling method (Altmann, 1974; Martin and Bateson, 1993). I also recorded the antler development of males (velvet, hard antlers or no antlers), hour, date, and GPS UTM co-ordinates. Each observation session lasted between 30 minutes and 1 hour. Animals within 50 m of each other were considered to be in the same group. All neighbouring groups were well separated by topographic features. I recorded group membership once a month for each deer.

Quantitative analysis of social organization

To describe huemul social organization, I used the program SOCPROG 2.3 (Whitehead, 2008b) in MATLAB 7.4 (The Mathworks Inc, Natick, MA, USA) (Whitehead, 2008b) to build a matrix of associations between all possible dyads. To estimate the strength of associations within dyads, a simple-ratio index of association was used to produce a symmetrical association matrix (Cairns and Schwager, 1987; Ginsberg and Young, 1992).

The simple ratio index has been suggested as the most appropriated method to estimate associations within dyads (Cairns and Schwager, 1987; Ginsberg and Young, 1992), when association is defined by the presence/absence of individuals in an observed group (Ginsberg and Young, 1992). The simple ratio index is calculated as:

$$\textit{Simple ratio of association} = \frac{x}{N - D}$$

where x is the number of observation periods during which animals Y and X were seen together, N is the total number of observation periods and D is the number of observation periods in which neither Y nor X are sighted (Ginsberg and Young, 1992).

The degree to which groups of animals are distinct units is an important element in social organization, because it measures preferential associations among all possible dyads (Bejder et al., 1998). The degree of association within huemul social units was examined by first establishing an association matrix among dyads that was displayed through an average-linkage cluster analysis of the average association between dyads that mostly associate with each other (Whitehead, 2008a). A dendrogram obtained from the average-linkage cluster analysis graphically represented the association matrix (Whitehead, 2008a; Whitehead, 2008b). Individuals were arranged on the Y-axis according to the cluster (social unit) they were mostly associated, with the strength of the associations ranging from 0 (the lowest) and 1 (the highest) on the X-axis. Figure 2.1 presents an example of a dendrogram of the average-linkage cluster analysis for killer whales (*Orcinus orca*) associations (Baird and Whitehead, 2000).
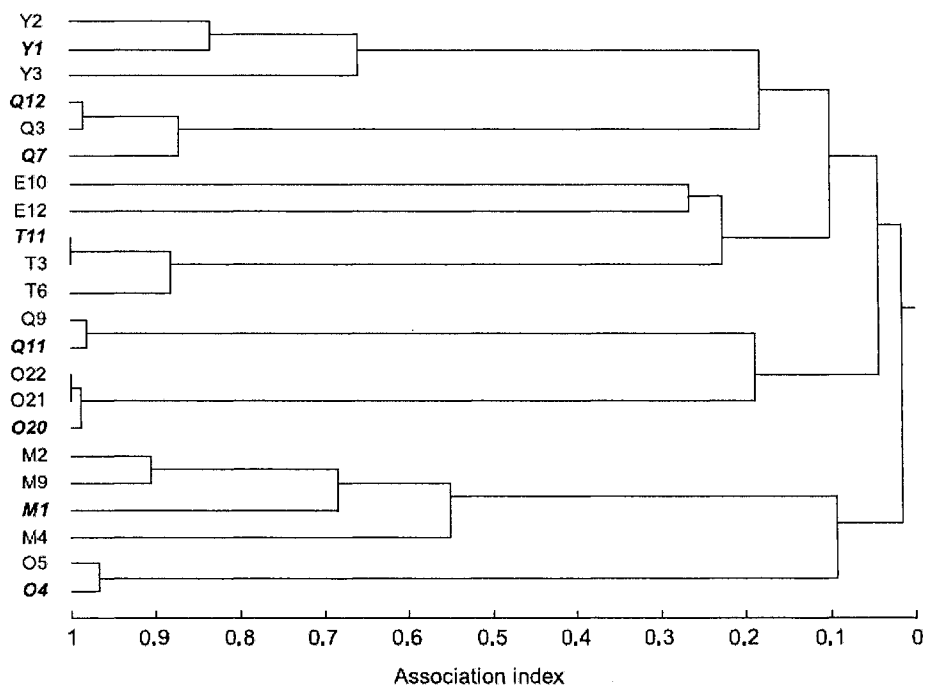
Figure 2.1. Dendrogram of cluster analysis for a killer whale population in British Columbia (Baird and Whitehead, 2000).

To test the fit of the observed data with the dendrogram, I calculated the cophenetic correlation coefficient (CCC), which estimates the accuracy of preserving the pairwise distances among raw

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data points (Sokal and Rohlf, 1962). In the clustering of biological information of association, the cophenetic similarity or cophenetic distance of two individuals X and Y is a measure of how associated those two individuals have to be in order to be grouped into the same cluster of a dendrogram (Sokal and Rohlf, 1962). A CCC ≥ 0.8 indicates that the dendrogram is an accurate representation of the observed data (Whitehead, 2008a).

Clusters of associated individuals were identified and corrected for *gregariousness*, which is defined as the mean number of associates for an individual (Pepper et al., 1999). Then, dyads that form a cluster were identified as the ones with at least twice of the average association index of the population (Bejder et al., 1998). I used *modularity*, which is the difference between the proportion of the total association within clusters and the expected proportion of associations, a network technique that visualizes the social system as a set of nodes representing individuals connected by line vectors that indicate their interactions (Newman, 2006), as an indicator of the strength of the association among individuals. Modularity identifies social units within the study population and provides a guideline for assessing at what level in the dendrogram to stop clustering (Whitehead, 2008a). Modularity expresses how much observed groups differentiate among each other. The association index which maximises modularity suggested the best division of clusters and it is also the association index when huemul began to form distinct social units. For example, in immature female sperm whales (*Physeter macrocephalus*) the maximum value of modularity was 0.63 at an association level of 0.1, then at that division of the clusters there was much more total association within clusters than would be expected for randomly determined clusters (Whitehead and Arborm, 1987). Maximisation of modularity was calculated according to association indices and then graphically determined by plotting modularity versus the association index (Whitehead, 2008a). Modularity (Q) was calculated as follows:

$$Q = \frac{\sum_{I,J} \alpha_{IJ} \delta(c_I, c_J)}{\sum_{I,J} \alpha_{IJ}} - \frac{\sum_{I,J} \hat{\alpha}_{IJ} \delta(c_I, c_J)}{\sum_{I,J} \hat{\alpha}_{IJ}}$$

where α_{IJ} is the association index between individuals I and J, $\hat{\alpha}_{IJ}$ is the expected value of α_{IJ} assuming random associations, $\delta(c_I, c_J) = 1$ if I and J are members of the same social unit, and $\delta(c_I,$

c_J) = 0 if I and J are members of different social units (Whitehead, 2008b). Modularity Q has an expected value between 0.0 and 1.0. Values > 0.3 indicate that the subdivision of the social units was satisfactorily established (Whitehead, 2008b).

The strength of the association of an individual to a social unit was estimated through the *eigenvector centrality method*, which is simply the appropriate element of the first eigenvector of the matrix of association indices (Newman 2004). The eigenvector is a measure of the strength of association of an individual within the social unit it was mostly associated with (Newman, 2004). Eigenvectors are linearly transformed into eigenvalues, which are used to interpret this parameter. High eigenvalues indicate that an individual is often seen with members of its social unit, values near zero indicate low association of an individual to its social unit.

I also created a sociogram where points representing individuals were arranged in a circle. Lines united those points if individual deer were associated, and the thickness of lines indicated the strength of the association indices for each dyad (Whitehead, 2008b).

After calculating the association between members of a dyad, an estimate of the *social differentiation*, the degree to which members of dyads within a population differ in their probability of association, was also calculated. *Social differentiation* is expressed as the coefficient of variation (CV) of the proportion of records when members of a dyad were seen together (true association index). *Social differentiation* measures variation in the social system, with CV ≤ 0.3 indicating rather homogeneous societies with similar indices of associations between dyads and no preferred companions among individuals; CV values ≥ 0.5 indicate well differentiated societies, where indices of associations between dyads are different and animals have preferred companions. Values of CV of > 2.0 reflect extremely differentiated societies with each animal seen with specific individuals most of the time (Whitehead, 2008b). The maximum likelihood method was used to estimate the correlation between the true and estimated (simple ratio of association) association indices (Whitehead, 2008b). The values of CV that determine the distinct levels of social differentiation are based in the correlation (r) value between the true and the estimated association indices as an indicator of the power of the analysis to detect the social system. Thus, 1.0 indicates a perfect fit of the observed and expected values of association indices and 0.0 indicates that the

system was very poorly detected (Whitehead, 2008b). Then r was calculated as follow (*sensu* Whitehead 2008c):

$$r = \frac{s}{CV(\alpha_{AB})}$$

where s is the *social differentiation* or the estimated CV of true association indices, and CV (α_{AB}) is the CV estimated from the simple ratio of association indices. For example, for 63 bottlenose whale (*Hyperodoon ampullatus*) the estimated correlation coefficient between the true and estimated association indices was 0.22, which suggested that the representation of the matrix of association indices will not reflect to any great extent and larger sample size is needed to identify the social system of this species (Whitehead 2008c).

An *association index* and *maximum association* were calculated within and between each sex-age class. The *association index* is the probability that a randomly chosen member of class X was associated with a randomly chosen member of class Y during any sampling period. *Maximum association* is the average of the strongest association index between each member of class Y and any member of class X (Baird and Whitehead, 2000). I used the permutation Mantel test to test the null hypothesis that associations between and within classes do not differ (Whitehead, 2008b). Results are expressed as a t-value with infinite degrees of freedom, the permutation P-value, and matrix correlation coefficient. If within-class associations are higher than between-class associations, t-value is positive, P-value is large, and the matrix correlation is positive (Whitehead, 2008b).

To determine the stability of associations over time between and within sex classes, temporal trends in association were displayed as lagged association rates or the probability of seeing together two individuals that were associated during a previous observation (Whitehead, 2008a). Temporal trends in association between dyads were examined by plotting the changes in average association rate or the probability of seeing together two individuals that were associated during a previous observation over time lags (termed as lagged association rate by (Whitehead, 1995; Whitehead, 2008a). This procedure estimates the probability that if two individuals are associated at some time, they will remain associated after various time lags (τ). The analysis was performed on all adults and

juveniles that associated during the study period, and it took into account mortalities: individuals that were not seen for at least six months during the study period were removed from the analysis. *The population lagged association rate g* (τ), the probability of association τ time units after a previous association averaged over all the associations (Whitehead, 2008a) was calculated as follows (Whitehead, 1995):

$$g(\tau) = \frac{\displaystyle\sum_{j,k/(t_k-t_j)=\tau} \sum_{X} \sum_{Y \neq X} a_j(X,Y) \cdot a_k(X,Y)}{\displaystyle\sum_{j,k/(t_k-t_j)=\tau} \sum_{X} \sum_{Y \neq X} a_j(X,Y) \cdot a_k(X,X)}$$

where $a_j(X, Y) = 1$ if X and Y were recorded as associated in time period j, $a_j(X, Y) = 0$ if they were not associated or if either was not identified during the sampling period, $a_k(X, X) = 1$ if X was identified in period k, and $a_k(X, X) = 0$ if X was not identified in period k (Whitehead, 2008a). The $g(\tau)$ was plotted against the time lag (τ) to describe how relationships between and within classes changes through time (Whitehead, 2008a). Figure 2.2 presents an example of killer whales lagged association rates (Baird and Whitehead, 2000).
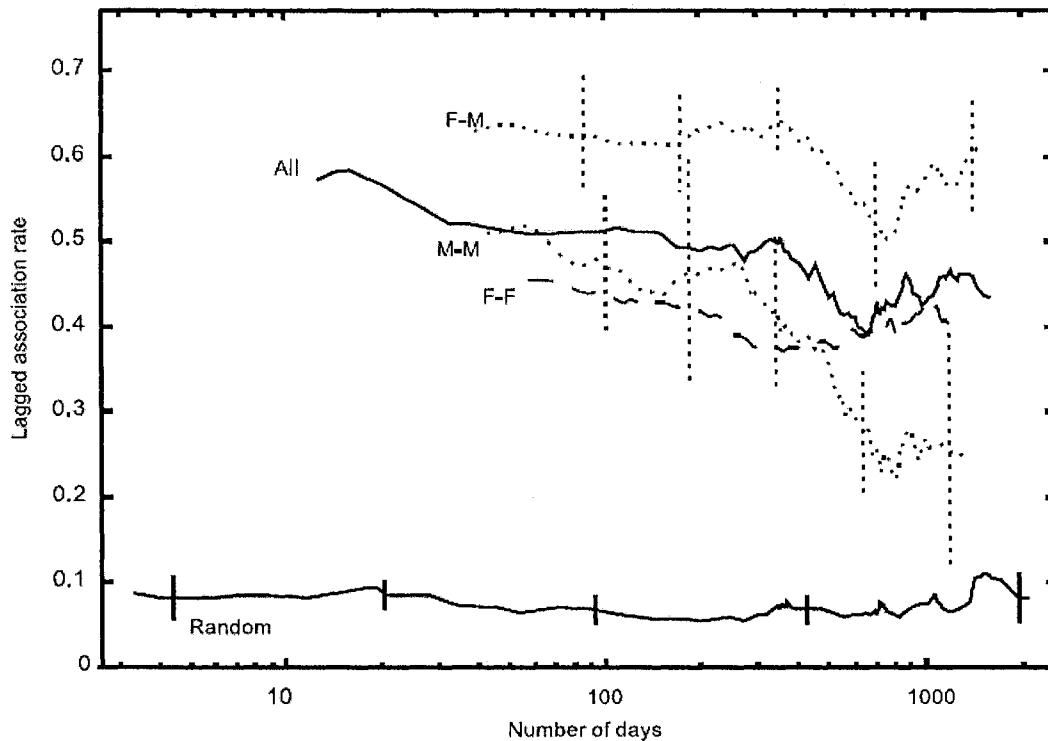
Figure 2.2. Lagged association rate from Baird and Whitehead (2000) data on killer whales in British Columbia. Lines represent lag association rates between females and males (F-M), males and males (M-M), females and females (F-F), and the null association rate (random).

Five different lagged association rate (LAR) models were fitted to the curves obtained from the plotted $g(\tau)$ for each relationship between and within classes to describe possible types of associations among individuals. The five LAR models, previously established by Whitehead (1995; 2008a), are pre-installed in SOCPROG 2.3. These LAR models fit various biologically plausible possibilities for the relationship of the probability of seeing animal Y with animal X at time τ $(T + t)$ if they were associated at time T (Whitehead, 1995; Whitehead, 2008a). Models (Fig. 2.3) were fitted by maximum likelihood (Whitehead, 2008a). To select the model that best fit the data, I used the Quasi-likelihood Akaike's Information Criterion (QAIC) as recommended by Whitehead (2007). Because of the non-independence of data points (associations of the same individuals throughout time), the summed log-likelihoods from different models cannot be used for formal

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likelihood ratio tests, so the QAIC provides a reasonable basis for model selection (Whitehead, 2007; Whitehead, 2008a) because it compensates for the poor fitting of models with large data sets (Anderson et al., 2000; Anderson et al., 2001; Whitehead, 2007; Whitehead, 2008a). Moving average for all model curves was set at 400 association rates per time lag arbitrarily widening the range of τ being considered, so that the denominator of the equation for $g(\tau)$ is above some minimum (Whitehead, 2008a). The desirable minimum depends on the situation that gave a reasonable precision of $g(\tau)$ and τ, with no excessively smooth curves (large moving average) that loose information or with exceedingly spurious peaks (small moving average) and depressions that confound the information obtained for further interpretation (Whitehead, 2008a).

The first candidate model (LAR 1) represents a social systems where associated individuals disassociate very quickly but then may reassociate again very briefly in a later month, with preferential reassociations between some pairs of individuals if $g = \beta1$ is greater than when animals associate randomly (Whitehead, 2008a). This model can also represent a situation where some, but not all, associations are permanent. The second model (LAR 2) covers an association type of constant companions (dyads that associate for several sampling periods) plus casual acquisitions (dyads that are rarely seen together) ($g = \beta2 + (1 - \beta2)e^{-\beta1\tau}$): some individuals associate permanently, but they can also have associations with other individuals that may be not very stable in time. Association rates, the probability of seeing together two individuals that were associated during a previous observation, initially fall exponentially, then level off. Here casual acquisitions of companions occur if the population is closed and the levelling off is equal the null association rate or random association between individuals. If the lagged association rate is above the null association rate, this model represents a system with permanent social units that associate temporarily, and preferred associations lasting over several sampling periods (constant companions), whereas other individuals are floaters who move between units (casual acquisitions). The third model (LAR 3) represents rapid dissociation of some dyads plus constant companions plus casual acquisitions ($g = \beta2 + \beta3e^{-\beta1\tau}$); this combines models LAR 1, with rapid disassociations between two sampling period, and LAR 2, in which association rates first decline but then recover to their previous level. This model is similar to LAR 2 but includes the possibility of rapid temporary disassociations among individuals. The fourth model (LAR 4) represents two levels of casual acquisitions ($g = \beta3e^{-\beta1\tau} + (1-\beta3)e^{-\beta2\tau}$) with disassociations at different lengths in time relative to the established sampling period. The shorter is probably a social disaffiliation of the

types discussed under LAR 2, however some strong associations eventually decay for reasons such as movements between permanent units, shifts in preferred companions, mortality, emigration, or a combination of these reasons. The fifth model (LAR 5) combines casual acquisitions of associated individuals that disassociate rapidly with the two levels of disassociations of LAR 4 ($g = \beta3e^{-\beta1\tau} + \beta4e^{-\beta2\tau}$); for example two individuals associate for some time, disassociate, and may or may not reassociate later, but eventually reassociation rates reach zero. This situation may arise through death, movements of individuals, or avoidance of previous associates.
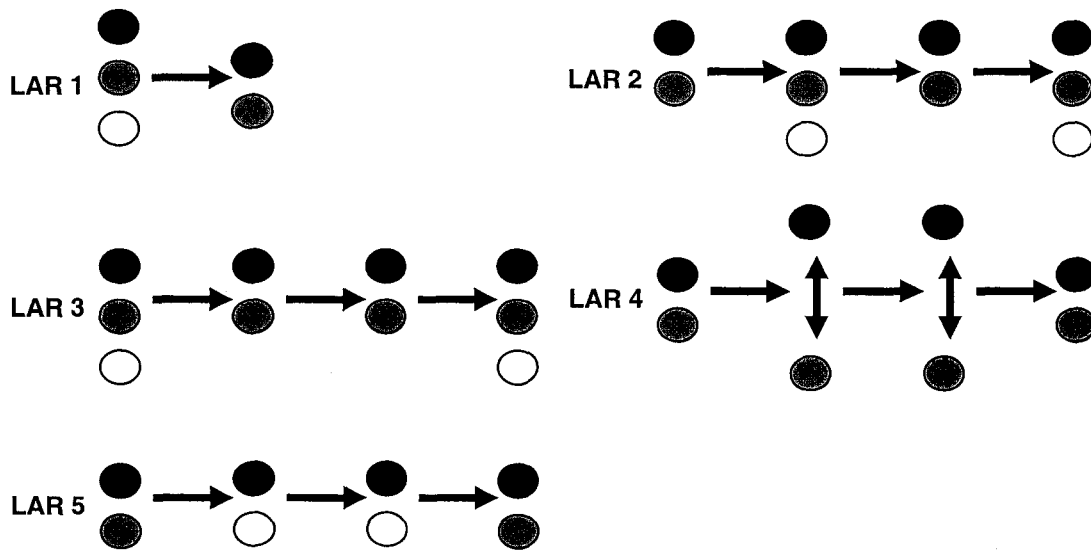
Figure 2.3. Diagrammatic representation of the five Lagged Association Rates (LAR) models. Circles of different colour indicate different individual deer, horizontal arrow represent the time elapsed between repeated observations of either member of the dyad. When circles are on top of each other deer remain associated, vertical arrows indicate no association at that sampling time. If one circle is not present after several observation periods, then that individual is not associated with the other(s).

Group characteristics

I calculated the typical group size (TGS) for each individual huemul. The arithmetic mean of the size of a group does not measure the average group experienced by animals (Jarman, 1974). TGS is calculated as follows (Jarman, 1974):

$$TGS = \frac{\sum_{i=1} n_i^2}{N}$$

where n is number of individuals in each group and N is the total number of animals in all groups. The mean typical group size (Jarman, 1974) and the mean number of individuals that can be possible companions, which were the animals that formed the same identified social unit according to modularity and the eigenvector centrality method, were calculated for each different sex-age class and compared with non-parametric one-way ANOVA (Kruskal-Wallis H test) (Zar, 1999).

Group composition was estimated considering the time individual males were observed with other individual males and females. Then the proportion of time that adult and juvenile males spent with other individuals was classified in the following categories: solitary; with at least one female; with at least one male; or with at least another deer of each sex. To look for seasonal differences in the time males spent alone or with females I used one-way ANOVA to compare the proportions of observations of solitary males and males in any group by season (Zar, 1999). Data were visually inspected for normality by examining normal probability plots and homoscedasticity was checked with Levene's test. Because data were proportions, they were arcsine-square root transformed to normalize their distribution (Zar, 1999). When group size varied significantly with season, seasons were compared using the *post-hoc* Sidak's test (Zar, 1999).

Huemul group size, determined using all observations from 2005, 2006, and 2007, included all individuals aged one year and older. To test for seasonal differences in group size, I used one-way ANOVA. Natural logarithm transformations normalized the distribution of group size (Zar, 1999). When group size varied significantly with season, seasons were compared using the *post-hoc* Tamhane-*T2* test because variances were not homogeneous (Huizingh, 2007).

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All statistical tests for group size and composition used SPSS 15.0 (SPSS, 2006). In nonparametric tests, the Monte Carlo estimate was used to obtain unbiased significance values without relying on the assumptions for the asymptotic method, sampling 10000 repeated measurements from the data set (Norušis, 1994).

Results

Social structure

During 33 months of study, 348 sampling days (approximately 30 min to 1 hour observation for each detected social unit) led to 1055 sightings of 41 individuals (Table 2.1). The mean of the simple ratio association index for males ranged from 0.00 to 0.03 that means a low association rate, for females it was from 0.01 to 0.03. The maximum simple ratio association index (with any other deer) for males ranged from 0.07 among territorial males to 0.47 for juveniles associating with other juveniles. For females, the maximum association index ranged from 0.10 to 0.47 (Table 2.1). Genetic analyses suggest that at least six of the seven female social units included mother-daughter pairs (P. Corti and A. Shafer, unpublished data), so it is highly probable they form kin related groups.

The dendrogram in Figure 2.4 shows eight huemul social units structured from the different dyad associations and separated by the modularity method. The dendrogram is an accurate representation of the true associations observed (CCC = 0.954). Association indices were weak, indicating that most deer spent short periods of time in small groups or were solitary, especially adult males. Figure 2.5 presents the variation of modularity values across different values of association indices. The maximum modularity (Q = 0.673), which successfully separated huemul social units, was at an association index of 0.033, indicating that at this association level huemul start to form identifiable and permanent social units.

Table 2.1. Individually known huemul observed from late March 2005 to December 2007. Deer first identified as fawns were only included after they became yearlings. Male class is based on social status (see section II.b). The mean of association is the average association for an individual with other deer and the maximum association is the strongest association index for each individual.

a) Males.

Individual	First Observation	Class	Last Observation	Mother/Father	N° Months Observed	Mean of Association	Maximum Association Index
11C*a	19/04/2006	Juvenile	27/02/2007	-	8	0.02	0.27
1A^a	02/11/2005	Juvenile	25/10/2007	19V/1V	17	0.03	0.47
22V	15/01/2006	Juvenile	15/10/2007	-/2V	10	0.03	0.47
23V	08/11/2005	Juvenile	25/11/2007	6C/2V	16	0.04	0.39
5N^a	26/11/2006	Juvenile	26/11/2007	24V/4V	10	0.01	0.31
11V	21/07/2005	Subordinate Adult	07/02/2007	-	11	0.01	0.12
10V	25/06/2005	Subordinate Adult	27/11/2007	-	20	0.02	0.13
21V	25/06/2005	Subordinate Adult	25/11/2007	-	16	0.02	0.15
5V	07/06/2005	Subordinate Adult	29/10/2007	-	17	0.01	0.10
7B*	10/03/2006	Subordinate Adult	20/08/2007	-	9	0.01	0.13
2B*	26/06/2005	Territorial Adult	25/11/2006	-	7	0.01	0.17
2N	25/03/2006	Territorial Adult	19/10/2007	-	15	0.02	0.34
2V	10/04/2005	Territorial Adult	02/04/2007	-	18	0.03	0.32
3A	12/12/2005	Territorial Adult	28/11/2007	-	15	0.01	0.07
4N	05/03/2006	Territorial Adult	18/11/2007	-	18	0.02	0.32
4V	04/06/2005	Territorial Adult	22/04/2007	-	17	0.00	0.07
17V	26/08/2005	Territorial Adult	22/11/2007	-	20	0.03	0.34
9V	22/07/2005	Territorial Adult	10/11/2007	-	20	0.02	0.21

[a] Individuals recognized as fawns when first seen, but included in the analysis when become yearlings.
* Individuals recognized through their natural marks.

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b) Females.

Individual	First Observation	Class	Last Observation	Mother/Father	Nº Months Observed	Mean Level of Association	Maximum Association Index
2A[a]	02/11/2005	Juvenile	16/11/2007	-/3A	13	0.03	0.47
5A	25/06/2005	Juvenile	25/11/2007	-	21	0.03	0.31
6B*	30/11/2005	Juvenile	15/10/2007	-	15	0.03	0.39
8C[a]*	06/11/2006	Juvenile	29/10/2007	12V/-	6	0.03	0.47
11B*	24/10/2006	Adult	25/07/2007	-	7	0.01	0.10
12V	25/06/2005	Adult	25/11/2007	-	21	0.03	0.22
14C	21/07/2005	Adult	16/11/2007	-	19	0.01	0.17
14V	25/03/2006	Adult	26/11/2007	1N/-	18	0.02	0.34
15V	27/06/2005	Adult	22/11/2007	-	21	0.03	0.32
16V	20/07/2005	Adult	18/11/2007	18V/-	22	0.03	0.28
18V	20/07/2005	Adult	19/11/2007	-	21	0.03	0.32
19V	22/08/2005	Adult	18/11/2007	-	20	0.03	0.42
1N	03/08/2006	Adult	01/12/2007	-	10	0.01	0.21
20V	17/11/2005	Adult	23/11/2007	-	22	0.02	0.32
24V	08/04/2005	Adult	17/11/2007	-	24	0.02	0.31
25V	26/06/2005	Adult	16/11/2007	19/-	21	0.02	0.42
3B*	28/07/2005	Adult	02/04/2007	-	8	0.01	0.10
3V	30/05/2005	Adult	18/11/2007	18V/-	20	0.03	0.34
4A	25/06/2005	Adult	28/11/2007	5A/-	17	0.03	0.31
4B*	25/06/2005	Adult	31/10/2007	-	10	0.02	0.21
6C	24/06/2005	Adult	25/10/2006	-	16	0.03	0.32
7N	23/10/2006	Adult	23/10/2007	8N/-	10	0.01	0.19
8N	02/04/2006	Adult	14/10/2007	-	13	0.02	0.21

[a] Individuals recognized as fawns when first seen, but included in the analysis when become yearlings.

* Individuals recognized through their natural marks.
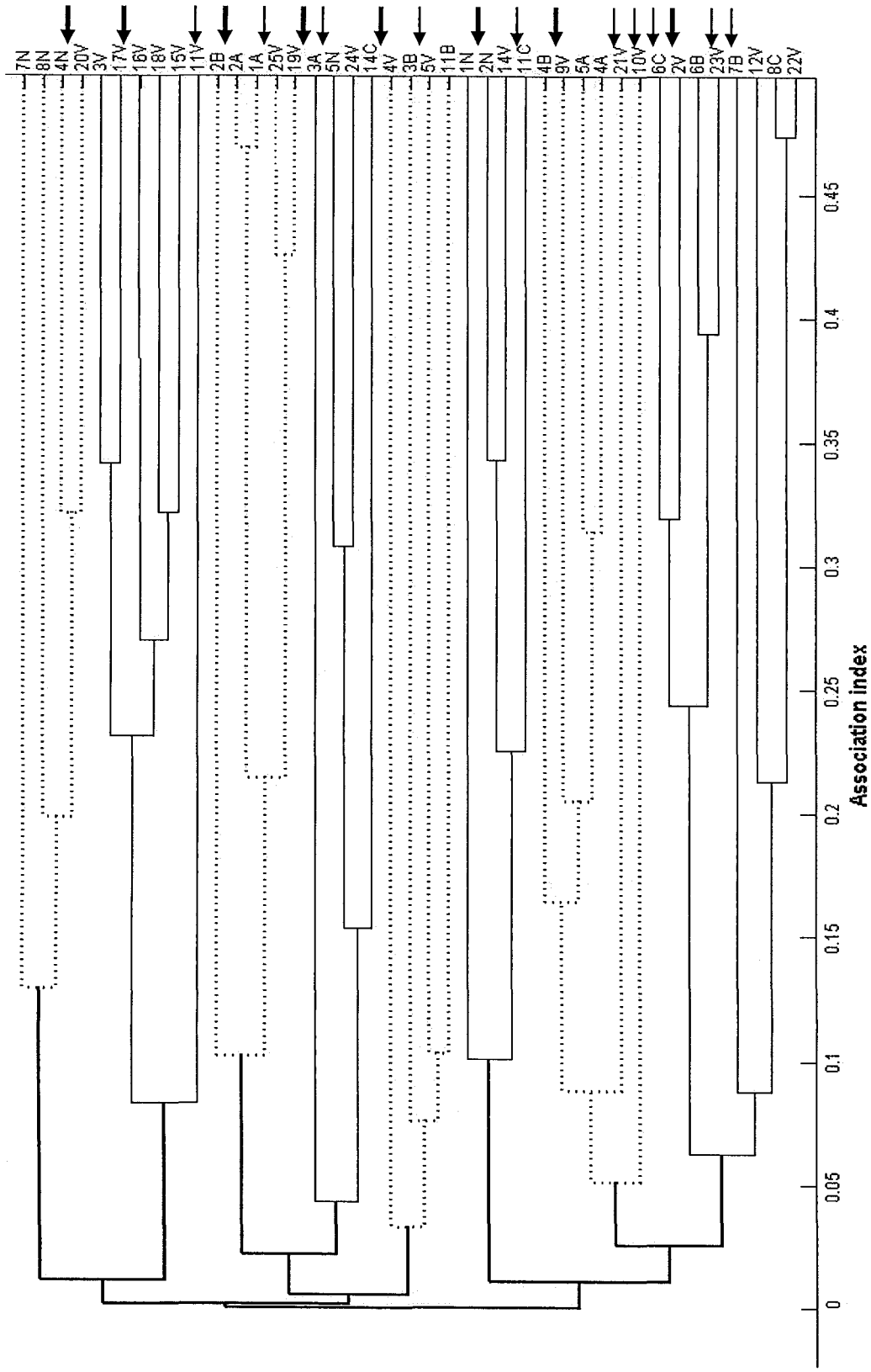
Figure 2.4. Dendrogram of the associations between huemul dyads observed for ≥6 months. Each type of line indicates a group of animals most commonly seen associated (a social unit) during the study. Territorial males are indicated with thick arrows, subordinate and juvenile males with small arrows.

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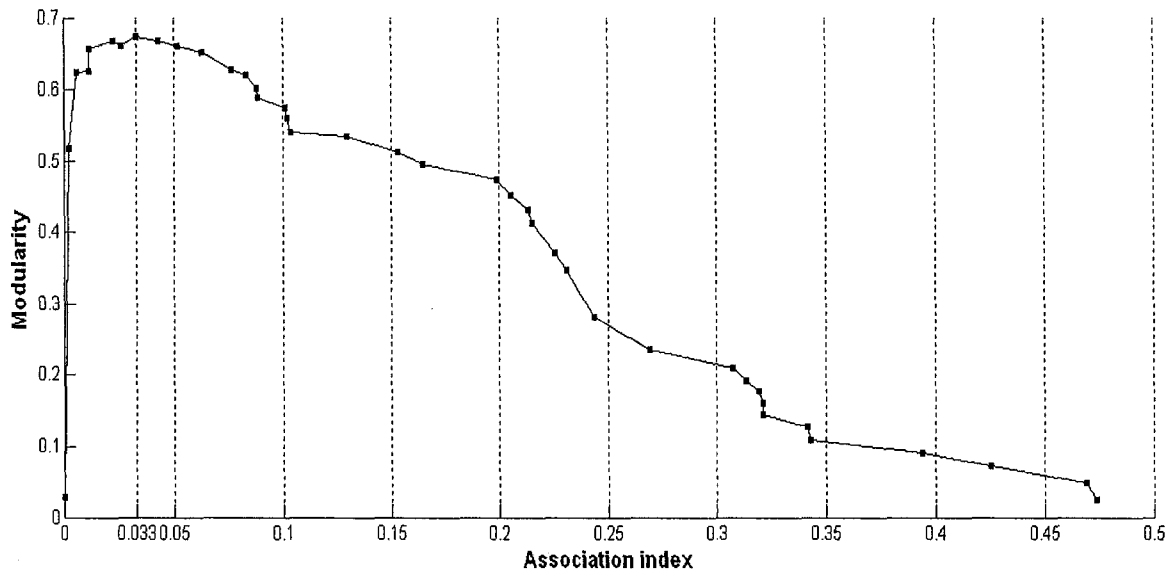
Figure 2.5. Modularity of the dendrogram, suggesting that the best division into clusters (social units) is with an association index of 0.033, at a maximum modularity of 0.673.

The eigenvector centrality method showed that the seven social units included one territorial male and several females, juveniles, and sometimes subordinate males (Table 2.2); the highest eigenvalues (highest association to a group) were for juveniles, adult females and territorial males, and the lowest were for subordinate males, who were the least associated individuals to particular social units. Social unit 7 over time included two territorial males because male 3A enlarged its territory towards the area of male 2B when the latter disappeared. Male 3A had the lowest eigenvalue for this cluster, indicating weak associations because he was mostly solitary. On the contrary 2B had one of the highest eigenvalues because he was permanently associated to other animals of this cluster before disappearing.

The cluster analysis (Fig. 2.4) was consistent with the eigenvector centrality analysis (Table 2.2) with the only exception of one social unit and one subordinate male. The social unit formed by male 3A, juvenile male 5V, and females 24V and 14C assigned to social unit 4 by cluster analysis was included in social unit 7 by the eigenvector centrality method. These animals were located in the same area of social unit 7, but were mostly solitary according to field observations, and their

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eigenvalues were smaller than for the other individuals at the social unit 7, indicating weak association. Subordinate male 10V was assigned to social unit 1 in the eigenvector centrality analysis and to social unit 7 in the cluster analysis. However in the eigenvector centrality analysis 10V presented a small eigenvalue indicating a weak association to social unit 1. Observations confirmed that 10V moved between the two social units.

The sociogram (Fig. 2.6) suggests that adult males, territorial or subordinates, were often associated with adult females or juveniles of both sexes, but rarely or never with other adult males. Subordinate adult males were apparently tolerated in the same area, but were not seen to socialize with territorial males. Females associated with other females as well as with territorial and subordinate males.

Social differentiation, estimated through the coefficient of variation of the true association indices (proportion of time a dyad spend together) was CV = 1.240, which indicates that there was considerable variation in association patterns between and within classes. The CV value also indicates that there were preferred associations among some individuals. The correlation coefficient between the observed and estimated association indices was 0.424, which indicates that the social system of huemul was satisfactorily represented by the model.

Table 2.2. Assignment of 41 huemul into groups based on the eigenvector centrality method. Social units were established through the modularity ($Q = 0.680$, see text) and corrected for gregariousness. Eigenvalues for each individual indicate the strength of its association to the assigned cluster/group. Negative and positive values are interpreted similarly.

Individual	Class	Eigenvalue	Social unit
10V	Subordinate male	-0.0481	
12V	Adult female	-0.1577	
22V	Juvenile male	-0.2176	
23V	Juvenile male	-0.2928	
2V	Territorial male	-0.2474	1
6B	Juvenile female	-0.2902	
6C	Adult female	-0.2698	
7B	Subordinate male	-0.1025	
8C	Juvenile female	-0.1884	
11V	Subordinate male	-0.1168	
15V	Adult female	-0.2896	
16V	Adult female	-0.2921	
17V	Territorial male	-0.2869	2
18V	Adult female	-0.2797	
3V	Adult female	-0.2632	
11C	Juvenile male	-0.3415	
14V	Adult female	-0.3847	
1N	Adult female	-0.2915	3
2N	Territorial male	-0.3809	
11B	Adult female	-0.4732	
3B	Adult female	-0.3145	
4V	Territorial male	-0.4747	4
5V	Subordinate male	-0.3334	

Individual	Class	Eigenvalue	Social unit
20V	Adult female	0.3951	
4N	Territorial male	0.3355	5
7N	Adult female	0.3823	
8N	Adult female	0.4154	
21V	Subordinate male	0.0013	
4A	Adult female	0.3245	
4B	Adult female	0.2556	6
5A	Juvenile female	0.3897	
9V	Territorial male	0.4276	
14C	Adult female	0.1171	
19V	Adult female	0.2072	
1A	Juvenile male	0.2520	
24V	Adult female	0.1523	
25V	Adult female	0.2282	7
2A	Juvenile female	0.2572	
2B	Territorial male	0.2510	
3A	Territorial male	0.0039	
5N	Juvenile male	0.1270	
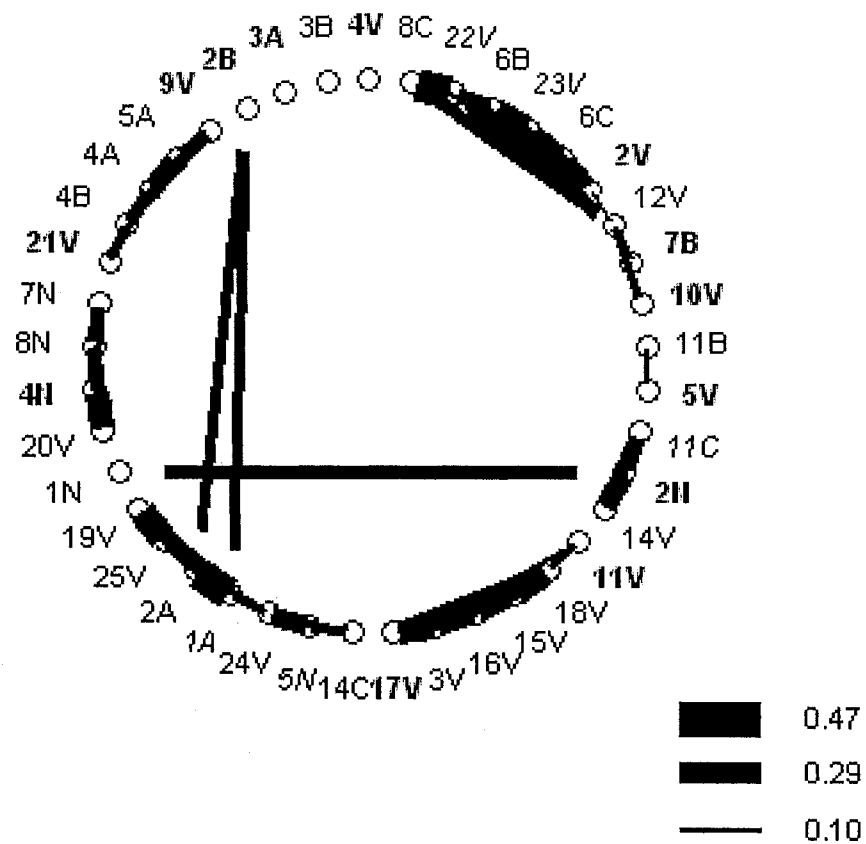
Figure 2.6. Sociogram of individual huemul observed for more than six months. The legend shows thickness for three values of association indices; lines linking individuals are proportional to the actual level of association. Linkages represent associations between dyads, which then form the different groups. Adult males in bold and juvenile males in italics.

Huemul association indices were stronger between than within sex classes (Mantel test comparing associations between and within classes, t = -1.962, P = 0.008 (with 1000 permutation), matrix correlation = -0.069) (Whitehead, 2008b) (Table 2.3). Hence, males associated more strongly with females than with other males.

Table 2.3. Indices of associations for huemul aged one year and older observed for at least 6 months, between and within sexes.

Associations	Mean association (SD)	Maximum association (SD)
Males – all others	0.0196 (0.0094)	0.2439 (0.1321)
Females – all others	0.0226 (0.0083)	0.2943 (0.1057)
Male – male	0.0116 (0.0087)	0.1159 (0.0958)
Male – females	0.0255 (0.0133)	0.2391 (0.1327)
Females – males	0.0255 (0.0119)	0.2526 (0.1057)
Females – females	0.0202 (0.0111)	0.2192 (0.1077)
Within classes	0.0164 (0.0109)	0.1738 (0.1138)
Between classes	0.0255 (0.0124)	0.2467 (0.1169)
Overall	0.0213 (0.0089)	0.2722 (0.1192)

The strengths of female-female and male-female associations decreased at similar rates, but only male association rate declined below the null association rate (Fig. 2.7). Male-female and female-female associations are similar and despite being weak, they were stable through time.
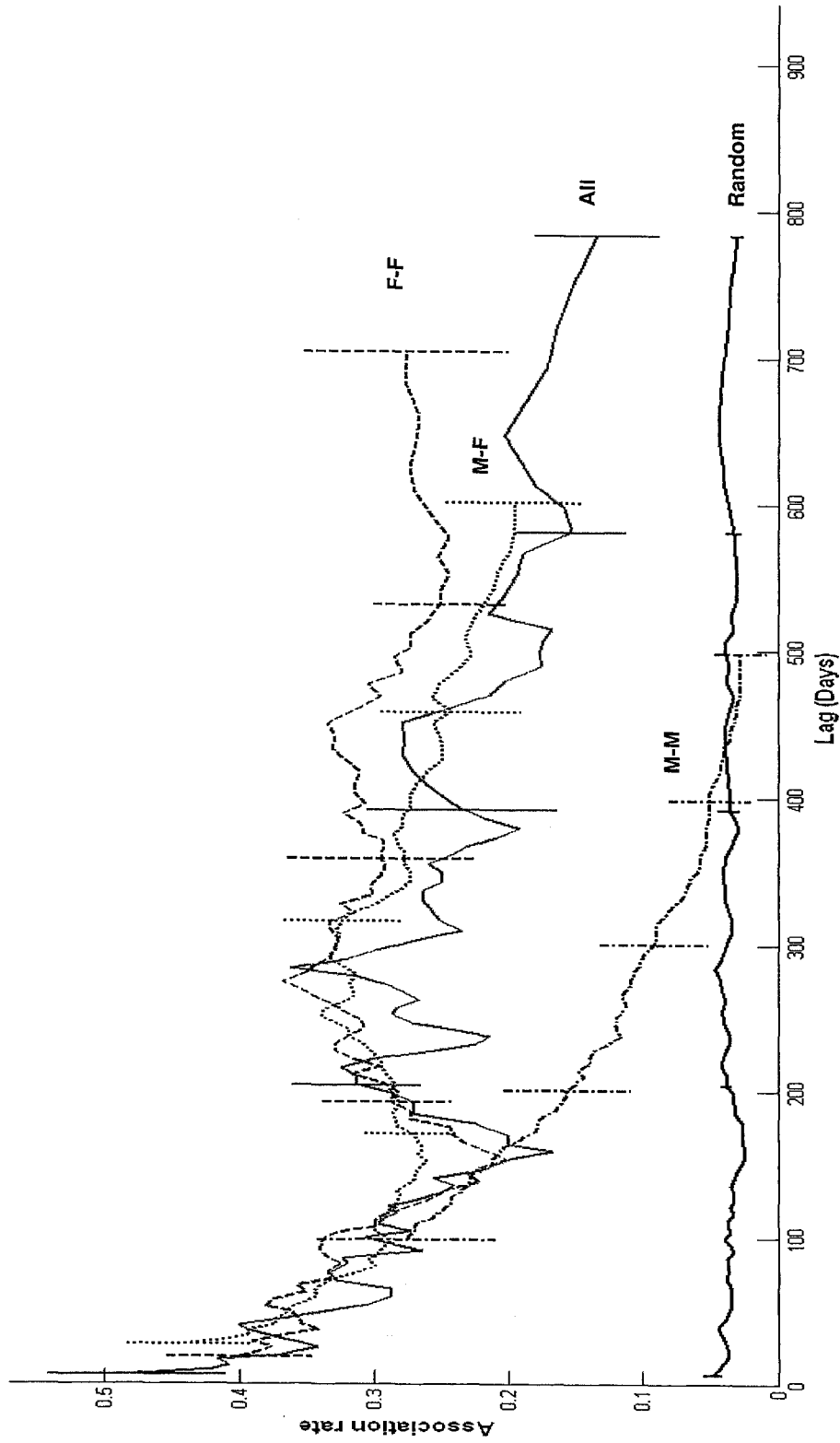
Figure 2.7. Lagged association rates determined for different pairwise associations between huemul sex classes. The lagged association rate shows the probability of association at time $T + t$ of two individuals that were associated at time T. The null lagged association rate is the expected results if deer associated at random with other deer. Jacknife standard error bars are shown in all lines. Moving averages were transformed to smooth out short-term fluctuations and highlight longer-term trends in the curves.

The best fitting LAR model of the five candidate models for the three possible sex associations (female-female, male-female, and male-male) was selected according to the QAIC value. All models presented a variance inflation factor smaller than five indicating no multicollinearity of equation coefficients (Whitehead, 2008b) (Table 2.4). For female-female associations the selected model was LAR 4 ($\beta 1$ = 0.6613, SE = 0.8256; $\beta 2$ = 0.00034229, SE =. 0.00025235; $\beta 3$ = 0.66285, SE = 0.034504) (Table 2.4.a.). The model represents two levels of disassociations over different time scales. Female huemul associated with some other females in permanent relationships. Model LAR 5 ($\beta 1$ = 0.6613, SE =. 0.8256; $\beta 2$ = 0.00034229, SE = 0.00025235; $\beta 3$ = 0.66285 SE = 0.034504) also received some support because the ΔQAIC was smaller than 2.

For male-female associations the best fit was for LAR 5 ($\beta 1$ = 0.60095, SE = 3.8656; $\beta 2$ = 0.0010461, SE = 0.0014971; $\beta 3$ = 0.61836, SE = 0.18193) (Table 2.4.b.). The model combines rapid disassociations with the two levels of disassociation. In this scenario males and females that share an area associate for some time, dissociate, and later reassociate as in a fusion-fission system, but eventually some males do not reassociate because they either die (territorial males) or they leave the area (subordinate males and juveniles). However LAR 4 also received some support ($\beta 1$ = 0.42206, SE = 1.343; $\beta 2$ = 0.00099289, SE = 0.00067441; $\beta 3$ = 0.69871, SE = 1.2869; $\beta 4$ = 0.37432, SE = 0.078117), because ΔQAIC was smaller than two.

For male-male associations, the best fit was for the model LAR 3 ($\beta 1$ = 0.0030624, SE = 1.5707; $\beta 2$ = -0.1351, SE = 37.1018; $\beta 3$ = 0.55096, SE = 37.1056) (Table 2.4.c.). The model represents rapid dissociation plus constant companions plus casual acquisitions: adult males rapidly disassociate after one sampling period, and their association rates decreased rapidly to zero. Permanent companions were exclusively juvenile males, including yearlings, which were in the area since birth.

Table 2.4. Models fit to data on the lagged association rates (*g*) of adult huemul and juveniles older than one year of age. τ is the time lag in days from an observed association. The lowest QAIC indicates the best fitting model, and the ΔQAIC (difference between QAIC of each candidate model and the best model) indicates the degree of support for the other models. Moving average was set at 400 associations in time lags for all models.

a) Female-female lag association rate models. Variance inflation factor is 2.335.

Model	Best fit	QAIC	ΔQAIC	
LAR 1	$g = 0.3082$	3969.1775	18.2947	No support
LAR 2	$g = 0.30414 + 069586e^{-0.43656\tau}$	3953.9378	3.055	No support
LAR 3	$g = 0.30431 + 0.79811e^{-0.50763\tau}$	3955.8307	4.9479	No support
LAR 4	$g = 0.66285e^{-0.6613\tau} + 0.33715e^{-0.00034229\tau}$	3950.8828	0	Best model
LAR 5	$g = 0.60281e^{-0.38519\tau} + 0.33247e^{-0.00030982\tau}$	3952.5370	1.654	Some support

b) Male-females lag association rate models. Variance inflation factor is 1.430.

Model	Best fit	QAIC	ΔQAIC	
LAR 1	$g = 0.29668$	4625.2806	81.8617	No support
LAR 2	$g = 0.28395 + 0.71605e^{-0.14323\tau}$	4581.5222	38.1033	No support
LAR 3	$g = 0.26724 + 0.31598e^{-0.026922\tau}$	4560.7095	17.2906	No support
LAR 4	$g = 0.61836e^{-0.60095\tau} + 0.38164e^{-0.0010461\tau}$	4544.5755	1.1566	Some support
LAR 5	$g = 0.69871e^{-0.42206\tau} + 0.37432e^{-0.00099289\tau}$	4543.4189	0	Best model

c) Male-male lag association rate models. Variance inflation factor is 2.084.

Model	Best fit	QAIC	ΔQAIC	
LAR 1	$g = 0.14238$	778.0231	140.0935	No support
LAR 2	$g = 0.061396 + 0.938604e^{-0.02025\tau}$	738.6612	100.7316	No support
LAR 3	$g = -0.1351 + 0.55096e^{-0.0030624\tau}$	637.9296	0	Best model
LAR 4	$g = 0.51973e^{-3.5191\tau} + 0.48027e^{-0.0063218\tau}$	645.3183	7.3887	No support
LAR 5	$g = 10.3068e^{-6.1356\tau} + 0.48015e^{-0.0063207\tau}$	647.3171	9.3875	No support

Typical group size did not vary among age-sex classes (Table 2.5) (Kruskal-Wallis test: H = 1.718, df = 2, P = 0.436). The number of possible companions according to the social units composition determined by the modularity method was also included and indicates the average number of individuals that can associate with a given deer. No significant differences were detected among means (H = 1.431, df = 2, P = 0.489). Territorial-subordinate male dyads were excluded because they were very rare.

There was no sexual segregation in any season (Fig. 2.8). Adult and juveniles males were either solitary or in groups with females. In summer, males were alone almost half the time (Fig. 2.8). Overall, excluding observations of solitary males, males were seen 58 ± 18% (mean ± SD) of the time in groups with one female, 29 ± 14% in groups with two females, and 13 ± 13% in groups with three or more females.

The time males (n = 18) spent alone varied among seasons (ANOVA: F = 3.183, df = 3, P = 0.029). They were more solitary in summer than in winter (*post-hoc* Sidak's test: P = 0.039), but there were no differences with other seasons ($P \geq 0.05$). Presence of males in groups did not vary seasonally (F = 1.926, df = 3, P = 0.134). For this analysis, observations of male-only groups were excluded because that rarely occurred (Fig. 2.6).

Table 2.5. Typical group size (*sensu* Jarman, 1974) of huemul of observed at Lago Cochrane National Reserve during 2005-2007. Mean and standard deviation are calculated for territorial males (n = 8), subordinate males (n = 10), and females (n = 23). Additionally, the mean and standard deviation of the number of possible companions or animals identified in the same social unit and that can interact with a given deer at a given class are also presented.

Huemul classes	Typical group size Mean ± SD	N° of companions Mean ± SD
Territorial males	2.49 ± 0.64	4.38 ± 2.00
Subordinate males	2.78 ± 0.42	5.40 ± 2.22
Females	2.57 ± 0.62	5.09 ± 1.81
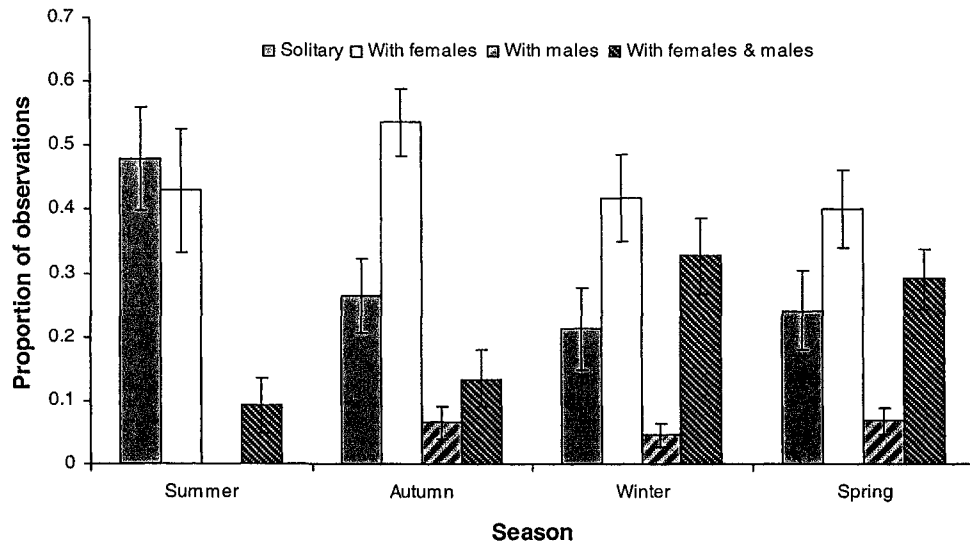
Figure 2.8. Mean proportion of adult huemul male (n = 18) present in groups by season, Chilean Patagonia, 2005-2007.

Group size varied by season (F = 7.294, df = 3, P < 0.0001) (Fig. 2.9). Groups in winter (n = 133) were larger than in summer (Tamhane-$T2$ *post-hoc* test: P = 0.007, n = 97), autumn (P = 0.002, n = 155), and spring (P < 0.001, n = 247) (Fig. 2.7), which did not present any differences among them ($P \geq$ 0.05).
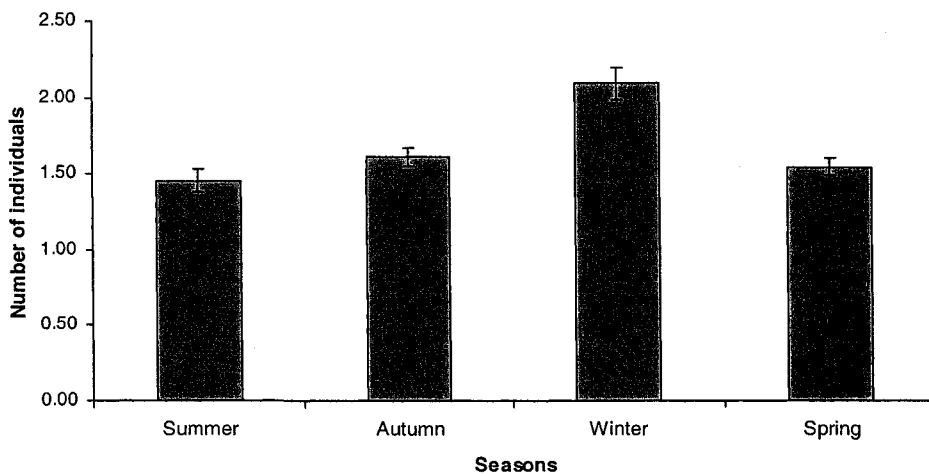
Figure 2.9. Mean ± SE huemul group size as a function of season. The analysis was limited to huemul older than one year. Observations (n = 632) were pooled by season across years (2005, 2006, 2007).

Discussion

The social organization of huemul is very different from that of most other temperate ungulates, that are sexually segregated outside the rut (Bleich et al., 1997; Bowyer et al., 1996; Corti and Shackleton, 2002; Kie and Bowyer, 1999; Miquelle et al., 1992; Pérez-Barbería and Gordon, 1999; Ruckstuhl, 1998; Stewart et al., 2000). Adult huemul males do not segregate from females socially or spatially, but avoid other males (Fig. 2.6). Male-only groups were absent. In roe deer (*Capreolus capreolus*), males also do not tolerate other adult males in their territory during the rut, but form bachelor groups outside the rut (Hewison et al., 1998; Liberg et al., 1998). The taruca (*Hippocamelus antisensis*), the closest relative of huemul (Gilbert et al., 2006), in the southern Peruvian Andes also forms mixed groups year-round (Merkt, 1987), but the open Andean highlands (Rundel and Palma, 2000) allow the formation of larger mixed groups (about 31 individuals (Merkt, 1987)) than in huemul.

Huemul social organization was characterized by small groups, usually including one territorial male and 1-2 females. When groups included more than one female, these appeared to be kin related (P. Corti and A. Shafer unpublished data). Huemul show relatively stable associations among females and between males and females, but male avoided each other and territorial males were never seen together. Association indices were low because deer of both sexes were often solitary. Although no similar analyses have been performed in other deer species to my knowledge, the long term associations between males and females resemble those described in Japanese serow, in which both sexes are territorial all year and males share their territory with one or two females (Kishimoto and Kawamichi, 1996), and in oribi antelopes, which keep territories year around and form small mixed groups with one dominant male, females, and juvenile males and females (Arcese, 1999).

When young males become adults, they are not longer tolerated by territorial males and presumably have to switch among different groups until they can establish a territory. Japanese serow presents similar behavioural patterns: young adults roam until they can establish a territory (Kishimoto and Kawamichi, 1996). Two subordinate males (10V and 21V) were not clearly assigned to a group because they moved between two neighbouring groups as 'floaters'. In general, subordinated males were weakly associated to groups, indicating their wide roaming behaviour. In roe deer, non-territorial males during the rut become floaters, in the territories of several other males (Liberg et al., 1998). A

similar behaviour has been reported for Japanese serow, but year-round (Kishimoto and Kawamichi, 1996). In huemul, it was common to see an adult male with a maternal group or even only with a yearling. Adult males and maternal groups often spent short periods of time together but did not form stable associations.

All individually identified huemul exhibited strong site fidelity as observed by Gill et al. (2008), but some subadults moved away from their natal ranges. Philopatry probably limited the variability of composition of social groups. Japanese serow (Kishimoto and Kawamichi, 1996) and oribi (Brashares and Arcese, 1999) also are highly philopatric and both species defend territories all-year-around. Huemul groups use well-defined areas, where one territorial adult male overlaps with a group of females, fawns and yearlings of both sexes. The association of females within social units is stable through time, and it has been suggested for other ungulates that this type of association allows matrilineal groups to defend resources against each other (Kishimoto and Kawamichi, 1996; Wronski and Apio, 2006). Future work should test the hypothesis that female groups are mostly made up of relatives.

Huemul is a middle-sized deer with very limited sexual dimorphism. The small differences in mass suggest similar metabolic requirements for both sexes (Demment and Van Soest, 1985). High-quality food is crucial for both sexes (Demment and Van Soest, 1985), but it is not uniformly distributed in huemul habitat, which is relatively stable throughout seasons and years (Daniels and Veblen, 2004; Veblen et al., 1981; Veblen and Schlegel, 1982). A patchy resource distribution should promote limited movements, high site fidelity, and possible territoriality (Jarman, 1974). Limited high-quality resources are not abundant, which would make them worthy of defense, discouraging the formation of large groups (Jarman, 1974). Brashares and Arcese (2002) showed that male oribi were most territorial where females had small home ranges because high-quality food was abundant and concentrated in those areas. The strong associations between males and females I documented in this huemul population support the theory that middle-sized and small ruminants with a territorial polygynous system do not show sexual segregation (Weckerly, 1998).

Groups were small from spring to autumn and similar to those reported by Povilitis (1983) during autumn in northern populations, but groups were larger in winter. Increased group size during winter, when food sources are more clumped and aggression among males decreases, has been also observed

in roe deer (Hewison et al., 1998) (section II.b). Huemul inhabit forested and rugged terrain that limits the visual communication required for the maintenance of large groups (Jarman, 1974; Jarman and Jarman, 1979).

Predation risk may also shape social behaviour (Bowyer et al., 1998; Brashares and Arcese, 2002). For adult huemul, the only natural predator is the cougar (Smith-Flueck and Flueck, 1997), a stalking, solitary predator (Pierce et al., 2000). This type of hunting strategy promotes a prey distribution where individuals attempt to decrease detection by the predator (Ballard et al., 2001; Pierce et al., 2000; Robinson et al., 2002). Huemul form small (Fig. 2.9), cryptic, and widely distributed groups in low-density populations (Chapter III), possibly in part as an antipredatory strategy (Brashares and Arcese, 2002; Jarman, 1974). The dilution effect of predator risk, however is important even in a group of two (Moody et al., 1996; Roberts, 1996), as the probability of being attacked is reduced by 0.5. Maternal groups might offer a dilution effect to huemul males, possibly explaining why males were often seen with females outside the breeding season.

Differences in the association values for individuals were related to sex, spatial location, male hierarchy (territorial or subordinate adult and juvenile), recent death of a territorial male, kin relationships of females, and high site fidelity. Huemul social behaviour offers new insights on ungulate ecology and evolution. It appears that the low sexual dimorphism, year-long male territoriality, the rugged landscape, and the influence of a single large predator species have shaped a distinctive social organization that contrasts with other deer species living in more complex predator-prey systems and more marked seasonality.

Huemul were formerly distributed over a latitudinal gradient of more than 20° along the Andes (Vila et al., 2006). It is likely that its social organization varied according to differences in habitat and other ecological variables (Brashares and Arcese, 2002; Coakes and Whitehead, 2004). For example, huemul in coastal Chilean Patagonia seem to migrate over longer distances (Frid, 1999) than observed in this study. Nevertheless, the isolation and fragmentation currently affecting this population (Chapter IV) is typical of most other remaining inland populations. The index of associations must vary between and within population of the species (Difiore and Rendall, 1994; Whitehead, 2008a). Quantitative information on the social organization of huemul populations in similar or different ecological

conditions would be extremely valuable, not only to increase our scientific knowledge on the plasticity of the social system of this deer, but also for its conservation and management.

SECTION II.b – HUEMUL MATING SYSTEM

Introduction

A species' mating system can vary substantially among populations, because it is the outcome of individual differences in reproductive strategies and tactics, rather than an intrinsic characteristic of a population or species (Clutton-Brock, 1989; Lott, 1991; Say et al., 2003). In addition to being affected by its environment, an animal's mating tactic may vary according to its body condition and social status (Clutton-Brock, 1989; Emlen and Oring, 1977; Hogg and Forbes, 1997; Pemberton et al., 1992). Studies of mating systems are critical from both fundamental and applied perspectives, because individual variance in reproductive success can affect the dynamics and the genetic variability of populations. Mating systems can limit the growth and size of a population (Fryxell and Lundberg, 1998), and have a major effect on effective population size (N_e). For example, in the polygamous Saiga antelope (*Saiga tatarica*), selective hunting of males reduced male density to the point where many females could not find a mate, with a negative effect on population growth (Milner-Gulland et al., 2003). Variance in male reproductive success can be drastically different according to mating system (Clutton-Brock, 1989; Clutton-Brock and Vincent, 1991). In polygamous systems many males fail to reproduce because a few males monopolize most females (e.g, fallow deer *Dama dama* (Say et al., 2003)), but in monogamous systems most males have similar success in siring offspring (e.g., Kirk's dik-dik *Madoqua kirki* (Komers, 1996)). Variation in male reproductive success affects effective population size, which directly determines the ability of a population to maintain genetic diversity (Caro, 2007). Consequently, knowledge of mating systems is fundamental for the management and conservation of threatened wildlife species (Berger, 1996; Bessa-Gomes et al., 2004; Brashares, 2003; Hogg, 2000; Höglund, 1996; Stephens and Sutherland, 2000).

Several causes for the decline of huemul have been suggested, including habitat loss and fragmentation, poaching, exotic diseases from livestock, and predation by domestic dogs and cougar (*Puma concolor*) (Diaz and Smith-Flueck, 2000; Frid, 1994; Povilitis, 1998; Smith-Flueck and Flueck, 2001a; Smith-Flueck and Flueck, 2001b). Intrinsic population characteristics that potentially might also affect its recovery, such as its mating system, however, have not been studied.

Current knowledge of huemul mating system is limited to non-systematic observations of unmarked individuals (Povilitis, 1983b, 1985). Povilitis (1983b) suggested that males adopt a tending strategy, defending one female until they mate and afterwards searching for other oestrous females. However, Gill et al. (2008) found that radio-collared males and females use small, similar-sized home ranges, with high site fidelity and very limited movements. High site fidelity throughout the year, together with limited dispersal and seasonal movement of adults, may result in both sexes remaining year-round in small and mixed groups. Under such conditions, adult males do not tolerate each other (Section II.a.). Living in small, mixed groups would facilitate mating in low-density populations that inhabit rugged and heavily forested discontinuous habitats, where the formation of large groups and location of partners are difficult (Jarman, 1974; Jarman and Jarman, 1979).

Huemul live in small and stable groups (Section II.a) at low densities (mean $\pm$ SD: 1.79 $\pm$ 0.33 /km^2) (Chapter III). Therefore, finding a mate is extremely important for individual fitness and for population persistence (Calabrese and Fagan, 2004). Inability to locate mates could lead to an Allee effect with a decline in birth rate at low density (Amarasekare, 1998; Courchamp et al., 1999; Stephens and Sutherland, 1999, 2000).

In huemul, male territoriality has been suggested (Flueck and Smith-Flueck, 2006; Serret, 2001), but not demonstrated. Male territoriality occurs in several other ungulates with similar ecological conditions and limited sexual dimorphism, as observed in huemul (Section II.a), including Japanese serow (*Capricornis crispus*) (Kishimoto and Kawamichi, 1996), oribi (*Ourebia ourebi*) (Brashares and Arcese, 2002), roe deer (*Capreolus capreolus*) (Hewison et al., 1998; Liberg et al., 1998), Indian muntjac (*Muntiacus muntjak*) (Odden and Wegge, 2007), and impala (*Aepyceros melampus*) (Jarman, 1974; Jarman and Jarman, 1979). Territorial males typically defend throughout all seasons stable home ranges overlapping the home range of a few females. At the same time, overlap with other territorial males is low or non-existent, and adult males almost never interact with one another (Section II.a.). Escalated fights between males are likely to be dangerous because the short and pointed huemul antlers can cause serious injuries or death (Geist, 1986), as in mountain goats (*Oreamnos americanus*) (Festa-Bianchet and Côté, 2008; Geist, 1964). Therefore, males may be selected to avoid fights unless they are likely to win. If territoriality is an effective strategy, males who hold territories should sire the most offspring (Liberg et al., 1998; Pemberton et al., 1992; von Hardenberg et al., 2000). Subordinate non-territorial males should adopt alternative mating tactics with lower success (Packer and Abrams, 1990;

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Pemberton et al., 1992). However, the measurement of male reproductive success requires molecular data, because behavioural observations may not accurately estimate it (Coltman et al., 1999; Hughes, 1998; Say et al., 2003).

Combining new knowledge on huemul social organization (Section II.a.), the limited published information on its mating system (Povilitis, 1983b), and the animals' ranging behaviour (Gill et al., 2008), I hypothesized that the huemul mating system should involve either stable monogamous pair bonds, or a polygynous territorial system in which one male will guard and mate with several females that live within the area it defends.

To assess these hypotheses I observed huemul behaviour over three consecutive years to quantify its mating system. I estimated home range sizes for males and females and their overlap, the proportion of time males and females spent together, and used molecular techniques to determine fawn paternity (Pemberton et al., 1992). Male-male interactions were also described to test the hypothesis of male territoriality.

Material and methods

Study area

The study area was located at the Lago Cochrane National Reserve (LCNR) (47°12'S, 72°30'W), Aysén District, Chilean Patagonia. Details of the study area are given in Chapter I.

Field data collection

I observed 41 (18 males, 23 females) individually identified huemul (the same studied in section II.a). Huemul classes that were recognised in the field included territorial males, satellite (subordinate) males, and females. Territorial males were adults older than four years that were regularly seen in specific areas from where they excluded other males and were never seen with other males of similar

social status (Section II.a.). Satellite males were subordinates, either young males between one and three years of age, or non-territorial adults that were partially tolerated by territorial males or chased whenever encountered (Section II.a.). Females included adults and yearlings.

Huemul were censused monthly from late March 2005 to December 2007. During peak rut (late March and early April), observations were more intensive, surveying the area daily to observe huemul interactions and courtship behaviour. The study included two rutting seasons (2006 and 2007). Deer could be readily located because 20 had telemetry devices and they held small home ranges with high site fidelity. Therefore, their location was highly predictable. When a group was encountered, the identity of each deer was recorded, together with group size and sex-age composition, behaviour of each individual, antler development of males (velvet, hard, or missing), habitat characteristics (floral composition at the group location), hour, date, and GPS-UTM coordinates. Animals within a radius of 50 m of each other were considered to be in the same group. Neighbouring groups were well separated by topographic features.

Spatial distribution of huemul

The spatial distribution of territorial males in relation to females and satellite males was assessed through the calculation of home ranges for the 41 individually known deer. For each age-sex class, overlap indices were calculated, for a total of 1945 dyads. An average of 25.7 location /individual was used in these analyses. Locations of observed, identified animals were considered independent because they were separated by at least two weeks (Machlis et al., 1985). Location fixes were established as UTM coordinates using a global positioning system (GPS) (eTrex Summit, Garmin Ltd., Kansas City, MO, USA), which was accurate to within 7 m. Adaptive kernel density estimation was used to build home ranges and measure overlap both between and within different huemul classes (Powell, 2000; Worton, 1989). For kernel analysis, a 300 × 300 m grid was used (Worton, 1989). Home range and overlap calculations in ArcView GIS 3.3. (ESRI, 2002) used the Animal Movement Analysis extension (Hooge and Eichenlaub, 2000).

Overlap was estimated for dyads in the same or in adjacent groups (Section II.a.). Overlaps of 95%, 80%, 60%, 50%, and 35% core areas were compared to quantify the decreasing overlap towards the

inner core between the home ranges of two animals. The proportion of home range overlap between two individuals was rarely equal (Powell, 2000), so two indices of overlap (*IO*) were estimated for each member of a dyad, using the formula:

$$IO(X) \ A \ over \ B = A_X \cap B_X \ / \ A_X \quad \& \quad IO(X) \ B \ over \ A = B_X \cap A_X \ / \ B_X$$

where X is the percentage of the core area used, and A and B are the individuals in the dyad. For territorial males, the 50% or less core range is generally considered as an exclusive area or centre of activity (Powell, 2000). These core areas represent portions of the home range in which the overlap between individuals should be minimal or none, and can be considered as defended territories or exclusive areas of mating; they are mostly at the inner core of the home range (Powell, 2000). All three huemul classes were included in this analysis to compare overlap within and between them. Females do not defend territories, but they were included in the analysis as a point of comparison with the two male classes. Spatial overlap between individuals was measured only while both were alive.

I used non-parametric one-way ANOVA (Kruskal-Wallis H test) to test for differences in 95% and 50% core areas among huemul classes. A Monte Carlo estimate was used to obtain unbiased significance values without relying on the assumptions for the asymptotic method, sampling 10000 repeated measurements from the data set (Norušis, 1994).

To test for differences in the amount of overlap both between and within classes, I used a univariate General linear Model (GLM) that provides regression analysis and analysis of variance for one dependent variable (amount of overlap) by one or more independent variables or factors (huemul classes) (Huizingh, 2007). Using the GLM approach, I tested the null hypotheses of no differences in core area overlap among the three huemul classes. I performed three different GLMs: 1) female overlap over other females, over satellite males, and over territorial males. 2) Satellite male overlap over females, other subordinate males, and over territorial males. 3) Territorial male overlap over females, over satellite males, and over other territorial males.

Normality of the data was evaluated by examining normal probability plots, while homoscedasticity was tested using the Levene's test. Estimates of overlap consisted of proportions, which were arcsine-square root-transformed to meet normality (Zar, 1999). In the case of significant differences ($\alpha = 0.05$)

in group size, seasons were compared using *post-hoc* Sidak's test or the Tamhane-*T2* test if variances were not homogeneous (Huizingh, 2007).

Male reproductive success

Microsatellite DNA analyses were used to establish paternity and to estimate reproductive success of individually identified males. I collected ear tissue samples from 21 hand-captured newborns, five chemically immobilized juveniles and 29 adults (14 males and 15 females), and from three animals found dead. Tissues were preserved in 70 % ethanol until DNA was extracted (Details of DNA extraction and genotyping are described in Chapter IV).

The program CERVUS 3.0.3 (Kalinowski et al., 2007) was used to determine paternity. This program takes into account genotyping error rate, incomplete sampling, and allele frequencies when calculating likelihoods of paternity (Kalinowski et al., 2007). Mothers were assigned from behavioural observations and candidate fathers were tested based on field observations. Mother-fawn relationships were known since the fawn was born or they were observed together, and molecular data were consistent with that relationship. Because of limited genetic variability, incomplete sampling, and probably genotyping error (Chapter IV), it was not possible to assign paternity at high confidence intervals. I assigned paternity if no DNA mismatches were observed in the tri- and pair-loci comparisons of the genotype of the offspring, the father and the mother (Marshall et al., 2002). Half of a fawn DNA profile will match the fragments from its mother's DNA profile; the remaining half will match the fragments from its father's DNA profile. If mismatches in the DNA fragments occur when testing for paternity, the putative father can be excluded (Pemberton et al., 1995; Wilson et al., 2005). Although this type of paternity assignment may generate a large bias in the results and uncertainty in establishing the true father (Marshall et al., 2002), but I reduced the bias as much as possible assigning only putative fathers those that were near the area of a mother.

Results were summarized as percentages of fawns sired by each male. In addition, the association index for each set of parents was presented (adapted from Section II.a.), and the average percentage of time that parents were observed together, the 10 months period before the rut when a fawn was conceived, was included. Spearman rank correlation (r_s) was used to relate siring success with time

spent with the mother and with the size of the male's home range (Zar, 1999). All analyses were considered significant at $\alpha = 0.05$ (one-tailed).

Male behaviour

Behavioural observations of male huemul were recorded during group observation periods throughout the study. I used instantaneous scan sampling at the start of each group observation (Altmann, 1974; Martin and Bateson, 1993), to record the following behaviours: lying, feeding, vigilant/walking, and agonistic and mating behaviour (courtship and marking). Courtship behaviour was considered to occur when males approached females with low stretch display, side kicking, or attempted to copulate. Observations were pooled by season.

Variation in male huemul behavioural activities, expressed as frequencies by season, was analyzed using contingency tables (Everitt, 1992). Adjusted (studentized) residuals for each cell in the tables were used for *post-hoc* tests to compare observed and expected values of the frequencies of each behaviour (Everitt, 1992). An adjusted residual ≥ 1.96, equivalent to a Z-score, is significant at $\alpha = 0.05$. A positive residual indicates an observed value greater than expected and a negative residual indicates an observed value smaller than expected (Everitt, 1992).

When observing agonistic displays among males I recorded the class and identity of interacting huemul. Antler development was recorded during each observation and classified as: velvet (growing); hard (fully grown, with no velvet); and cast (not present).

Results

The rut started in early March, when males were observed following females, presenting courtship behaviour, and trying to mount them. It ended in early May in both 2006 and 2007. Most attempted or successful copulations ($n = 8$) were seen at the end of March and the beginning of April.

Spatial distribution

Huemul of the three sex-age classes had similar-sized 95% core areas (Kruskal-Wallis test: $H = 1.854$, $df = 2$, $P = 0.407$) and 50% core areas ($H = 2.217$, $df = 2$, $P = 0.340$) (Fig. 2.10). One territorial male (3A) was an outlier at the 95% core level because it increased its home range soon after a neighbouring territorial male (2B) disappeared. Females presented one outlier and one extreme value. The outlier was a female that moved widely, and the extreme value (female 24V) increased its home range after male 1V, who was often seen with her, was poached. At the 50% core level, the extreme value is for the same female (24V). The satellite male with large core area is an adult (21V) that moved extensively between the home ranges of two territorial males.

Home-range overlap differed among huemul classes. Overlap of female with other sex-age classes varied according to class (GLM; $F = 8.842$, $df = 2$, $P < 0.0001$), being greater with satellite males than with other females (Sidak's test: mean difference ± SE = 1.465 ± 0.360, $P < 0.0001$) or with territorial males (1.362 ± 0.358, $P = 0.007$). No differences were observed between females-females and females-territorial males (-0.104 ± 0.386, $P = 0.990$). Overlap of satellite males with other sex-age classes varied ($F = 12.058$, $df = 2$, $P < 0.001$). The overlap among satellite males was less than that between satellite males and females (Tamhane-*T2* test: -2.460 ± 0.457, $P < 0.0001$), or between satellite males and territorial males (-1.657 ± 0.576, $P = 0.013$), but there were no differences between the overlap of satellite males with females and with territorial males (0.803 ± 0.527, $P = 0.338$). Overlap of territorial males over different huemul classes were different ($F = 31.156$, $df = 2$, $P < 0.001$). The overlap among territorial males was smaller than the overlap of territorial males with females (Tamhane-*T2* test = -4.407 ± 0.436, $P < 0.0001$) and with satellite males (-4.951 ± 0.580, $P < 0.0001$), but there were not differences in overlap between territorial males and females and territorial males and satellite males (0.544 ± 0.618, $P = 0.761$).

Overlap decreased as one compared increasingly central parts of each animal's range. Females showed the largest overlap within and between sex-age classes. Overlap was lowest among territorial males, followed by overlap among satellite males (Fig. 2.11). Territorial males avoid each other, with almost zero overlap at 50% and 35% core ranges. Satellite males overlapped more with each other than with territorial males but overlap levels were also close to zero at 35% core adaptive kernel home range (Fig. 2.11).

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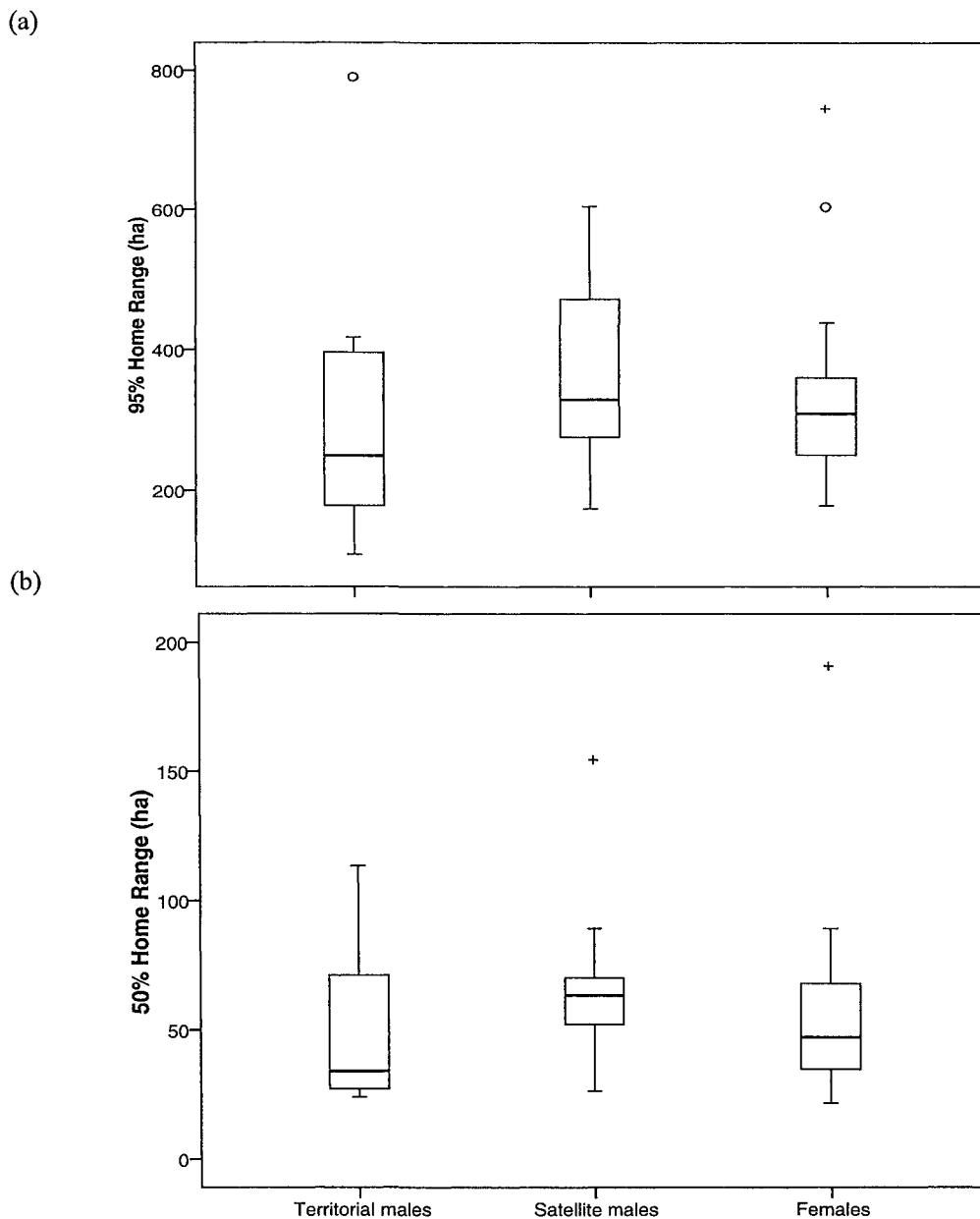
Figure 2.10. Home range sizes for three huemul classes at Lago Cochrane National Reserve, Chilean Patagonia, for (a) 95% core area and (b) 50% core. The lower and upper edges of the box are the respective 25th and 75th percentiles, while the horizontal bar within the box is the median range size. The whiskers represent the lower 10th and upper 90th percentiles, excluding outliers (o) and extreme values (+). No significant differences were detected among huemul classes.
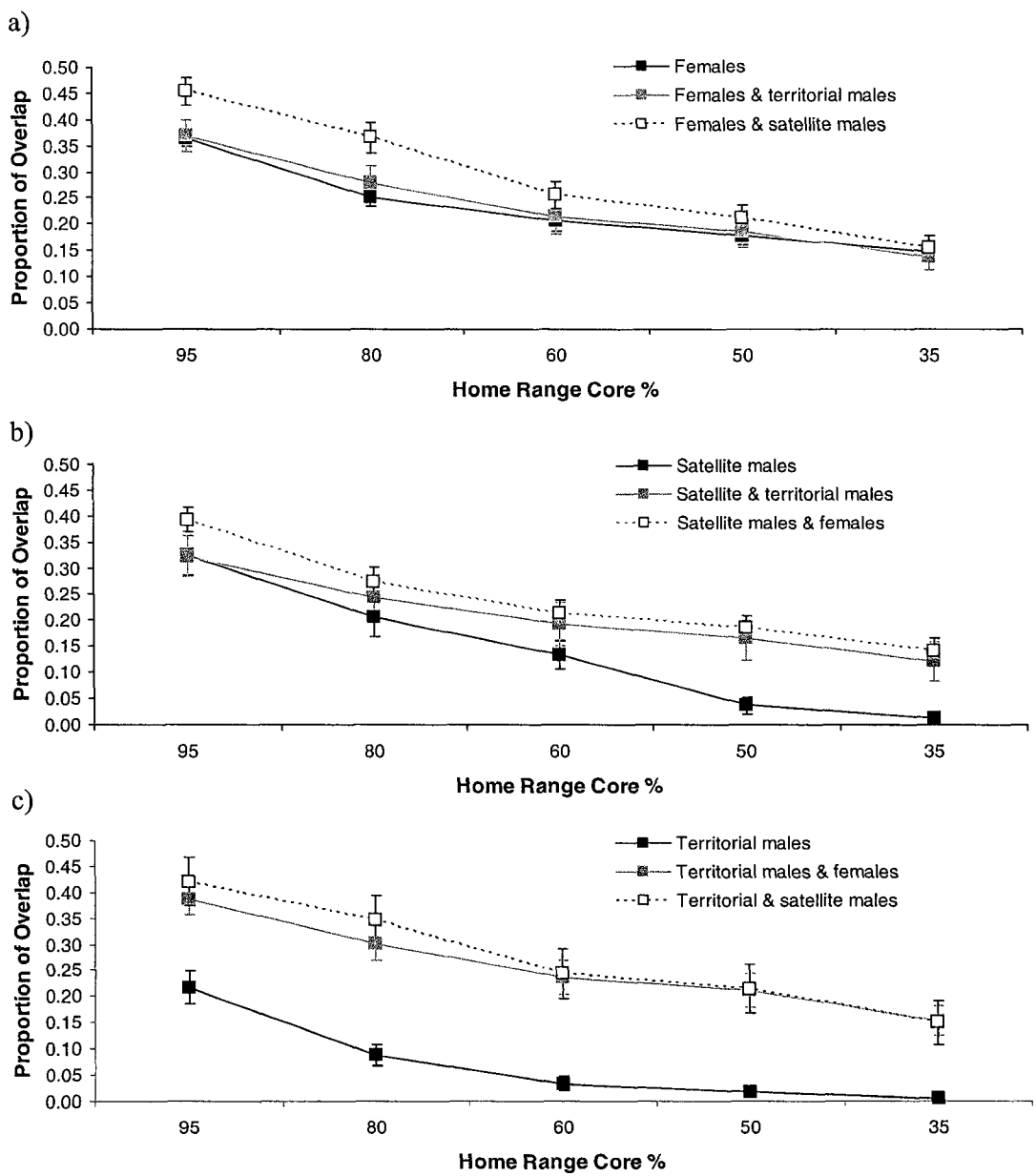
Figure 2.11. Home range overlap within and between sex-age classes for: (a) females, (b) subordinate males, and (c) territorial males. The overlap indicates how much one class overlaps other classes at increasingly central home range cores. Each point represents the mean overlap for the respective core area with its standard error bars.

With two exceptions, territorial males were spatially segregated from one another; satellite males, however, overlapped widely with territorial males (Fig. 2.12). At the 50% core area level, one dyad of territorial males using the same area included 17V and 4N (overlap of 17V over 4N = 0.09; 4N over 17V = 0.27) because at the beginning of the second year of study 4N enlarged its home range over part of the home range of 17V, while 17V was still alive. Males 4V and 2V also overlapped (4V over 2V = 0.12; 2V over 4V = 0.09) in an area that had many shrubs apparently rich in food resources.
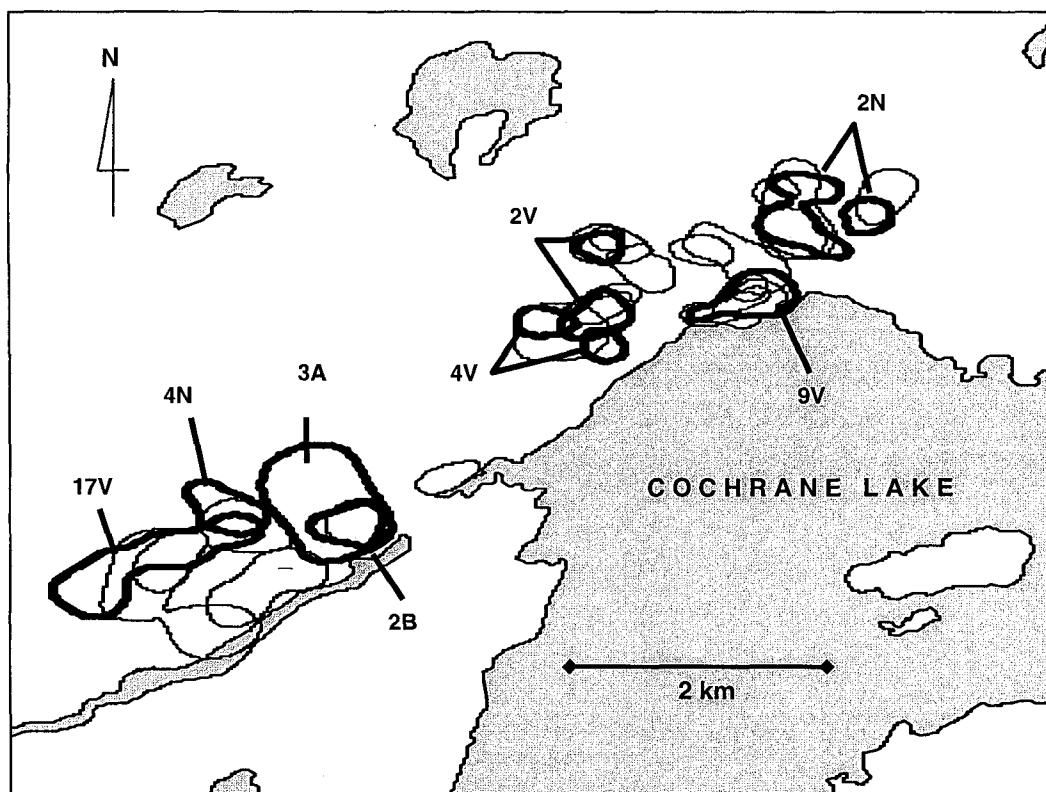
Figure 2.12. Geographic distribution of home ranges of territorial male (thick lines) and satellite male (light lines) huemul in the study area during 2005-2007. The ID of each territorial male is indicated. Each area represents the 50% home range core. Lago Cochrane National Reserve, Chilean Patagonia.

Male reproductive success

Using 14 microsatellite loci with more than one allele (Chapter IV), I assigned a single father in 20 of 23 (87 %) cases. In three cases, multiple potential fathers living close to the mothers had no mismatches. Eight males were identified as fathers (Table 2.6). Two that sired 44% of offspring had the largest home ranges among territorial males. Male 21V had a large range, but he was a satellite male who overlapped two territories. Of 16 adult males sampled between 2005 and 2007, only 50% (seven territorial and one satellite) were known to sire at least one offspring. All males that did not sire offspring were satellites, with the exception of one territorial male (2N). The proportion of time the father was observed with the mother (mean ± SD: 18.46 ± 18.58%) 10 month before the rut was lower than the time it spent alone or with other deer during the same period (81.54 ± 18.11%). The father's index of overlap with the mother's home range varied from 0.00 to 0.90 for 95% core areas (mean ± SD: 0.42 ± 0.23), and 0.00 to 0.67 for 50% core areas (0.28 ± 0.28).

The size of the 50% core range of males was correlated with siring success (Fig. 2.13). The most successful male enlarged his territory, increasing his fitness over other males in the area. Five females (3B, 5A, 13B, 14V, 20V) had offspring sired by males inhabiting neighbour areas. In three cases (3B, 5A, 20V), they were directly observed leaving their areas and moved into the area of other male, before returning to their ranges after mating.

Table 2.6. Number of fawns determined to be sired between 2005 and 2007 by huemul males, the percentage of time the father was seen with the mother during the 10 months before the rut of fawn conception, the size of the father's 95% and 50% core area, and its overlap with the mother's core areas. When there were more than one potential father the home range was not included for correlation analysis. Male 1V was poached at the beginning of the study, and no home range could be calculated. Mothers in parentheses were not sampled for DNA and their maternal status was determined through behavioural observations.

Father	Mother	Offspring	Three way genotype comparisons	Association index between father & mother	% Time father observed with mother	Home range of father (ha) (95% - 50%)	Index of father overlap with mother (95% - 50%)
17V	16V	11T	no mismatches	0.06	22.22	417.67 - 84.35	0.55 - 0.67
	18V	25T	no mismatches	0.07	22.22		0.60 - 0.57
	16V	35T	no mismatches	0.06	57.14		0.55 - 0.67
3A or 17V	24V	43T	3A and 17V one mismatch	0.03, 0.02	10.00, 9.09	--	--
3A	19V	17T	no mismatches	0.03	50.00		0.21 - 0.20
	(3B)	13T	no mismatches, one less pairwise mismatch	0.05	50.00		0.30 - 0.00
	25V	09T	no mismatches	0.00	0.00		0.31 - 0.00
	20V	05T	no mismatches	0.00	8.33	789.89 - 113.69	0.12 - 0.00
	(1B)	2A	no mismatches	--	--		--
	19V	41T	no mismatches	0.03	9.09		0.21 - 0.20
3A or 4V	(11B)	21T	Both no mismatches	0.00, 0.04	0.00, 0.00	--	--
4V	24V	5N	no mismatches	0.00	--	163.09 - 24.88	0.39 - 0.00
	5A	19T	no mismatches	0.00	0.00		0.00 - 0.00

Table 2.6. Continuation.

Father	Mother	Offspring	Three way genotype comparisons	Association index between father & mother	% Time father observed with mother	Home range of father (ha) (95% - 50%)	Index of father overlap with mother (95% - 50%)
2V	5A	07T	no mismatches	0.03	11.76	194.11 - 34.28	0.45 - 0.00
	(13B)	22V	no mismatches	0.00	--		--
	12V	15T	no mismatches	0.04	11.11		0.63 - 0.50
1V or 2V	(6C)	23V	1V and 2V have one pairwise mismatch	0.00, 0.07	--	--	--
1V	19V	1A	no mismatches	0.00	--	--	--
4N	8N	37T	no mismatches	0.06	45.45	223.20 - 29.45	0.90 - 0.51
	8N	9T	no mismatches	0.06	25.00		0.90 - 0.51
9V	4A	39T	no mismatches	0.05	26.67	373.81 - 33.75	0.57 - 0.48
21V	14V	01T	no mismatches	0.04	0.00	455.37 - 69.97	0.34 - 0.27
21V	14V	27T	no mismatches	0.04	11.11		0.34 - 0.27
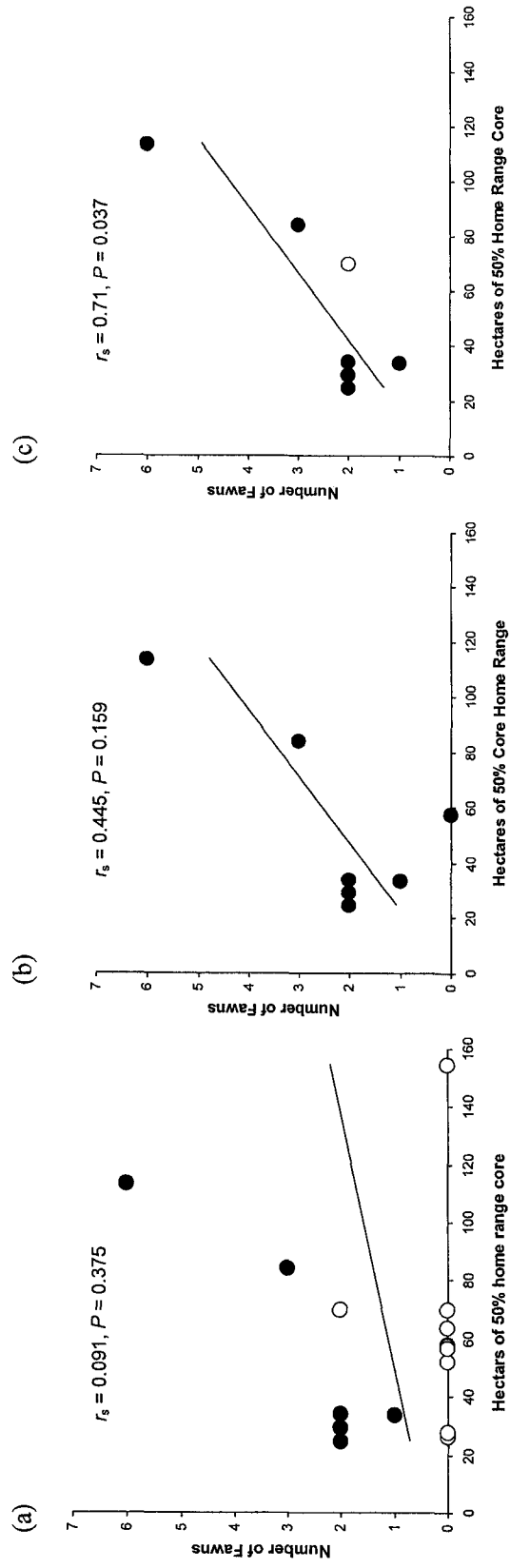
Figure 2.13. Spearman's rank-correlation coefficients between 50% core area size and number of sired fawns by territorial (●) and satellite (○) huemul males. (a) All potential candidate fathers. (b) Territorial males only. (c) All males that sired at least one fawn. One adult male that sired at least one fawn was excluded because it was poached before its home range could be determined. Males younger than two years were excluded.

Male behaviour

I saw no sexual behaviour during winter (Fig. 2.14). Male behaviour varied across seasons ($\chi^2 =$ 70.467, $df = 9$, $P < 0.0001$). Males were more active in autumn, when they spent less time lying than in other seasons (Adjusted residual: $Z = -3.6$, $P = 0.0003$). Lying was more frequent in spring ($Z = 4.2$, $P < 0.0001$), while there were no departure from expected in summer ($Z = 0.9$, $P = 0.368$) or winter ($Z = -1.6$, $P = 0.110$). Vigilant/walking behaviour showed no seasonal differences (summer, $Z = 1.1$, $P = 0.271$; autumn, $Z = 1.3$, $P = 0.194$; winter, $Z = -0.4$, $P = 0.689$; spring, $Z = -1.6$, $P = 0.110$). Males fed more frequently in winter ($Z = 4.8$, $P < 0.0001$) and less in summer ($Z = -2.6$, $P = 0.009$), but feeding did not differ from expected in autumn ($Z = -1.2$, $P = 0.230$) or spring ($Z = -1.6$, $P = 0.110$). Not surprisingly, mating behaviour was observed more often than expected in autumn ($Z = 5.4$, $P < 0.0001$) and less often in winter ($Z = -5.4$, $P < 0.0001$). The occurrence of mating did not differ from expected in spring ($Z = -1.6$, $P = 0.110$) or summer ($Z = 1.2$, $P = 0.230$).

Agonistic interactions between males

The few interactions seen during the rut were agonistic: 4V chased 5V; 9V chased 21V and 10V; 4N fought and chased 6N (not included in the analyses due to small sample size); and 17V fought and chased 6N. In each case, winners were territorial and losers were satellite or subordinates. Once I observed 2V and 9V parallel walking (Hoem et al., 2007) at the apparent boundaries of their territories; no other aggression was observed after this behaviour. Indications of agonistic encounters during the rut included 4V with a broken right forelimb, and 1A with its right rear leg and left antler broken. Male 1A, a satellite, had been seen the previous day with female 20V, who the following day was with territorial male 3A.

In late spring, I twice saw territorial males chasing satellite males. In addition, in the early autumn prior to starting my study (2004), I found an adult male killed by a deep perforation that extended into the lungs, apparently caused by the antler of another male. During a previous study in an area adjacent to my study site, an adult male was also killed by another male (Gill et al., 2008).

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Huemul retain hard antlers for 8 months ($n = 18$ males observed). They shed velvet in late spring (November - December), at least 3 to 4 months before the rut (late March and April). Antlers are cast in late June and July, start to grow in early August and are fully grown within about two months. Fawns grow their first antlers, short and unbranched, at about 10 months of age (16 male fawns reaching one year old).
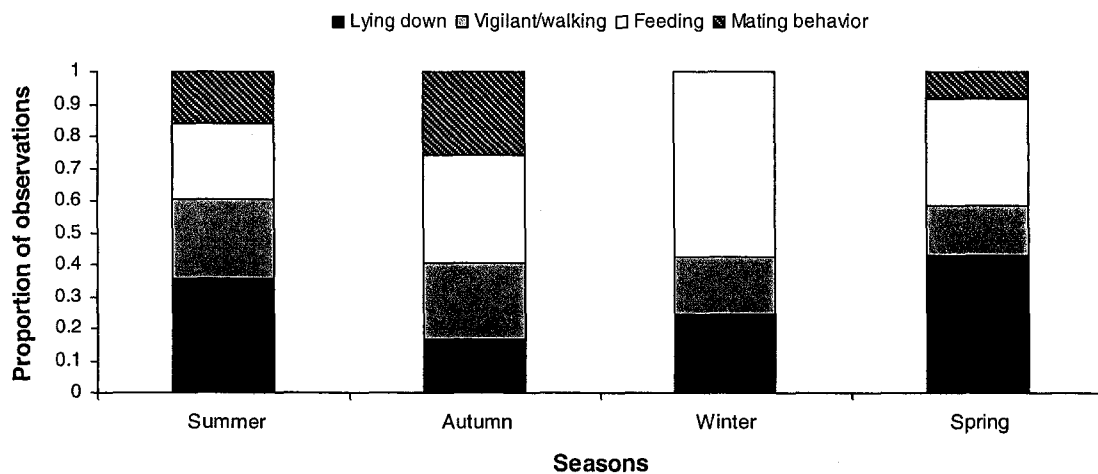
Figure 2.14. Seasonal changes in activities of male huemul, as proportions of observations for 18 individuals in 2005-2007. Activities were grouped as: lying (ruminating or resting), vigilant and walking (standing or moving), feeding, and mating behaviours (courtship, copulation attempts, fighting, and marking). Observations made over the 33-month study were pooled for summer ($n = 56$), autumn ($n = 109$), winter ($n = 115$), and spring ($n = 149$).

Discussion

The huemul mating system resembles that of territorial polygamous ungulates with limited sexual dimorphism (Brashares and Arcese, 1999, 2002; Liberg et al., 1998). Huemul males are only about 3% heavier than females, although they have antlers and develop muscle hypertrophy during the rut (P. Corti, unpublished data), together with dark face marks (Povilitis, 1983b, 1985). Although testes were not measured, they appear smaller in comparison with ungulates where sperm competition is common

(Hogg, 1988; Preston et al., 2003), suggesting that sperm competition likely plays a minor role in their mating system.

The huemul birth season is not as synchronous as in many other temperate ungulates (Côté and Festa-Bianchet, 2001; Festa-Bianchet, 1988; Gregg et al., 2001; Leslie et al., 1999; Rachlow and Bowyer, 1991). Most births in my study area occurred from mid-November to the first week of December; however, two fawns were born in late December and early January. The same length of parturition season has been observed in other huemul populations (Diaz and Smith-Flueck, 2000), suggesting a long mating season, as observed in this study (Leslie et al., 1999; Rutberg, 1987). The long mating season and consequently the long birthing season could be due to the comparative stability of the environment that huemul inhabit (Veblen et al., 1981; Veblen and Schlegel, 1982). Alaback (1991) compared the forest ecology of the southwest coast of South America with the northwest coast of North America and found milder winter seasons in the south than those in the northern hemisphere.

The huemul mating system was polygynous, with males defending several oestrous females sequentially (Section II.a.). Both sexes showed strong site fidelity, which probably relates to resources distribution (Switzer, 1993). Each male that holds a stable home range overlaps extensively with a group of females and their offspring. Theory suggests that territoriality occurs when food density is sufficiently high that an effective defence of the home range is possible (Liberg et al., 1998; Maher and Lott, 1995, 2000; Wronski and Apio, 2006). Female associations that follow matrilineal structures have been suggested as a strategy to defend access to high quality foraging patches against other matrilineal groups (Wrangham, 1980; Wronski et al., 2006). Future research should determine whether huemul females in fact form matrilineal groups and behave aggressively towards other female groups.

Territorial males avoided each other, but tolerated subordinate males. The behavioural data required to clearly establish the presence of a territorial system are difficult to obtain (Powell, 2000), especially in a forest-dwelling and cryptic species like the huemul. I saw very few interactions among putatively territorial males and therefore I cannot establish conclusively that they defend areas against other males. However, the degree of home range overlap can be used to deduce territorial behaviour (reviewed in Powell, 2000). The almost complete lack of overlap for 50% core areas of males strongly suggests territoriality (Powell, 2000). Even for 95% core areas, putatively territorial males had very low overlap, suggesting strong spatial avoidance.

Territorial males appeared to avoid other territorial males, especially the 50% core areas. Adult males kept their territories until death (n = 3 males that died during the study), suggesting life-long territoriality. A similar territorial mating system has been described for roe deer (*Capreolus capreolus*) (Liberg et al., 1998). Huemul satellite males roam within the territory of one or more territorial males. The parentage analysis suggests that this strategy may allow some access to oestrous females. Alternative mating strategies by subordinate males have been also observed in red deer (*Cervus elaphus*) (Pemberton et al., 1992) and in low-ranking bighorn sheep males (*Ovis canadensis*) (Pelletier et al., 2006).

The parentage analysis revealed that putative territorial males sired most fawns, but satellite male 21V sired two fawns with female 14V in consecutive years. He was observed using a coursing and blocking strategy (Hogg and Forbes, 1997) to sequester females away from territorial males. Territorial male 2N, who apparently sired no fawns, was mostly associated with female 1N, who produced no fawns during the study period. The only other female within 2N's range was 14V, and she mated with satellite male 21V. In this case, the satellite male entered the territory of 2N to mate with 14V. Three other females (3B, 5A, 20V) had offspring sired by males inhabiting neighbour areas. These females moved into the neighbour male area and returned to their range after mating. Some female roe deer (*Capreolus capreolus*) leave the male's territory in which they live to mate with another male (Lovari et al., 2008; Richard et al., 2008).

Because males that sired at least one fawn spent little time with the mothers over the previous year, it seems unlikely that the mixed groups of huemul are a form of prolonged mate guarding. Instead, males may join females in small groups to obtain some antipredator protection through the dilution effect (Moody et al., 1996; Roberts, 1996).

The importance of keeping a large core area year-round in the home range of a huemul male was reflected in their activity budgets through the year. Mating behaviour such as marking was observed all year, with the exception of winter. The same behaviour of marking trees and shrubs in spring with pre-orbital glands, far in advance of the rutting season, has been described in roe deer to establish early their territories (Hewison et al., 1998). Although rarely seen, the few observations of aggression among males supported territoriality, together with the appearance of mating behaviour about six months in advance of the rutting season. Mating behaviour, which includes fighting and marking in addition to copulation and courtship displays, starts soon after antlers are fully grown and velvet is shed in late

spring. Adult huemul kept hard antlers for at least seven months and cast them in mid-winter, when no mating behaviour was observed and deer formed larger groups (Section II.a). Red deer (*Cervus elaphus*) shed their velvet in late summer when preparing for the autumn rut (Suttie and Fennessy, 1992). The time between antler casting and regeneration varies substantially among deer species (Brown, 1992). In Old World deer the new antler begins growing as soon as the previous antler is cast. In North American deer, antler regeneration is delayed up to several months after casting (Goss et al., 1992). Huemul antlers start growing immediately after casting. Two close relatives of huemul, the lesser pudu (*Pudu puda*) (Bubenik et al., 1996) and pampas deer (*Ozotoceros bezoarticus*) (Pereira et al., 2005), also display similar patterns of rapid antler growth and prolonged mating behaviours, which have been associated with two testosterone peaks, a main one in the rut and a minor one in spring that promotes rapid velvet shedding (Bubenik et al., 1996; Pereira et al., 2005).

Male huemul obtained copulations by defending a single oestrous female inside their territories and performing displays as described by Povilitis (1985). Satellite males were never seen to challenge tending males for access to females. The satellite tactic may have a higher cost than defending a territory, decreasing survival (Pelletier et al., 2006) and increasing the risk of being killed by a territorial male. I saw no direct interactions between territorial males. Huemul antlers are excellent for attack, but offer limited defence against similar weapons. Fighting could result in serious injuries or death, as occurs in mountain goats (*Oreamnos americanus*) (Festa-Bianchet and Côté, 2008; Geist, 1964).

Male huemul defend territories all year and overlap with a group of philopatric females with whom they share space, but impede the access of other males. A large territory may increase siring success simply by encompassing the ranges of more females. However, there must be an upper limit to territory size, where the cost of keeping a large area is greater than the payoff of siring more offspring (Maher and Lott, 2000). A mating system where reproductive success is mostly limited to a few territorial males can be detrimental in very small populations, because it reduces effective population size. When the same few males gain access to females for several consecutive years, the potential for inbreeding may also increase, especially when populations are severely fragmented with no dispersal (Chapter IV).The mating system of several ungulate species can vary according to environmental and ecological variables, particularly the distribution of forage and of females (e.g., oribi, (Brashares and Arcese, 2002); roe deer, (Hewison et al., 1998; Liberg et al., 1998); impala (Jarman, 1974; Jarman and Jarman,

1979)). Although the mating system described here should be similar in other huemul populations under similar conditions, it is important to investigate variation of this species' behaviour in areas with different environmental conditions.

CHAPTER III

HUEMUL POPULATION DYNAMICS: CAUSES OF MORTALITY AND POPULATION
TRENDS

Introduction

Knowledge of huemul (*Hippocamelus bisulcus*) ecology and demography is limited. Most of its historic range has been disturbed by habitat loss and fragmentation when forest habitats were cut or burned to create livestock and farm lands (Diaz and Smith-Flueck, 2000; Flueck and Smith-Flueck, 2006; Frid, 2001; Povilitis, 1998; Smith-Flueck and Flueck, 1997). These events diminished suitable huemul habitats and likely increased the distances between remaining populations, possibly increasing mortality of dispersers as reported in many species (Diffendorfer, 1998; Namba et al., 1999; Van Vuren, 1998). Huemul are not known to disperse farther than 5-7 km (Gill et al., 2008), making it difficult to maintain population connectivity in severely fragmented habitats.

The ecosystems that huemul inhabit have also been perturbed by exotic species such as European hares (*Lepus europaeus*), which were introduced into southern South America in the early 1900s, red deer (*Cervus elaphus*), introduced in the 1950s, and wild boar (*Sus scrofa*), introduced in 1925 (Jaksic et al., 2002). Exotic species can alter the relationship of huemul with their natural predators by providing alternative prey. Predator populations subsidized by introduced species can exert high predation rates on native prey (Sinclair et al., 1998). For example, the culpeo fox (*Pseudalopex culpaeus*), endemic to South America, has specialized its diet on European hares (Johnson and Franklin, 1994; Novaro et al., 2004); the same specialization has occurred with cougars (*Puma concolor*) (Franklin et al., 1999; Iriarte et al., 1991) in areas of high hare abundance (Jaksic et al., 2002). Finally, the introduction of large numbers of domestic livestock into Patagonia, especially through extensive sheep farming, has also increased the number of prey available for local predators (Bank et al., 2002; Franklin et al., 1999; Iriarte et al., 1991).

As remaining natural areas become smaller and more fragmented, it becomes increasingly important to understand the ecological and evolutionary dynamics of small populations of endangered animal so that they may be effectively protected until restoration of natural areas can allow expansion of their ranges (Lande, 1988). However, our knowledge of the dynamics of endangered species is limited by the difficulty of obtaining sufficient data (Caughley and Gunn, 1996). Consequently, the estimation of demographic parameters of declining and small populations is crucial for the recovery of endangered species, because they can point to specific factors affecting the risk of extinction of a population

(Caughley, 1994; Caughley and Gunn, 1996; Sinclair et al., 2006). Huemul deer has a high risk of extinction (IUCN, 2008), but its demographic parameters are unknown. Thus, the relative roles of different vital rates in the observed population decline remain speculative.

Demographic models can play an important role in the management of endangered species (Norris and McCulloch, 2003). For example, estimates of population growth rates can quantify changes in the numbers of individuals through time (Sinclair et al., 2006). Yearly changes in specific vital rates, such as survival and reproduction, are responsible for these changes and determine current and future population dynamics (Caswell, 2001; Stearns, 1992). Elasticity values derived from matrix models can be used to predict the impact that a proportional change in each vital rates would have on population growth (de Kroon et al., 2000; Gaillard et al., 1998a; Gaillard et al., 2000). High elasticity by itself does not imply that a parameter should be the target of population recovery (Coulson et al., 2005). Instead, one should consider both elasticity and the empirical values of change over time in each vital rate (Coulson et al., 2005; Gaillard et al., 2000).

The objectives of this study were to determine basic demographic parameters for a huemul population in Chilean Patagonia, including estimates of population density and trend, fertility and recruitment rates, age- and sex-specific survival rates as well as estimates of the causes and temporal distribution of mortalities. I then parameterized an age-structured matrix model (Caswell, 2001; de Kroon et al., 2000) to determine the relative sensitivity of estimates of lambda to changes in different vital rates. I sought to document the structure and dynamics of an endangered ungulate population, providing estimates of vital rates that are critical to future population viability analyses and recovery plans; and to present the first data on the population dynamics of huemul based on monitoring marked individuals. Such data on endangered species in South America are particularly scarce. Data on the population dynamics should enable managers to focus attention where it is most immediately needed to preserve this endangered deer.

Material and methods

Study area

The study area was located at the Lago Cochrane National Reserve (LCNR) (69.25 km^2, 47°12'S, 72°30'W), Aysén District, Chilean Patagonia. The Reserve was established in 1967 to protect southern beech forests dominated by lenga (*Nothofagus pumilio*) and one of the last remaining huemul populations in Chile. The huemul population of LCNR is part of a larger population of approximately 120 individuals (H. Velásquez pers. comm.; P. Corti unpublished data), which extends beyond the limits of the Reserve, but is thought to be isolated from other populations because the surrounding unsuitable habitats prevent connectivity (Chapter IV). More details about the study area are presented in Chapter I.

Other wild herbivorous mammals in the LCNR include guanacos (*Lama guanicoe*) and exotic European hares. The guanaco population in the immediate neighbouring areas is estimated at about 2000 individuals (P. Corti unpublished data). Hares are abundant and observed easily almost everywhere. Confirmed huemul predators are culpeo foxes and cougars. Domestic dogs enter the Reserve sporadically from the nearby town and ranches. Several scavenger bird species, such as Andean condor (*Vultur gryphus*), caracara (*Polyborus plancus*), and black-chested buzzard eagle (*Geranoaetus melanoleucus*) are readily observed in the study area. Cattle and sheep ranches are common around the study area.

Huemul capture and monitoring

Between 2005 and 2008, 55 huemul were captured and marked with ear tags. The age of animals older than one year was estimated at capture from incisor eruption (Dimmick and Pelton, 1994) or was known for individuals monitored from birth. My sample of marked individuals included 29 adults older than three years (15 males/14 females), three two-year-olds (2 males/1 female), two male yearlings, and 21 fawns (15 males/6 females). Further capture details are described in Chapter I. Newborn fawns were captured by hand in late spring. All captured huemul received an ear tag with a colour and unique

number to identify them. Eighteen adults were fitted with very high frequency (VHF) radio collars with mortality/motion sensors (Sirtrack Ltd., Havelock North, New Zealand). This sample included two adult females with VHF collars from a previous project (Gill et al., 2008). Eighteen fawns were fitted with VHF radio-ear tags (Sirtack) that transmitted for seven hours a day and lasted for approximately 12 to 14 months. In addition, 33 deer (two adult males, five adult females, three juvenile males, two juvenile females, three yearlings, and 18 fawns) were identified by natural markings (scars, body shape, coat coloration, colour distribution of the rump patch), through their associations with other tagged individuals, and from their locations within the study area.

Locations of known individuals (with and without telemetry devices) were recorded by direct observations every 20 days or once a month between March 2005 and June 2008. Locations were taken using a handheld Global Positioning System (eTrex Summit, Garmin Ltd., Kansas City, MO, USA), and later plotted in ArcView 3.3 (ESRI, 2002).

Delineation of huemul use area

I pooled all locations to establish the boundaries of the area occupied by deer between 2005 and 2008. Using all recorded locations, I estimated a multi-annual population range using the 95 % adaptive kernel (Worton, 1989) within the animal movement extension (Hooge and Eichenlaub, 2000) in ArcView 3.3 (ESRI, 2002). I used this estimate of population range to estimate density.

Huemul population size, sex ratio, and density

Within the LCNR, all deer were individually identifiable after January 2006, so I estimated the number of huemul through direct counts, conducted each year in November just before the parturition season. All individuals seen were sexed and aged. The sex ratio for each year was then estimated for all animals older than two years. Population density was estimated for all animals older than one year.

Causes of mortality

Mortality was detected in three ways: 1) when the signal from a motion-sensitive telemetry collar indicated that a huemul was dead, I investigated the site as soon as possible (13 deaths); 2) since tagged and untagged animals were located at least once a month, the probability of finding a dead individual without a telemetry device was high, especially for adults, where detection was often aided by the presence of scavenger birds (11 deaths); and 3) animals that disappeared were assumed to be dead ($n =$ 17). For this last category, the last time an individual was seen was used as the date of death. Cause of death was determined from evidence found at the site, or from direct observation of the event (e.g., dog attacks). Suspected cases of predation were classified into cougar, culpeo fox, and domestic dog categories, based on tracks, scats, hair, distribution and disposition of the carcass (cougars generally buried their prey), and from predator sightings. Deaths not attributed to predation were classified as caused by humans (poaching) or accidents. In 41.5% of cases it was difficult to determine the cause of death, especially for animals that disappeared, and I classified the cause as unknown. I then grouped mortality causes according to the following classes: 1) adults (all individuals older than two years); 2) yearlings (males only since no female mortality was registered for this class); and 3) fawns (younger than one year). I then classified mortality by seasons and finally examined the seasonal distribution of different causes of mortality.

Survival

I estimated survival probabilities (Φ) of LCNR huemul for different sex-age classes using known-fate mark-recapture models in program MARK (White et al., 1999). These models estimate the probability of surviving an interval between sampling occasions and assume that the resighting probabilities of identified individuals are 1 at each sampling period (White et al., 1999). Thus, the status (dead or alive) of all animals is known at each sampling occasion (White et al., 1999). A total of 39 monthly intervals (from April 2005 to June 2008) were used to estimate sex- and age-specific survival. The probability to survive an entire year is then the product of 12 monthly survival probabilities (Blums et al., 2002).

Survival estimates in MARK are derived via maximum likelihood (White et al., 1999). The number of estimable parameters (k) (i.e., survival probabilities of different sex- and age-classes) were used to

compute the Akaike's Information Criterion corrected for small sample sizes ($AIC_c = AIC + (2k$ $(k+1)/(n-k-1))$, for a set of candidate models (Anderson et al., 2000; Burnham and Anderson, 2002), where n is the sample size. The model with the smallest AIC_c value explains the greatest amount of variation using the fewest variables and is selected as the model most supported by the data (Anderson et al., 2000; Burnham and Anderson, 2002). Akaike's weights (ω) were computed as reflections of relative evidence supporting each model and these weights sum to one for all the models in the model set (Burnham and Anderson, 2002). AIC_c ω were also used to estimate the likelihood value (Model L) of a model given the set of models (Burnham and Anderson, 2002). The Model L is the AIC_c ω for the model of interest, divided by the AIC_c ω of the best model, and provides the relative strength of evidence of this model within the candidate models considered (Burnham and Anderson, 2002). The deviance in these models was the difference between the -2 log-likelihood of a current model and the -2 log-likelihood of the saturated model (White et al., 1999).

Candidate models for the AIC_c selection procedure

The estimation of biologically meaningful survival probabilities required a stepwise exploration of available data (Table 3.1). First, I evaluated whether there were differences in survival probabilities among animals with and without telemetry devices according to age class. I then evaluated possible differences in survival among sex- and age-classes $\Phi(g)$, possible temporal trends $\Phi(t)$, as well as an interaction between class and time $\Phi(g*t)$. Since this analysis did not detect differences in survival between adults (older than three years) and juveniles (two-year-olds), the two age groups were pooled for further analyses. Finally, I evaluated possible differences in survival probabilities by sex for fawns, yearlings, and adults. Models were tested against null models $\Phi(.)$ with similar survival probabilities.

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Table 3.1. Stepwise exploration of huemul survival probabilities (Φ) in program MARK. 1) test for differences in monthly survival probabilities between huemul with and without telemetry devices; 2) test for differences in survival probabilities among sex-age classes $\Phi(g)$, over time $\Phi(t)$, and the interaction between class and time $\Phi(g*t)$; 3) tests to compare survival probabilities of a) fawns, b) yearlings, and c) adults grouped by sex. All models were tested against their respective null models $\Phi(.)$ which assumed constant survival probabilities. The number of parameters used for each model is also indicated (k).

Models	Parameters (k)	Biological meaning
1. Telemetry (8 models) Fawns, Yearlings, Juveniles, Adults		
$\Phi(.)$	1	Monthly survival is the same for deer with and without telemetry devices.
$\Phi(g)$	2	Monthly survival is different for deer with and without telemetry devices.
2. Sex-age class and time (4 models)		
$\Phi(.)$	1	Monthly survival is the same among all sex- and age classes.
$\Phi(g)$	6	Monthly survival is different among sex- and age-classes.
$\Phi(t)$	39	Monthly survival varies over time but not with sex-age class.
$\Phi(g*t)$	234	Differences in survival among groups depend on the month.
3. Sex within classes (6 models) Fawns, Yearlings, Adults		
$\Phi(.)$	1	Monthly survival is the same between males and females.
$\Phi(g)$	2	Monthly survival is different between males and females.

Fertility

I estimated fertility directly from observations of marked females with a fawn (within the first 15 days of birth). Huemul females produce one fawn per year, after a gestation of about 200 to 220 days (Diaz and Smith-Flueck, 2000). Fawns are born over approximately one month in late November and December (Diaz and Smith-Flueck, 2000; P. Corti unpublished data). Huemul newborns stay hidden for the first 10 to 15 days of life, when they can be hand-captured. The age of primiparity is unknown, but it is assumed to be three years (Diaz and Smith-Flueck, 2000; Serret, 2001). In this study, three females were observed with their first fawns at three years of age, and one at four years.

Age structure

Combining information obtained during capture and the basic information on reproduction, I grouped huemul into four age classes: fawns (0-1 year), yearlings, two-year-olds, and adults (older than three years).

Matrix Models

Matrix models have been used extensively to evaluate the dynamics of age-structured populations (Caswell, 2001). Assuming an annual birth-pulse reproduction, I used survival and fertility data for each of the four age classes to parameterize the following population projection matrix (A):

$$A = \begin{bmatrix} 0 & 0 & 0 & F_3 \\ p_0 & 0 & 0 & 0 \\ 0 & p_1 & 0 & 0 \\ 0 & 0 & p_2 & p_3 \end{bmatrix},$$

where F_3 is adult female fertility, p_x is the age-specific survival probability, and the matrix columns represent age classes (fawns, yearlings, two-year-olds, adults). The matrix predicts parameters of huemul populations in December, just after parturitions. The finite rate of increase (λ) of these matrices is the dominant eigenvalue of the projection matrix A, and the stable age distribution (w) corresponds to the right eigenvector (Caswell, 2001). The left eigenvector yields the reproductive value (v), the contribution of each age class to population growth (Caswell, 2001). Reproductive values have been standardised to 1, so that they represented relative contributions of each age class.

To assess how changes in fertility or age-specific survival would influence population growth, I performed a sensitivity analysis. The sensitivity of a population's growth rate to a change in matrix element a_{ij} (fertility of adult females and survival of all classes) is defined as the partial derivative of λ with respect to a_{ij} (Caswell, 2001):

$$\text{Sensitivity} = \frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i w_j}{<w, v>}$$

where v_i and w_j refer to the ith and jth elements of the age-specific reproductive value and stable age distribution vectors, respectively, and where $<w, v>$ is the scalar product of w and v (i.e., $<w, v> = v1$ $w1 + v2\,w2 + ... + v_n\,w_n$).

Finally, I estimated elasticity values as the proportional change in population growth rate as a function of proportional changes in vital rates such as age-specific survival or reproduction (Benton and Grant, 1999; de Kroon et al., 2000) as (Caswell, 2001):

$$\text{Elasticity} = e_{ij} = \frac{a_{ij}}{\lambda}\frac{\partial\lambda}{\partial a_{ij}} = \frac{\partial\log\lambda}{\partial\log a_{ij}}$$

These elasticities indicate the relative importance of a possible change in a given age-specific vital rates for changes in λ (Benton and Grant, 1999; de Kroon et al., 2000). Elasticities are easy to interpret and translate into management decisions when a measure of variability is added (i.e., stochastic models with standard errors and confidence intervals) (Benton and Grant, 1999; de Kroon et al., 2000). In general, such analyses allow to assess the relative effectiveness of different management scenarios implemented to increase population size (Benton and Grant, 1999; Gaillard et al., 2000; Mills et al., 1999). All matrix analyses were performed using Microsoft Excel and the Excel add-in PopTools (Hood, 2005).

Model parameterization

I parameterized the matrix model using estimates of age-specific survival and fertility rates from data collected in the field. To account for uncertainty in parameter values, I compared three scenarios where I varied estimates of age-specific survival probabilities according to the results from the detailed analyses conducted in program MARK (Table 3.2).

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Table 3.2. Vital rates of the huemul population (±SE) at Lago Cochrane National Reserve used in matrix models simulating three different scenarios of age-specific survival. 1) Model 1 uses female survival probabilities for all age-classes except fawns. Estimates of fawn survival pool data from both sexes (optimistic scenario); 2) Model 2 is based on all survival estimates from females only; and 3) Model 3 assumes a reduction in female adult survival using the estimate obtained when both sexes were pooled (worst case scenario). Survival probabilities were obtained using known-fate models in program MARK and fertility estimates were directly observed from the ratio of females with fawns within 15 days of birth.

Parameters	Model 1	Model 2	Model 3
Fertility	0.72 (± 0.11)	0.72 (± 0.11)	0.72 (± 0.11)
Survival			
0-1 year (fawns)	0.34 (± 0.07)	0.11 (± 0.09)	0.11 (± 0.09)
1-2 years (yearlings)	0.77 (± 0.20)	0.77 (± 0.20)	0.77 (± 0.20)
2-3 years (juveniles)	0.95 (± 0.03)	0.95 (± 0.03)	0.91 (± 0.03)
≥ 3 years (adults)	0.95 (± 0.03)	0.95 (± 0.03)	0.91 (± 0.03)

I incorporated stochasticity into the matrix models following Wittmer et al. (2007b). To account for uncertainty in estimates of vital rates, I sampled both fertility and adult survival from a specified probability distribution that reflected temporal variation for a vital rate. Values for vital rates were selected using the Excel function NORMINV (probability, mean, standard_dev), where 'probability' is a uniform random number from 0 to 1 (selected using RAND()), and 'mean' and 'standard_dev' were the estimate and standard error of each vital rate (Table 3.2). Each set of randomly selected vital rates for the population was then used to construct a time-invariant matrix population model. I repeated the procedure 1000 times, re-sampling from the probability distribution of each vital rate for each replicate.

Statistical analyses to determine confidence intervals for the estimated λ's, age distribution values, and reproductive values were conducted using the percentile method from the 1000 repeated measurements (Efron and Tibshirani, 1993).

Results

Area used by huemul

I determined the space used by the LCNR huemul population including all identified individuals older than one year of age ($n = 72$) located from 2005 to 2008 (Fig 3.1). The area used was ca. 19.93 km^2, which represents 29% of the reserve.

Huemul population size, sex ratio, and density

The total population size of the Reserve was estimated through individual identification of all huemul in the area. November counts for 2005, 2006, and 2007 suggest an increase after the first year (Table 3.3), but not all animals were identified during the first year. Sex ratios in all years were biased towards females. Mean ($\pm$ SE) density was 1.79 ($\pm$ 0.19) huemul/km^2.
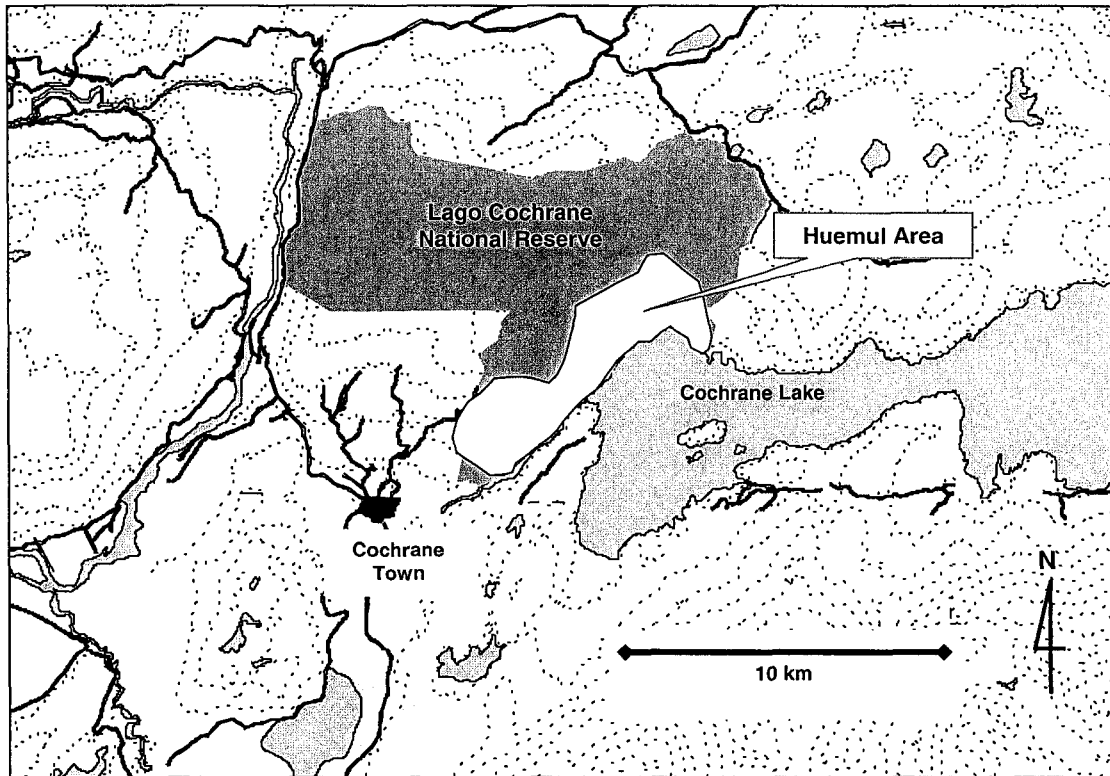
Figure 3.1. Map of the study area showing the area used by huemul (from 95% adaptive kernel utilization distribution) in the Lago Cochrane National Reserve, Chilean Patagonia. The grey area represents the total area of the Reserve. The dotted area was used by huemul between 2005-2008.

Table 3.3. Estimated huemul population size in the Lago Cochrane Reserve in November 2005, 2006, and 2007. Sex ratio was calculated for individuals older than two years and population density for huemul older than one year. Fertility was the number of fawns born each year divided by the number of females older than three years.

	2005	2006	2007		
Adults (≥ 3 years)					
Male	9	12	10		
Female	14	19	20		
Juveniles (2 years)					
Male	1	1	4		
Female	2	1	2		
Yearlings					
Male	1	5	2		
Female	1	2	1		
Fawns					
Male	4	3	4		
Female	1	2	0		
Total population	33	45	43		
Sex ratio (F:M)	1.60:1	1.54:1	1.57:1	**Mean**	**SE**
Density (huemul / km^2)	1.41	2.01	1.96	1.79	0.19
				Mean	**SE**
Fertility *	0.94	0.55	0.68	0.72	0.11
Fawns	15	11	15		
Females	16	20	22		

* Fertility estimates include two-year-old females because at the time of count they become three-year-olds and can have a fawn. Fawns are all the ones born at the end of November and December of each year after the census was carried out.

Causes of mortality

Forty-one deaths of huemul were recorded between 2005 and 2008 (Fig. 3.2). Ten adults (7 males, 3 females) died, including a male captured during a previous project (not used for later analyses). Mortality in adult males was relatively evenly distributed among causes: two were poached; two were killed by cougars; one was killed by dogs; and two disappeared from the study area. One adult female was killed by a cougar and two by dogs. For yearlings, mortality causes were unknown: two males disappeared. For fawns (sexes pooled), causes of mortality were multiple: cougars, three individuals; culpeo foxes, five; dogs, five; disappearances 13; poaching, two; and one fawn fell into a rock crevasse.

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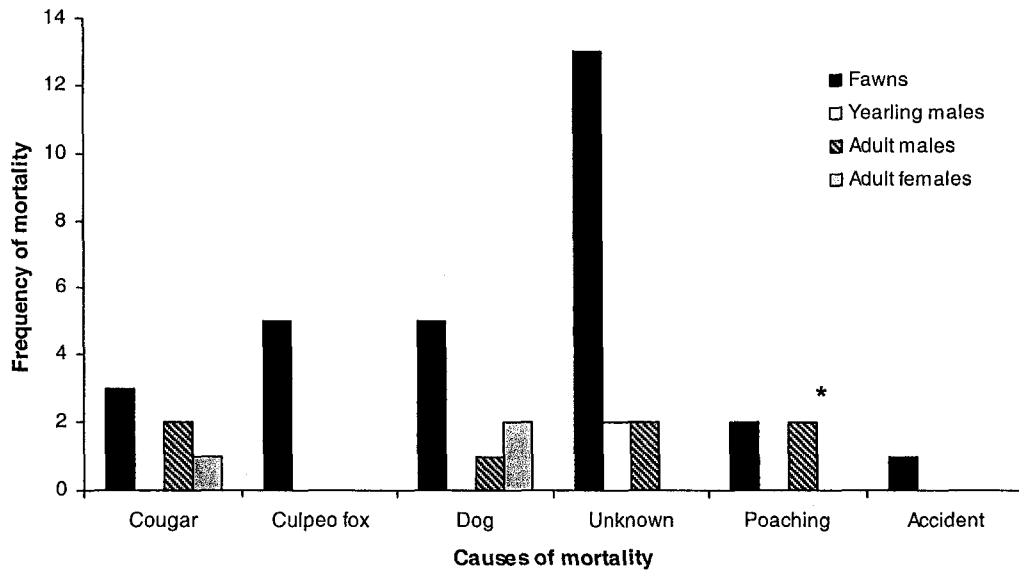
Figure 3.2. Causes of huemul mortality by sex-age class (male and female fawns were pooled) at Lago Cochrane National Reserve, Chilean Patagonia. (*) One poached male from a previous project was included because the radio collar was found during the study period.

Fawn deaths mostly occurred in summer, during their first three months of life, and in spring just when they reached one year of age, while no fawn mortality was observed in winter (Fig. 3.3). Causes of mortality did not show a clear seasonal pattern (Fig. 3.4).
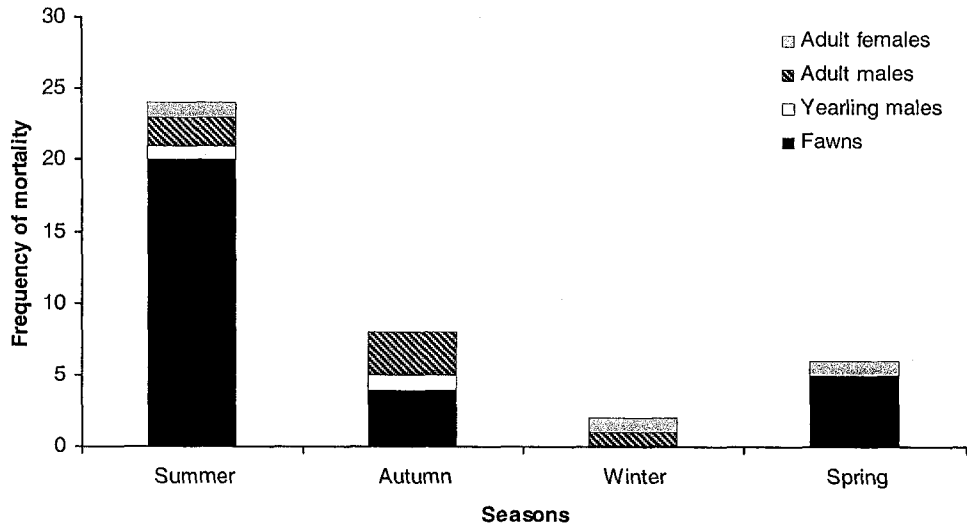
Figure 3.3. Seasonal mortality of huemul from March 2005 to June 2008 in Chilean Patagonia. Individuals that died of different causes were pooled by age and sex classes.
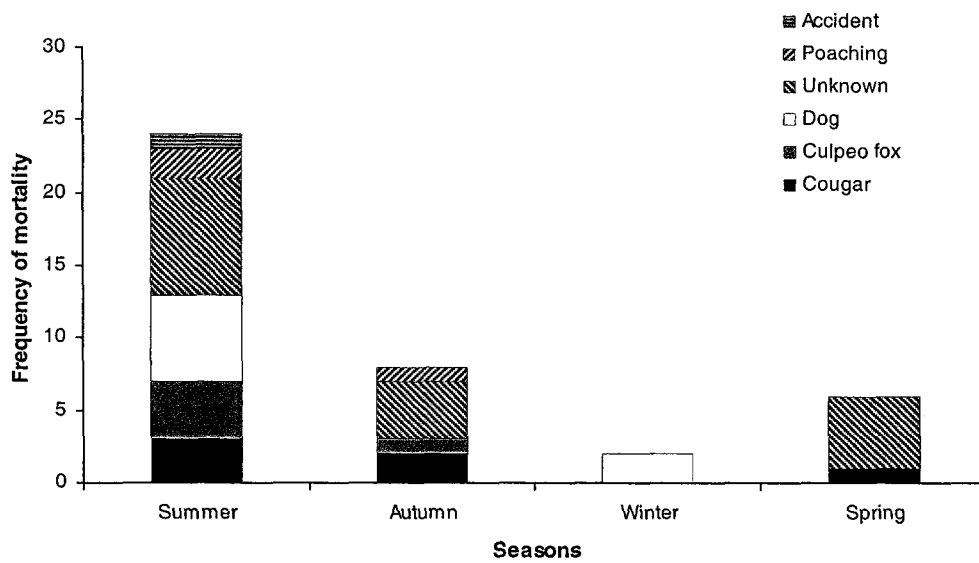
Figure 3.4. Causes of huemul mortality by season (all age and sex classes pooled) at Lago Cochrane National Reserve, Chilean Patagonia.

For both fawns and adults, there was no indication that survival differed between animals with and without telemetry devices (Table 3.4 and Table 3.5). Therefore, individuals with and without telemetry devices were pooled for all further analyses.

Table 3.4. Estimates of monthly survival rates of huemul of different age classes with and without telemetry devices (radio-collars or radio-ear tags) in the Lago Cochrane National Reserve, Chilean Patagonia.

	With telemetry device Survival rates ± SE (*n*)	Without telemetry device Survival rates ± SE (*n*)
Fawns	0.90 ± 0.03 (18)	0.93 ± 0.02 (31)
Yearlings	1.00 (6)	0.98 ± 0.01 (14)
Juveniles (2 years)	1.00 (3)	0.99 ± 0.01 (10)
Adults (≥ 3 years)	0.99 ± 0.003 (18)	0.99 ± 0.004 (24)

Table 3.5. Models comparing survival between individuals with and without telemetry devices $\Phi(g)$ for 4 age-classes of huemul. When compared to a null model $\Phi(\cdot)$, there were no detectable differences in survival probabilities ($\Delta AIC_c < 2$). AIC_c weights, model likelihood (Model L), number of parameters in the models (*k*), and model deviance.

Model	AIC_c	ΔAIC_c	$AIC_c \omega$	Model L	*k*	Deviance
Fawns (*n* = 49)						
$\phi(\cdot)$	173.94	0	0.65	1.0	1	58.38
$\phi(g)$	175.21	1.27	0.35	0.53	2	57.63
Yearlings (*n* = 20)						
$\phi(\cdot)$	32.30	1.69	0.30	0.43	1	16.90
$\phi(g)$	30.61	0	0.70	1.0	2	15.21
Juveniles (*n* = 13)						
$\phi(\cdot)$	13.52	0.86	0.39	0.65	1	6.48
$\phi(g)$	12.66	0	0.61	1.0	2	5.62
Adults (*n* = 42)						
$\phi(\cdot)$	118.13	0	0.59	1.0	1	42.72
$\phi(g)$	118.89	0.76	0.41	0.69	2	41.47

A second step evaluated if survival probabilities differed among sex and age classes, as well as time. The fully parameterized model $\Phi(g*t)$ had a deviance of zero indicating good fit (Table 3.6). Results

indicated that differences in survival probabilities were best explained by sex- and age-classes (Table 3.7). However, the survival of two-year-olds and of adults did not differ. Thus, these age-classes were pooled for subsequent analyses.

Table 3.6. Models comparing survival probabilities of huemul sex and age classes $\Phi(g)$, over time $\Phi(t)$ and the interaction between groups and time $\Phi(g*t)$. Model comparisons were based on AIC_c values. AIC_c weights, model likelihood (Model L), number of parameters in the models (k), and model deviance.

Model	AIC_c	ΔAIC_c	$AIC_c \omega$	Model L	k	Deviance
$\phi(g)$	610.28	0	1.00	1.0	6	159.93
$\phi(t)$	626.78	16.50	0	0	39	109.52
$\phi(\cdot)$	651.18	40.90	0	0	1	210.86
$\phi(g*t)$	941.59	331.31	0	0	234	0

Table 3.7. Estimates of huemul survival rates between March 2005 and June 2008, according to age and sex class, in the Lago Cochrane National Reserve, Chilean Patagonia. Sample sizes by year are summarised in Table 3.3.

Sex-age classes		Survival rates	
		Monthly (±SE)	Yearly (±SE)
Adult survival (≥ 3 years)		0.992 (±0.003)	0.908 (±0.029)
Two-year-olds		0.993 (±0.007)	0.917 (±0.079)
Adults (≥ 2 years)			
	Females	0.996 (±0.002)	0.954 (±0.026)
	Males	0.986 (±0.005)	0.843 (±0.054)
	Overall	0.992 (±0.003)	0.909 (±0.027)
Yearlings			
	Females	0.978 (±0.022)	0.769 (±0.203)
	Males	0.983 (±0.012)	0.817 (±0.117)
	Overall	0.982 (± 0.010)	0.803 (± 0.101)
Fawns			
	Females	0.829 (±0.059)	0.106 (±0.090)
	Males	0.949 (±0.018)	0.534 (±0.119)
	Overall	0.914 (±0.017)	0.339 (±0.073)

Finally, I evaluated if survival probabilities of males and females differed within age classes (Table 3.8). For fawns, the survival of males was higher than that of females (Table 3.7), and the best model for fawn survival considered the sexes separately ($\Phi(.)$, $\Delta AIC_c > 2$, Table 3.8.a). For yearlings, survival was similar for males and females ($\Delta AIC_c \sim 2$, Table 3.8.b). The survival of adult females was slightly higher than that of males (Table 3.6); although the difference in AIC compared to a model that assumed equal survival of the sexes was small ($\Delta AIC_c < 2$) (Table 3.8.c).

Table 3.8. Model selection for differences in survival between huemul sex classes within each age class at Lago Cochrane National Reserve, Chilean Patagonia, 2005-2008.

a) Fawns:

Model	AIC_c	ΔAIC_c	$AIC_c \omega$	Model L	k	Deviance
$\phi(\cdot)$	108.26	3.51	0.15	0.17	1	55.83
$\phi(g)$	104.75	0	0.85	1.0	2	50.28

b) Yearlings:

Model	AIC_c	ΔAIC_c	$AIC_c \omega$	Model L	k	Deviance
$\phi(\cdot)$	32.05	0	0.73	1.0	1	18.94
$\phi(g)$	34.05	2.00	0.27	0.37	2	18.89

c) Adults:

Model	AIC_c	ΔAIC_c	$AIC_c \omega$	Model L	k	Deviance
$\phi(\cdot)$	118.74	1.86	0.28	0.39	1	45.73
$\phi(g)$	116.88	0	0.72	1.0	2	41.86

Matrix model and population trends

The three matrices yield different results with respect to lambda (Table 3.9). The scenario based on observed female survival for yearling and adults, and pooled fawn survival, predicts that the huemul population should increase by approximately 8% per year. The other two scenarios project either a

stable or declining population trend, and in both cases the confidence intervals overlap zero. However, in model two, 460 of 1000 runs indicated a declining population (Fig. 3.5). In model three, 744 out of 1000 runs resulted in $\lambda \leq 1.0$. Confidence intervals of stable age-distributions and reproductive values overlap for all 3 models suggesting that they were not significantly different.

Table 3.9. Lambda, age distribution, and normalized reproductive values (with 95% confidence intervals) for huemul estimated using a matrix model. Model input parameters are summarized in Table 3.2.

	Model 1	Model 2	Model 3
Lambda	1.08 (1.00-1.16)	1.00 (0.92-1.09)	0.97 (0.88-1.06)
Age distribution			
0-1 year (fawns)	0.33 (0.26-0.38)	0.39 (0.31-0.47)	0.39 (0.31-0.47)
1-2 years (yearlings)	0.10 (0.07-0.14)	0.04 (0-0.09)	0.04 (0-0.09)
2-3 years (juveniles)	0.07 (0.04-0.10)	0.03 (0-0.07)	0.03 (0-0.08)
>3 years (adults)	0.50 (0.44-0.59)	0.54 (0.47-0.63)	0.54 (0.46-0.63)
Reproductive value			
0-1 year (fawns)	0.07 (0.04-0.10)	0.03 (0-0.06)	0.03 (0-0.07)
1-2 years (yearlings)	0.23 (0.14-0.28)	0.26 (0.16-0.34)	0.26 (0.17-0.34)
2-3 years (juveniles)	0.33 (0.29-0.39)	0.35 (0.31-0.41)	0.34 (0.30-0.40)
>3 years (adults)	0.38 (0.34-0.43)	0.37 (0.32-0.42)	0.36 (0.32-0.42)
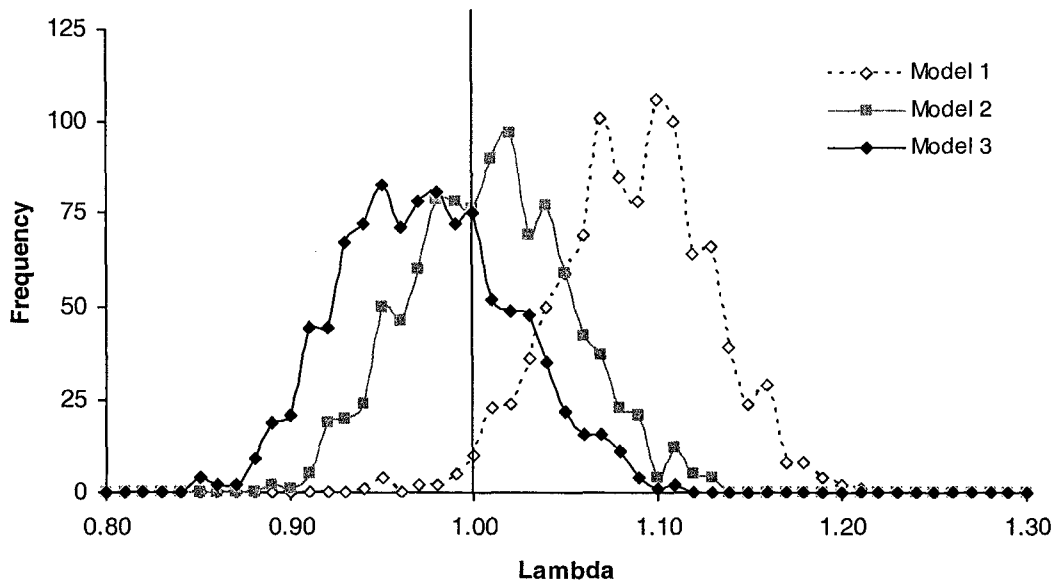
Figure 3.5. Frequency distribution of lambda (λ) for 3 different matrices calculated from 1000 repeated random measurements of λ around the estimated variance.

Matrix elasticities

In all models, λ was most sensitive to changes in survival of adult females (Fig. 3.6). The elasticities of other parameters (adult female fertility, survival of fawns, and survival of yearlings) were similar and low. In the first model, fertility has a slightly higher impact on λ when compared to the other two models. The relative importance of adult survival is highest in the two models assuming low fawn survival.

■ Adult fertility　　□ Fawn survival　　⊟ Yearling survival　　◫ Juvenile survival　　◫ Adult survival
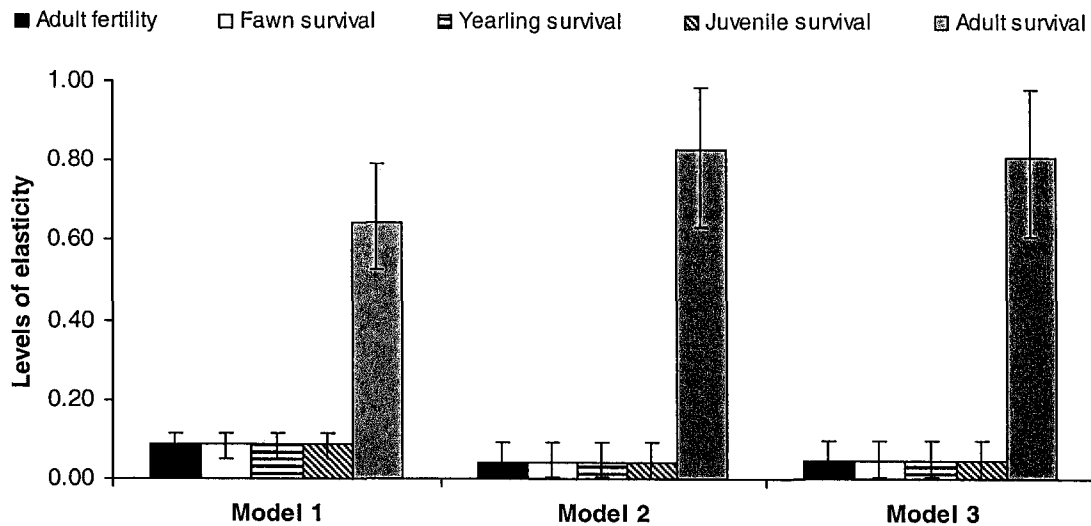
Figure 3.6. Elasticities (± 95% confidence intervals) for vital rates for huemul estimated from three different matrix models (see Table 3.2 for parameter values). Lago Cochrane National Reserve huemul population, Chilean Patagonia 2005-2008.

Discussion

Huemul density in the study area was low, similar to densities reported for other areas far from my study site (1 huemul/km² (Smith-Flueck and Flueck, 1997)). The low density of huemul contrasts with other related deer species of similar size like mule deer that can exist at greater densities (e.g., 21-76 deer/km² (White et al., 1989)), or forest dwellers like roe deer (e.g., 5-12 deer/km² (Hewison et al., 1998)).

Causes of mortality in this huemul population were multiple. Adults died mostly from predation by cougars and dogs, poaching, and unidentified causes. Most mortality occurred in summer and autumn, unlike most reports for deer in the northern hemisphere where most deaths are during winter, due to low body condition and starvation (Unsworth et al., 1999; White and Bartmann, 1998; White et al., 1996). Although these results might suggest that forage availability in existing habitat was not a limiting factor, this issue deserves more attention because many mortality causes were unknown. The

relationship of huemul with habitat quality has been inadequately studied. In addition, the fertility of this population was low compared to other deer species of similar size (e.g., white-tailed deer *O. virginianus* (DelGiudice et al., 2007)) which could suggest food limitation or a senescent population (Gaillard et al., 2000).

For yearlings, most mortality occurred in summer and autumn, and during spring for fawns about to become yearlings, coinciding with weaning. The study area was surrounded by unsuitable habitat that might have increased the risk of mortality when yearlings attempted to emigrate. Fragmentation may contribute to the lack of immigration when young left their natal areas through unsuitable habitats (Van Vuren, 1998). Fawn mortality was also mostly in summer, just after birth. While predation by foxes and dogs was a common cause of mortality, fawns were affected by all mortality causes that could be identified in the study area. Middle-sized predators like culpeo fox (adult body mass 6-12 kg (Novaro et al., 2004)) can only kill huemul younger than six months of age. Indeed, mortality of fawns was most severe until they reached six months. Poaching and dogs attacks appeared to be stochastic events. Dogs killed huemul of all age and sex classes, while poaching mostly affected adult males. The majority of adult male disappearances from the study area occurred in early summer. Although the causes of disappearance were unknown, poaching was suspected because that is the time when most people enter the Reserve, both locals and tourists.

Estimates of the mean annual survival of huemul in this population were similar to those of other ungulates, where adult survival is stable and high, and where fawn and yearling survival is lower and variable in time (Gaillard et al., 1998a; Gaillard et al., 2000). Estimated elasticities confirmed that changes in adult female survival have the greatest potential effect on population growth rate, as is the case for most ungulate populations (Gaillard et al., 2000). It is often inferred that management should focus on the parameters with the largest elasticities (de Kroon et al., 2000). However, elasticity calculated from matrix projections will have no consequence for population dynamics if the vital rate in question does not vary over time. Instead, the vital rate with the highest combination of variability and elasticity should have the greatest effect on changes in population growth. For example, survival of adult female roe deer is rather high and stable through time, suggesting that year-to-year changes in adult survival play a relatively minor role in changes in population growth (Gaillard et al., 1998b). Instead, juvenile survival has low elasticity, but because it varies substantially from year to year, it has a major influence on variation in population size (Gaillard et al., 1998b).

During the last two years of the study, only male fawns survived. The few female fawns born died before reaching one year of age. The study population has probably been maintained mostly because adult huemul have high survival rates. Simulations showed that a slight decrease in adult female survival would make this population prone to decline. The recruitment rate of yearling huemul was very low, for unclear reasons as most causes of death were unknown. It is likely that the male-biased fawn sex ratio was due to demographic stochasticity. The low recruitment of young females may have been more important than the mortality of adult females in driving population dynamics during the study. Stochastic variability in fawn sex ratio would not be a problem if the population was large enough to persist until the pattern is reversed; however, this population is rather small, as are most remaining huemul populations (Flueck and Smith-Flueck, 2006; Povilitis, 1998; Redford and Eisenberg, 1992; Vila et al., 2006). Despite the high survival of adult females, a run of years with minimal recruitment could lead to a sudden decline as the population becomes older. Although I did not monitor enough known-age adults to determine the onset of senescence, studies of other ungulates strongly suggest that both increased mortality and reduced fertility should be expected among older females, beginning at about 9-12 years of age (Gaillard et al., 2000). The low recruitment of females is a conservation concern in a small population, and if this situation persists, the population will be driven to extinction (Caughley, 1994).

The huemul ecosystem is disturbed, not only by habitat loss, but also by the introduction of exotic species such as the European hare, and by extensive domestic sheep and cattle farming. These new alternative prey could artificially sustain a high number of predators, especially culpeo fox that can prey heavily on huemul fawns. The huemul population could decline because the high density of hares subsidizes the fox population, a phenomenon referred to as 'apparent competition' (Bonsall and Hassell, 1997; Sinclair et al., 1998; Wittmer et al., 2005b).

'Apparent competition' among herbivores has been observed in woodland caribou (*Rangifer tarandus caribou*), where changes in habitat structure caused by logging allow an increases in moose, which increase the number of wolves that in turn increase predation on caribou (Wittmer et al., 2005a; Wittmer et al., 2007a; Wittmer et al., 2005b). Apparent competition has been also observed in the relationship between mule deer and white-tailed deer. Increasing numbers of white-tails in western North America have allowed cougars to maintain high population numbers, while preying heavily on mule deer (Robinson et al., 2002). Recovery efforts for caribou and mule deer must address both

alternative prey abundance and predator abundance. I suggest that recovery of huemul will also require a multispecies perspective, in a scenario which will also include more abundant guanaco, domestic livestock, and dogs.

Factors that alter the predator-prey system in huemul are just starting to be quantified. It may be impossible to reduce the density of European hares or to control culpeo fox, because the hares will likely allow a very rapid increase in the population of foxes. Cougar predation was not as important as fox or dog predation. In the study population, dog predation is likely to increase as the human population of the nearby village continues increasing and subsidizing the dog population. Dogs can reduce female survival, which has very high elasticity and, if decreased, would quickly lead to a decline in population size.

Although huemul may have co-inhabited with canids during the Pleistocene, including the dire wolf (*Canis dirus*) (Dundas, 1999), and past predation pressures might continue to influence current antipredation behaviours (Blumstein and Daniel, 2002), they do not exhibit an effective antipredator strategy against cursorial predators like dogs. In contrast, the low density and scattered distribution of huemul make them an unprofitable prey for cougars in comparison to the more abundant guanaco (Bank et al., 2002; Franklin et al., 1999; Iriarte et al., 1991). Guanaco prefer open steppe habitat which is not suitable for huemul (Gill et al., 2008), and in the absence of exotic prey species it may be more profitable for cougars to hunt in the steppe and seek high-density guanacos than search for rare huemul in forested habitats.

Increasing the huemul population size in the LCNR and adjacent areas will depend on augmenting the survival probabilities of young, fawns and yearlings, but also on the space available. Adult males are territorial and huemul live in small groups, factors that may also limit population growth (Chapter II). Thus, space and connectivity of habitats are fundamental to management strategies to increase or at least stabilize this huemul population in the long term. It is necessarily to continue monitoring and tagging huemul individuals because more time and larger sample sizes are required to decrease some of the large confidence intervals (i.e., yearling survival) and to know if our management actions are being successful. It is also essential to determine if the very low female recruitment that I documented was indeed a stochastic event as I proposed, or whether it will continue and thereby lead to a population

decline. This will increased the accuracy required to adequately predict the future fate of this huemul population.

CHAPTER IV

HUEMUL POPULATION GENETICS: IMPLICATIONS FOR CONSERVATION AND
MANAGEMENT

Introduction

The huemul (*Hippocamelus bisulcus*) population has decreased dramatically in both size and distribution since the arrival of European settlers 500 years ago (Diaz and Smith-Flueck, 2000; Flueck and Smith-Flueck, 2006; Redford and Eisenberg, 1992; Vila et al., 2006). Such drastic reduction in numbers and distribution, plus the fragmentation of remaining populations through habitat loss, might have reduced their genetic variability.

Small populations in fragmented habitats can lose genetic variation through drift (Hedrick et al., 1996; Kretzmann et al., 2001; Newman and Tallmon, 2001; Nunney, 1999). In addition, when there are few breeders the probability of mating between related individuals increases, resulting in inbred progeny (Dudash and Fenster, 2000). Habitat fragmentation may also cause a rapid reduction in population size inside remaining patches, leading to a reduction in genetic variability (Keller and Waller, 2002; Kirkpatrick and Jarne, 2000; Luikart and Cornuet, 1998; Maudet et al., 2002). If mechanisms for purging deleterious alleles are inadequate, mating among close relatives can lead to inbreeding depression, and in extreme cases to local population extinction (Keller and Waller, 2002).

Connectivity between populations is crucial for the persistence of many species; thus, habitat fragmentation can accelerate extinction (Primack, 2006). Habitat fragmentation can reduce population size, because patches are smaller than their former contiguous habitat (López and Pfister, 2001). Breeding structure can be altered by fragmentation, as potential mating partners may be separated by unsuitable habitat (Banks et al., 2007). Habitat fragmentation can increase mortality of dispersing animals that traverse unsuitable areas (Aars and Ims, 2000). Fragmented populations may have reduced growth rates and high extinction risk because of inbreeding depression, lowered genetic diversity, and high probabilities of fixing deleterious mutations, relative to pre-fragmentation population structure (Banks et al., 2007; Dudash and Fenster, 2000; Newman and Tallmon, 2001; Saccheri et al., 1998).

The relative role of genetic factors in species extinctions is controversial (Caro and Laurenson, 1994; Frankham et al., 2002; Lande, 1988). 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; Lande, 1988). Small populations have low genetic variability; but this does not

imply that low genetic variability affects extinction risk. However, low genetic variation makes species susceptible to disease and environmental changes (Spielman et al., 2004a; Wilson et al., 2006). Recent investigations have suggested that most threatened taxa have lower genetic diversity than closely related taxa that are not threatened, suggesting reduced reproductive fitness and elevated extinction risks (Spielman et al., 2004b).

Management for the conservation of endangered species like huemul, which live in small populations and in fragmented habitats, may require several manipulative actions. These actions include: 1) the translocation of individuals to aid recovery of small and declining populations; however, this action implies high risk of disease transmission among populations (Cunningham, 1996) and possible loss of locally adapted genes (Edmands and Timmerman, 2003); 2) repopulation of areas in which the species was formerly present, after identifying and removing the causes of local extirpation (e.g., Singer et al., 2000b); and 3) the establishment of corridors to connect isolated populations within patchy landscapes (Gurd et al., 2001; Lindenmayer and Nix, 1993; Mech and Hallett, 2001). Conservation plans for recovering endangered species must also consider measurements of genetic diversity. Modern molecular tools can evaluate a population status, level of isolation, and the success of recovery actions (Frankham et al., 2002; Reed et al., 2002). It is important to detect the cause of decline at its earliest stage, and to monitor fluctuations in effective population size and sex ratio to detect changes in the genetic health of a population (Lippé et al., 2006). During population declines, loss of genetic diversity can be influenced by environmental or demographic stochasticity, or both (Dennis, 2002; Engen et al., 2003; Kendall and Fox, 2002). Disruption of important gene interactions, when the action of one gene is modified by one or several other genes, can negatively affect the expression of a particular characteristics if one of the interacting genes is lost (Hoelzel, 1999; Lippé et al., 2006).

Huemul fit both the small- and declining-population paradigms in Conservation Biology (Caughley, 1994). The small-population paradigm deals with the effect of smallness on population persistence, while the declining- population paradigm deals with the causes of declines (Caughley, 1994). Factors that produce population declines and small sizes are numerous and mostly associated with human interventions, 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 (Corti et al., 2005; Diaz and

Smith-Flueck, 2000; Flueck and Smith-Flueck, 2006; Frid, 1994, 2001; Povilitis, 1983a, 1998; Smith-Flueck and Flueck, 1995), but so far none has been investigated.

Research on the social organization of huemul (Section II.a), together with its mating system (Section II.b), population dynamics (Chapter III), and ranging behaviour (Gill et al., 2008), allows stronger inferences about the consequences of habitat fragmentation for this deer. Huemul territorial males defend a group of females in a specific area and sire most offspring (Section II.b). Because of high site fidelity and no long migration movements (Gill et al., 2008), isolated huemul populations are prone to loose genetic variability.

Here, I present the first evaluation of the genetic variability of a huemul population using microsatellite DNA markers (Kretzmann et al., 2001; Luikart et al., 1998; Mech and Hallett, 2001). Preliminary results using mitochondrial DNA (DNA_{mt}) suggested low variability (Jara, 2005). To understand the impact of population reduction and current habitat fragmentation on genetic variation, I studied one population of huemul isolated by unsuitable habitat (ranch land, human settlements, and steppe). Habitat fragmentation and the isolation of this population began at least 80 years ago when settlers started cutting and burning the southern beech forest (*Nothofagus* spp.) to create cattle pastures (Donoso and Otero, 2005).

Because I examined an isolated population with limited genetic variability, I predicted a high level of inbreeding, reflected by a positive (F_{IS}) value (Weir and Cockerman, 1984). I tested if the huemul population suffered a recent bottleneck due to population reduction and habitat fragmentation. I looked for possible heterozygosity excess as described by Cornuet and Luikart (1996). Populations that have experienced a recent and severe reduction 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 expected at mutation drift equilibrium, because the latter is calculated from the current allele number that have been reduced (Cornuet and Luikart, 1996). Finally, I used simulations to predict future genetic diversity and suggest conservation targets.

Material 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) (69.25 km²), Aysén District, Chilean Patagonia, northeast of the town of Cochrane (ca. 3000 inhabitants). The Reserve was created in 1967 to protect lenga southern beech (*Nothofagus pumilio*) forest and the remaining huemul population that currently uses ca. 20 km² of the southern part of this protected area (Chapter III).

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 until 2004, when additional lands became a wildlife protection area. To the west, there are cattle ranches and the town, and to the 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. 4.1). The population appears isolated, surrounded by unsuitable habitats that prevent connection with other populations. The total population size is estimated at about 120 individuals (Chapter III). Within the Reserve, the greatest number of huemul counted was in November 2006, including 33 individuals older than two years of age, with a sex ratio of 1.54:1 (F:M), seven yearlings and five fawns older tan six months (Chapter III). Although the northern limit is now protected and free of domestic livestock, there is a valley of steppe habitat, running east-west and ca. 5 km wide, that is avoided by huemul (Gill et al., 2008). The nearest population is in the next mountain range north of that valley, at approximately 10 km from the Cochrane Lake population (Fig. 4.1).
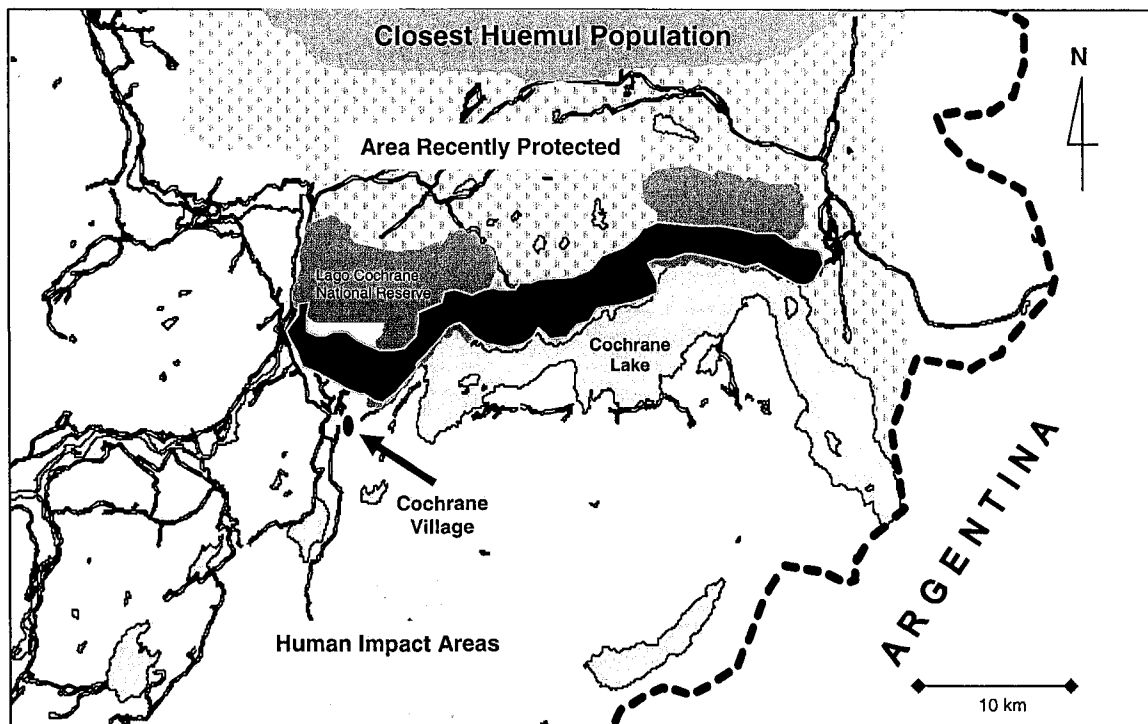
Figure 4.1. Location of the huemul study population at Cochrane Lake, Aysén District, Chilean Patagonia. The dark grey dotted area is used by huemul along the northern shore of Cochrane Lake. The limits of major human impacts (ranching, urban areas with dogs, agricultural areas, logging and exotic tree plantations) are indicated by the left-sided slanted lines. The possible range of the closest huemul population is also shown. The light grey dotted area was recently transformed from cattle and sheep ranching to a protected private area. The grey patches indicate the Lago Cochrane National Reserve and areas within the Chilean government protected areas system. The arrow indicates Cochrane village.

DNA extraction and genotyping

I collected 58 tissue samples from live-captured (n = 55) or dead huemul (n = 3) inside the Reserve between 2005 and 2007. Samples were stored in 70% ethanol until DNA extraction.

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DNA was extracted using the DNeasy™ Blood & Tissue Kit (Qiagen, Inc., Valencia, CA, USA). Because quantities of DNA did not differ significantly (5-50 ng/μl) between samples, I used the initial genomic DNA extraction as a template in all polymerase chain reactions (PCR). Two separate PCRs were conducted. I used a previously optimized cervid multiplex PCR (Anderson et al., 2002), consisting of fourteen primers 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. One primer of the pair was fluorescently labelled to make it distinguishable from the other (fluorescent tags: 6-FAM, TET, PET or HEX), and each set was diluted to an optimal working concentration. The multiplex PCR began with an initial 15 minute denaturation at 95°C, followed by 33 cycles of 30 seconds at 94°C, 90 seconds at 60°C, and 60 seconds at 72°C. The run ended after 30 minutes at 72°C. Twenty-four additional primers were screened individually. The 10 μl PCR reaction contained 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 minute denaturing period at 94°C, followed by 38 cycles of 30 seconds at 94°C, 60 seconds at 49°C, and 60 seconds at 72°C.

The microsatellite amplicons (pieces of DNA from natural or artificial amplification events) were run (co-loaded when possible) on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a GS500LIZ size standard (genetic fragments of known size) (Applied Biosystems). All bands were detected, scored, and manually verified using GENEMAPPER version 4.0 (Applied Biosystems) (Rinehart, 2004).

Genetic analysis

I used GENEPOP version 3.4 (Raymond and Rousset, 1995) to test for Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium for each locus across all individuals. GENEPOP implements a Markov chain process to create an equilibrium distribution of genotype frequencies expected under Hardy-Weinberg Proportions (HWP) for samples with the same allelic counts as the observed data. Following a specified number of iterations, a *P*-value is computed for an exact test of HWP in the population (Guo and Thompson, 1992). Markov chain parameters specified in GENEPOP consisted of

1000 dememorizations, where the initial state of the Markov chain is 'forgotten' (*sensu* Guo and Thompson, 1992), followed by 1000 batches of consecutive observations, with 1000 iterations of each batch. 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). I quantified genetic variability by assessing the number of alleles (A), together with expected (H_E) and observed heterozygosity (H_O). From these data, I calculated Wright's inbreeding coefficient, F_{IS} (= 1- (H_O/H_E)) (Weir and Cockerman, 1984), using GENEPOP and FSTAT version 2.9.3.2 (Goudet, 2000). Positive values of this coefficient suggest that deer mate with closer kin than expected at random; negative values indicate outbreeding and if value is zero mating within the breeding group is considered random (Weir and Cockerman, 1984). Null alleles were identified using CERVUS 3.0.3 (Kalinowski et al., 2007) and loci that contained them were removed from the analyses. Null alleles produce deviations in the HWE in the microsatellite marker, and they occur when a mutation happens in the complementary sequence of the primers impeding the PCR amplification of alleles (Pemberton et al., 1995). Samples were genotyped multiple times to ensure reliability.

Bottleneck tests and simulation of loss of variability

I tested for a population bottleneck using BOTTLENECK version 1.2.0.2 (Cornuet and Luikart, 1996; Piry et al., 1999). This program 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). A Sign test and Wilcoxon signed-rank test were used to test for significant heterozygosity excess (Piry et al., 1999).

To assess the chance of maintaining 90% of observed genetic variation over the next 100 years (as suggested by Frankham et al., 2002), I used BOTTLESIM version 2.6 (Kuo and Janzen, 2003). Using present genetic diversity, BOTTLESIM simulates future population genetic parameters based in the current observed conditions (i.e., OA, the observed number of alleles, as well as H_O) under different bottleneck scenarios. The LCNR and nearby areas along the north shore of Cochrane Lake might contain about 120 individuals (Chapter III); accordingly, genetic diversity estimates over 100 years

were simulated retaining 100, 90, 75, 50, and 25% of the current population. I performed 1000 iterations with constant parameters (lifespan = 15 years, age at maturity = 3 years, completely overlapping generations, random mating, dioecious reproduction, and assumed sex ratio of F:M: 1.5:1).

Results

Genetic diversity

Of 38 microsatellite loci screened (Annexe 4.1), six did not amplify, 16 were monomorphic and 16 were variable and amplified from 56 individuals (Table 4.1). Two samples taken from dead huemul did not amplify. Two loci, BL6 and N, deviated from Hardy-Weinberg Equilibrium (HWE) (BL6 more heterozygotes than expected and N less heterozygotes than expected, $P < 0.001$). Possible null alleles were detected for BL6. 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 ($P > 0.05$) in these 14 loci. The constructed genotype matrix was 98.72% complete with only 20 alleles missing from a total of 1568 (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.

Table 4.1. Summary of sample size (N), allele number (A), expected (H_E) and observed heterozygosity (H_O), and coefficient of inbreeding (F_{IS}) for each locus (calculated in GENEPOP and FSTAT) for huemul from lake Cochrane, Chile. References indicate in which species the primers for each locus were developed.

Locus	N	A	H_E	H_O	F_{IS}	Reference
RT27	55	2	0.071	0.073	-0.028	(Wilson et al., 1997)
Q	56	2	0.419	0.339	0.149	(Jones et al., 2000)
INRA011	56	2	0.473	0.500	-0.063	(Vaiman et al., 1992)
ILSTS011	50	2	0.489	0.540	-0.077	(Brezinsky et al., 1993)
BM203	56	2	0.404	0.411	-0.050	(Bishop et al., 1994)
BM1225	55	2	0.467	0.509	-0.074	(Bishop et al., 1994)
BBJ11	53	2	0.440	0.453	-0.038	(Wilson and Strobeck, 1999)
BBJ2	56	2	0.207	0.196	0.056	(Wilson and Strobeck, 1999)
BL25	56	2	0.164	0.179	-0.086	(Bishop et al., 1994)
BM6438	56	2	0.018	0.018	0.000	(Bishop et al., 1994)
BM6506	56	2	0.260	0.232	0.088	(Bishop et al., 1994)
RT30	56	2	0.369	0.375	-0.027	(Wilson et al., 1997)
RT5	56	3	0.589	0.500	0.177	(Wilson et al., 1997)
RT7	56	2	0.446	0.446	0.017	(Wilson et al., 1997)
Average		2.071	0.344	0.341	0.009 (weighted)	

Bottleneck tests and simulations

Under the three mutation models, 10 loci showed significant heterozygosity excess (Q, INRA011, ILSTS011, BM203, BM1225, BBJ11, BM6506, RT30, RT5, RT7). Overall, both the Sign and Wilcoxon signed-rank tests showed evidence of a recent bottleneck ($P < 0.05$ for both under IAM; $P = 0.06$, $P < 0.05$ for TPM; $P = 0.07$, $P = 0.06$ for SMM), because the heterozygosity excess observed for the 10 out of 14 loci is not expected to occur by chance in a population under mutation-drift equilibrium. Simulated levels of genetic diversity projected with BOTTLESIM showed a continued decrease in genetic diversity in the next 100 years (Fig. 4.2). H_O values decreased faster than observed allele diversity (OA), with a minimum of 75% (90 individuals) of the population required to retain 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. 4.2). To conform to general conservation goals of retaining 90% of the genetic diversity over a 100-year period, the population size would therefore need

to remain at a value above > 90 deer. Simulations were also run with the observed sex ratio of 1:1 (F:M), and the same trend was observed.

a)

b)
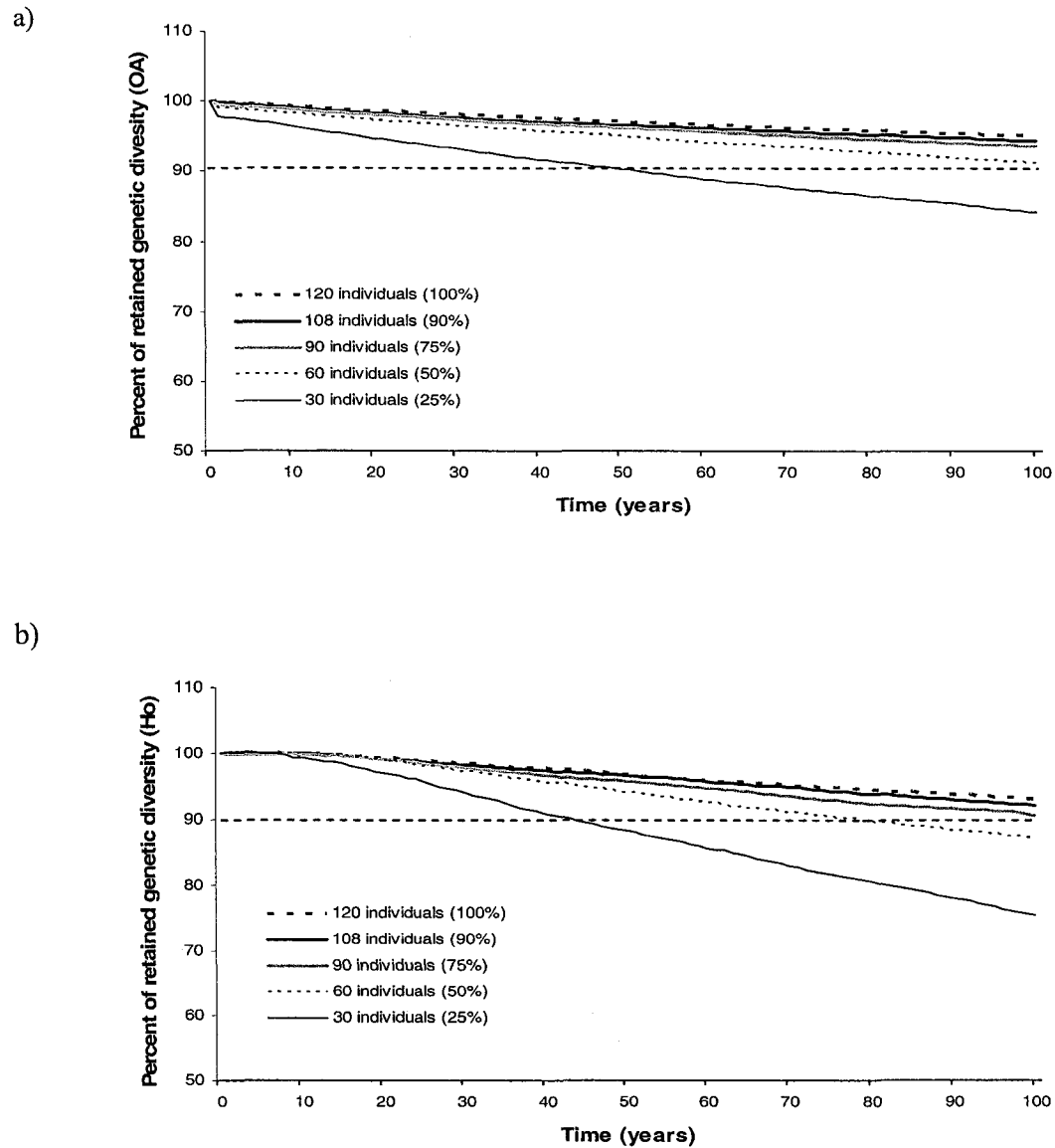
Figure 4.2. Simulated genetic diversity of the huemul population in the Lago Cochrane National Reserve over 100 years using BOTTLESIM. At least 75% (90 individuals) of the current population is required to maintain 90% of current genetic diversity over the next 100 years. Both (a) the observed number of alleles (OA) and (b) the observed heterozygosity (H_O) were projected to decline (sex ratio: 1.5:1 F:M).

Discussion

The huemul population of LCNR showed very low genetic diversity, but still random mating (F_{IS} = 0.009). Although the value of F_{IS} is low, the inbreeding coefficient is probably underestimated as I only took into account variable loci (Frankham et al., 2002). Although it is generally not valid to compare microsatellite variation between species because the loci are usually biased towards polymorphism in the species from which they were developed, compared to other ungulates that also inhabit mountainous environments such as the mountain goat (*Oreamnos americanus*) (Mainguy et al., 2005) and Alpine ibex (*Capra ibex*) (Maudet et al., 2002), huemul appears to be very genetically depauperate. Mountain goats and Alpine ibex have low genetic variability compared to other ungulates (Mainguy et al., 2005; Maudet et al., 2002), but both presented higher mean number of alleles (mountain goat: 3.4; Alpine ibex: 3.16) and higher mean H_E (0.50; 0.40) per locus than the studied huemul population. When compared to the 'inbreeding standard', the cheetah (*Acinonyx jubatus*) (Marker et al., 2008), the huemul has far less variation according to average number of alleles (3.7) and H_E (0.64). Compared to other endangered species with low genetic variability (reviews in Frankham et al., 2002), the huemul has one of the lowest among mammals. Two related Neotropical deer, the pampas deer (*Ozotoceros bezoarticus*) and the marsh deer (*Blastocerus dichotomus*) (Gilbert et al., 2006), also have been screened for genetic variability through microsatellite markers (Cosse et al., 2007; Leite et al., 2007 respectively). Pampas deer showed much higher diversity (mean alleles per locus = 15 and H_O = 0.703) (Cosse et al., 2007) than huemul at LCNR. Marsh deer showed low diversity (mean alleles per locus = 2 and H_O = 0.263); although the determination of genetic variability parameters for marsh deer included several monomorphic loci (Leite et al., 2007)

Eight of the loci amplified in this study for huemul have been amplified in mule deer (*Odocoileus hemionus*), where they show much higher variability (S. Nakada unpublished data). The microsatellites used here were not develop specifically for mule deer, so this fact reduces the bias in my comparison across this two species. The number of alleles was significantly higher in mule deer (mean alleles ± SD: huemul = 2.13 ± 0.35; mule deer = 6.63 ± 2.88; Wilcoxon-signed rank test: Z = 2.555, P = 0.011). These data lends support to my findings indicating low genetic variability of this huemul population.

With the evidence of a recent bottleneck and projected decline in genetic diversity, the F_{IS} value might increase (Dobson and Zinner, 2003; Frankham et al., 2002; Kristensen and Sorensen, 2005). Bottlenecks in Alpine ibex populations have been successfully detected despite a low number of alleles (Maudet et al., 2002). Maudet et al. (2002) also used primers developed for other ungulates, indicating that the Wilcoxon-singed rank test was robust enough to detect a recent bottleneck.

The simulations do not take into account gene flow between populations, but gene flow in this population is likely quite small or non-existent, because it is surrounded by unsuitable habitat and known dispersal movements of huemul are limited (about 5-7 km (Gill et al., 2008)). My results showed a faster decline in H_O than OA, likely as a result of the reduced allelic richness in the huemul. In a similar 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 trends are 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 endangered fish (Lippé et al., 2006). The arctic foxes (*Alopex lagopus*) in Scandinavia have lost approximately 25% of the microsatellite alleles, however, the level of heterozygosity was significantly higher than expected from the simulations indicating that a probably bottleneck occurred due to strong population reduction caused by overhunting in the early 20th century (Nyström et al., 2006). In the other hand, the Kerguelen mouflon (*Ovis aries*) presented an unexpected high variability because of natural selection, despite the founder population was two individuals (Kaeuffer et al., 2007).

As the total huemul population is estimated to be less than 2000 (Flueck and Smith-Flueck, 2006; Vila et al., 2006), this species has suffered a drastic decrease from its historic population size and many local subpopulations are now extirpated (Redford and Eisenberg, 1992). The last glaciation may have affected the genetic structure of the huemul metapopulation when it became isolated in glacial refugia during the Quaternary (Hewitt, 2000). Hence, the possibility exists that the huemul metapopulation was fragmented along the Andes during glacial advances. The arrival of European settlers some 500 years ago slowly began the reduction of the huemul population numbers and size, increasing in speed when human settlement augmented (Lomolino and Channell, 1995). Huemul populations in central Chile, about 1100 km north of my study population, possibly first lost connectivity amongst themselves and were extirpated.

Although the exact date of the bottleneck for the study population is difficult to determine, a large human impact started approximately 80 years ago when the first settlers arrived near the study area, which was rarely used by native South Americans (Diaz et al., 2007). The entire area was used for cattle ranching and forest logging for firewood, until the population was protected in 1967 when the LCNR was created; an apparent gradual increase in the huemul population was observed until it reached its current size (C. Galaz pers. comm.). With the clearing of land for human settlement and cattle ranching, huemul populations became increasingly fragmented, and exposed to livestock-related diseases and poaching (Corti et al., 2005; Flueck and Smith-Flueck, 2006; Povilitis, 1983a; Smith-Flueck and Flueck, 1995). These cumulative effects resulted in a major population decline and are likely partly responsible for the current low genetic diversity.

Fragmentation alters inter-population dynamics in the landscape matrix (Aars and Ims, 2000; López and Pfister, 2001; Namba et al., 1999). Individual movements among the patches of a landscape matrix decrease when fragmentation increases (Hanski and Gilpin, 1997; With et al., 1997). The situation observed at LCNR is likely repeated in the majority of inland huemul populations, that are isolated and fragmented (Flueck and Smith-Flueck, 2006; Vila et al., 2006). As a result, most populations are likely inbred (Banks et al., 2007; Frankham et al., 2002).

The social and mating system of huemul (Chapter II) likely contributes to the reduction of genetic diversity and the increase in inbreeding in isolated populations (Banks et al., 2007). Huemul live in small mixed groups and territorial males defend a few females (Chapter II). Both sexes are highly philopatric, use a small home range of ca. 350 ha, defended by males all year and for several years. Females seemed to prefer to mate with territorial males and a few males appear to sire most fawns (Chapter II), although 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). Of 16 sexually mature males sampled (older than two years) and present during the rutting season of 2005, 2006 and 2007, half sired no offspring, and 44% of fawns were sired by two males (Chapter II).

Some aspects of huemul mating behaviour suggest selection for inbreeding avoidance. For example, some females left their home ranges to mate with neighbouring territorial males. However, mate

selection as a strategy for inbreeding avoidance may not be effective in small and isolated populations where most individuals are related (Dudash and Fenster, 2000). Dispersal is one of the main behavioural strategies to minimize the risk of inbreeding (Pusey and Wolf, 1996; Wahlstrom and Liberg, 1995), but no dispersal or long distance movements were observed in the study population, or in other huemul populations (Gill et al., 2008). Habitat fragmentation has likely forced the observed low genetic variation in the huemul population by decreasing population size and restricting dispersal.

When huemul habitat is fragmented by human activities, populations become isolated, and are pushed into an ecological and evolutionary trap (Schlaepfer et al., 2002). Small groups are likely adaptations to forested and rugged environments (Jarman, 1974) and may be effective to avoid cougar predation, find mates and reproduce. Those adaptations, however, may be no longer helpful when populations become separated by unsuitable habitat. Thus, huemul are 'trapped' by their adaptations to a continuous forested habitat (Schlaepfer et al., 2002), because they do not disperse through large gaps of unsuitable habitat. This 'trap' can lead to local extirpation. The situation worsens if additional environmental and demographic stochasticity is added (Keller and Waller, 2002), as occurred in the study population, where most fawns born in 2005-2007 were males (Chapter III). Populations in western coastal Patagonia, where adverse weather and rugged landscapes limit human colonization (Corti et al., 2005; Frid, 1994), may still remain connected and genetic diversity there is probably higher than in population that suffered fragmentation.

The very limited genetic variability of this huemul population underlines the urgency for genetic screening of other populations in a variety of ecological conditions (Frid, 1994). This assessment will determine the current genetic make-up of huemul populations and whether what was observed at LCNR is a general characteristic of this species. Conservation of huemul should consider its behaviour and evolutionary history to maintain connectivity among populations. Management plans should seek to maintain 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. Although some simulations (e.g. > 75% individuals retained) showed a relatively slow decline, these estimations were based on the current low levels of diversity. Although it is prudent for the LCNR population to maintain its current numbers, we have to take into account that huemul with their historical small population size might have purged their deleterious recessive alleles through natural selection, and may have a low risk of inbreeding depression as observed in ungulates living in islands

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(i.e. Kaeuffer *et al.*, 2007). In addition, conservation of habitat corridors to facilitate gene flow, enlargement of current protected areas or creation of buffer zones to gain more space (Boyd et al., 2008; Brashares et al., 2001; Woodroffe and Ginsberg, 1998), and possible translocations should be addressed to offset inbreeding, while considering the low dispersal and social organization of the species (Chapter II).

Annexe 4.1. The 38 microsatellite loci screened in the huemul (*Hippocamelus bisulcus*). The Table presents the loci names, number of alleles, chromosome location, and original references indicating in which species the primers for each locus were developed.

Loci	Number of Alleles	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)
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 et al., 1994)
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)

CHAPTER V

CONCLUSIONS

Five hundred years ago, huemul deer (*Hippocamelus bisulcus*) were distributed along the Andes cordillera from 34°S to the Strait of Magellan in the rugged mountains and southern beech forests of Chile and Argentina (Cabrera and Yepes, 1960; Redford and Eisenberg, 1992). Huemul numbers and distribution declined rapidly since the arrival of European settlers, and now its population stands at less than 2000 deer. Most of the remaining large populations are in the fjordlands of Chilean coastal Patagonia (Frid, 1994), where habitat conditions do not appear to be optimal because the animals are smaller and have smaller antlers than deer in inland populations (P. Corti, pers. obs.).

The causes of huemul decline have been mainly speculative and based on few data. Some authors attributed the rapid decline to cattle diseases such as foot and mouth, or exotic parasites (Simonetti, 1995; Texera, 1974), or to habitat loss, predation, and poaching (Diaz and Smith-Flueck, 2000; Povilitis, 1998). None of these factors, however, have been studied. The reasons for the continuing decline are unknown. What is clear is that much huemul habitat has been altered or destroyed by human activities. Because the same trend of habitat degradation is continuing, most huemul populations now live in fragmented habitats (Flueck and Smith-Flueck, 2006; Povilitis, 1998).

Currently, this species is the only endangered deer in South America (IUCN, 2008). Despite the fact that it is also the national symbol of Chile, together with the Andean condor (*Vultur gryphus*), most efforts to prevent its extinction have consisted of environmentalist campaigns by non-governmental organizations (NGOs) which lack the scientific knowledge necessary for its conservation and management (Flueck and Smith-Flueck, 2006).

I faced an immense challenge, logistically, politically, and economically, when I began this research project in 2005 by individually tagging several huemul. My efforts allowed me to initiate the first long-term study on the ecology and conservation of huemul. Long-term studies of individually tagged animals are essential to understand the key environmental factors affecting the life history of individuals and their long-term effects on population dynamics (Festa-Bianchet and Côté, 2008). This kind of study has been conducted only on one South American ungulate – the guanaco (*Lama guanicoe*), at Torres del Paine National Park, Chilean Patagonia (Bank et al., 2000; Bank et al., 2002; Franklin et al., 1997; Franklin and Johnson, 1994; Sarno and Franklin, 1999).

My study population is probably representative of the environmental and demographic conditions of several other inland huemul populations. These populations are mostly isolated and fragmented, separated from each other by unsuitable habitat that has been transformed into agricultural lands or monoculture plantations of exotic conifers (Armesto et al., 1994), making huemul vulnerable to poaching and dog predation. My aim was to increase our knowledge about intrinsic and extrinsic factors affecting huemul conservation and recovery, by studying key traits such as social organization and mating system, population dynamics, and genetic variability. Together, these provide baseline scientific knowledge needed for huemul conservation and management.

My analyses suggest that the huemul population at Lago Cochrane National Reserve is stable or slightly declining (Chapter III). The long-term viability of this population is limited without immigration, because the closest neighbouring population is separated by unsuitable steppe habitat (Gill et al., 2008).

The social organization and mating system of huemul, particularly year-long territoriality by males (Chapter II), also make the management and recovery of isolated populations challenging. If all suitable space is occupied by territorial males, territoriality may limit population growth and consequently restrict the potential management strategies to increase population size. Females apparently form stable groups that appear to include related individuals. Any translocation attempts need to consider these characteristics before moving animals across populations. If new animals introduced in areas with current huemul presence were excluded by residents, they may face a higher risk of mortality. In the case of males they might be killed if one of them is introduced in the territory of other male. Ideally, entire social units should be moved in areas formerly inhabited by huemul.

Undetermined factors and predation were the primary causes of mortality in the study population (Chapter III). The high mortality (69%) during the first month of life suggests that predation, especially by culpeo fox, causes the early disappearance of fawns. Further work on mortality is needed, through monitoring and tagging more newborn fawns. Post-weaning mortality is probably related to the lack of suitable habitat for establishment of adults and limited potential for emigration. The possible projected population decline is likely a consequence of small decrease in adult female survival, demographic stochasticity, and artificial subsidies to predators in the study area. Introduced exotic prey have become extremely abundant (e.g., European hare (Jaksic et al., 2002)) and altered the huemul's ecosystem.

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Culpeo fox have almost certainly increased due to the abundance of introduced hares. Predators that depend on another primary and more abundant prey species can cause the extinction of rare prey species through incidental kills (Sinclair et al., 1998). The alteration of the predator-prey system will be difficult if not impossible to reverse, and so alternative management plans are needed. Additional factors that have adversely affected huemul demography likely include habitat loss caused by conversion to cattle and sheep ranching through forest logging and burning, and the large numbers of domestic sheep (ca. 25000 in the neighbouring areas of the huemul population; D. Jara pers. com). In addition, huemul may have evolved with few interactions with humans (Diaz et al., 2007), so their behaviour towards people is naïve, making them easy prey for modern human hunters, similar to what has been observed in island bird populations (e.g., the New Zealand takahe *Porphyrio hochstetteri* (Lettink et al., 2002)).

I suggest that conservation strategies that do not increase the number and size of protected areas with suitable habitats for huemul will have little impact on the species' recovery. Some conservation strategies that may superficially appear beneficial can be detrimental if they ignore huemul ecology. If detailed pedigrees are not maintained, captive breeding can also increase homozygosity through inbreeding and the fixation of deleterious alleles, as well as produce animals that lose necessary survival traits for the wild because of their habituation to humans (Lynch and O'Hely, 2001; Snyder et al., 1996). However, if management is properly performed and all possible precautions are taken, captive breeding and reintroductions should not create inbreeding, nor introduce diseases, and so can be beneficial for a specie's conservation (Soorae and Seddon, 1998). Each captive breeding facility should provide a delimited space for each social unit.

Translocations, for example, can introduce new diseases and swamp the recipient population with the possible loss of local adaptations (Cunningham, 1996; Edmands and Timmerman, 2003). Each potential reintroduction site should be assessed thoroughly for its suitability, including size, habitat types, presence of exotics, and current and future land use. In addition, when reintroducing huemul into area from where they have been extirpated, my results suggest translocating entire female groups rather than individual animals, to mimic the social organization of my study population (Chapter II). An organization structure should be established well in advance for each reintroduction site including a management plan to carefully monitor the population and its surrounding ecosystem after the animals are released into the wild.

Special attention must be given to local socioeconomic situations, community participation, and staff training for management, research, and wildlife protection. A successful example of such a reintroduction program is that of the Przewalski horse *(Equus ferus przewalskii)* which have been released into the wild in Mongolia (Van Dierendonck and De Vries, 1996) and are no longer considered Extinct-in-the-wild but downgraded to Critically Endangered (IUCN, 2008).

Increasing the size of current protected areas and maintaining connectivity among them will help preserve huemul in a larger area by ensuring the survival of emigrants through dispersal corridors (Van Vuren, 1998). Buffer zones around protected areas, created with the participation of local communities, are also essential to ensure huemul survival and their movements through habitat where human activities predominate (Gurd et al., 2001; Reading et al., 1999). Mongolia is an example where protected areas are being enlarged. Almost 11% of that country is protected, but the goal is to place 30% of its territory under protection. For this objective, the Mongolian authorities are creating additional protected areas, expanding existing reserves, increasing the types of protected areas, and creating habitat corridors and buffer zones (Reading et al., 1999).

The isolation my study population (Chapter IV), a social organization apparently leading to low density (Chapter II), the increasing conflict with anthropogenic factors (habitat alteration, dog predation, and poaching), and the presence of exotic species (Chapter III), are common among inland huemul populations. These are crucial factors in this species' survival. In addition, in species with a polygamous system, there is high variance in male reproductive success (Owen-Smith, 1993; Say et al., 2003). This becomes important when populations are reduced in size and when individual territories are maintained throughout the year by males, and there is no immigration. The effect can be to reduce genetic variability if a few males monopolize most females (Chapter II and IV). Due to its influence on genetic variability and population demography, a detailed understanding of the mating system of huemul is essential to formulate effective conservation strategies (Berger, 1996; Hogg, 2000).

The results presented in this dissertation point the way to further important research, to increase our knowledge of the life history and population dynamics of mountain ungulates that inhabit forest environments. My results also generated new questions:

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1) From a behavioural ecology viewpoint, it is necessary to assess if the social organization and mating system of the study population also occurs under different environmental conditions. It is also important to investigate the potential genetic effects of such conditions.

2) For population dynamics, my results show the need for continued monitoring of the study population to provide baseline data for comparison with other populations. The systematic monitoring of tagged animals will allow us to modify and improve recovery strategies rapidly identifying demographic and environmental stochastic events. Similar studies should be initiated in other huemul populations. We also need detailed investigations of the relationships between huemul and its predators and of the impacts on huemul populations of alternate prey populations.

3) The low genetic variability of the study population strongly indicates the need for an extensive genetic survey of other populations, to assess if the observed condition is a normal feature of this species or is an outcome of its current fragmentation.

My research is an important contributed to our knowledge of huemul behaviour, ecology, and conservation. We now have a strong foundation for comparing other huemul populations. We now know the potential factors and mechanisms that promoted the decline of extirpated populations. In addition, the new information on behaviour of huemul, which evolved in a combination of forest and mountainous environments, has contributed to the theory of ungulate biology by bringing new information of how social organization and mating system are shaped in conditions which differ from those largely studied in ungulates of the northern hemisphere.

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