SÉNESCENCE, SÉLECTION SEXUELLE ET DYNAMIQUE DE POPULATION DU BOUQUETIN DES ALPES (*CAPRA IBEX*)

par

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de l'obtention du grade de docteur ès sciences (Ph.D.)

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Le __17 mars 2005____,
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A mi cabrita

und an meine kleine Maus

SOMMAIRE

Le principal objectif de cette thèse est d'étudier les variations intra et inter individuelle pour deux traits phénotypiques reliés à la valeur sélective, et d'analyser la dynamique d'une population sauvage de bouquetin des Alpes (*Capra ibex*). Le premier chapitre analyse la relation de deux traits phénotypiques reliés à la valeur sélective (le gain de masse corporelle, et l'intensité d'infection parasitaire gastro-intestinal) et d'un caractère sexuel secondaire (la croissance des cornes) avec l'âge, des facteurs environnementaux, la survie, et avec l'hétérozygotie, ceci au sein d'un large échantillon de bouquetins des Alpes mâles en libertés et marqués individuellement. A travers la croissance corporelle et les comptages fécaux d'œufs de nématodes gastro-intestinaux, il a été mis en évidence un phénomène de sénescence et de coût pour la reproduction. En analysant un large échantillon de squelettes de bouquetins mâles trouvés morts dans l'hiver pour cause de famine, nous avons trouvé que le début de la sénescence était caractérisé par la longueur de segments de croissance annuels âges spécifiques des cornes, mais pas par leur asymétrie. Enfin, des corrélations entre l'hétérozygotie et la valeur sélective ont été mises en évidence pour la croissance des cornes, mais pas pour la masse corporelle et l'intensité d'infection parasitaire. Le deuxième chapitre traite de l'importance relative de la densité dépendance et de la variabilité stochastique du climat sur la dynamique d'une population d'ongulé de montagne, à travers l'analyse de série temporelle de 45ans de recensements de bouquetins des Alpes collectés dans le Parc National du Grand Paradis en Italie. Pendant les 28 premières années de l'étude, le nombre total de bouquetins a varié entre 2600 et 4000 sans tendance visuelle apparente. Au cours de cette période, il est apparu que des oscillations avec une périodicité de 3 à 8ans étaient présentes. Dès 1982, les comptages de bouquetin ont augmentés régulièrement pour atteindre un pic de 5000 en 1993 et ensuite décroître. Nous avons montré que la taille de population du bouquetin était limitée à la fois par la densité dépendance et par l'épaisseur de neige. Un modèle basé sur ces deux facteurs et ajusté pour les 19 premières années de données, réussi avec succès à prédire l'augmentation et le déclin subséquent de la taille totale de la population pour les 20 dernières années de l'étude. Cette thèse conclue avec un troisième chapitre présentant deux techniques innovatrices utiles pour l'étude de populations d'ongulés en liberté.

SUMMARY

The main objectives of this thesis are to investigate within and between-individual variation in fitness related phenotypic traits and to analyse the population dynamics of a wild population of Alpine ibex (*Capra ibex*). The first chapter analyzes the relationship of two fitness-related phenotypic traits (body mass gain and the intensity of gastrointestinal parasite infection) and one secondary sexual trait (horn growth) with age, environmental factors, survival and heterozygosity in a large sample of individually tagged free-ranging Alpine ibex males. Evidence for senescence and costs of reproduction in body growth and fecal counts of gastrointestinal nematode eggs was found. Analysing a large sample of male ibex skulls, found dead in winter from starvation, we found that the onset of senescence was signaled by the length of age specific yearly horn growth segments, but not by their asymmetry. Finally evidence for heterozygosity-fitness correlations was found for horn growth, but not for body mass and the intensity of parasite infection. The second chapter investigates the relative importance of density dependence and of stochastic climatic variability in the population dynamics of a mountain ungulate, analysing a 45 year long time series of Alpine ibex censuses collected in the Gran Paradiso National Park, Italy During the first 28 years of the study, the total number of ibex ranged from about 2600 to about 4000 with no visually apparent trend. During this period oscillations with periodicities of about 3 and 8 years appeared to be present. From 1982 onwards, ibex counts increased steadily and peaked at almost 5000 in 1993, decreasing afterwards. We show that the ibex population size was limited by both density dependence and deep snow. A model based on these factors fit to the first 19 years of data was sucessfull in forecasting the increase and subsequent decline in total population size over the final 20 years of the study. The thesis concludes with a third chapter presenting two innovative techniques useful in the study of free-ranging populations of ungulates.

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INTRODUCTION

INTRODUCTION

The good, the bad and the ugly

In all good old western movies there is usually a good guy, a bad guy and ...the ugly one. Similarly, individuals in biological populations can have "good genes", "bad genes" and some may even be "ugly", signalling their low quality or condition through the reduced expression of morphological traits. Between-individual variation in phenotypic traits, and therefore in ability to deal with environmental and metabolic challenges, is a prerequisite for natural and sexual selection. The central argument of life history theory is the existence of trade-offs in resource allocation between different fitness components (Stearns, 1992; Roff, 1993). Strong selective pressures in favour of optimal resource allocation strategies during each stage of life is thus predicted. For example, when resources are scarce, bighorn ewes *Ovis canadensis* reduce maternal investment in their lambs in favor of their own mass gain, suggesting a trade-off between current reproductive effort and future reproductive potential (Festa-Bianchet and Jorgenson, 1998).

Evolutionary ecologists are increasingly realizing that parasite resistance is among the main phenotypic traits that can be subject to trade-offs in the life history of individuals. Parasites have the potential to decrease host survival, fecundity and body growth (Festa-Bianchet, 1989; Gulland, 1992; Mulvey *et al.*, 1994) and trade-offs between immune defence and life history traits have been documented in a variety of taxa (Forbes, 1993; Sorci and Clobert, 1995; Agnew *et al.*, 2000). Parasites and hosts are in an evolutionary arms race: the first trying to optimise the exploitation of hosts, the latter evolving to minimise the effects of parasite virulence (Ebert and Hamilton, 1996). Parasites may affect host fitness directly, reducing its lifespan and/or its fecundity, or indirectly through the metabolic costs of maintaining the immune system, which may reduce resource allocation to other life history traits (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000). Altered trade-offs between life history traits may also be an adaptive response of the infected host to minimise the costs of parasitism, rather than a parasite-driven effect (Forbes, 1993; Agnew *et al.*, 2000).

Ungulates and their macroparasites appear to be particularly suitable models to study the life history consequences of parasitism in free-ranging populations (Grenfell, 1992), because the parasites affecting this group are usually well studied and their life cycles well characterised. Furthermore, the temporal variation in fitness components for different sex-age classes in ungulate populations has been quite well quantified through long-term studies on marked individuals (Gaillard *et al.*, 2000). Systematic, long-term studies on the effects of macroparasites on life history traits of free-ranging ungulates, however, remain rare. Demonstrating a direct effect of parasites on survival or fecundity of hosts is not easy. Correlations between parasite loads and fitness components have been shown in organisms as different as reptiles (European common lizards *Lacerta vivipara*: Sorci and Clobert, 1995), birds (pied flycatchers *Ficedula hypoleuca*: Merino and Potti, 1996), and mammals (bighorn sheep *Ovis canadensis*: Festa-Bianchet, 1991; marmots: *Marmota marmota*, Arnold and Lichtenstein, 1993 and *M. flaviventris*, Vanvuren 1996; deer mice *Peromyscus maniculatus*: Fuller and Blaustein, 1996). A correlation between parasite burden and host fitness does not, however, necessarily imply a cause-effect relationship, because it could be due to a third, latent factor influencing both variables (Shipley, 2000). For example, a correlation between parasite load and host survival would arise if some individuals, due to a congenital unfavourable condition, had both reduced survival and reduced immunity, and thus higher parasite burdens.

Only few studies have investigated the effects of endoparasites on fitness components of free-ranging mammals experimentally. Munger *et al.* (1991) removed bot fly larvae *Cuterebra angustifrons* from white-footed mice *Peromyscus leucopus* in one population, and experimentally infected mice with tapeworms *Hymenolepis citelli* in another population, but could not find any effect on survival or fecundity. Samson *et al.* (1987) did not find any difference in survival between bighorn lambs experimentally infected with larvae of *Protostrongylus* spp. and controls. The experimental infection of hosts used in these two studies, however, may provide unreliable results, because the number of larvae to which the treated animals were exposed may have been not sufficient to cause deleterious effects. Murray *et al.* (1997; 1998) tested experimentally if parasitism and nutritional status interacted to affect susceptibility to predators (and thus survival) and reproduction in snowshoe hares *Lepus americanus.* They showed that hares treated with antihelmintic drugs had higher survival than untreated controls when food was limited (Murray *et al.*, 1997). Gastrointestinal nematodes, however, appeared not to affect directly the host's body mass, fat storage and reproduction (Murray *et al.*, 1998). The only published large-scale parasite manipulation on a free living ungulate has been conducted on a feral population of domestic sheep *Ovis aries* on the Island of St. Kilda, Scotland (Gulland, 1992; Gulland and Fox, 1992). In that study, antihelmintic treatment appeared to improve survival of ewes and male lambs (female lambs were not treated) but not of adult rams (Gulland, 1992).

Another phenotypic trait important for the life history strategy of individuals, is body mass. Body mass appears to be a key factor in the life history of mammals in general and of large temperate herbivores in particular (Festa-Bianchet *et al.*, 1997; Réale and Festa-Bianchet, 2000). Small mass has been associated with decreased juvenile survival (Festa-Bianchet *et al.*, 1997; Loison *et al.*, 1999) and decreased fertility (Svendsen and White, 1997). In most studies to date, unfavorable weather (Gaillard *et al.*, 1996; Post *et al.*, 1997; Post and Stenseth, 1999), and density-dependent depletion of resources (Leberg and Smith, 1993; Vincent *et al.*, 1995) have been considered responsible for growth reductions in ungulates. A possible impact of parasites on host body mass in ungulates has only been inferred from correlations between parasite load and host body weight or through the experimental infection of captive individuals, with partially contradictory results. For example, Segonds-Pichon *et al.* (1998), found a negative correlation between faecal egg counts (an index of gastrointestinal parasite load) and body mass in male and female roe deer *Capreolus capreolus* fawns. On the contrary, Mulvey *et al.* (1994), found a greater mean body weight for adult females infected with liver flukes *Fascicoloides magna,* than for uninfected individuals, in harvested white-tailed deer *Odocoileus virginianus*. Captive moose calves *Alces alces*, experimentally infected with winter ticks *Dermacentor albipictus,* accumulated less mass than untreated controls, and the effect appeared to increase with tick infestation intensity (Addison *et al.*, 1994). Weight gains were also lower in untreated semicaptive reindeer *Rangifer tarandus* calves compared with calves treated with antihelmintic drugs (Arneberg and Folstad, 1999). Because variations in body mass may have important consequences for individual fitness in northern ungulates, a better understanding of the factors affecting within and between variabilty in this trait, is extremely important.

A great phenotypic variabilty is particularly to be expected in the expression of secondary sexual ornaments, such as horns and antlers in ungulates, as these traits are particularly costly to maintain and their growth is condition-dependent (Anderson, 1994). According to the "good genes" hypothesis, as the expression of secondary sexual traits is costly in terms of fitness for males (it is a handicap), it is used by females to assess their potential partner's viability, or by contestants to evaluate their opponent's fighting ability, driving the evolution of exaggerated secondary sexual ornaments (Zahavi, 1975). Antler size in red deer *Cervus elaphus* appears to be associated with sperm quantity and quality (Malo *et al.* 2005) as well as with lifetime reproductive success (Kruuk *et al.* 2002). In mature bighorn sheep *Ovis canadensis* rams, seasonal reproductive success increased with horn size (Coltman *et al.* 2002). Again, the "good guys" with "good genes", and thus able to afford the costs of growing and maintaining elaborate displays, win at the end of the story (and reproduce) while the bad and ugly ones don't. As secondary sexual traits are condition dependent and costly to maintain, they can be considered a form of reproductive effort (Nur & Hasson, 1984). A trade-off between life history traits and secondary sexual characters can therefore be expected (Gustafsson *et al.* 1995). For example, Gustafsson *et al.* (1995) found a reduction in the size of a secondary sexual character (the male's forehead patch) in the year following increased reproductive effort in male collared flycatchers *Ficedula albicollis.*

Both naturally and sexually selected phenotypic traits, typically undergo senescence, a process of deterioration with age resulting in an increased mortality rate (Comfort, 1964, Kirkwood & Austad, 2000). Three main hypothesis have been proposed to explain the evolution of senescence: a) the mutation-accumulation hypothesis (Medawar, 1952) attributes senescence to the accumulation of deleterious mutations, with effects on fitness acting at later ages; b) the antagonistic pleiotropy hypothesis (Williams, 1957; Hamilton, 1966) assumes the existence of pleiotropic alleles with positive effects on fitness early in life but bearing fitness costs at later ages; c) the disposable soma hypothesis (Kirkwood & Rose, 1991) assumes an optimisation of resource allocation in which repair of somatic damage is traded against current reproductive investment. Life history theory predicts that as they age individuals should reduce the allocation of resources to bodily repair and maintenance in favour of reproduction, leading to an acceleration of ageing (Cichoń, 1997, 2001). Sexually selected traits, that regrow each year or that grow through life shoud be sensible indicators of the onset of senescence. For example, it has recently been proposed that disposable soma senescence in molariform teeth is mediated by sexual selection in red deer (Carranza *et al.* 2004). Senescence is to be expected to also strongly affect immune defense, as the immune system is costly to mantain (Saino *et al.* 2003; Cichoń *et al.* 2003).

Both the "good genes" hypothesis of sexual selection and life history theory assume individual variability in the ability to cope with various fitness costs. Some of this between-individual variability in the expression of phenotypic traits is assumed to be due to heritable differences in genetic quality among individuals. Evidence of heritable genetic variation in phenotypic traits in free ranging populations is growing in the scientific literature (Kruuk *et al.* 2000), as is the evidence for direct associations between individual genetic variation (usually expressed as multilocus heterozygosity of selectively neutral microsatellites) and fitness-related traits (Coltman and Slate, 2003). Heterozygosity-fitness correlations were usually interpreted as evidence of inbreeding depression (Pemberton, 2004). Two recent papers (Balloux *et al.* 2004; Slate *et al.* 2004) however, challenge this view suggesting instead that these correlations are likely due to associative overdominance with loci directly affecting fitness. Both hypotheses predict genetic effects on variability in phenotypic traits. The quantification of the contribution of genetic effects, and their effect on the evolution of fitness related traits is a promising topic which researchers are only starting to explore.

Up to now I have considered individuals, the different processes by which individuals optimize their life history strategies and the intrinsic and extrinsic factors affecting the expression of phenotypic traits. Individuals, however, constitute populations, and the intrinsic and extrinsic factors affecting individual life histories have strong effects on population dynamics. Variation in life history is a key factor in the dynamics of populations. Density dependence and environmental stochasticity play an important role in shaping both individual life history strategies and the dynamics of free-ranging populations. For example, high density usually causes increased mortality in juvenile and yearling ungulates, reducing recruitment and thus driving the dynamics of populations (Gaillard *et al.* 2000). Inter-annual variations in weather conditions have also been shown to affect population dynamics in a variety of taxa (Saether *et al.* 2004; Garel *et al.* 2004, Schwartz & Armitage, 2005). In many ungulates, high yearly variability in juvenile survival appears to play a predominant role in population dynamics, regardless of whether the major causes of mortality are density-dependent or due to environmental stochasticity (Gaillard *et al.* 1998).

Outline of the dissertation

The main goal of this thesis is the analysis of within and between-individual variation in fitness related phenotypic traits (body mass and fecal egg counts of gastrointestinal parasites) and a secondary sexual trait (horn growth) in Alpine ibex (*Capra ibex*) males. From the individual level I will move to the population level, analysing, in the second part of this dissertation, the dynamics of the Alpine ibex population in the Gran Paradiso National Park, Italy. The thesis is divided into three chapters. Each chapter is composed of papers ready to be submitted or published in international peer-reviewed journals, introduced by remarks on their theoretical and empirical background to explain how they fit within the general framework of the thesis. For co-authored papers, my contribution in the realisation of each paper is specified.

The first chapter analyses the relationship of two fitness-related phenotypic traits (body mass gain and the intensity of gastrointestinal parasite infection) and one secondary sexual trait (horn growth) with age, environmental factors, survival and heterozygosity in Alpine ibex. In **section Ia** I explore within and between-individual variation in body mass and fecal counts of gastrointestinal parasites (an index of parasite infection and resistance). The main goal of this section is to test if in Alpine ibex males the costs of reproduction lead to a reduction in immune defences and if changes in body mass and fecal egg counts suggest senescence with increasing age. Life history theory predicts that in large mammals with high adult survival and polygynous mating systems where male-male competition for oestrous females is high, such as Alpine ibex, males should mature after they reach asymptotic weight (Stearns, 1992). In Alpine ibex the attainment of asymptotic weight appears to be particularly late: males continue to increase in body weight until 12 years of age (See **Section Ia** and Giacometti *et al.*, 1997). Assuming that male reproduction does not peak until asymptotic body mass is reached, the observed pattern suggests that males should limit any risk-taking until they are about 9-10 years old. By comparing individuals treated with anthelmintic drugs with untreated controls, I also tested the hypothesis of a direct effect of gastrointestinal parasites on weight gain during summer. Parasites can negatively affect the body condition of their host and thus may constrain the host's ability to use available resources for growth or reproduction (Sorci *et al.*, 1996; Møller, 1997). As a consequence, the host's optimal resource allocation - the one which maximizes its fitness - between life history traits may be altered in the presence of parasites.

The main goal of **section Ib** is to test if the size and asymmetry of age-specific yearly horn growth segments were correlated with annual survival probability. If horn growth or asymmetry could predict the onset of senescence in Alpine ibex males, they could be indicators of male quality. I also tested the relative importance of size and asymmetry as predictors of individual survival. The horns of Alpine ibex males are a secondary sexual ornament that is likely under strong sexual selection because of its use in male-male competition. Ibex horns grow considerably during the entire life of the individual (up to 110 cm in length) and are presumably costly to produce. Each year a new horn segment is grown, and growth stops in winter, forming a very distinctive ring around the horn. Therefore age- and year-specific growth rates can be measured and compared. Alpine ibex horns appear to be ideal models to study the evolution of exaggerated sexual ornaments, and to analyze the effects of life-history trade-offs and senescence on secondary sexual traits. **Section Ic** analyses the effects of individual heterozygosity on all traits previously analyzed in Section **Ia** and **Ib** in Alpine ibex. Specifically, I predicted a strong effect of heterozygosity on the size of yearly horn growth segments, in accordance with the "good genes" hypothesis of the evolution of secondary sexual ornaments. Heterozygosity-fitness correlations were predicted as well for body mass gain and for parasite resistance and intensity. Associations between locus-specific genotypes and macroparasite burdens was shown in Soay sheep (Gulland *et al.*, 1993; Paterson *et al.*, 1998). In the same population, sheep with low individual heterozygosity (assessed by microsatellite variation) suffered from high susceptibility to gastrointestinal nematode parasites, particularly at high population density (Coltman *et al.*, 1999). In years when the population crashed, over-winter mortality was higher for parasitized and also for relatively homozygous sheep than for non-parasitized and heterozygous individuals. Mortality was independent of heterozygosity for individuals treated with anthelmintic drugs, indicating that higher susceptibility to parasites was the direct cause of mortality of homozygous sheep. Parasite-mediated selection in favour of genetic variation should be particularly strong at the major histocompatibility complex (MHC). The MHC is a multigene family, encoding receptors involved in antigen presentation to the immune system, that appears to be directly responsible for parasite resistance (Amills *et al.*, 1998; Edwards and Hedrick, 1998; Paterson *et al.*, 1998). Among the loci analysed in **Section IIIc** we specifically test one microsatellite locus (OMHC1) known to be situated in proximity of the major histocompatibility complex and therefore in physically linkage with MHC loci directly involved in parasite resistance. The Soay sheep studies were conducted in an insular population of feral domestic ungulates. More research is needed to determine if those results apply to wild populations of large mammals. The potential link between low heterozygosity and susceptibility to infectious disease is also of great conservation concern. A better understanding of this interaction is thus particularly important for small populations of endangered species. It is important as well for those species – such as Alpine ibex - that underwent through recent demographic bottlenecks and thus may have low levels of genetic variation.

In the second chapter the population dynamics of Alpine ibex in the Gran Paradiso National Park are analysed thanks to an exceptionally long series of census data collected over more than 40 years by the Park wardens. **Section IIa** explores the correlation between the spring and the summer censuses conducted each year by the park wardens. The second goal of this section is to verify, with power spectrum analysis, the previously suggested hypothesis of recurrent cycles in the population dynamics of alpine ibex.

The main goal of **section IIb** is to test the relative importance of density-dependence and climatic factors on ibex population dynamics. Using an out-of-sample prediction test we assess the long-term predictive power of a simple model incorporating snow depth and population density. A second goal of **section IIb** is to test if changes in ibex population dynamics were due to variations in recruitment or in adult survival.

The third chapter presents two novel techniques, developed during this Ph.D., useful in the study of the ecology of large herbivores. The first technique, presented in **section IIIa,** is a method to weigh free-ranging ungulates without handling using a remotely controlled platform scale on which the animals step voluntary to lick salt. The technique allowed me to obtain repeated weights of the same individuals over the summer and to estimate summer body mass gain, a trait likely correlated with individual fitness in ungulates. I used this technique to measure body mass and to study its variation within and between individuals in two of the papers presented in Chapter I. The second paper, in **section IIIb**, analyses the usefulness of faeces as a source of DNA for microsatellite analysis in mountain ungulates. It shows that faeces collected in winter are most suitable for DNA extraction. The development of indirect non-invasive methods for the collection of DNA is of growing interest as molecular tools are increasingly used by behavioural ecologists. The use of noninvasive DNA sampling methods may become particularly important in the study of endangered species for which live capture is too risky or too difficult. Even thought it was not necessary to use DNA extracted from faeces for the study presented in section Ic, the technique presented in **section IIIb** could prove to be extremely useful in further development of this research, for genotyping kids and yearlings (which are extremely difficult to capture) possibly eventually allowing a study on paternity and the direct measurement of male reproductive success.

The thesis ends with a general conclusion, summarising the major results of this research, discussing the implications these results have for the conservation and management of Alpine ibex, discussing how these results fit within a wider framework, and suggesting future directions for research. In **annex I**, I present a paper not directly connected to the main topics of this thesis. The paper tests the hypothesis of a direct effect of incisor-arcade size on body mass gain and survival in Bighorn sheep *Ovis canadensis*. Contrary to the hypothesis of Illius *et al.* (1995), we show that variation in incisor-arcade size is due to individual variation in body size and age rather than to directional selection.

CHAPTER I

Variation in fitness-related traits, sexual selection and senescence

SECTION IA

Body mass and nematode fecal egg counts in male Alpine ibex (*Capra ibex*) : evidence for senescence and costs of reproduction

Achaz von Hardenberg, Bruno Bassano, Francesca Cattaneo and Marco Festa-Bianchet

Body mass and parasite resistance against gastrointestinal nematodes are important fitness related phenotypic traits in large herbivores. Life history theory predicts a trade-off in allocation of resources between body growth and reproductive effort. Individuals should decrease investment in growth in favour of current reproduction after reaching reproductive maturity. Relative allocation to parasite resistance should decrease with age, as more resources are directed to reproduction. A reduction in investment in immunity, however, may accelerate senescence. This paper investigates evidence for senescence and costs of reproduction in body growth and fecal egg counts in male Alpine ibex. I was responsible for all phases of the study. I partecipated to all capture and marking operations, organized data collection and collected myself a substantial proportion of the fecal samples and of body mass data. Fecal egg counts were done mostly by F.C. and by assistants, but I also helped in the lab. I performed all statistical analyses, litterature review, planned which hypotheses to test, and wrote the manuscript.

Body mass and nematode fecal egg counts in male Alpine ibex (*Capra ibex*) : evidence for senescence and costs of reproduction

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Abstract

The relationship between age, body mass, and fecal egg counts (FEC) of gastrointestinal nematodes was studied in Alpine ibex (*Capra ibex*) in the Gran Paradiso National Park (North-western Italian Alps). FEC analyses were performed on fresh faeces collected every two weeks over five summers (2000 - 2004) from individually marked males. Males were repeatedly weighed from June to September each year using an electronic platform scale baited with salt. Individual weights were adjusted to August 1 of each year. Counts of parasite eggs in the faeces varied little during summer. Between-individual variability in FEC was higher than within-individual variability in all five years of study suggesting individual differences in parasite resistance, possibly of genetic origin. Body mass increased with age peaking at 12 years, suggesting late maturity in this population. The number of nematode eggs in faeces also increased with ibex age but appeared to peak after 12 year of age. Reduced parasite resistance may be a cost of reproduction for older males or a sign of senescence. A direct effect of parasite counts on summer weight gain was not apparent. Eighteen males were treated with a single dose of the antiparasite drug Ivomec in early summer. This treatment was sufficient to maintain faecal egg counts significantly lower than in untreated males until September. There was no difference in FEC one year later between treated and control individuals. This result suggests a low rate of reinfection during summer, possibly due to unfavourable environmental conditions for nematode larvae at high altitude.

Key words: Fecal egg counts, nematodes, ibex, body mass, senescence, cost of reproduction

Introduction

Body mass is a key factor in the life history of ungulates (Festa-Bianchet *et al.*, 1997; Reale and Festa-Bianchet, 2000). Small mass has been associated with lower juvenile survival (Festa-Bianchet *et al.*, 1997; Loison *et al.*, 1999) and decreased fertility (Svendsen and White, 1997). In most studies to date, changes in individual body mass for ungulates have been attributed to weather (Post and Stenseth, 1999; Post *et al.*, 1997) and density-dependence (Leberg and Smith, 1993; Vincent *et al.*, 1995). Parasites may also negatively affect the body condition of their host and thus may constrain, possibly in interaction with other factors such as density, the host's available resources (Sorci *et al.*, 1996; Møller, 1997). Parasite infection may lead to a reallocation of resources among competing requirements such as growth, reproduction or immunity (Lochmiller and Deerenberg, 2000; Sheldon and Verhulst, 1996). In other cases, reallocation of resources to the immune system could lead to changes in infection. For example, Festa-Bianchet (1989) showed that lactating bighorn ewes had greater lungworm fecal egg counts than non-lactating ewes, suggesting reduced investment into immunity, in favour of current reproduction. A trade-off between current reproductive effort and immunity, however, has never been reported for male ungulates.

Murray *et al.* (1997; 1998) tested experimentally if parasitism and nutritional status interacted to affect susceptibility to predators (and thus survival) and reproduction in snowshoe hares *Lepus americanus.* When food was limited, hares treated with anthelmintic drugs had higher survival than untreated controls (Murray *et al.*, 1997). Treatment, however, did not affect mass, fat storage or reproduction (Murray *et al.*, 1998). Investigations of the possible impact of parasites on host body mass in ungulates have provided somewhat contradictory results. Segonds-Pichon *et al.* (1998), found a negative correlation between faecal egg counts of gastrointestinal parasites and body mass in roe deer *Capreolus capreolus* fawns. On the contrary, Mulvey *et al.* (1994) found a greater mean body weight for adult female white-tailed deer *Odocoileus virginianus*, infected with liver flukes *Fascicoloides magna,* than for uninfected individuals. Captive moose *Alces alces* calves, experimentally infected with winter ticks *Dermacentor albipictus,* accumulated less mass than untreated controls, and the effect appeared to increase with the intensity of tick infestation (Addison *et al.*, 1994). Weight gains were also lower in semicaptive untreated reindeer *Rangifer tarandus* calves compared with calves treated with anthelmintic drugs (Arneberg and Folstad, 1999). Because variations in body mass may have important consequences for individual fitness in temperate ungulates, a better understanding of the potential impact of parasites on the weight of their hosts is extremely important. Here we investigate the relationship between age, summer weight gain, and gastrointestinal helmintic parasitism, in free-ranging, individually tagged male Alpine ibex (*Capra ibex*) in the Gran Paradiso National Park (North-western Italian Alps). The objective of this study was to test the hypothesis that in male Alpine ibex there is a trade off between current reproduction and immune defence. We expected that the intensitiy of gastrointestinal parasitism, measured by fecal egg counts, would increase with age, as we expect that males would be more active in reproduction as they age. By comparing individuals treated with antihelmintic drugs with untreated controls, we also tested the hypothesis of a direct effect of gastrointestinal parasites on weight gain during summer, and explored the patterns of infection during summer in the alpine environment.

Methods

Study area and population

The study was conducted in the Levionaz basin, Valsavaranche valley, Gran Paradiso National Park (GPNP; North-western Italian Alps; 45° 25' N, 07° 34' W). Most of the study area is above the tree line, between 2300 and 3500 m a.s.l. and is characterised by high-altitude alpine meadows (mainly *Festuca varia*), moraines, rock cliffs and glaciers. The study area is used by Alpine ibex from late May to December. The winter range (elevation 1700 to 2300 m a.s.l.) is characterised by pastures near villages and forest of *Larix decidua, Picea abies* and *Pinus cembra*. During this study about 200 adult ibex were counted in the study area. Alpine chamois *Rupicapra rupicapra* is the only other mountain ungulate found in the study area.

Capture and marking

Alpine ibex males aged 3-16 years were captured with a Dan-Inject® dart-gun (9 between 1992 and 1998, 7 in 1999, 11 in 2000, 27 in 2001, 15 in 2002 , 9 in 2003 and 5 in 2004). A mixture of xylazine HCl (Rompun®, 20-40 mg), a muscle relaxant, and ketamine (Inoketam®, 50-100 mg), a sedative, was used following Peracino and Bassano (1988; 1990). Age of captured ibex could be determined with precision by counting the annual horn growth rings (annuli) (Ratti and Habermehl, 1977). Animals were released after completing the marking, sampling and morphometric measurement operations, in the same place were they were captured. As recommended by Bassano and Peracino (1995) before releasing them, the captured animals were injected with Atipemazol, an antidote, which acts as an antagonist of ketamine, reducing the risks of hypothermia and accelerating recovery. Sedated individuals were marked uniquely with different combinations of coloured ear-tags (Allflex®) or with differently coloured radio-collars. The entire operation, from capture to release usually took not more than half an hour. Two of 83 captured males died during capture operations, similar to the low capture mortality rate (2%) reported for ibex in the Gran Paradiso National Park area from 1985 to 2004 (Bassano *et al.* 2004).

Anthelmintic treatment

Eighteen males aged 3-7 years were treated with 0.8-1.2 ml of injectable antihelmintic drug (Ivomec®) at capture in May-June in 2001, 2002 and 2003. We injected 0.02 ml of Ivomec® per kg of weight of the captured individual as suggested by the producer. Summer weight gain and FEC of treated individuals were compared with untreated controls of the same age.

Body mass data measurements

Individual males were repeatedly weighed from June to September each year with an electronic platform scale baited with salt (Bassano *et al.* 2003). Most (83%) body mass measurements were collected from June to early August, when mass gain appeared linear when plotted against date. After the beginning of August ibex appeared to be much less interested in the salt offered at the scale making body mass measurements rare. We therefore adjusted body mass to the 1st of August (mid summer). The strong linearity of growth in body mass was confirmed by fitting linear regression lines to those individuals with at least four repeated mass measurements during the same summer (n=26), with mass as the dependent variable and date (considering the 1st of May as day 1) as the independent variable. Regression coefficients were extremely high (mean $r^2= 0.963\pm0.015$). To adjust individual weights to August 1 (day 92), we fitted linear mixed effects models by restricted maximum likelihood, implemented in the NLME package of S-PLUS 2000 (Insightful Corp.; Pinhero & Bates, 2001). Separate models were fitted for each year of the study. We specified the fixed effects as fixed = Weight ~I(Day number - 92) and the random effects and grouping structure as random = ~I(Day number - 92|ID, where ID is the identification code for each tagged individual. The formula I(Day number - 92) specifies that data were centered at day 92. Predicted weights and slopes (growth rates) were then extracted using the coef.lme function. The predicted weights and slopes are empirical Bayes estimates.

Fecal egg counts

Faecal egg counts (FEC) can be considered an index of the combined effects of intensity of Trichostrongyle parasitism and parasite resistance influencing parasite fecundity (Coltman *et al.* 1999). FEC were estimated twice a month from all individually tagged males in Levionaz in 2000-2004 (n= 228 ibex-years). Animals were observed in the field from a distance of 10-50 meters and faeces were collected within 1-5 minutes of defecation. When possible, at least 20 grams were collected for each individual. Faecal pellets were kept in plastic bags at 4°C prior to analysis. Faecal egg counts (FEC) were done following a modified McMaster technique (Ministry of Agriculture, Fisheries and Food, 1971) and were expressed as number of eggs per gram of fresh faeces (EPG). At least two counts per faecal sample were done in order to account for measurement error. The number of helminth eggs was determined to the nearest 20 eggs/gram of faeces.

Data analysis

We followed Lessels & Boag (1987) to estimate repeatability of measurements with unbalanced group sizes. Within and between-year repeatability was estimated excluding from the sample those individuals for which we had FEC measurements only in one year.

To account for repeated measurements of the same individuals in different years, we fitted linear mixed effects models (LME) implemented in the NLME package of S-PLUS 2000 (Pinhero and Bates 2001). The LME function in S-Plus also allows one to fit models with heteroscedastic within-group errors. We fitted LME models with the appropriate variance function whenever the within-group errors appeared to have unequal variances. We followed the model building approach suggested by Pinhero and Bates (2001) for all fitted LME models. The significance of fixed terms was assessed using conditional F tests while for LME models with different random terms, we chose the model with the lowest Akaike information criterion (AIC) in accordance with Pinhero and Bates (2000). To normalize the error terms, we transformed the FEC data in Ln(EPG +1) prior to all analyses. Treatment contrasts were used to asses differences within factors. Age was centered at 8 years to reduce multicollinearity whenever a quadratic term was included. Maximum values in quadratic growth functions were determined with equations 20.9 and 20.10 in Zar (1998).

Results

Within season repeatability in fecal egg counts

Variability in FEC from June to September in untreated ibex, was greater between individuals than within individuals in all years of the study (Table 1). Within individual repeatability of FEC measurements varied among years (Table 1).

Table 1. Repeatability of FEC measurements for individual adult ibex males from June to September in the Levionaz basin, Gran Paradiso National Park, Italy. *r* refers to repeatability estimated following Lessels and Boags, (1987).

Year	***Df***	***F***	***P***	***r***
2000	18, 88	2.247	0.0067	0.182
2001	36, 213	4.406	≤ 0.0001	0.366
2002	48, 247	4.372	≤ 0.0001	0.359
2003	42, 90	1.999	0.0031	0.245
2004	49, 216	5.754	≤ 0.0001	0.468

Within-individual repeatability of fecal egg counts and body mass across years

We found significant variation in mean yearly FECs (2000-2004) between untreated individuals, after accounting for age differences (ANCOVA: $F_{59,144} = 3.053$, $P \leq 0.0001$; including only untreated individuals). Repeatability of mean yearly FECs was comparable to the average within-year repeatability (r = 0.375). Variability between individuals was larger than within individuals also for body mass estimated on the 1st of August in each year, accounting for age differences (ANCOVA: $F_{47,92} = 28.40$, $P \leq 0.0001$) and for summer mass gain rate (ANCOVA: $F_{47,93} = 2.18$, $P = 0.0007$); Repeatability of individual body mass from one year to the next, after accounting for age differences, was high (r = 0.903) but was low for summer mass gain rate (r =0.268).

Variability in fecal egg counts

Visual inspection of individual plots of ln(EPG +1) *vs.* date suggested no evident trend in FECs from June to September in any year of the study. This result was by confirmed fitting a LME model, with Ln(Epg +1) as the dependent variable, Date, Age and Year as independent variables, ID as a grouping factor and a general positive-definite within-group error structure. The best fitting model had a random intercept and a random slope for Age ($AIC = 2563.8$ for the model with a random slope for Age *vs.* $AIC = 2577.1$ for a model with a random intercept only; Log-likelihood ratio = 17.89, $P = 0.0001$). Only Age and Year entered the model, while Date was rejected (Table 2). Based on these results, we used the arithmetic mean of FEC measured for each individual from June to September as a yearly index of individual parasite infection in all further analyses. There was a significant increase in FEC from 12 years of age onwards (Fig. 1). Fitting a LME model, we found a positive correlation between mean yearly FEC from the same males in successive years (Fig. 2) (β =0.41). This relationship remained significant after accounting for Age and Year of sampling (Table 3). Age had a positive effect on Ln(EPG+1) in the following year (β = 0.08; Table 3).

Table 2. Wald test for the significance of terms included in a LME model with fecal output of parasite eggs (Ln(EPG +1)) of Alpine ibex as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and Year, Age and Date as independent variables, Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004.

	Df	***F***	***P***
Terms included in the model:			
Intercept	1, 980	10874.60	≤0.0001
Year	4, 980	28.64	≤0.0001
Age	1, 980	25.75	≤0.0001
Rejected terms:			
Date	1, 980	0.63	0.4279

Table 3. Wald test for the significance of terms included in a LME model with mean yearly fecal output of parasite eggs (Ln(EPG +1)) in year 2 as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and Year, Age and Ln (EPG + 1) in year 1 as independent variables, in Alpine ibex in Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004.

	Df	***F***	***P***
Terms included in the model:			
Intercept	1, 67	18739.97	$\leq$0.0001
Year	3, 67	9.35	$\leq$0.0001
Age	1, 67	65.43	0.0004
Ln(EPG + 1) year 1	1, 67	17.45	$\leq$0.0001

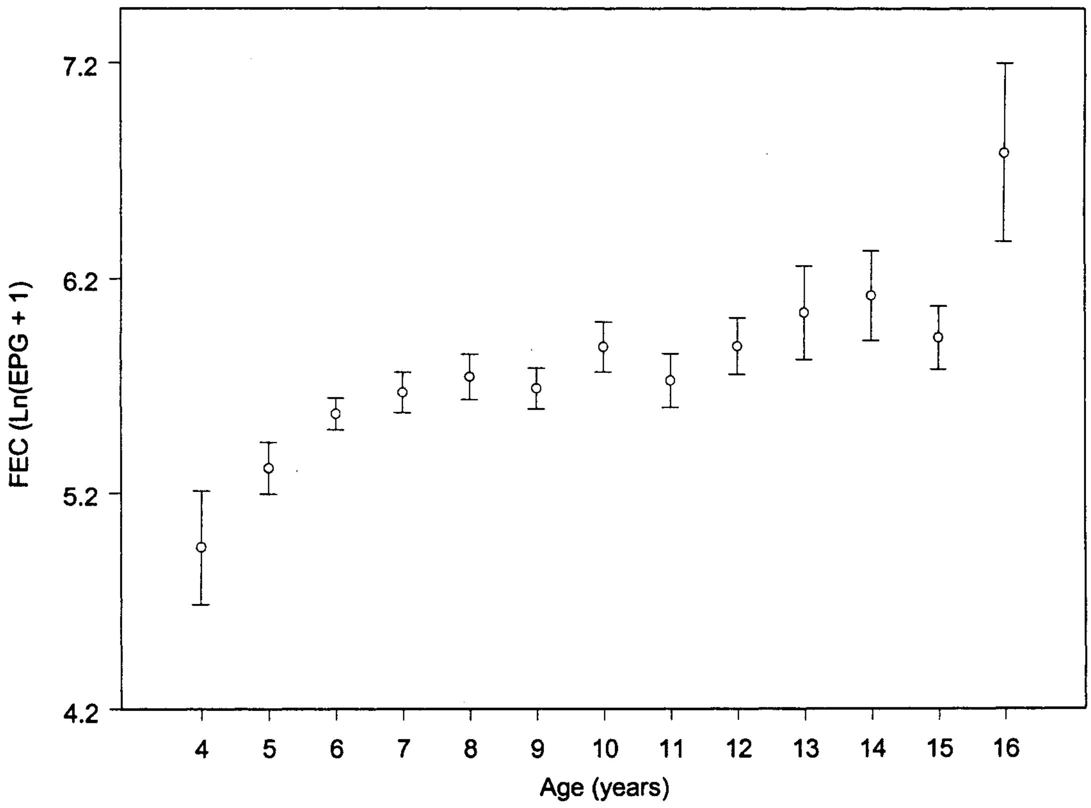

Figure 1. Age and fecal egg counts of nematode parasites (ln(EPG +1)) in Alpine ibex males in Levionaz 2000-2004

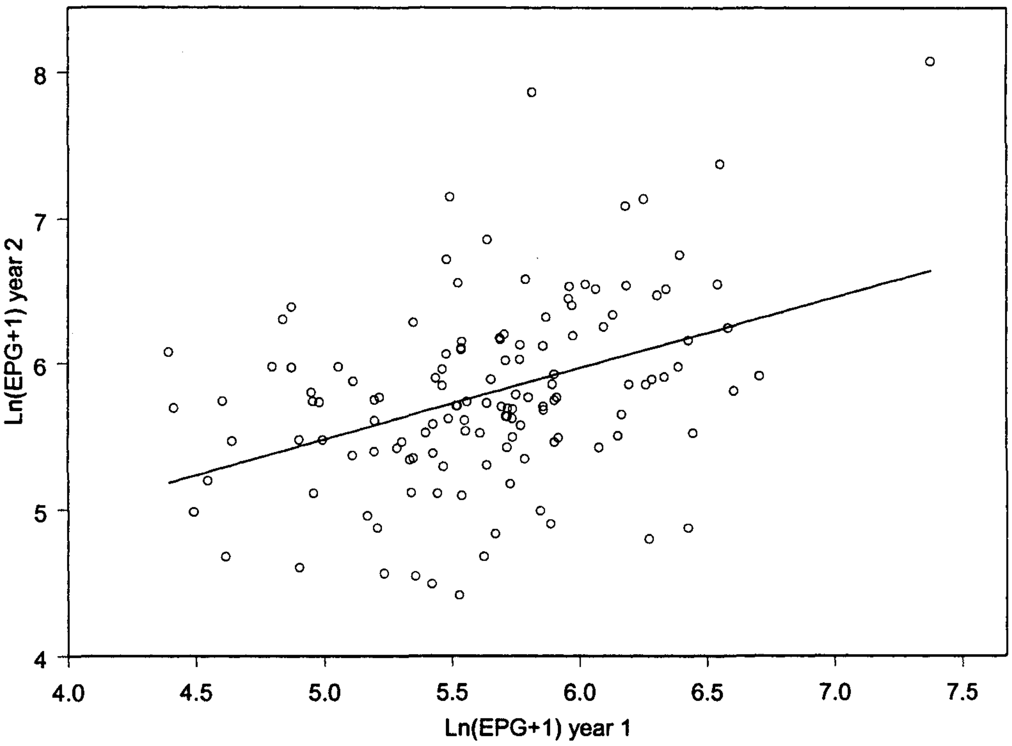

Fig. 2. Mean Ln-transformed FEC from individual male Alpine ibex in Levionaz in successive years, 2000-2004

Body mass

Body mass increased as a quadratic function of age (Body mass ~ Age + Age^2) with ID as a grouping factor and a random intercept (Table 4). Body mass adjusted to August 1 peaked at 12 years of age (Max. Y = 93.0 Kg ; Fig. 3) and then declined. Ln(EPG+1) did not affect body mass (Table 4).

Table 4. Wald test for the significance of terms included in the LME model with Body mass of Alpine ibex adjusted to August 1 as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and Year, Age, Age^2 and Ln(EPG+1) as independent variables, Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004.

	B	***Df***	***F***	***P***
Terms included in the model:				
Intercept	115.33	1, 89	5674.76	≤ 0.0001
Age	14.67	1, 89	190.93	≤ 0.0001
Age^2	-0.60	1, 89	137.22	≤ 0.0001
Rejected terms:				
Ln(EPG +1)	-	1, 89	1.39	0.241

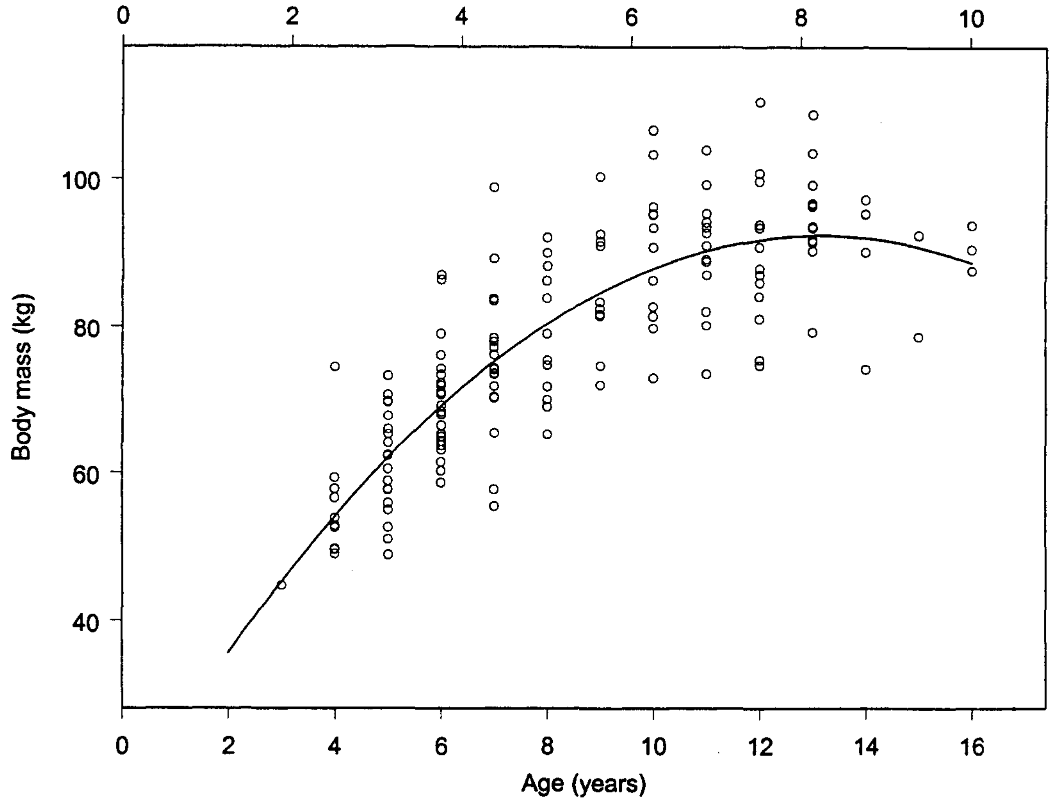

Fig. 3. Age and body mass adjusted to August 1 for Alpine ibex males in Levionaz 2000-2004

Antihelmintic treatment

Anthelmintic treatment reduced the mean number of parasite eggs by about 75% (Table 5 and Fig. 4). There was, however, no difference in mean FEC one year later between treated and control individuals (difference = 0.308 ± 0.189 Ln(EPG+1); Confidence intervals: lower = -0.146, upper = 0.761). Treatment effects interacted with age, appearing to be stronger for older individuals (Table 6 and Fig. 4). Antihelmintic treatment did not, however, affect body mass adjusted to August 1 in the year of treatment (Table 6), although effect size did go in the expected direction (+ 0.22).

Table 5. Wald test for the significance of terms included in the LME model with fecal output of parasite eggs (Ln(EPG+1)) of Alpine ibex as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and age, treatment and the interaction between the two as independent factors (individuals aged 3-7 only). Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004.

	B	***Df***	***F***	***P***
Terms included in the model:				
Intercept	5.43	1, 55	4410.36	$\leq$0.0001
Age	-0.18	1, 55	25.97	$\leq$0.0001
Treatment	1.21	1, 55	83.46	$\leq$0.0001
Age:Treatment	-0.38	1,55	26.51	$\leq$0.0001

Table 6 . Wald test for the significance of terms included in the LME model with Body mass of Alpine ibex as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random interce and age and treatment and the interaction between the two as independent factors (individuals aged 3-7 only). Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004.

	B	***Df***	***F***	***P***
Terms included in the model:				
Intercept	19.46	1, 38	2309.27	$\leq$0.0001
Age	8.18	1, 32	269.56	$\leq$0.0001
Rejected terms:				
Treatment	-	1, 32	0.96	0.33
Age:Treatment	-	1,32	0.07	0.78

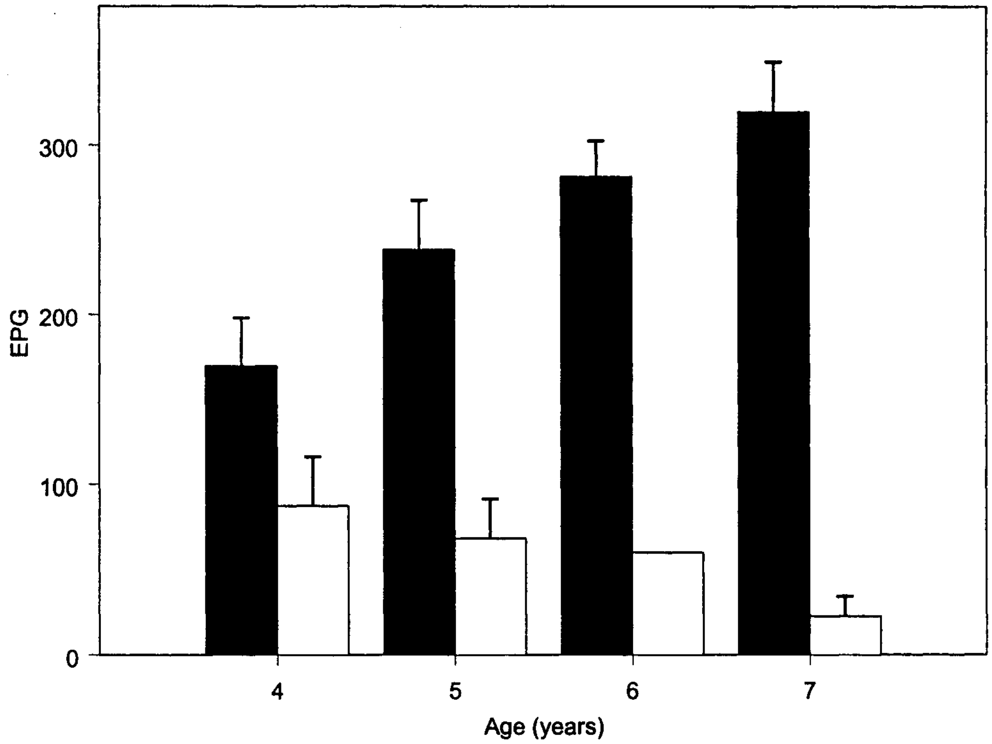

Fig. 4 – Effect of anthelmintic treatment on fecal counts of nematode parasites (EPG) at different ages in Alpine ibex, Levionaz, Gran Paradiso National Park, Italy. White squares rappresent the mean EPG for individuals treated with anthelmintic drugs. Black squares are the mean EPG for untreated controls. Error bars are standard errors.

Discussion

Sources of variation in fecal egg counts

Between-individual variation was larger than within-individual variation both for FEC and body weight, suggesting individual differences in parasite resistance and body condition in the Gran Paradiso Alpine ibex population. The fact that mean FEC in one year were positively correlated with mean FEC the following year for the same individual, further suggests strong inter-individual differences in gastrointestinal parasite intensity. Sources of variation which can contribute to differences in parasite loads and thus FECs among individuals include population density, environmental variability, sex, age, body mass and genotype (Wilson *et al.* 2002). Stochastic variation in the number of infective larvae that an ibex picks up while foraging may contribute as well to between-individual variabilty in parasite loads.

We could exclude population density, environmental variability and sex as factors contributing to inter-individual variability in FECs, as this study was based on males from one single population affected by the same density and environmental conditions.

Interindividual variability remained significant after accounting for age. Body mass could be excluded as a source of interindividual variability as we found no significant correlation between body mass and FECs after accounting for age effects in both variables (Figure 5).

Considerable heritable variation in FECs of gastrointestinal nematodes has been shown in domestic sheep, cattle and goats (Stear and Murray, 1994) as well as in a free-ranging population of Soay sheep (Smith *et al.* 1999). Furthermore, locus specific associations with parasite resistance (Patterson *et al.* 1998) and correlations between FECs and heterozygosity both in domestic and in unmanaged ungulate populations (Slate *et al.* 2004) further suggest significant additive genetic variation in parasite resistance in ungulates. To demonstrate additive genetic variation in a trait usually offspring-parent regression is used, in which the mean offspring values are regressed on the values of the parents (Falconer and Mackay, 1996).

Alternatively, in sib analysis, ANOVA methods are used to partition the variability in a trait explained by the identity of the parent (Falconer and Mackay, 1996). Our results suggest a genetic component in interindividual variability in FECs in Alpine ibex, but are insufficient to demonstrate it, as we lack the pedigree data necessary to perform offspring-parent regressions or sib analyses.

Age, body mass and FECs

Life history theory predicts that in large mammals with high adult survival and polygynous mating systems where male-male competition for oestrous females is high, such as Alpine ibex, males should mature after they reach asymptotic weight (Stearns, 1992). In Alpine ibex the attainment of asymptotic weight appears to be particularly late: males increased in body weight until 8.5-10.5 years of age in a reintroduced population in Switzerland (Giacometti *et al.*, 1997) while in Levionaz body mass increased until 12 years of age. This result suggests a prolonged investment in future reproductive success and late maturity of males in this population. The earlier attainment of asymptotic weight in the Swiss population may be due to the fact that this population is hunted.

In the swiss population larger males may be shot at younger ages, causing a possible selective effect of hunting on body mass (Coltman *et al.* 2003). Also lower density may contribute to the fact that the Swiss ibex grow faster. In Levionaz, instead, where the structure of the ibex population includes a large proportion of old, dominant males (> 10 years of age), younger males have possibly less chance to access reproduction, and therefore may be forced to pursue a conservative reproductive strategy, continuing to invest in body mass up to 12 years of age. In mountain ungulates dominance has been shown to be strongly related with age (Hass, 1991; F. Pelletier, *in prep.*). Preliminary results on dominance for access to salt, confirm a strong relationship between dominance and age in the Levionaz ibex population (A. von Hardenberg, *unpublished*). The onset of breeding and mass asymptote, as predicted by life history theory (Stearns, 1992), however, may not coincide exactly in mountain ungulates. For example, two-year-old bighorn rams succesfully father lambs (although rarely), yet asymptotic mass is at 6-7 years of age (M. Festa-Bianchet, *pers. comm.*). Information on male reproductive success in Alpine ibex would therefore be needed to conform our hypothesis.

Fecal egg counts increased gradually with age from 4 to 15 years, rapidly increasing afterwards, after asymptotic body mass was reached. Increased parasite egg output in older males, may be caused by an increased reproductive effort. If mass is important in determining access to mates, males should invest more in reproduction after reaching asymptotyc mass. A trade-off between current reproduction and immunity was shown for female ungulates (Festa-Bianchet, 1991), but we are unaware of any evidence of such a trade-off in free-ranging male ungulates in the litterature. Alternatively, the increase in FECs at old age may also have been influenced by senescence. Senescence is a process of phenotypic deterioration in the years before death (Comfort, 1964). Evidence of genetic or phenotypic correlates of senescence in natural populations is rare, because most individuals die, from exogenous causes, before starting to senesce (Comfort, 1964, but see also: Nesse, 1988; Gaillard et al., 1994). In the present study we show that individual body mass decreased after 12 years of age. Senescence in horn growth has already been shown in the Gran Paradiso ibex population (Jacobson *et al.*, 2004). Toigo (1997) showed senescence in survival in Alpine ibex, starting at 12 years of age, and Jacobson *et al.* (2004) suggested that mortality of older age classes (over 12 years of age) may be important in the dynamics of the Gran Paradiso Alpine ibex population. Senescence in immune function has been shown also in barn swallows (*Hirundo rustica*; Møller and De Lope, 1999; Saino *et al.* 2003) but not, to the best of our knowledge, in large herbivores. Our results do not permit us to discriminate between reproductive costs or senescence as factors explaining the increase in FECs with age. While it is possible that both factors contribute to the phenomenon, further studies are necessary to better understand the relationship between immune function, age and body weight in Alpine ibex.

Our results suggest also that male Alpine ibex possibly participate actively in reproduction for a very short period of time (possibly only few years). Males likely attempt to breed well before age 12, but they may not have much success until about 12 years of age. Therefore individual males appear to have limited possibilities for monopolizing reproduction, and only at very late age. This hypothesis is supported by recent genetic analyses showing a low inbreeding coefficient in this population despite high heterozygosity (von Hardenberg *et al. in prep.*).

Anthelmintic treatment

One single dose of anthelmintic drug administered at the beginning of summer was effective in reducing mean FECs in treated individuals compared to untreated controls. This result suggests a low rate of reinfection during summer, possibly due to unfavourable environmental conditions for nematode larvae at the high altitudes (2300-3200 m a.s.l.) inhabited by the Levionaz ibex population in summer. Because there were no difference in mean FEC one year later between treated and control individuals, it seems likely that infection happens mostly during March-May when ibex males pasture at lower altitudes (1700-1900 m. a.s.l.), with possibly more favourable conditions for nematode larvae. The efficiency of the antihelmintic treatment appeared to improve with age. Contrary to expectations, treated individuals did not gain more mass than untreated controls. While our results suggest that gastrointestinal parasites do not cause measurable effects on summer weight gain in ibex males, they are not conclusive due to low power of statistical tests caused by a small sample size of treated individuals although effect size did go in the expected direction. Further studies, with a larger sample of treated individuals, would be needed to verify this hypothesis.

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SECTION IB

Horn growth but not asymmetry heralds the onset of senescence in male Alpine ibex

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The evolution of exaggerated secondary sexual traits has puzzled evolutionary biologists since Darwin proposed the concept of sexual selection. Theories of sexual selection predict that secondary sexual traits should be costly to produce, so that they may function as reliable indicators of the quality of the bearer. If they are costly to produce, secondary sexual traits, such as the horns and antlers of ungulates, should thus be particularly susceptible to senescence. It has also been hypothesized that trait asymmetry should be a better indicator of individual quality than trait size for sexually selected traits. In this paper we tested if the length and asymmetry of age-specific yearly horn growth segments in male Alpine ibex were good predictors of mortality in successive years and thus an indicator of phenotypic senescence. I was responsible for all phases of this study. I planned and organized data collection and measured a substantial proportion of the horns. I performed all statistical analyses, literature review, planned which hypotheses to test, and wrote the manuscript.

Horn growth but not asymmetry heralds the onset of senescence in male Alpine ibex

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Abstract

Senescence is accelerating phenotypic deterioration with old age. For traits that grow throughout life, like the horns of some ungulates, senescence may be expressed as a decrease in annual growth rates, or an increase in asymmetry, in the years preceding death. Age-specific yearly horn growth segments of 378 male Alpine ibex (*Capra ibex* L.) that died from natural causes were analysed in the Gran Paradiso National Park (Italian Alps). Horn annuli displayed fluctuating asymmetry. The hypothesis if asymmetry and size of horn annuli predicted annual survival probabilities was tested. Between 5 and 11 years of age, individuals that grew shorter annuli than the population average had a greater probability of mortality over the following years than males with greater rates of horn growth. Horn asymmetry and mortality rates were not significantly correlated. Annulus size, reflecting the onset of senescence, appeared to be a better indicator of individual quality than annulus asymmetry.

Introduction

The age of onset of senescence, a process of phenotypic deterioration in the years before death (Comfort, 1964), may vary among individuals because of genetic and environmental differences (Mysterud *et al.*, 2001). Evidence of genetic or phenotypic correlates of senescence in natural populations is rare, because most individuals die, for exogenous causes, before starting to senesce (Comfort, 1964). Most studies have consequently concentrated on establishing if senescence occurs in natural populations (Nesse, 1988; Promislow, 1991; Gaillard *et al.*, 1994; Sibly *et al.*, 1997; Loison *et al.*, 1999), while little is known about individual differences in its onset (Bérubé *et al.*, 1999; Gaillard *et al.*, 2000).

Exaggerated secondary sexual traits, such as the horns and antlers of ungulates and long tail feathers and bright coloration in birds, are particularly costly to maintain and their growth is condition-dependent (Andersson, 1994). The growth of secondary sexual traits should therefore be a sensible phenotypic indicator of senescence between individuals (Møller & De Lope, 1999). In the barn swallow (*Hirundo rustica*), for example, the size of the tail feathers increased and then decreased with age although not significantly (Møller & De Lope, 1999).

The development of the left and the right sides of a bilateral trait is under the control of the same genes. In an individual of high phenotypic quality, their size is predicted to be identical (Møller & Swaddle, 1997). Non directional random deviations from bilateral symmetry is called *Fluctuating Asymmetry* (FA; Palmer, 1994). Fluctuating asymmetry may be a sensitive indicator of developmental stability if it reflects ability to resist stress during development (Zakharov, 1992). FA has been used to identify populations under environmental or genetic stress (e.g. Clarke, 1993; Alados *et al.*, 1995; Vollestad & Hindar, 1997; but see also Anne *et al.*, 1998; Lens *et al.*, 1999; and Floate & Fox, 2000) and over the last decade it has been proposed that departures from bilateral symmetry could be an indicator of developmental stability at the individual level. Individual asymmetries, especially of sexually selected traits (Møller, 1994; Markusson & Folstad, 1997; Putman *et al.*, 2000) may be correlated with individual fitness and therefore could be an index of individual quality. Meta-analysis studies have recently also suggested that, for sexually selected traits, asymmetry is a better predictor of mating success than size (Thornhill & Møller, 1998).

Asymmetry in sexually selected traits that regrow each year or that grow throughout life, should be particularly sensitive to the decline in individual quality related to the onset of senescence. Yet to our knowledge, the only test of a correlation between age and asymmetry was reported for barn swallows (Møller & De Lope, 1999).

Studies of the relationship between asymmetry in ungulate horns and antlers and various indicators of individual quality have produced contradictory results (Arcese, 1994; Malyon & Healy, 1994; Folstad *et al.*, 1996; Møller *et al.*, 1996; Markusson & Folstad, 1997; Lagesen & Folstad, 1998; Pelabon & Joly, 2000; Côté & Festa-Bianchet, 2001; Ditchkoff *et al.*, 2001; Kruuk *et al.*, 2003). No study has compared the relative importance of size and asymmetry of sexual ornaments as potential indicators of the onset of senescence in ungulates.

We tested whether horn size or asymmetry are better predictors of senescence in Alpine ibex (*Capra ibex,* L. 1758) from the Gran Paradiso National Park (GPNP, North-western Italian Alps), taking into account climatic, density and year effects. We chose environmental factors likely to affect horn growth, as shown in reintroduced populations of Alpine ibex (Giacometti *et al.* 2002). The same environmental variables tested in this study have been shown to affect the population dynamics of Alpine ibex in GPNP (Jacobson *et al.*, in press) No large carnivores exist in the GPNP, and winter starvation appears to be the most important factor limiting the population growth rate (von Hardenberg *et al.*, 2000, Jacobson *et al.* in press). Alpine ibex are fully protected in GPNP since its institution in 1922. The spectacular horns of male ibexes can reach 110 cm in length and grow throughout life, forming a distinct growth segment (annulus) each year. The GPNP wardens have systematically collected and catalogued the skulls of ibex found dead during winter since the 1950s, providing a unique opportunity to investigate the relationship between horn growth patterns and natural longevity. Because ibex horns grow a new annulus each year, they allow the study of developmental stability of the same genotype over time. In this study, we examined the horns of male Alpine ibex found dead at different ages, and predicted that the length of horn growth annuli would decrease and their asymmetry would increase in the years just before death.

Methods

Collection studied

We examined ibex skulls collected between 1988 and 1997, at ages ranging from 5 to 15 years. The date, locality and cause of death were recorded for each ibex. The exact age at death in years was determined by counting the clearly separated annuli (Ratti & Habermehl, 1977). Knowing the date of death and the age, we calculated the cohort to which each individual belonged.Death of individuals in this sample can be attributed with confidence to winter starvation, as skulls of individuals found dead under avalanches were not included in the sample and no large predators are present in the GPNP. Climate data was obtained from the Serrù meteorological station within the GPNP, at an elevation of 2240 m a.s.l. Total population size of ibex in the GPNP was obtained from the yearly censuses conducted by the park wardens (Jacobson *et al.* in press).

Measurements

The length of each annulus was measured for both horns, along a central line on the back of the horn. For each pair of annuli, we calculated annulus length as the mean value of the left and the right annulus and absolute asymmetry as the unsigned value of the difference between the left and the right annulus. Measurements were performed using a calliper to the nearest 0.5 mm. We did not consider annuli grown in the first (horn tip) or the last year of life, because the first annulus was often broken or worn, and growth of the last annulus was often incomplete when the ibex died.

FA analysis

To test for the presence of true FA in horn annuli and in total horn length we followed Palmer (1994), comparing FA to measurement error and testing for directional asymmetry and antisymmetry. The mean of the signed asymmetry of annuli grown from 3 to 12 years of age did not deviate from zero, indicating the absence of directional asymmetry (DA) (Table 1).

Table 1. Descriptive data of annuli size and asymmetry.

[(R+L)/2] = mean annulus length (mm); b = slope of the regression line of unsigned asymmetry vs. annulus size;(R-L)=signed asymmetry. Total length = total horn length at death.1) Significant departures ($P<0.05$) from an expected value of 0 are represented with an asterisk. 2) A negative kurtosis is indicative of platykurtosis, a positive one of leptokurtosis. P values were corrected for multiple tests using the Bonferroni sequential method (Rice, 1989).

	Mean				Mean			(R-L)	(R-L)
Annulus	[(R+L)/2]	s.e.	*b*[1]	s.e.	(R-L)[1]	s.e.	N	Skew[1]	Kurtosis[1,2]
2	71.77	0.60	0.006	0.008	0.38*	0.13	374	-0.18	2.40*
3	73.08	0.66	0.007	0.006	0.14	0.12	378	-0.31	1.01*
4	74.04	0.67	0.022*	0.006	0.02	0.12	371	-0.16	1.03*
5	72.22	0.66	0.002	0.006	0.16	0.11	352	-0.05	0.42
6	68.82	0.60	0.015	0.007	0.09	0.12	336	-0.35	1.30*
7	62.75	0.58	0.012	0.009	0.28	0.14	317	-0.10	0.71
8	52.30	0.62	0.022*	0.008	0.32	0.13	295	0.24	0.18
9	42.43	0.63	0.022	0.010	0.20	0.15	251	0.07	0.81
10	32.98	0.62	0.044*	0.013	0.48	0.17	181	0.32	0.87
11	27.08	0.71	0.046*	0.016	0.17	0.18	111	0.16	1.00
12	20.70	1.28	0.045	0.040	0.03	0.33	38	-0.44	1.79
Tot.length	486.33	6.94	0.011*	0.002	1.69*	0.49	377	0.11	0.15

The annulus grown at two years of age exhibited DA and was thus not considered in further analyses involving asymmetry. Total horn length also exhibited DA: the right horn was slightly longer than the left. The amount of DA in total horn length, however, is biologically insignificant (mean [R-L] averaged only 0.3% of total horn length; Table 1). The skew of the signed asymmetry of annuli grown at all ages did not deviate from zero (Table 1). Platykurtosis (indicative of antisymmetry) was not present in any trait. Leptokurtosis, however, was detected for annuli grown from 2 to 4 and at 6 years of age (Table 1). Because leptokurtosis can arise due to individual differences in developmental stability (Leung & Forbes, 1997), both normal and leptokurtic distributions of asymmetry values can be considered indicative of FA (Leung & Forbes, 1997; Møller & Swaddle, 1997). Absolute asymmetry was positively related to trait size for annuli grown at 4, 8, 10 and 11 years of age and for total horn length (Table 1), but, because the relationships between trait size and unsigned asymmetry were generally weak, we did not apply any size correction (as suggested by Palmer (1994)), and used instead unsigned absolute asymmetry as an indicator of individual asymmetry in all analyses. In order to ensure the appropriate table-wide probability of type 1 error, we applied the Bonferroni sequential adjustment (Rice, 1989) to each set of related tests for annuli grown at different ages (e.g. DA, regression of unsigned asym. vs annulus size, kurtosis, skew) as suggested by Palmer (1994),

Measurement error

FA must be distinguished from measurement error (Palmer, 1994). Because of the large sample size (6142 annuli measurements) and the long time needed to precisely take the measurements, we could not repeat all measurements twice as is usually recommended (Palmer, 1994). Therefore, each observer measured a sub-sample of 53 annuli twice. We then did a two-way ANOVA on the signed values of asymmetry with annulus nested within observer. The test (among-individuals nested in observers: $F_{156,159} = 4.86$, $p<0.00001$), indicated that measurement error (taking into account inter-observer variability) was smaller than among-individual variability. The ANOVA among observers ($F_{2,159} = 1.08$, $p=0.34$) suggested no observer bias. Measurement error accounted for 17.1 % of total variability in annulus asymmetry (expressed as the variation remaining unexplained after calculating repeatability of annulus asymmetry as: (s^2_{within}/ s^2_{within} + s^2_{among}) x 100; Palmer & Strobeck, 2003).

Statistical Analysis

The length of horn annuli was modelled using Linear Mixed Effects models (LME; Pinheiro & Bates, 2001) with individual identity fitted as the random effect, to avoid pseudo-replication due to horn annuli measured at different ages on the same individual. Age, climatic variables, total population size and the number of years before death, were fitted as fixed predictor terms. The following climatic variables were tested: Average snow depth (cm); Number of days of snow depth above mean; Average daily maximum temperature in winter (°C); Average daily minimum temperature in winter (°C); Average daily maximum temperature in summer (°C); Average daily minimum temperature in summer (°C); Total precipitation in spring (mm); Total precipitation in winter (mm); Total precipitation in summer (mm). More details on the climatic variables used, can be found in Jacobson *et al.* (in press).The significance of the fixed terms was assessed with conditional F tests. Survival at age *x* was modelled using Generalized Linear Models (GLMs) with a binomial error structure and a logit link function. Length and absolute asymmetry of annuli grown at ages *x-1*, *x-2* and *x-3* were fitted as fixed predictor terms. Year was fitted to control for environmental variability among years. To avoid non-independence of the data, we did separate GLM analyses for annuli grown at ages *x-1* and *x-2* including each individual in the sample only once. The significance of the fixed terms was assessed by the difference in deviance when removing the fixed terms from a full model including all terms (Crawley, 1993). Goodness of fit was explored in all analyses by examining the deviance residuals graphically. All models were fitted using S-PLUS 2000 (MathSoft Inc.).

Results

Annulus length was best described by a quadratic function of Age in a Linear Mixed Effects (LME) model with Individual Identity fitted as a random effect (Intercept: β_0= 57.79; Age: β = 7.44, $F_{1,2613}$ = 1789.19, p <0.0001; Age^2: β = - 0.81, $F_{1,2613}$ = 1284.02, p <0.0001). Annulus length was also affected by the environmental factors total precipitation in spring (β = 1.57, $F_{1,2613}$ = 24.93, p <0.0001) and average daily minimum temperature in winter (β = 0.65, $F_{1,2613}$ = 19.29, p <0.0001). Other climatic variables and population size were not significant. We found that, even taking into account age and environmental effects, annulus length grown at all ages was a function of the number of years until death (β = 1.10, $F_{1,2613}$ = 37.84; p <0.0001), suggesting senescence in horn growth in the years preceding death (Fig. 1). The annulus grown of one year was significantly shorter for individuals that died in the following year than for survivors, at all ages considered with the exception of the annulus grown at 8 years of age (Table 2a). The annulus grown two years earlier was shorter for individuals that died than for surviving ibex at all ages, although the difference between animals that died and survived was significant only for the annulus grown at 4 years of age (Table 2b). Annulus asymmetry one or two years before death did not differ between surviving and dying ibex at any age (Table 2a,b).

We used generalised linear models (GLMs) to asses the ability of length and absolute asymmetry of the annuli grown at age *x-1* and *x-2* to predict survival at age *x*. The length of the annulus grown at 5, 7, 9, 10 and 11 years of age was a good predictor of survival in the following year (Table 3). The length of the annulus grown at 6 years of age had a nearly significant effect (P=0.08). We also found a positive association with survival two years later for annuli grown at 4, 8 and 9 years (Table 3). Asymmetry had a significant effect on survival in the following year at 11 years of age only when surviving males unexpectedly had more asymmetrical horns than males that died (Table 3). Annulus length or asymmetry at age *x-3* or earlier was unrelated to survival (results not shown).

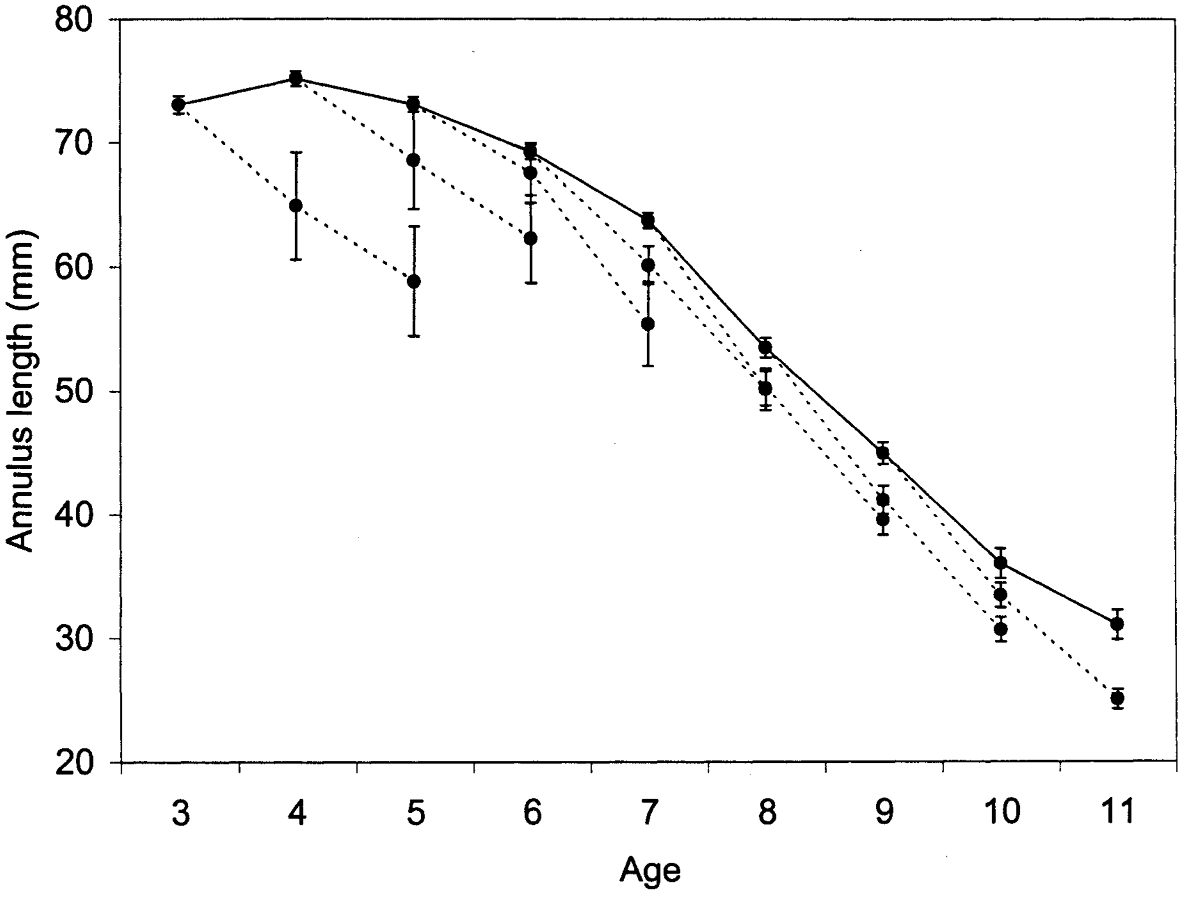

Figure 1. Length (± s.e.) of horn annuli grown at 3 to 11 years of age by Alpine ibex males found dead in the Gran Paradiso National Park, Italy. The solid line indicates mean annulus length at age x for ibex that survived to at least to age x+3. The dashed lines connect the mean lengths (± s.e.) of annuli grown at ages x-1 and x-2 by ibex that died at age x, for individuals that died at 6 to 12 years of age.

Table 2. Mean annulus length and asymmetry of dead and surviving male ibex.

a) Ibex males that survived and died one year after growing each annulus; b) Ibex males that survived and died two years after growing each annulus. % refers to the percentage by which the mean annulus length of ibex that died was smaller than the mean annulus length of surviving individuals. All measures are in mm (±s.e.). *t Length* and *t Asym.* refer to the *t* value for the difference in annulus length and asymmetry between dead and surviving ibex.

a)		Died			Survived				
Age	N	Length	Asym.	N	Length	Asym.	*t* Length	*t* Asym.	%
5	16	58.9 (4.4)	1.5 (0.3)	336	72.9 (0.6)	1.6 (0.1)	-4.48**	-0.31	19.2
6	19	62.3 (3.5)	1.5 (0.3)	317	69.2 (0.6)	1.7 (0.1)	-2.67*	-0.39	10.0
7	22	55.5 (3.4)	2.0 (0.3)	295	63.3 (0.6)	1.9 (0.1)	-3.44**	0.24	12.3
8	43	50.2 (1.7)	1.9 (0.2)	252	52.7 (0.7)	1.7 (0.1)	-1.39	0.66	4.7
9	70	39.6 (1.2)	1.8 (0.2)	181	43.5 (0.7)	1.6 (0.1)	-2.85*	0.94	9.0
10	69	30.7 (1.0)	1.7 (0.2)	112	34.4 (0.8)	1.8 (0.1)	-2.94*	-0.51	10.8
11	73	25.0 (0.8)	1.3 (0.1)	38	31.1 (1.2)	1.5 (0.2)	-4.38**	-0.93	19.6

b)		Died			Survived				
Age	N	Length	Asym.	N	Length	Asym.	*t* Length	*t* Asym.	%
4	16	64.9 (4.3)	1.2 (0.4)	336	75.2 (0.6)	1.7 (0.1)	-3.35**	-1.31	13.7
5	19	68.6 (3.9)	1.6 (0.3)	317	73.1 (0.6)	1.6 (0.1)	-1.60	-0.09	6.2
6	22	67.6 (2.4)	1.2 (0.3)	295	69.3 (0.6)	1.6 (0.1)	-0.73	-1.42	2.5
7	43	60.2 (1.5)	1.5 (0.2)	252	63.8 (0.6)	1.9 (0.1)	-2.28	-1.46	5.6
8	70	50.3 (1.4)	1.8 (0.2)	181	53.6 (0.8)	1.7 (0.1)	-2.18	0.68	6.2
9	69	41.2 (1.1)	1.7 (0.2)	112	45.0 (0.9)	1.6 (0.1)	-2.55	0.66	8.4
10	73	33.5 (1.0)	1.9 (0.2)	38	36.1 (1.2)	1.8 (0.2)	-1.55	0.32	7.2

* $P<0.01$, ** $P<0.05$, after correcting P values using the Bonferroni sequential method (Rice, 1989).

Table 3. GLM models of the survival of Alpine ibex males.

Survival probability at age *x* is compared to year of study, horn annulus length and asymmetry (Asym) one year (*x-1*) and two years (*x-2*) earlier. Probability levels are based on the difference in deviance between the full model (including all fixed factors) and a model excluding each term. For variables measured one and two years earlier, and for ibex aged from 6 to 12 years, the deviance of the full model is followed by the increase in deviance after removing each fixed factor from the full model.

Age (*x*)	Term	d.f.	*x-1*	Deviance	*P*	*x-2*	Deviance	*P*
6	Full model	331	5	53.09		4	55.26	
	Year	344		+59.51	<0.00001		+63.60	<0.00001
	Length	332		+7.41	0.006		+5.51	0.02
	Asym	332		+1.77	0.18		+0.06	0.80
7	Full model	316	6	86.12		5	88.85	
	Year	328		+53.07	<0.00001		+54.16	<0.00001
	Length	317		+3.07	0.08		+0.42	0.51
	Asym	317		+0.40	0.53		+0.03	0.85
8	Full model	298	7	97.65		6	104.18	
	Year	309		+50.79	<0.00001		+52.36	<0.00001
	Length	299		+6.20	0.01		+0.03	0.87
	Asym	299		+1.41	0.23		+0.54	0.46
9	Full model	277	8	169.83		7	168.66	
	Year	287		+71.24	<0.00001		+67.86	<0.00001
	Length	278		+0.33	0.57		+0.11	0.74
	Asym	278		+0.02	0.90		+1.36	0.24
10	Full model	234	9	198.02		8	203.98	
	Year	243		+87.01	<0.00001		+87.06	<0.00001
	Length	235		+9.99	0.002		+5.81	0.02
	Asym	235		+2.32	0.13		+0.15	0.70
11	Full model	165	10	146.48		9	148.85	
	Year	173		+81.14	<0.00001		+80.96	<0.00001
	Length	166		+6.76	0.009		+4.99	0.02
	Asym	166		+2.12	0.14		+0.15	0.83
12	Full model	96	11	86.38		10	98.78	
	Year	103		+30.04	0.00009		+30.41	0.00008
	Length	97		+13.84	0.0002		+2.42	0.12
	Asym	97		+4.59	0.03		+0.66	0.42

Discussion

In Alpine ibex, horn growth is relatively constant for the first 6 years of life and declines afterwards following a negative sigmoid curve (Nievergelt, 1966; Giacometti, 1988). This typical pattern was shown also by our sample.

Environmental factors, such as spring and summer rainfall and winter minimum temperature, influenced annuli growth in this study. Rainfall and temperature also affect horn growth in transplanted Alpine ibex populations, probably through their effect on plant growth and consequently the availability of food resources (Giacometti *et al.*, 2002). Even after accounting for environmental effects, however, annulus length decreased in the years approaching death independently of age. This result suggests that heterogeneity in yearly horn growth is related to heterogeneity in the onset of senescence.

Phenotypic deterioration in horn growth begins one or two years before death. The reduction of yearly horn growth in the two years preceding death appeared to be age-independent, indicating non-random mortality affecting low quality males. Various factors could contribute to the variability in the age of death for winter starvation, including variability in individual genetic quality, diseases, accidental injury and poor condition following overexertion during the early-winter breeding season. We cannot support one cause over the others, but the deterioration in horn growth in the years preceding death suggests that accidental events, overexertion or disease in the year of death can be ruled out. The occurrence of chronic diseases, reducing the ability of individuals to assimilate nutrients and to store energetic resources, may lead to death after one or two years due to winter starvation (Dean & Thorne, 1982). Reduced resistance to disease due to a deterioration in various functions of the immune system has been associated to senescence in humans and domestic mammals (review in Miller, 1996) and recently in barn swallow (Saino *et al.* 2003). Individual ibex of low genetic quality, predisposed to disease, may therefore suffer reduced condition and be forced to reduce investment in horn growth.

Horn annuli of Alpine ibex showed true fluctuating asymmetry, and therefore provided a good opportunity to explore developmental instability. Yet asymmetry failed to predict ibex survival. Our results suggest little or no effect of senescence on the developmental stability of horn growth, supporting recent studies that question whether FA in sexually selected traits is always an indicator of fitness (reviewed in Bjorksten *et al.*, 2000).

Møller and Pomiankowski (1993) predicted that sexual ornaments should present a negative relationship between absolute asymmetry and trait size. The rationale behind this hypothesis is that only high-quality individuals should be able to sustain the costs of large ornaments while being able to maintain symmetry. Our results do not support this hypothesis: we found a weak positive relationship between absolute asymmetry and trait size for Alpine ibex horn annuli. Positive or flat relationships between absolute asymmetry and trait size were also found in the horns of mountain goats (*Oreamnos americanus*)(Côté & Festa-Bianchet, 2001) and in the antlers of moose (*Alces alces*)(Solberg & Sæther, 1993), white-tailed deer (*Odocoileus virginianus*)(Ditchkoff *et al.*, 2001) and red deer (*Cervus elaphus*)(Kruuk *et al.*, 2003)..

The lack of negative relationship between individual asymmetry in the horns of male Alpine ibex and fitness components may be due to a weak and possibly undetectable relationship between FA and developmental stability, as suggested by several recent studies (Whitlock, 1996; Van Dongen, 1998; Whitlock, 1998). The undetectability of differences in individual asymmetry may possibly be due to relatively large FA in this population. Asymmetry has been shown to increase with inbreeding (review in Møller & Swaddle, 1997). Alpine ibex has been shown to present among the lowest genetic variability in wild populations of vertebrates (Maudet *et al.*, 2002), possibly due to a strong demographic bottleneck at the beginning of the 19th century, when less than one hundred individuals survived in the Gran Paradiso area (Couturier, 1962). Alternatively the lack of a negative relationship between individual asymmetry and survival may be due to insufficient selective pressure for symmetry. Horns are used as weapons during male-male combat (Lundrigan, 1996), while the importance of female choice in the mating system and in the evolution of secondary sexual traits in Alpine ibex as in other ungulates, is still unclear. If large horns in this species evolved only through male-male competition, the symmetry of the horns might be less important than their size.

According to the handicap principle (Zahavi, 1975; Zahavi & Zahavi, 1997) exaggerated secondary sexual traits in males can evolve if they are costly to produce and so reliably advertise male quality. Even though most studies to date have shown that this "good genes" model explains ornament evolution under female mate choice, the handicap principle could apply also to intra-sexual advertisement among males in species where sexual ornaments have evolved as weapons (Zahavi & Zahavi, 1997). Ditchkoff *et al.* (2001) provided indirect evidence that antler development of white-tailed deer males may be a honest signal of genetic quality, being associated with characteristics of genes at the major histocompatibility complex (MHC) level. Fitzsimmons *et al.* (1995) showed that large-horned bighorn rams (*Ovis canadensis*) were more heterozygous than small-horned rams aged 6-8 years. Both MHC genes and heterozygosity are believed to be indirectly related to fitness components (reproductive success and survival). Coltman *et al.* (2002) found a strong correlation between horn length and reproductive success among older bighorn rams. Effects of horn length on lifetime and seasonal reproductive success have been shown also in feral sheep (Preston *et al.* 2001), and Kruuk *et al.* (2002) found a positive relationship between antler size and mating success in red deer. To the best of our knowledge, this is, however, the first demonstration of a direct correlation between horn length and survival in ungulates. Our results provide support to the “good genes” hypothesis showing that only high quality males can afford to grow large horns, while low quality males reveal their reduced survival chances, and thus possibly their “bad genes”, through their horns. Low-quality males have shorter horns than high-quality males of the same age. Horn growth in Alpine ibex males thus appears to be a strictly honest (Kokko, 1997) advertisement of male quality.

Finally, our results have important implications for the conservation and management of ibex populations. All ibex populations, nowadays spread over the Alps, derive from reintroductions from the only population surviving, at the beginning of the 19th century, in the Gran Paradiso area. Many of these reintroduced populations are small, some including less than ten individuals, and therefore in need of additional transplants, while new reintroduction projects are planned in suitable areas. Maudet *et al.* (2002) identified the populations with the highest genetic variability, and thus the most suitable to provide individuals for transplants. Within these populations horn growth patterns, being easily measurable indicators of senescence, could be used to identify individuals most suitable for reintroduction purposes.

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SECTION IC

Heterozygosity and development of a secondary sexual trait in Alpine ibex *Capra ibex*

Achaz von Hardenberg, David Coltman, Bruno Bassano, Marco Festa-Bianchet,
Gordon Luikart and Paolo Lanfranchi

Correlations between individual heterozygosity, measured at microsatellite markers, and fitness related traits has been interpreted as evidence for either inbreeding depression or associative overdominance with loci directly affecting fitness. Both hypotheses imply measurable differences in genetic quality of individuals. According to the "good genes" hypothesis, strong genetic effects should be particularly evident in secondary sexual traits, such as the horns of Alpine ibex. The main goal of this paper was to investigate evidence for fitness-fitness correlations in two traits under viability selection (body growth and parasite resistance) and one sexually selected trait (horn growth). I was responsible for all phases of the study. I participated in all ibex capture and marking operations, measured most horns, organized data collection and collected a substantial proportion of fecal samples and weight measurements of marked ibex. I performed all statistical analyses, literature review, planned which hypotheses to test, and wrote the manuscript.

Heterozygosity and development of a secondary sexual trait in Alpine ibex *Capra ibex*

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Abstract

Multilocus heterozygosity (MLH) is the standardized average observed heterozygosity among all microsatellite marker loci in one individual. Correlations between MLH and fitness traits, termed heterozygosity-fitness correlations (HFC), have been reported in various vertebrate populations. HFCs are generally thought to reflect inbreeding depression, i.e. the reduction in fitness in inbred individuals. Alternatively, HFCs can arise due to associative overdominance of neutral microsatellite loci with loci directly affecting fitness traits. Secondary sexual traits, such as horn growth in ungulates, should present relatively strong HFCs because they are costly to develop. We investigated the relationship between individual heterozygosity and fitness-related traits (parasite resistance measured by fecal egg counts, summer weight gain and horn growth) in free-ranging Alpine ibex *Capra ibex* males in the Gran Paradiso National Park, Italy. We also explored if individual inbreeding coefficients estimated from microsatellite data are a better indicator of individual genetic variability than MLH. Contrary to expectations, inbreeding coefficients did not appear to be a better indicator of individual genetic variation than MLH. As predicted, we found evidence of heterozygosity-fitness correlation in horn growth but not in body mass or fecal egg counts. The slope of the correlation between MLH and horn growth was age-dependent. We also found lower fecal counts of parasite eggs for heterozygotes compared to homozygotes at the ETH10 microsatellite locus. Our results suggest that the horns of Alpine ibex males are a honest signal of male genetic quality.

Introduction

Inbreeding depression is the decrease in fitness of offspring born from matings among related individuals (Charlesworth and Charlesworth, 1987). Inbreeding depression has been typically measured by regressing individual inbreeding coefficients with fitness-related phenotypic traits. Inbreeding coefficients are usually estimated from complete pedigree data, which are rarely available for wild animals. Alternatively, researchers have used individual multilocus heterozygosity (MLH), the standardized average observed heterozygosity among all microsatellite marker loci in one individual, as a proxy for inbreeding coefficients.

Associations between marker heterozygosity and the phenotypic value of fitness-related traits, hereafter termed heterozygosity-fitness correlations (HFC), have been widely reported in wild vertebrate populations in recent years (review in Coltman and Slate, 2003).

Three main hypotheses have been proposed to explain HFC: (a) the direct overdominance hypothesis, whereby marker loci are directly affecting fitness; b) the associative overdominance hypothesis, whereby marker loci are in linkage disequilibrium with loci directly influencing fitness traits; c) the identity disequilibria hypothesis, whereby marker heterozygosity reflects genome-wide heterozygosity. Hypothesis (a) is likely possible only for allozyme markers, while it can usually be ruled out for non-functional and selectively neutral microsatellite markers. Hypothesis (b), which is also termed a local effect, assumes that individuals heterozygous at a marker locus, physically linked to a overdominant or dominant locus affecting fitness, tend to be heterozygous also at the latter locus. The identity disequilibria hypothesis (c) assumes that individuals heterozygous at marker loci tend to be heterozygous also at unlinked fitness-influencing loci. Identity disequilibrium is expected to arise only in structured populations showing variance in inbreeding, therefore positive HFC are assumed to indicate inbreeding depression (Coltman and Slate, 2003). Therefore hypothesis (c) is also referred to as the inbreeding hypothesis.

Microsatellites loci are characterised by simple short sequences of bases. Because most microsatellite loci lie outside of expressed genes, it is assumed that they are selectively neutral (Scribner and Pearce, 2000). Mutation rates for microsatellites are extremely high because of slipped-strand mispairing in DNA replication, making them ideal markers for assessing the genetic variability of individuals. Microsatellite polymorphism is due to variation in the length of sequence repeats. Even though MLH has been widely used as an indicator of inbreeding, it is not equivalent to the true inbreeding coefficient, which measures the probability that two alleles at a locus are identical by descent. Instead, MLH scores all homozygous loci that are identical by state; identical alleles are not necessarily inherited from a common ancestor. Slate *et al.* (2004) in a large sample of domestic sheep, showed that MLH was only weakly correlated with inbreeding coefficient, even in populations where inbreeding is common. In the same study, the inbreeding coefficient, estimated from pedigree data, but not MLH, detected evidence of inbreeding depression in morphological traits (Slate *et al.*, 2004).

Recently developed methods allow the estimation of individual inbreeding coefficients (f) from microsatellite data in the absence of complete pedigrees (Ritland, 1996, Lynch & Walsh, 1998). We will use the notation f' to indicate inbreeding coefficients estimated from microsatellite data to distingush them from true inbreeding coefficients (f) estimated from pedigree data. Such an estimated inbreeding coefficient should, in theory, be superior to MLH in detecting inbreeding depression, as this index also includes information on allele frequency.

Here we investigate the relationship between individual heterozygosity (MLH and f') and the following fitness-related traits: fecal counts of parasite eggs (FEC, an index of infection intensity), summer body mass (a determinant of winter survival and reproductive success in mountain ungulates; Festa-Bianchet *et al.*, 1997; Coltman *et al.* 2002) and horn growth (a sexually selected trait related to reproductive success in mountain ungulates; Coltman, 2002) in a free-ranging population of alpine ibex *Capra ibex* in the Gran Paradiso National Park (GPNP), Italy. We do so using an unusually large sample of 32 microsatellite loci. Alpine ibex suffered a narrow bottleneck at the beginning of the 19th century when they were apparently reduced to less than 100 individuals.

We predicted that MLH would be negatively correlated with FEC, and positively correlated with body mass and horn size. According to the handicap principle (Zahavi, 1975; Zahavi & Zahavi, 1997) exaggerated secondary sexual traits in males, such as horns in Alpine ibex, can evolve if they are costly to produce and so reliably advertise genetic quality. Secondary sexual traits are usually more phenotypically variable than other traits (Pomiankowski and Møller, 1995). A comparison of standardized linear selection gradients on quantitative traits in natural populations, suggested that sexual selection is stronger than viability selection (Hoekstra *et al.*, 2001). Moreover, it has been shown that additive genetic variation is higher in sexually selected traits than in non-sexually selected traits (Pomiankowski and Møller, 1995) Secondary sexual traits, can therefore be expected to be more strongly affected by genetic effects (inbreeding depression or local effects) than other morphometric and physiological traits. We therefore expected that the effects of genome-wide genetic diversity (i.e. MLH and *f'*) would be stronger on horn growth than on body mass and FECs. Finally, we used a recently developed statistical estimator to explore the correlation between MLH and inbreeding, estimated from microsatellite data, in the GPNP Alpine ibex population, and to estimate the power of this correlation.

Methods

Study area and population

The study was conducted in the Gran Paradiso National Park (GPNP; North-western Italian Alps; 45° 25' N, 07° 34'). Samples for genetic analyses were collected in 3 of the 5 main valleys of GPNP (Orco, Cogne and Valsavarenche), from 116 live-captured and tagged and from 37 untagged animals found dead of starvation during winter. Preliminary analyses, using Bayesan assignement tests implemented in STRUCTURE (Pritchard *et al.* 2000), showed no evidence of population substructure within the Gran Paradiso National Park among the three valleys (A. von Hardenberg, *unpublished*). Data on gastrointestinal parasitism and mass gain during summer were collected, on 83 tagged alpine ibex males, in the study area of Levionaz, Valsavaranche (GPNP) (Section Ia). Most of the Levionaz study area lies above the tree line, between 2,300 and ca. 3500 m a.s.l. and is characterised by high-altitude alpine meadows (mainly *Festuca varia*), moraines, rock cliffs and glaciers. The study area is used by the study population of Alpine ibex during summer and fall, from the end of May to December. The winter range (at 1700 to 2300 m a.s.l.) is characterised by pastures (near villages) and a mixed forest of *Larix decidua, Picea abies* and *Pinus cembra*. During this study about 200 adult ibex were found in the study area.

Field methods

Details about capture and marking methods are provided in Section Ia. Individual males in Levionaz were repeatedly weighed from June to September each year with an electronic platform scale baited with salt (Bassano *et al.* 2003). Body mass was adjusted to the 1st of August of each year, as described in Section Ia.

Faecal egg counts

Faecal egg counts (FEC) can be considered as an index of the combined effects of intensity of gastrointestinal nematode parasitism and host resistance influencing parasite fecundity (Coltman *et al.* 1999). FEC were estimated twice a month from all individually tagged males in Levionaz in 2000-2004 (n= 228 ibex-years). Animals were observed in the field from a distance of 10-50 meters and faeces were collected within 1-5 minutes of defecation. When possible, at least 20 grams were collected for each individual. Faecal pellets were kept in plastic bags at 4°C prior to analysis. Faecal egg counts (FEC) followed a modified McMaster technique (Ministry of Agriculture, Fisheries and Food, 1971) and were expressed as number of eggs per gram of fresh faeces (EPG). At least two counts per faecal sample were done in order to account for measurement error. Number of helminth eggs was determined to the nearest 20 eggs/gram of faeces. Individuals experimentally treated with antihelmintics (Section Ia), were excluded from analyses in the year of treatment. The arithmetic mean of FEC measured for each individual from June to September was used as a yearly index of individual parasite infection in accordance with von Hardenberg *et al.* (Section Ia).

Horn growth measurement

Horn growth was measured on individuals at capture and on those found dead. A total of 109 males were measured. The length of each yearly growth annulus was measured for both horns, along a central line on the front of the horn using a precision calliper to the nearest 1 mm. The average length of the left and right annuli was used for analysis. More details on horn growth measurement can be found in von Hardenberg *et al.* (2004).

Microsatellite analysis

A tissue sample for microsatellite analysis was collected from each live-captured animal and from carcasses of animals found dead during winter. Using a sterile blade, we cut a tiny piece of tissue (< 2 mm^3) from one ear and stored it at room temperature in tight collection tubes with 1 to 5 ml of 95% (w/v) ethanol solution. DNA was extracted from about 20 mg of tissue using the Dneasy^{TM} Kit (QIAGEN GmbH, Germany) following the manufacturer's instructions. We amplified with polymerase chain reaction (PCR) 39 polymorphic microsatellite loci: MAF209, MAF36, OarHH35, BMS1350, TGLA441, Texan4, IDVGA30, BM81124, OarHH62, McM73, CSSM47, BM4208, RT1, HUJ1177, McM152, OarJMP29, BM121, BM1225, HEL1, SR6Q, SR26, ETH10, BM1818, SR25, ILST029, INRA185, FCB48, AE54, TGLA122, TGLA126, SR8, HAUT27, ILST030, SR,24, SR15, BM4505, OMHC1, OarkP6, TGLA387. Details on methods used for PCR and electrophoresis are described in Maudet *et al.* (2001; 2002).

Data analysis

Hardy-Weinberg proportions and gametic disequilibrium for all possible pairs of loci were tested using the program GENPOP version 3.4 (Raymond and Rousset, 1995). Individual standardized multilocus heterozygosity (MLH) was calculated as the ratio of the heterozygosity of an individual to the mean heterozygosity of those loci at which the individual was typed. The standardization avoids confounding because of possible systematic differences in loci used between individuals (Coltman *et al.*, 1999). Individual inbreeding coefficients (f), were estimated from microsatellite data, using SPAGeDi version 1.1 (Hardy and Vekemans, 2002). These coefficients estimate the relatedness between two alleles within an individual at a locus averaged over all loci, therefore the sign of expected relationships with fitness related traits is opposite to what expected for MLH (for example, a negative relationship with horn size, rather than the positive one expected for MLH).

To account for repeated measurements of the same individuals in different years, we fitted linear mixed effects models (LME) implemented in the NLME package of S-PLUS 2000 (Pinhero and Bates, 2001) to test the relationship between MLH or f and FEC and body mass. The LME function in S-Plus also allows models to be fitted with heteroscedastic within-group errors. We fitted LME models with the appropriate variance function whenever the within-group errors appeared to have unequal variances. We followed the model building approach suggested by Pinhero and Bates (2001) for all fitted LME models. The significance of fixed terms was assessed using conditional F tests. For LME models with different random terms, we chose the model with the lowest Akaike information criterion (AIC). To normalize the error terms, we transformed the FEC data to Ln(EPG +1) prior to all analyses. Treatment contrasts were used to assess differences within factors. Age was centered at 8 years to reduce multicollinearity whenever a quadratic term was included. To minimize the risk of type I error from multiple tests, all probabilities were corrected with the sequential Bonferroni method (Rice, 1989). The correlation between MLH and mean inbreeding was estimated using *equation 4* in Slate *et al.* (2004). Power analyses were done following Zar (1994).

Results

Hardy-Weinberg and linkage disequilibrium

Six out of 39 loci (BM81124, OarJMP29, BM121, SR25, TGLA126 and SR24) deviated significantly from Hardy-Weinberg proportions after Bonferroni correction. We therefore removed them from all further analyses. Furthermore, two loci (OMHC1 and TGLA387) were in linkage disequilibrium (and thus highly correlated) and we therefore excluded one (TGLA387) from analyses. These procedures left us with 32 loci for most analyses.

Fecal egg counts

We found no significant relationship between MLH and FEC, nor between f' and FEC, after accounting for identity, age and year effects (Tables 1 and 2). Analysing the effect of each single locus on FEC (Table 3), heterozygotes had lower mean FEC than homozygotes in 11 out of 32 loci, but for only one locus (ETH10) the difference was significant after Bonferroni correction.

Table 1. Wald test for the significance of terms included in a linear mixed effects (LME) model with fecal output of parasite eggs (Ln (EPG+1)) of Alpine ibex as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random slope for Age (*AIC* = 244.87 for the model with a random slope for Age *vs*. *AIC* = 246.35 for a model with a random intercept only), and Age, Year and MLH as independent variables, Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004. Standard errors in parenthesis. Model is based on 164 observations from 53 individual ibex.

	Coeff.	***Df***	***F***	***P***
Terms included in the model:				
Age	+ 0.21 (0.08)	1,105	20.23	$\leq$0.0001
Year		4,106	20.22	$\leq$0.0001
Rejected terms:				
MLH	+ 1.54 (0.09)	1,51	0.76	0.39
Age:MLH	- 0.14 (0.08)	1,105	2.88	0.09

Table 2. Wald test for the significance of terms included in a LME model with fecal output of parasite eggs (Ln (EPG+1)) of Alpine ibex as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random slope for Age (*AIC* = 242.84 for the model with a random slope for Age *vs*. *AIC* = 244.97 for a model with a random intercept only), and Age, Year and inbreeding coefficient *f'* as independent variables, Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004. Standard errors in parenthesis. Model is based on 164 observations from 53 individual ibex.

	Coeff.	***Df***	***F***	***P***
Terms included in the model:				
Age	+ 0.06 (0.01)	1,105	17.78	$\leq$0.0001
Year		4,105	20.09	$\leq$0.0001
Rejected terms:				
f'	- 0.86 (1.19)	1,51	2.18	0.15
Age:*f'*	0.04 (0.14)	1,105	0.09	0.76

Table 3. Difference in body mass, FEC and Horn annulus length between heterozygotes and homozygotes at 32 microsatellite loci for adult male Alpine ibex (*Capra ibex*) in Levionaz (Gran Paradiso National Park, Italy). A positive sign indicates a larger value in heterozygotes compared to homozygotes. Standard error in parenthesis. Values and significances have been estimated with Linear mixed effects models with ID as grouping factor, a general positive-definite within-group error structure, and the following fixed terms structures: Body mass ~ Age + Age^2 + Year + Locus; FEC ~ Age + Year + Locus; Annulus length ~ Age + Year + Locus. Significance levels were corrected using the Sequential Bonferroni Method. Significant results are undelined. The model could not be computed for HUJ1177 on horn annulus size because of singularity in backsolving.

Locus	FEC (Ln(EPG+1))	Body mass (Kg)	Horn annulus length
MAF209	+ 0.007 (0.05)	- 0.71 (1.21)	+ 0.44 (0.97)
MAF36	- 0.08 (0.06)	- 0.46 (1.5)	- 2.18 (1.12)
OarHH35	- 0.04 (0.05)	- 1.48 (1.18)	- 0.30 (1.02)
BMS1350	- 0.001 (0.05)	+ 0.33 (1.38)	- 0.61 (0.97)
TGLA441	+ 0.08 (0.05)	+ 0.64 (1.82)	- 1.45 (0.94)
Texan4	+ 0.13 (0.07)	+ 0.64 (1.82)	+ 1.72 (1.59)
IDVGA30	+ 0.006 (0.05)	+ 0.35 (1.46)	+ 0.72 (1.17)
OarHH62	- 0.066 (0.06)	+ 0.69 (1.48)	+ 1.06 (0.92)
McM73	+ 0.03 (0.05)	- 0.88 (1.24)	+ 0.42 (0.95)
CSSM47	+ 0.04 (0.06)	- 0.11 (1.35)	+ 0.64 (1.12)
BM4208	+ 0.05 (0.06)	+ 2.03 (1.47)	- 0.56 (1.11)
RT1	+ 0.02 (0.05)	- 1.17 (1.23)	+ 0.72 (1.15)
HUJ1177	+ 0.01 (0.07)	- 2.11 (1.46)	--
McM152	- 0.008 (0.06)	- 0.76 (1.34)	+ 1.15 (1.00)
BM1225	- 0.18 (0.11)	+ 0.78 (2.65)	- 0.60 (1.6)
HEL1	+ 0.025 (0.05)	- 1.72 (1.12)	+ 0.33 (1.02)
SR6Q	+ 0.05 (0.07)	+ 1.18 (1.63)	- 1.45 (1.66)
SR26	+ 0.011 (0.07)	- 0.01 (1.60)	+ 0.65 (1.05)
ETH10	- 0.15 (0.04)	- 0.88 (1.10)	+ 1.89 (0.93)
BM1818	+ 0.03 (0.05)	+ 3.13 (1.27)	+ 1.40 (1.02)
ILST029	+ 0.04 (0.05)	+ 2.30 (1.17)	- 0.59 (0.96)
INRA185	- 0.02 (0.07)	- 0.68 (1.65)	- 1.63 (1.09)
FCB48	+ 0.01 (0.05)	- 0.21 (1.14)	+ 0.45 (1.06)
AE54	+ 0.002 (0.05)	+ 1.30 (1.13)	+ 1.06 (0.92)
TGLA122	- 0.06 (0.05)	- 0.32 (1.14)	- 0.96 (0.94)
SR8	+ 0.04 (0.08)	- 1.18 (1.60)	- 0.12 (1.07)
HAUT27	- 0.03 (0.05)	+ 1.34 (1.17)	- 0.11 (0.96)
ILST030	- 0.08 (0.05)	- 0.23 (1.38)	- 1.00 (0.99)
SR15	+ 0.10 (0.05)	- 0.67 (1.21)	+ 1.06 (0.97)
BM4505	+ 0.05 (0.05)	- 2.93 (1.07)	0.25 (0.96)
OMHC1	+ 0.10 (0.04)	+ 0.08 (1.09)	+ 0.03 (0.97)
OarkP6	+ 0.05 (0.07)	+ 1.20 (1.58)	+ 1.97 (0.99)

Body mass

Body mass was not related to MLH or f after accounting for Identity, Age and Year effects (Tables 4 and 5). Analysing the effect of each single locus on Body Mass (Table 3), for 14 of 32 loci heterozygotes were heavier than homozygotes, but no difference was significant after correcting P-values with the Sequential Bonferroni method.

Horn growth

Neither MLH and f' on horn annulus length had significant effects as single factors (Table 6 and 7) on horn growth, but their interactions with Age had significant positive effects on horn annulus length (Table 6 and 7). The slope of the regression between MLH and horn annulus length progressively increased with age (Figure 1). A similar pattern was found for f'. Regressing MLH against horn annulus length at each age, we found a significant positive relationship at 9 years of age. The length of horn annuli grown at 6-8 and at 10-12 years of age presented a positive relationship with MLH, although no relationship was statistically significant (Table 8). Inbreeding coefficient f' was negatively related with horn annulus size for annuli grown at 7-12 years of age although no relationship was statistically significant (Table 8). Analysing the effect of each single locus on horn annulus length, we found that in 17 of 31 loci, heterozygotes had longer mean horn annuli than homozygotes, although no difference was statistically significant (Table 3).

MLH-inbreeding correlation

Mean f' of the population was $f = 0.025$. The correlation between MLH and mean inbreeding, estimated using *equation* (4) in Slate *et al.* (2004), was low ($r(MLH,f) = -0.09$; power = 0.1974). For this correlation to be significantly different from 0 with a power of 0.8, would require a sample of at least N = 965.

Table 4. Wald test for the significance of terms included in a LME model with Body mass (Kg) as the dependent variable, Identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and Age, Age^2, Year and MLH as independent variables for male Alpine ibex in Levionaz (Gran Paradiso National Park, Italy). Standard errors in parenthesis. Model is based on 122 observations from 50 individuals.

	Coeff.	*Df*	*F*	*P*
Terms included in the model:				
Age	+ 16.33 (1.86)	1,65	121.82	≤ 0.0001
Age^2	-0.70 (0.06)	1,65	135.60	≤ 0.0001
Year		4,65	13.70	≤ 0.0001
Rejected terms:				
MLH	- 3.19 (13.49)	1,48	0.33	0.57
Age:MLH	- 0.05 (1.19)	1,65	0.002	0.96

Table 5. Wald test for the significance of terms included in a LME model with Body mass (Kg) as the dependent variable, Identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and Age, Age^2, Year and f' as indipendent variables in Alpine ibex males in Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004. Standard errors in parenthesis. Sample size was 122 observations from 50 individuals.

	Coeff.	***Df***	***F***	***P***
Terms included in the model:				
Age	+ 16.23 (1.15)	1,65	121.89	≤0.0001
Age^2	-0.69 (0.06)	1,65	136.23	≤0.0001
Year		4,65	13.76	≤0.0001
Rejected terms:				
f'	9.22 (18.22)	1,48	0.03	0.86
Age:f'	- 1.36 (1.99)	1,65	0.47	0.50

Table 6. Wald test for the significance of terms included in a LME model with horn annulus length (mm) as the dependent variable, Identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and Age, Year, MLH and the interaction term Age:MLH as indipendent variables in Alpine ibex males in Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004. Standard errors in parenthesis. Sample size was based on 750 observations from 109 individuals.

	Coeff.	*Df*	*F*	*P*
Terms included in the model:				
Age	- 5.79 (1.87)	1,623	747.59	≤ 0.0001
Year		16,623	4.59	≤ 0.0001
MLH	8.51 (5.00)	1,107	0.171	0.67
AGE:MLH	3.54 (0.92)	1,623	14.83	0.0001

Table 7. Wald test for the significance of terms included in a LME model with horn annulus length (mm) as the dependent variable, Identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and Age, Year, *f'* and the interaction term Age:*f'* as indipendent variables in Alpine ibex males in Levionaz (Gran Paradiso National Park, Italy), 2000 to 2004. Standard errors in parenthesis. Sample size was based on 750 observations from 109 individuals.

	Coeff.	***Df***	***F***	***P***
Terms included in the model:				
Age	- 5.54 (0.25)	1,623	743.41	$\leq$0.0001
Year		16,623	4.51	$\leq$0.0001
f'	4.22 (6.26)	1,107	1.89	0.17
AGE: *f'*	- 3.91 (1.35)	1,623	8.30	0.0041

Table 8. Relationships between MLH and *f* and annulus size (Ln transformed) grown at different ages in Alpine ibex life captured or found dead in winter in the Gran Paradiso National Park. P (Bonf.) is the P value after Bonferroni sequential correction (Rice, 1989). Significant results are underlined.

Annulus	**Value**	***St.Err.***	***F***	***Df***	P	***P(Bonf.)***	R^2
MLH							
2	-0.2765	0.1445	3.662	1,107	0.0583	NS	0.03309
3	-0.1087	0.1079	0.2141	1,105	0.3158	NS	0.009584
4	-0.0825	0.1141	0.2237	1,100	0.4716	NS	0.005196
5	-0.0604	0.1219	0.2453	1,81	0.6217	NS	0.003019
6	0.0573	0.1164	0.4921	1,64	0.6243	NS	0.00377
7	0.1194	0.1243	0.9233	1,56	0.3407	NS	0.01622
8	0.1724	0.1288	1.339	1,52	0.1864	NS	0.03333
9	0.4113	0.129	10.16	1,47	0.0025	0.05	0.1778
10	0.3738	0.2063	1.8114	1,43	0.0771	NS	0.0709
11	0.5119	0.4097	1.2495	1,30	0.2211	NS	0.04947
12	0.0167	0.4994	0.0334	1,23	0.9736	NS	4.86E-05
f'							
2	0.2607	0.1869	1.947	1,107	0.1658	NS	0.01787
3	0.2753	0.1368	4.047	1,105	0.0468	NS	0.03712
4	0.2136	0.1524	1.964	1,100	0.1642	NS	0.01926
5	0.3124	0.1612	3.755	1,81	0.0561	NS	0.04431
6	0.0748	0.1588	0.2219	1,64	0.6392	NS	0.003455
7	-0.1637	0.1853	0.7808	1,56	0.3807	NS	0.01375
8	-0.2675	0.1901	1.979	1,52	0.1654	NS	0.03666
9	-0.4975	0.198	6.313	1,47	0.0155	NS	0.1184
10	-0.3996	0.3093	1.669	1,43	0.2033	NS	0.03736
11	-0.5953	0.6137	0.9409	1,30	0.3398	NS	0.03041
12	-0.2488	0.8406	0.08763	1,23	0.7699	NS	0.003796

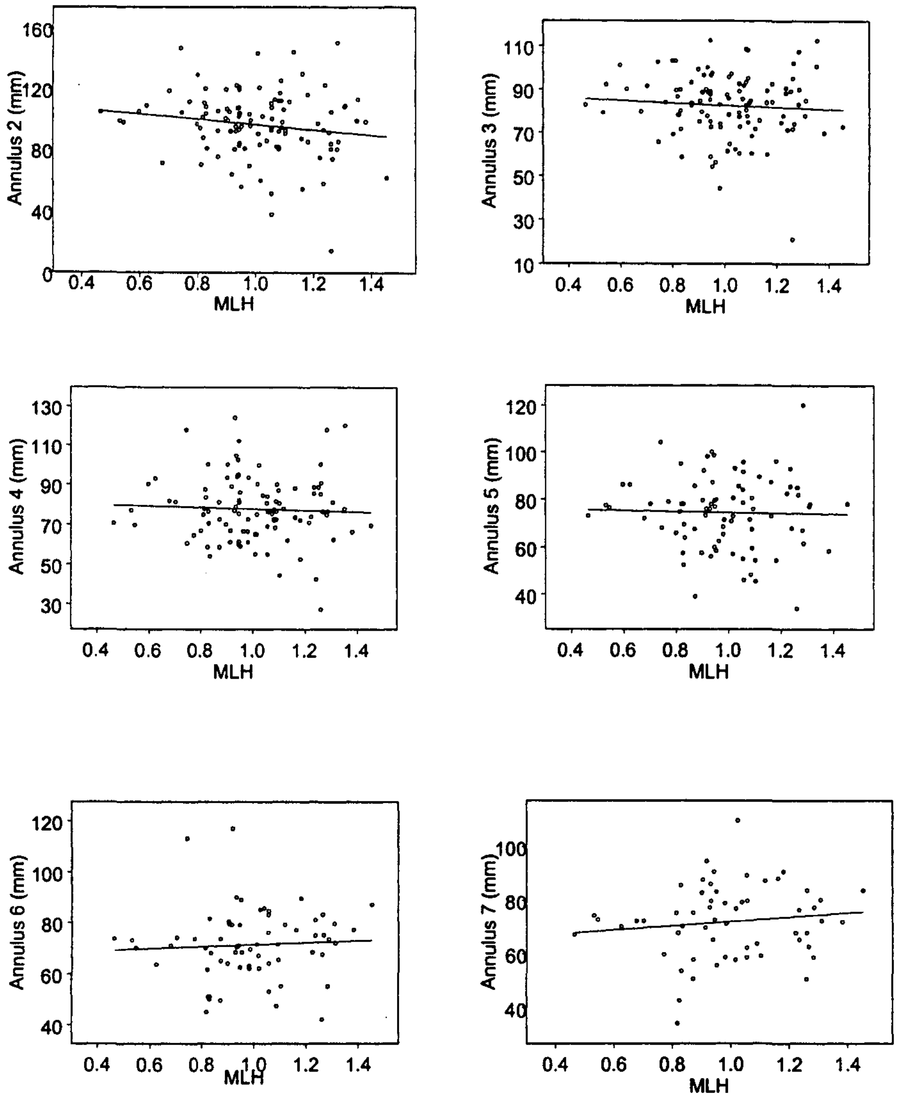

Figure 1a. Relationship between MLH and horn annuli grown at 2-7 years of age in male Alpine ibex in Gran Paradiso National Park (Italy).

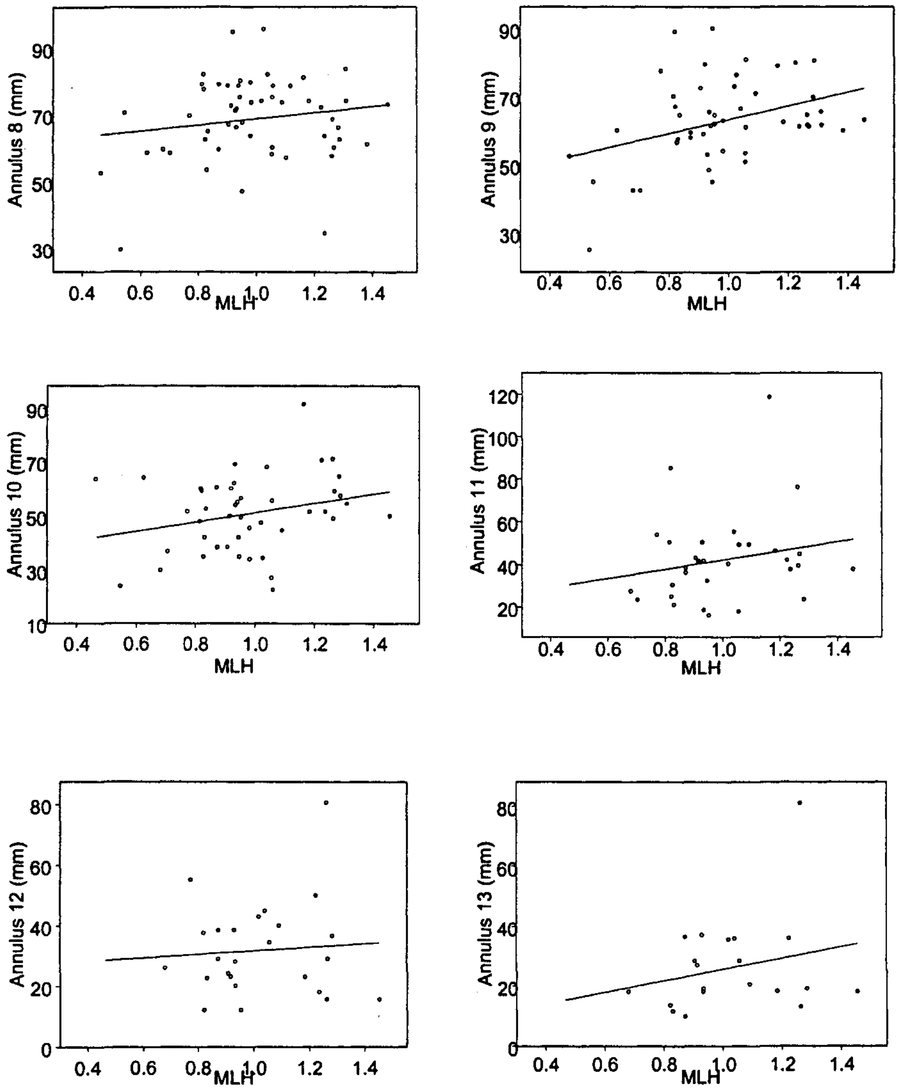

Figure 1b . Relationship between MLH and horn annuli grown at 8-13 years of age in male Alpine ibex in Gran Paradiso National Park (Italy).

Discussion

We found no relationship between MLH or individual inbreeding (f', estimated from microsatellite data) and faecal egg counts or body mass in Alpine ibex males. Moreover, trends in the data were typically opposite to expectations, except for the relationship between f' and body mass. These results are not surprising given the recent meta-analysis of HFC in wild populations which revealed a weak effect size for MLH (mean r = 0.0274) (Coltman & Slate, 2003) and estimated that a minimum sample size of at least 600 individuals as required to obtain a nominal power of 80 % for detecting HFC. The same study revealed an even lower mean effect size of MLH on physiological traits (including parasite resistance) only (mean r = 0.0075) or on morphometric traits (including body mass) only (mean r = 0.0052) (Coltman & Slate, 2003). Moreover, HFCs are expected to decrease with age, because usually growth and survival differences are maximal early in life (David, 1998). Because of difficulties in capturing young animals, we were limited to individuals aged 3-15 years. If very low heterozygosity was associated with small body mass or high levels of gastrointestinal nematode infestation, it is possible that highly homozygous individuals did not survive to the age classes included in our study and therefore were not sampled. In Soay sheep the effects of both MHC loci and MLH were age-dependent (Paterson et al., 1998; Coltman et al. 1999).

Despite the lack of an overall effect of heterozygosity on FEC and body mass, heterozygotes at locus ETH10 had significantly lower FECs than homozygotes. Such a significant effect was not found for any other locus, and the sign of the difference between heterozygotes and homozygotes appeared to be randomly distributed among loci. It is possible that ETH10 is in linkage disequilibrium with loci directly selected for parasite resistance in Alpine ibex, in accordance with the associative overdominance hypothesis. Associative overdominance is at least partially responsible for an association between birth weight and MLH in Red deer Cervus elaphus (Slate and Pemberton, 2002).

Among the loci we analyzed there was also OMHC1, which is linked to a marker associated with FEC in Soay sheep (Patterson *et al.* 1998). In Alpine ibex, however, we found no significant effect of OMHC1 on FEC or any other analysed trait.

Despite the lack of an association between individual genetic variation and FECs or Body mass, as expected MLH was positively correlated with the length of horn annuli grown at 6-13 years of age and the slope of the relationship between horn annulus length and MLH increased significantly with age. The relationships between individual inbreeding coefficient (*f'*) and the length of horn annuli grown at 7-13 years of age, was negative as predicted. There is evidence that horns and antlers reliably advertise male quality in ungulates. Coltman *et al.* (2002) found a correlation between horn length and reproductive success in mature bighorn rams *Ovis canadensis*. Effects of horn length on lifetime and seasonal reproductive success have also been shown in feral sheep (Preston *et al.*, 2001), and Kruuk *et al.* (2002) found a positive relationship between antler size and mating success in red deer. Antler size in Red deer has also been shown to correlate positively with sperm quantity and quality (Malo *et al.*, 2005). In Alpine ibex, males with annuli shorter than the population average had reduced survival chances over the following two years (von Hardenberg *et al.*, 2004).

Our results, support the hypothesis that horns are honest signals of male genetic quality in Alpine ibex (von Hardenberg *et al.*, 2004). Indirect evidence of a relationship between secondary sexual traits and genetic variability in ungulates was provided also by Ditchkoff *et al.* (2001) who showed that antler development of male white-tailed deer was associated with characteristics of genes at the major histocompatibility complex (MHC) level. Fitzsimmons *et al.* (1995) showed that large-horned bighorn rams were more heterozygous than small-horned rams aged 6–8 years.

Correlations between MLH, based on microsatellite markers, and fitness related traits have usually been interpreted as evidence of inbreeding depression (Pemberton, 2004). Two recent papers however, show that individual heterozygosity is only weakly correlated with individual inbreeding coefficients (Slate *et al.*, 2004, Balloux *et al.*, 2004). In this study we found that the correlation between MLH and mean inbreeding coefficient (f) was extremely low, in accordance with Slate *et al.* (2004). Heterozygosity-fitness correlations are therefore most likely due to the local effects of specific microsatellites in linkage disequilibrium with loci directly affecting fitness traits, unless correlation of heterozygosity at different loci within individuals can also be detected. We found that the horn annulus grown at 9 years of age was larger in heterozygotes than in homozygotes for most loci (75 %), even thought not statistically significant, possibly suggesting evidence of inbreeding depression, rather than a local effect, on horn growth.

Theoretically, f' is a better measure of inbreeding than MLH, because it includes information on allele frequency. Inbreeding coefficients (estimated from pedigree data) but not MLH, detected inbreeding depression for morphological traits in a population of domestic sheep (Slate *et al.*, 2004). Contrary to predictions, however, we found that individual inbreeding coefficients (estimated with marker-based regression methods) were not a better index of individual genetic variability than MLH. The inherent, possibly inevitable, inaccuracy of the indirect regression approach used to estimate f in the absence of pedigree information (Ritland, 1996; Lynch & Walsh, 1998) may have caused too much noise around the inferred parameters, restricting their usefulness. Similarly, Thomas *et al.* (2002) showed that several indirect marker-based methods were poor estimators of heritability of body weight in free-ranging Soay sheep *Ovis aries*.

In conclusion we found evidence of heterozygosity-fitness correlation in a secondary sexual trait (horn growth) but not in body mass or in resistance to gastrointestinal parasites in Alpine ibex. It is possible that this HFC was due to inbreeding depression rather than to a local effect, despite the poor correlation between multilocus heterozygosity and inbreeding coefficient. The selective pressure on secondary sexual traits may be strong enough to leave such a signal of genetic quality which, in other traits, such as body mass and parasite resistance may be evident only at younger ages. We also found lower fecal egg counts for heterozygotes compared to homozygotes at a single microsatellite locus. The possibility that this result may be due to associative overdominance with loci directly involved in parasite resistance needs further explorations. Inbreeding coefficients, estimated from microsatellite data, did not appear to be a better indicator of individual genetic variation than MLH. We therefore suggest that inbreeding coefficients in HFC studies should be used only if they can be calculated from pedigree data.

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CHAPTER II

Ibex in the snow: climate and population dynamics

SECTION IIA

Preliminary analysis of the temporal variability of the Alpine ibex population in the Gran Paradiso National Park

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The quantification of temporal variability in abundance of a natural population is the first step to understand its dynamics. Spectral analysis can be used in population ecology to identify periodic oscillations in the time series of population counts. This paper presents a preliminary analysis of the population dynamics of Alpine ibex in the Gran Paradiso National Park. We analysed the correlation between the counts performed at the beginning and the end of summer each year, and tested the previously hypothesized presence of recurrent periodicities in the dynamics of this population. My contribution to this paper included participation in statistical analysis, the interpretation of results, and writing of the manuscript. I also organized and proofread for accuracy the census database.

Preliminary analysis of the temporal variability of the Alpine ibex population in the Gran Paradiso National Park

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Abstract

We study changes in size of the population of Alpine ibex (*Capra ibex ibex*) in the Gran Paradiso National Park, in the Western Italian Alps, by analysing a long series of censuses conducted in the past forty years. Spring and fall counts are shown to be strongly correlated with each other. Spectral analysis of the ibex abundance data indicate two main oscillations, with approximate periods of 3 and 8 years. Meteorological data suggest that the size of the ibex population is limited by winter snow cover.

Keywords: Alpine ibex, Population dynamics, Climatic effects

1. Introduction

Ungulate populations appear to be limited by endogenous, density-dependent factors, by the action of predators, and by stochastic factors such as weather (*e.g.*, Picton, 1984; Sinclair, 1989; Skogland, 1991; Grenfell *et al.*, 1992; Escos *et al.*, 1994; Murdoch, 1994; Turchin, 1995; Langvatn *et al.*, 1996; Clutton-Brock *et al.*, 1997; Post *et al.*, 1997; Saether, 1997; Forchhammer *et al.*, 1998; Gaillard *et al.*, 1998). Given this multiplicity of causes and effects, a proper identification of the key factors that affect a given ungulate population is crucial for its sensible management.

The first step to understand the dynamics of a natural population is the quantitative assessment of its temporal variability. Here, we discuss some preliminary results of the analysis of the population dynamics of the Alpine ibex, *Capra ibex ibex*, in the Gran Paradiso National Park (PNGP). As the Alpine ibex population in the PNGP is censused twice a year, we investigate if there are significant differences between the two yearly censuses. An earlier analysis of the Alpine ibex population of PNGP was presented by Bassano *et al.* (1992), who detected apparent periodicities in the ibex abundance. In the present paper we discuss their results, in light of new analyses of the data set. Furthermore, we consider the possible role of weather in limiting the Alpine ibex population at PNGP.

The Gran Paradiso National Park was founded in 1922 in the Western range of the Italian Alps, and it is the oldest protected area in Italy. The park is located between Piedmont and Valle d'Aosta (Italy), at 45° 25' N, 7° 34' E, partially bordering on the Vanoise National Park in France. As a mountain park, the PNGP is characterized by large, scarcely populated areas that are mostly located above the tree-line. Most of the park (42,389 hectares, representing 58.6% of the total park surface) is composed of alpine pastures, moraines and unproductive land. Hunting is forbidden in the park, and human activities are in general limited.

Two mountain ungulates live in the park, the Alpine chamois *(Rupicapra r. rupicapra)* and the Alpine ibex (*Capra ibex ibex*). The protection of the Alpine ibex, endangered by excessive hunting pressure, was the main reason for creating the Gran Paradiso National Park, formerly a royal hunting reserve. Both ungulates are now fully protected in the park, and the Alpine ibex is protected also outside the PNGP. No predators are present in the park area and thus ibex mortality is due to age, winter starvation and disease.

Alpine ibex live mainly above the tree-line at altitudes between about 2,000 and 3,500 meters, descending to the lower elevations during late winter and spring. Ibex forage on grass, mosses and lichens, and are most active at dawn and dusk. Typically, adult males live separated from the herds of females and subadults. During the mating season, December through January, males fight with each other by loudly and spectacularly clashing horns, in order to gain access to females. Females give birth to one, and rarely two, kids the following June. In general, females first reproduce when three years old. Before this age, young males and females stay with adult females in small herds. Although individuals as old as 20 years have been observed, few ibex survive beyond 14 years.

2. Censuses of the Alpine ibex population at PNGP

During the last forty years, the Alpine ibex population of the PNGP has been censused twice a year, namely in late spring and early fall. Each census has recorded the number of adult males (*M*), adult females (*F*), male and female yearlings (*Y*), and male and female kids (*K*). These latters were counted only in spring until 1984, and both in spring and fall from 1985 on. Censuses are conducted along trails and from fixed locations within each surveillance area. Each park warden is assigned an average area of about 1,050 hectares. Most of these areas are treeless, making observation easier. This effort has led to a long time series of ibex abundance in the Park, which provides important information on the dynamics of this population. Here, we analyze the time series of ibex abundance recorded from 1956 to 1997.

Figure 1a shows the total ibex abundance over the study period, as separately given by the spring and fall counts (however, we always used the spring counts for kids, due to the fact that fall counts are available only for the last 12 years). The total ibex population,

$T_n=M_n+F_n+Y_n+K_n$, where n indicates the year, fluctuated around 3,300 individuals until the mid eighties, when an apparent eruption of the population took place, leading to a total of about 5,000 individuals. After about ten years, the total number of ibex started decreasing.

Figure 1b and 1c show the abundances in the four age/sex classes discussed above, as given by the spring and fall censuses respectively. During the population increase, the number of newborns and yearlings did not increase significantly, suggesting that the increase in the ibex population was due to higher survival rate of non-reproducing adults rather than to increased recruitment. This interpretation is confirmed by the study of the age distribution in the ibex population, which indicates that there was a larger proportion of old individuals during the eruption (Bassano *et al.*, 1992).

An important question is whether there are significant differences between spring and fall censuses. Figures 2a-d compare the number of males, females, yearlings and newborns counted in the fall with those counted in the previous spring.

In general, fewer adults and yearlings are seen in fall than in the spring, while the opposite is observed for newborns. The ratio between fall and spring counts for adults and yearlings is approximately constant, and it is independent of the class (males, females or yearlings). Presumably, the difference between the results of spring and fall censuses for adults and yearlings can be ascribed to a wider dispersion (also outside the park) in late summer and early fall. Later in the year, ibex concentrate in the park. Conversely, the larger number of kids counted in the fall can be due to late births.

Linear regression of the fall versus spring counts gives slopes 0.78±0.03, 0.77±0.04 and 0.78±0.05 for males, females and yearlings respectively, and 1.19±0.33 for newborns. The correlations between fall and spring counts are respectively 0.98, 0.95, 0.94 for the adults and yearlings, and are highly significant (Student t-test: $p<0.001$). For newborns, the correlation is 0.71 and the Student t-test gives p=0.007, presumably due to the smaller number of data points available and to possibly larger censusing errors. In any event, these results indicate strong correlation between spring and fall counts of adults and yearlings, suggesting that one count per year is enough to monitor fluctuations in ibex abundance.

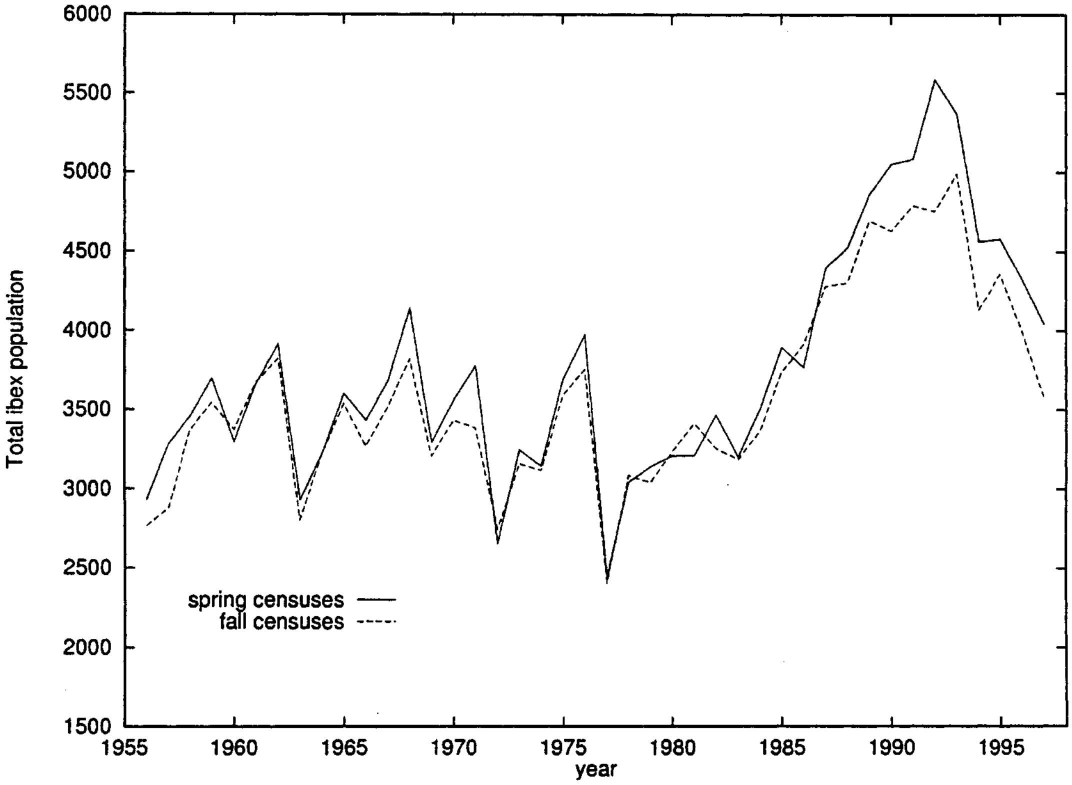

Figure 1a. Time series of the total ibex abundance, as separately obtained from spring and fall counts.

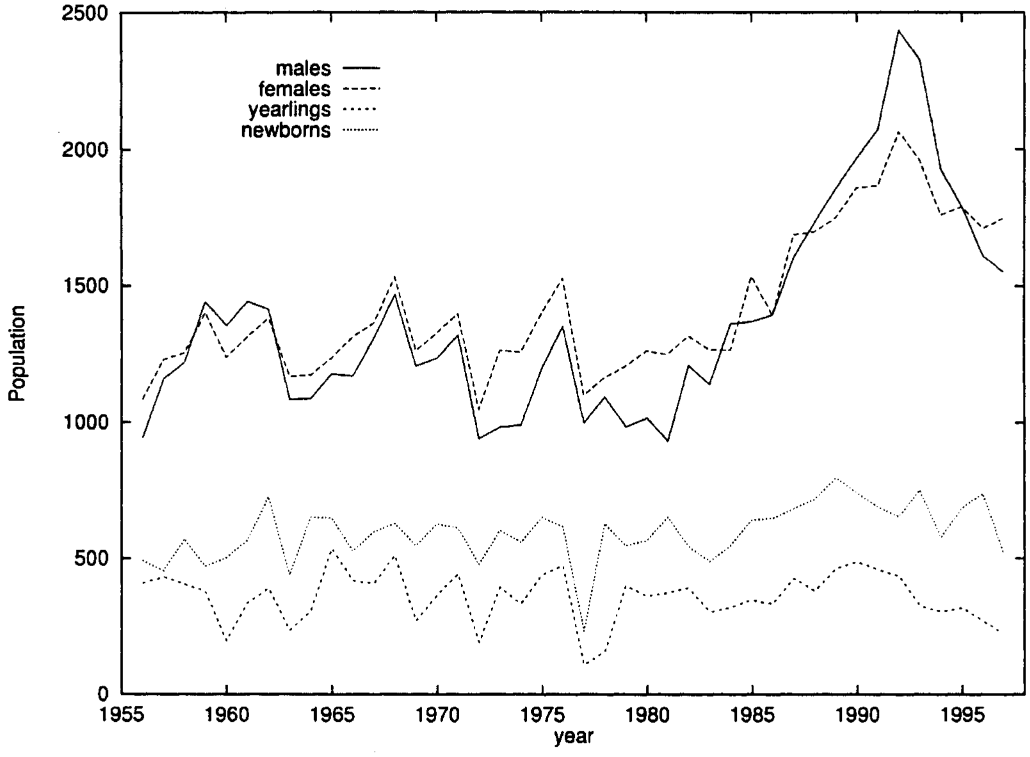

Figure 1b. Time series of the abundances in the four age/sex classes considered (males, females, yearlings, newborns) as obtained from spring counts.

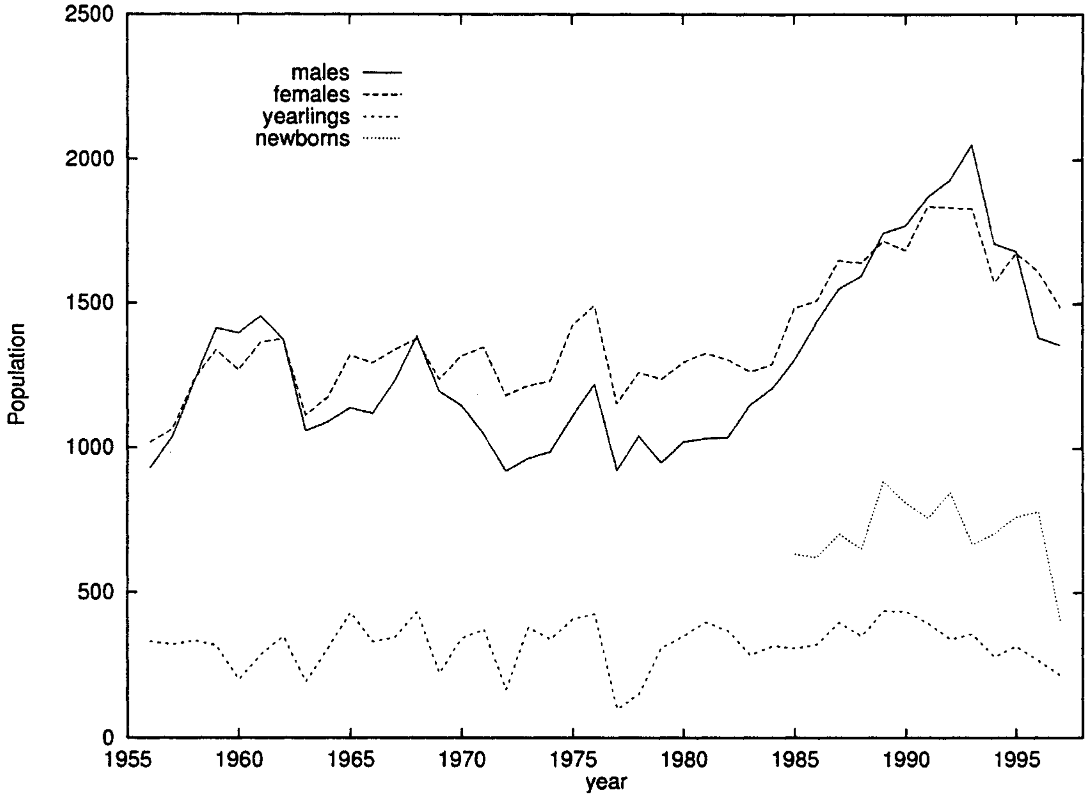

Figure 1c. Time series of the abundances in the four age/sex classes considered (males, females, yearlings, newborns) as obtained from fall counts.

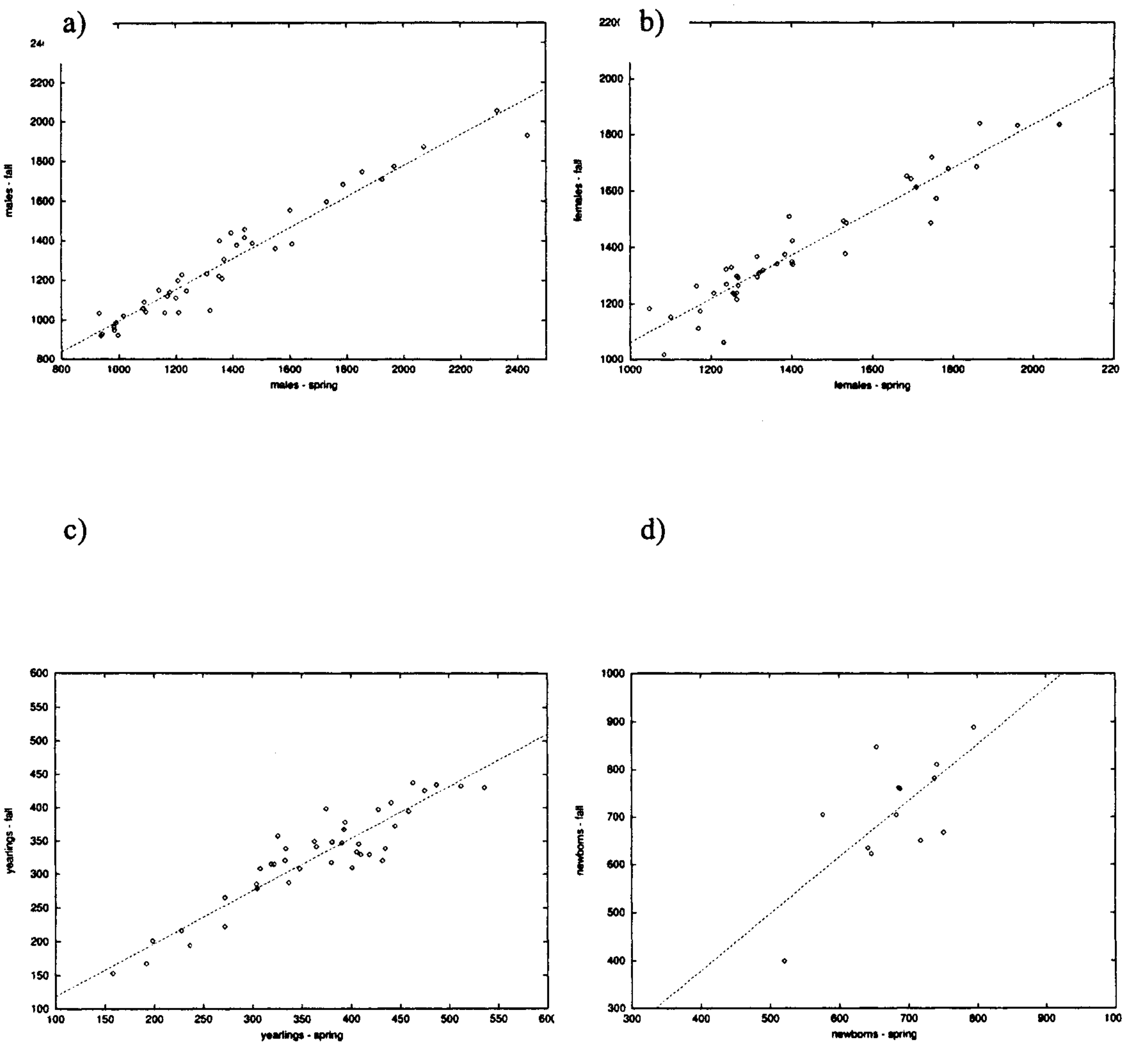

Figure 2. A comparison of fall and spring counts of ibex in the PNGP during 1956-1997 for males (a), females (b) , yearlings (c); and kids (d) for the period 1985-1997.

3. Spectral analysis

In spectral analysis, a generic time series, X_n , $n=1,...,N$, recorded at unit (e.g. yearly) intervals, is decomposed into the sum of different sinusoidal components with period N/k, where $k=1,...,N/2$. From this, we can write

$$X_n = \sum_{k=1}^{N/2} A_k \cos(2\pi \tfrac{k}{N} n + \phi_k)$$

Where A_k is the amplitude of the component with period N/k and ϕ_k is its phase. The quantity $|A_k|^2$, where the vertical bars indicate the absolute value, provides a measure of the variance explained by the component with period N/k (see, e.g., Box & Jenkins 1976). The power spectrum is herein defined as $P(k)=2|A_k|^2/N$ and it provides information on how the variance is distributed among the various components. A significant peak of $P(k)$ at a given period N/k implies the presence of an energetic oscillation with this periodicity in the signal considered.

In the study of Bassano *et al.* (1992), apparent periodicities of about 8 yr and 3 yr in the ibex abundance were detected. With such a small number of data points, however, power spectra have very low frequency resolution and almost null significance, and must be interpreted cautiously. Moreover, the sudden increase during the last ten years makes the time series strongly non-stationary, leading to spectra that are dominated by low-frequency components (see, *e.g.*, Balmforth *et al.*, 1999). For this reason, we have computed the spectra (by a standard Fast Fourier Transform routine) only for the first 25 years of measurement (1956-1980), during which the total population was stationary on average. Figure 3 shows the spectra obtained from this first segment of data, for both spring and fall counts. Two spectral peaks, at about 8 yr and 3 yr, are apparent, confirming the earlier inferences.

It is interesting to compare the spectra of the total population with those of specific sex-age classes. Figures 4a,b show the spectra, for the first 25 years, of the time series of the total number of adults, M_n+F_n, and of the total number of yearlings and kids, Y_n+K_n, as obtained by the spring counts. The spectrum of the adult abundance shows evidence of a an approximate 8-yr periodicity, while there is less evidence of a 3-yr periodicity. Conversely, the spectrum of the yearlings and newborns shows increased spectral power at a periodicity of about 3 yr, and no sign of the longer 8-yr periodicity. Although the indications of the spectral analysis must be regarded as speculation for such a short series, these results suggest the presence of two different mechanisms, associated respectively with the 3-yr and 8-yr periodicities, that affect different age classes.

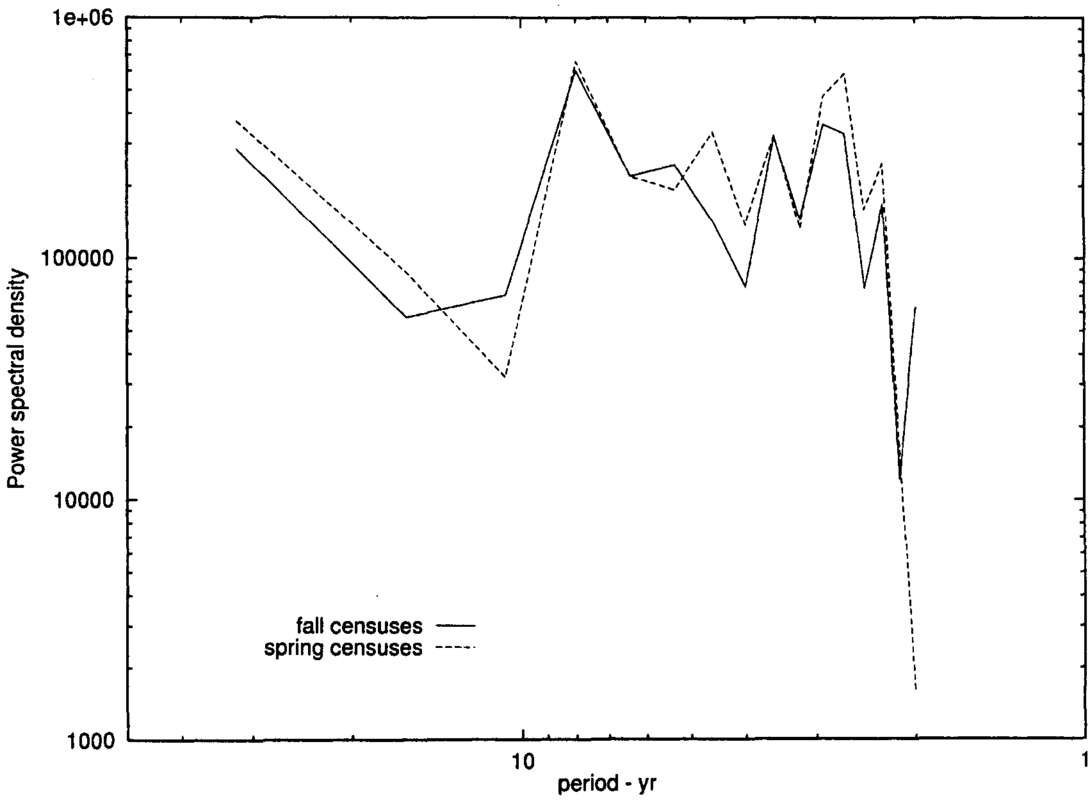

Figure 3. Power spectra of the total ibex abundance data in the period 1956-1980, as obtained from spring and fall counts.

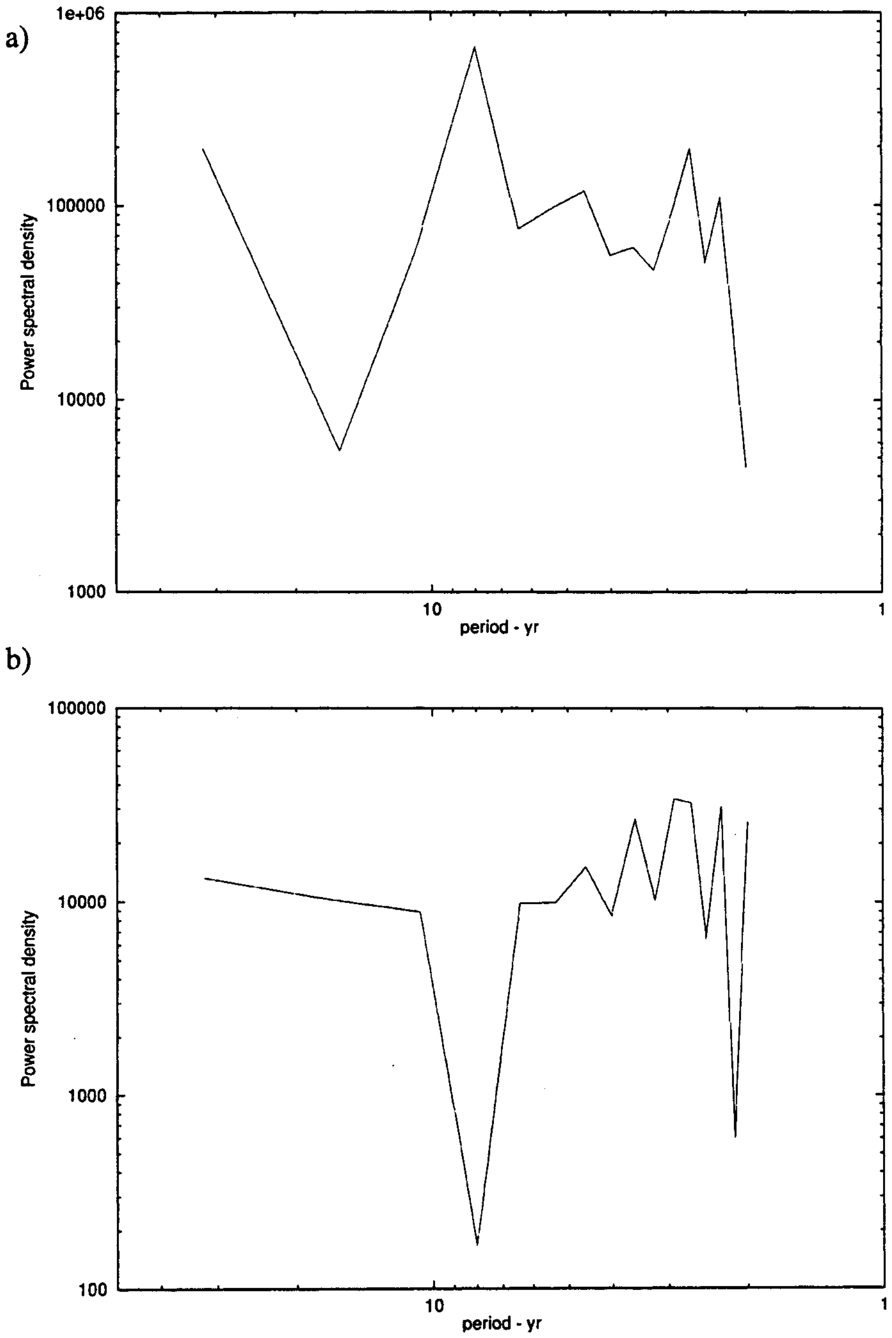

Figure 4. (a) Power spectrum of the abundance of adult ibex (males and females) in the period 1956-1980. (b) Power spectrum of the abundance of kid ibex (yearlings and newborns) in the period 1956-1980. Ibex abundance is obtained from spring counts.

4. Weather effects on population dynamics

The most striking aspect of the ibex abundance data (Figure 1a), is the sudden increase in the total population during the period 1985-1995. Its cause, then, should be properly determined. First we note that large carnivores, like lynx, have been absent from the study area since the end of the last century, so it is not possible to ascribe the growth of the ibex population to decreased predation pressure. Analogously, hunting has not been allowed in the Park since its creation, and thus one cannot invoke a changed hunting pressure on the population. Potential causes may include a decreased impact of diseases, decreased competition from domestic stock, and weather effects. To explore the role of weather, we considered the time series of rainfall intensity, temperature and snow cover recorded daily at two stations, located respectively at Teleccio lake, 1866 meters above sea level, and at Serrù lake, 2240 meters above sea level. The comparison of meteorological data with ibex abundances suggests that the mean winter snow cover S_n, as obtained by averaging the snow depth measured at each station from October to May, is probably the most important climatic factor. The value S_n is an average over the months of October, November and December of the year $n-1$ and over the months of January through May of the year n. Figure 5 shows the time series of the total ibex abundance (as measured in spring) together with that of the snow cover S_n. An anticorrelation is visible, with more ibex seen when the winter snow cover is smaller. A more detailed quantitative exploration of the possible climatic limitation of the ibex population is in progress, and will be reported elsewhere. Here we just note that the results shown in figure 5 suggest a significant effect of the depth and/or duration of the snow cover on the ibex population. The most plausible mechanism is through weakening and starvation of older ibex during winter months. In winter, Alpine ibex have serious difficulties in finding food, and many reach the end of that season in physically precarious conditions. A higher, or longer, snow cover may thus lead to the death of the more stressed individuals. Starting in the mid eighties, for several years the snow cover was smaller than in the previous period. This climatic fluctuation may thus be the ultimate cause of the eruption in the ibex population. Since snow seems to affect survival more than natality, during periods of reduced snow one expects a larger proportion of old animals, as observed during the period 1985-92 by Bassano *et al.* (1992).

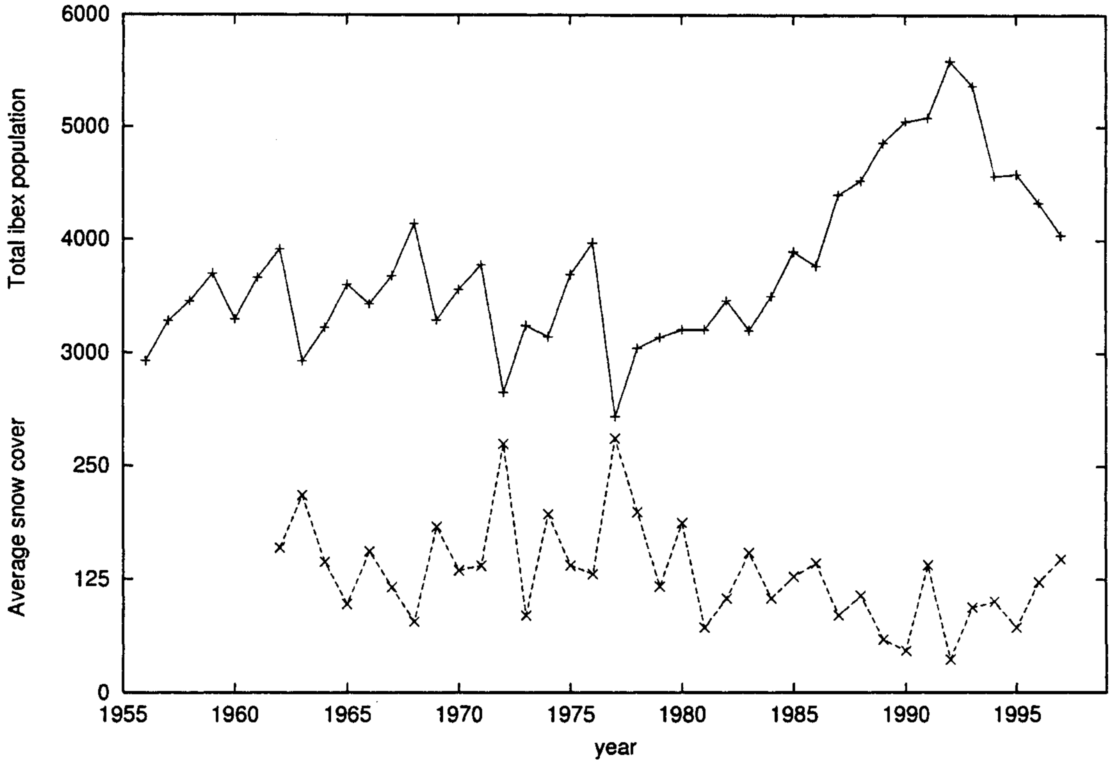

Figure 5. Time series of the total ibex abundance (spring counts) and winter snow cover at Serrù.

5. Summary and conclusions

Ibex abundance data from the Gran Paradiso National Park indicate a strong correlation between spring and fall censuses. Spectral analysis of the ibex abundance has suggested the presence of two periodicities at about 8 and 3 yr, associated respectively with the adult and kid ibex subpopulations. A preliminary comparison between ibex abundance and meteorological variables suggest an important role of climate as a limiting factor for the ibex population. In such a situation, the ibex population may undergo large-amplitude fluctuations in phase with climatic variability, as observed in the period 1985-95. Further work is in progress to quantitatively characterize the population dynamics of Alpine ibex at PNGP and the role of limiting climatic factors.

Acknowledgements

This research could not have been done without the dedicated effort of the PNGP wardens, who conducted the censuses in often difficult environmental conditions. We thank Marco Festa-Bianchet for constructive comments on the paper.

SECTION IIB

Climate forcing and density dependence in a mountain ungulate population

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The relative contribution of density dependence and of extrinsic stochastic environmental noise in population dynamics is the subject of considerable debated in ecology. There is increasing empirical evidence that changes in climate may have strong effects on the population dynamics of ungulates. Most studies, however, have used climatic variables to study variation in historic population time series. Very few studies have attempted to test the predictive power of specific population models. This paper first estimates the independent contributions of weather (snow depth) effects and density dependence on the population dynamics of Alpine ibex in the Gran Paradiso National Park. The effects of snow depth and density dependence were then included in a simple model which was tested using an out-of-sample prediction test. I contributed extensively to this paper, organizing and proofreading the ibex census database, participating in the choice of hypotheses to be tested, contributing in the biological interpretation of results and in the writing of the manuscript.

RUNNING HEAD Climate Effects in Mountain Ungulates

Climate Forcing and Density Dependence in a Mountain Ungulate Population

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Abstract

Population models in ecology are rarely validated by comparing their predictions to long-term observations of changes in population size. We have used a variety of analytical tools to examine a 45-year time series of annual censuses of Alpine ibex (*Capra ibex*) in the Gran Paradiso National Park in northwestern Italy. This ibex population grew from about 3300 to almost 5000 individuals in the 1980s during a decade of anomalously mild winters, and then began to decline in the 1990s. By 1997, the population size had returned to previous levels. Adult survival apparently increased and adult sex ratio may have changed to slightly favor males during the increase in population density. Yearly changes in total population were correlated with seasonal average snow depth and population density over the 39 years for which climate data were available. Our results show that the ibex population size was limited by both density dependence and deep snow. A model based on these factors fit to the first 19 years of data was used to forecast subsequent changes in total population based on initial population size and yearly snow depth. The model was able to predict the increase and subsequent decline in total population size over the final 20 years of the study, but failed to reproduce population levels after the eruption. Our results suggest that the 1980s episode of population growth was primarily driven by increased adult survival, rather than increased recruitment.

Key words: climate changes and population fluctuations; density dependence; ibex, Alpine; Italy, Gran Paradiso National Park; model validation; population dynamics; time series, population data.

Introduction

Species that live in Arctic or Alpine environments can be particularly sensitive to changes in climate (Post *et al.*, 1999) and may thus face an increased extinction risk (Derocher and Stirling, 1998,1995). It is therefore essential to test the ability of population models not only to explain historic patterns of population fluctuations, but also to predict future changes (Forchhammer *et al.*, 1998a), based on climate projections. Reviews of studies based on long-term monitoring of marked individuals in ungulate populations suggest that juveniles are the age class most likely to be affected by both extrinsic and density-dependent processes, while the survival of adult females is generally unaffected by changes in density or in environmental conditions (Gaillard *et al.*, 1998,2000). Severe winter weather is generally thought to affect juvenile survival by increasing the probability of starvation, through a combination of greater thermoregulatory costs and decreased forage availability because of deep snow (Forchhammer *et al.*, 1998b; Goodson *et al.*, 1991; Portier *et al.*, 1998; Loison and Langvatn, 1998). A rather consistent result of ungulate studies is that high population density increases the age of primiparity, so that fewer age classes of females contribute to recruitment (Gaillard *et al.*, 1998; Fowler, 1987; Langvatn *et al.*, 1996; Gaillard *et al.*, 2000; Jorgenson *et al.*, 1993; Swihart *et al.*, 1998). One goal of our research is to determine whether changes in ibex population size were due to variations in recruitment, as might be expected from these previous works, or to changes in adult survival.

The processes that control animal abundance are of fundamental interest to ecologists. Recently, several studies of ungulates have suggested that an interaction of year-to-year changes in weather and density-dependent changes in survival and reproduction may explain changes in population density in environments where large predators are very rare or absent (Forchhammer *et al.*, 1998b; Langvatn *et al.*, 1996; Sæther, 1997; Post and Stenseth, 1999). Other studies suggest that even in the presence of healthy predator populations or human harvests, climate and density may play an important role in ungulate population dynamics (Post and Stenseth, 1998; Coulson *et al.*, 2000). Studies of feral sheep populations that show strong density-dependent fluctuations have suggested that changes in climate may synchronize the dynamics of populations on separate islands in the same geographical area (Grenfell *et al.*, 1998). Despite the wealth of information that has accumulated, however, tests of the predictive power of specific models using time-series data on population size are rare: researchers have mostly used climatic variables to explain historic variation in population size or in specific vital rates (Solberg *et al.*, 1999; Coulson *et al.*, 2000). The scarcity of attempts to predict population changes stems partly from the rarity of studies that have accumulated the many years of demographic data required by this exercise. For example, the ability of population viability analysis (PVA) models to predict population dynamics over several years is subject to considerable debate (Coulson *et al.*, 2001b; Brook *et al.*, 2000). Here we take advantage of the unusual opportunity of having a 45-year time series of Alpine ibex (*Capra ibex*) counts to test the ability of a simple model based on snow depth and population density to predict changes in total ibex abundance. We first estimate the independent effects of snowfall and density on year-to-year changes in population size for Alpine ibex in the Gran Paradiso National Park, Italy. Having demonstrated that both of these factors affect the population dynamics, we then develop a simple model incorporating both factors. We assess the long-term predictive power of this model using an out-of-sample prediction test: the model parameters are determined using only the first 19 years of data, then the model predictions for the following 20 years of population change are compared with actual changes in ibex abundance.

Numerous techniques have been proposed for statistical detection of density dependence, but these all have potential pitfalls which constrain their usefulness (Holyoak, 1993; Dennis and Taper, 1994; Wolda and Dennis, 1993; Fox and Ridsdill-Smith, 1995; Pollard *et al.*, 1987). To compensate for differences in the strengths and weaknesses of these various techniques, in this paper we have used multiple statistical tests for density dependence. In addition, we have shown the generalization of these tests to the detection of population dependence on extrinsic environmental factors such as climatic conditions.

Methods

Study area and population

The Gran Paradiso National Park (GPNP) was established in 1922 in northwestern Italy (45° 25'N, 7° 34'E), in part to protect the only surviving population of Alpine ibex. The park is bounded on the west by the Parc National de la Vanoise in France. The GPNP is composed entirely of mountainous terrain and is sparsely populated by humans. Alpine pastures, moraines, cliffs, glaciers and rock account for 59% of its 720 km^2 area. Ibex use elevations ranging from about 800 m above sea level to beyond the upper limit of vegetation at about 3200 m. There is no hunting of ibex either inside or outside the park in either Italy or France, and human activities in general are severely limited inside the GPNP. Although there have been a few sightings of both lynx (*Lynx lynx*) and wolf (*Canis lupus*) in recent years, large predators have been absent for most of the past 100 years. There were no reported sightings of wolves during the period of this study, and the first lynx sighting was in 1995. Predation is believed to be a negligible factor in the dynamics of this ibex population, as most ibex die of senescence, starvation or disease (Bassano *et al.*, 1992). The GPNP has been the source of ibex for many reintroductions in the Alps. Yearly removals for transplants never exceeded 1% of the park's ibex, and were of 0 - 0.5% in most years, and are thus assumed to have had a negligible impact on the population. The only other wild mountain ungulate in the park is the chamois (*Rupicapra rupicapra*).

Adult ibex are highly sexually dimorphic. Males weigh about 95-100 kg, about twice as much as females (Giacometti *et al.*, 1997). Like most sexually dimorphic ungulates, ibex are sexually segregated outside the rut, with males four years of age and older in bachelor groups and females, juveniles and subadult males in nursery groups (Gross *et al.*, 1995). Females may produce their first kid at two years of age, although age of primiparity may be delayed in high-density populations, as in most other ungulates (Gaillard *et al.*, 2000). Twins are almost never observed in the GPNP, but have been documented in captivity (Stüwe and Grodinsky, 1987). Ibex are unique among ungulates studied thus far in that the survival of prime-aged adult males does not appear to be lower than that of adult females. Adults of both sexes up to about 10 years of age have been shown to enjoy 95-99% annual survival (Toïgo *et al.*, 1997).

Data

In this study, we attempt to model the variability of the total number of animals counted in the autumn census at GPNP from 1956 to 2000. The GPNP census data and methodological details appear in Appendix A. Daily meteorological observations of minimum and maximum temperature, precipitation, and snow depth were available for most of the study period from two stations within the GPNP. Data for the Serrù station, at an elevation of 2240 m, were available from 1962. Observations at Teleccio lake, elevation 1866 m, were available from 1959. The daily meteorological observations at these two stations were aggregated into seasonal quantities to provide indicators of each year's climate. We also computed the number of days in each winter when the snowpack was deeper than one of two level values, set at the mean winter snow depth for each station (level 1) and at the mean plus one standard deviation (level 2).

Population models

Our aim in this work is to construct simple models for the changes in total ibex population (the number of animals aggregated across gender and age classes). The variable of canonical interest is the relative change in total population size. If the number of individuals in the total population is designated by *n*, then the relative change is y = 1/n dn/dt = d(ln n)/dt = dx/dt, where following convention, we define *x* as ln *n*. For *N* discrete observations, this becomes

$$y_i = x_{i+1} - x_i = \ln(\frac{n_{i+1}}{n_i}), \qquad (1)$$

where the index $i = (1, 2,..., N - 1)$. We wish to determine whether y is affected by climate or density (Dennis and Taper, 1994; Wolda and Dennis, 1993; Vickery and Nudds, 1984; Bulmer, 1975; Pollard *et al.*, 1987). Several simple models of density dependence have been proposed, and here we only consider two basic formulations. The stochastic Gompertz model takes the form:

$$n_{i+1} = n_i \exp\left(a + b \ln n_i + \sigma \epsilon_i\right), \tag{2}$$

in which ϵ_i and σ represent the stochastic contribution from noise and unmodeled processes. Taking the natural log of (2) and recalling the definitions (Eq.1) we have

$$y_i = a + bx_i + \sigma \epsilon_i \tag{3}$$

In contrast, the stochastic Ricker model assumes an exponential density dependence:

$$n_{i+1} = n_i \exp(a + bn_i + \sigma \epsilon_{i.}), \text{ or} \tag{4}$$

$$y_i = a + bn_i + \sigma \epsilon_i \tag{5}$$

In both the Gompertz and Ricker growth models, a population is said to be density-dependent if b is significantly different from zero, as would be expected in the presence of competition for limited resources. Populations with no limiting factors may be expected to grow according to the stochastic growth equation,

$$y_i = a + \sigma \epsilon_i \tag{6}$$

which describes perturbed exponential growth. In the following we explore models that account not only for the effects of density, but also for the effects of climate on the population growth.

Tests for density dependence

Much effort has been devoted to developing statistical methods to detect density dependence in natural mammal populations for which the available time series are limited in duration, corrupted by observational errors, and subject to external influences. We chose three representative methods to detect density dependence. We test whether the null hypothesis that the *b* parameter of Eqs. 3 or 5 is zero against a two-sided alternative. The observed value of *b* is examined to determine how likely it is that it may have been generated by a stochastic process represented by the null model (Eq. 6). The probability distribution function (PDF) of the test statistic under the null hypothesis is estimated by resampling methods such as the parametric bootstrap, jackknife, and random shuffle.

Bulmer's first and second tests (Bulmer, 1975), which assume a Gompertz growth model, attempt to detect excessive correlation between y and x. The two test statistics, R and R^*, are measures of this correlation, and their associated confidence intervals are given by functional fits to the empirically-determined PDF of the null model (Bulmer, 1975). The two test statistics differ in how the compromise between detection and consideration of observational error is set: R is the more powerful of the two tests, but R^* is preferable when there are considerable errors in the data (Bulmer, 1975). The Bulmer technique has been criticized as being ineffective when there is a trend present in the data (Slade, 1977; Vickery and Nudds, 1984). However, despite more than two decades of research to find more robust methods, Bulmer's tests are still valued for their simplicity (Fox and Ridsdill-Smith, 1995).

The randomization technique of Pollard *et al.* (1987) attempts to address the deficiencies of Bulmer's tests. In their work, the test statistic was the likelihood ratio between the stochastic Gompertz density-dependent model (Eq. 3) and a stochastic density-independent model (Eq. 6). Confidence limits were established by generating a large number of surrogate datasets and computing the test statistic for each. The surrogate data were generated by shuffling the x values in (Eq. 3), whereas the y values remained in their original order. This action destroys any serial correlation between x and y. Pollard *et al.* (1987) performed their test by instead shuffling the y values and generating the x series from that shuffled series, but this modification allows us to test multivariate models of density dependence. The number of possible reorderings of x is $(n - 1)!$, which for $n = 44$ is greater than 10^{46}. The chance that two reordered sets in 100,000 are identical is correspondingly small, therefore we do not attempt to assure uniqueness of the shuffled sets.

The technique of Dennis and Taper (Dennis and Taper, 1994; Wolda and Dennis, 1993) has been used to test the stochastic Ricker model expressed by (Eq. 5). The test statistic was again the likelihood ratio between the density-dependent and density-independent models. The method used a ``parametric" bootstrap to establish the empirical distribution of the likelihood ratio by explicitly evaluating the null model. In this case, the null model, the stochastic growth equation (Eq. 6), is fitted to the observed population changes by computing the maximum-likelihood value of the growth parameter α_n. The variance of the null model residuals, σ^2_n, is then computed. Each surrogate dataset is generated by starting at the observed initial population level, and allowing the population to grow stochastically via Eq. 6 using the values of α_n and σ_n and independent Gaussian deviates produced by a random number generator. The confidence interval method proposed by Dennis and Taper can be applied to other proposed forms of density dependence, such as the Gompertz model.

Whereas both methods can be used to estimate the PDF of the test statistic of a density-independent population, the shuffling technique is more parsimonious than the parametric bootstrap since it makes no assumption about the explicit form of the null model. Since the random shuffling technique uses the actual data, the distribution of each surrogate dataset is guaranteed to have the same one-point statistical moments as the original one. These properties are not necessarily preserved for surrogate datasets generated by a parametric bootstrap.

We have evaluated the Gompertz and Ricker models using both techniques for determining confidence limits. In addition, we present results in which the test statistic is not a likelihood ratio, but rather is the value of the b parameter. Use of a model parameter as the test statistic presents an additional concern, because the maximum likelihood methods used to determine the model parameter values are only valid to the extent that the data are distributed normally about their modeled values. This means that the significance levels reported for such tests are only valid if the model residuals are Gaussian. For this work, we proceeded heuristically by exploring significance levels and rejecting models for which the residuals were not acceptably Gaussian.

Model evaluation

Our stochastic formulations, e.g. Eq. 3, and the standard least squares methods used to obtain the maximum likelihood parameter values for them, assume that the residuals are independent samples of a normally-distributed random variable with constant variance σ^2, as expressed by the term $\sigma\,\epsilon_i$. This stochastic process is intended to represent both observational error and processes which are omitted or misrepresented by the model. As a result, one way of identifying a model which fails to resolve critical processes is to examine its residuals for systematic variance. Model parameters obtained using least squares methods are by definition those that minimize the variance of the residuals, but they cannot be considered maximum likelihood parameters if the residuals fail to meet the aforementioned criteria.

We applied five tests to the model residuals. The first two compared the skewness and kurtosis of the residuals following the t-test technique outlined by Sokal and Rohlf (1995: Sec. 7.9). Following Tong (1990), the autocorrelation function (ACF) of the residuals was examined to test the assumption of residual independence. Periodic oscillations or lagged dependencies which are not addressed by a model may appear as significant peaks in the autocorrelation function. Since the ACF members beyond lag 0 of a white noise sequence should themselves be Gaussian I.I.D., Tong recommends that no more than 5% of those elements should exceed the level of $\pm 1.96/\sqrt{N}$, where N is the number of data points in the ACF.

Another test that Tong recommends is the computation of the Lin-Mudholkar (LM) normality statistic (Lin and Mudholkar, 1980), which is based on the principle that the mean and variance of a Gaussian distribution are independent. For normally-distributed residuals, the LM statistic is itself expected to be normally distributed with mean of zero and unit variance, and its significance can therefore be obtained from the normal cumulative distribution function (CDF) with those parameters.

Another common technique for assessing goodness-of-fit is to examine the departures of the empirical cumulative distribution function from the CDF of the normal distribution of which the residuals are assumed to be samples (e.g., Stephens, 1974). We chose to use a variant of the Kolmogorov-Smirnov test proposed by Lilliefors (1967) for cases in which the mean and variance of the residuals are not known in advance. With the exception of the examination of the ACF, which is an informal procedure, all of these measures of normality were tested against two-sided alternatives at the $a = 0.05$ level.

The time series of residuals r is related to the observed population changes y and the modeled population changes m as $y_i = m_i + r_i$, so the variance of y can be computed as

$$\mathrm{var}(y) = \mathrm{var}(m) + \mathrm{var}(r) + 2\mathrm{cov}(m, r). \tag{7}$$

This expresses the apportioning of variance of y among the deterministic variance $\mathrm{var}(m)$, the variance of the residuals $\mathrm{var}(r) = \sigma^2$, and the model-residual covariance $\mathrm{cov}(m, r)$. This covariance term tends to zero in the limit of an infinitely-long series of independent residuals, and for models whose residuals are acceptably Gaussian it becomes negligibly small. A convenient way to quantify model performance is to compute the proportion of observed variance which it explains

$$R^2 = \frac{\mathrm{var}(m)}{\mathrm{var}(y)}. \tag{8}$$

This multiple R^2 can be used to compare the explanatory power of different models, but it does not provide an unbiased means of comparing models having different numbers of free parameters. We instead use the Akaike Information Criterion (AIC), an unbiased estimator of the expected log likelihood l of a given model, corrected for the number of free parameters p in the model: AIC = $-\ 2l + 2p$ (Sakamoto *et al.*, 1986). Akaike (1974) suggests that when comparing two models, the one with the lower AIC should be selected.

Results

Changes in ibex population and in snow depth

During the first 28 years of the study period, the total number of ibex seen in the autumn census counts averaged about 3400 and ranged from about 2600 to about 4000 with no visually apparent trend. Although oscillations with periodicities of about 3 and 8 years appear to be present (von Hardenberg *et al.*, 2000), the time series is too short to make a determination of the significance of those cycles. From 1982 to 1990, ibex counts increased steadily and peaked at almost 5000 in 1993. The population then began to decline, and by 1997 it had returned to levels characteristic of those before this event. Changes in different sex-age classes generally paralleled each other over time (Fig. 1), although in later years the proportion of yearlings appeared to decline.

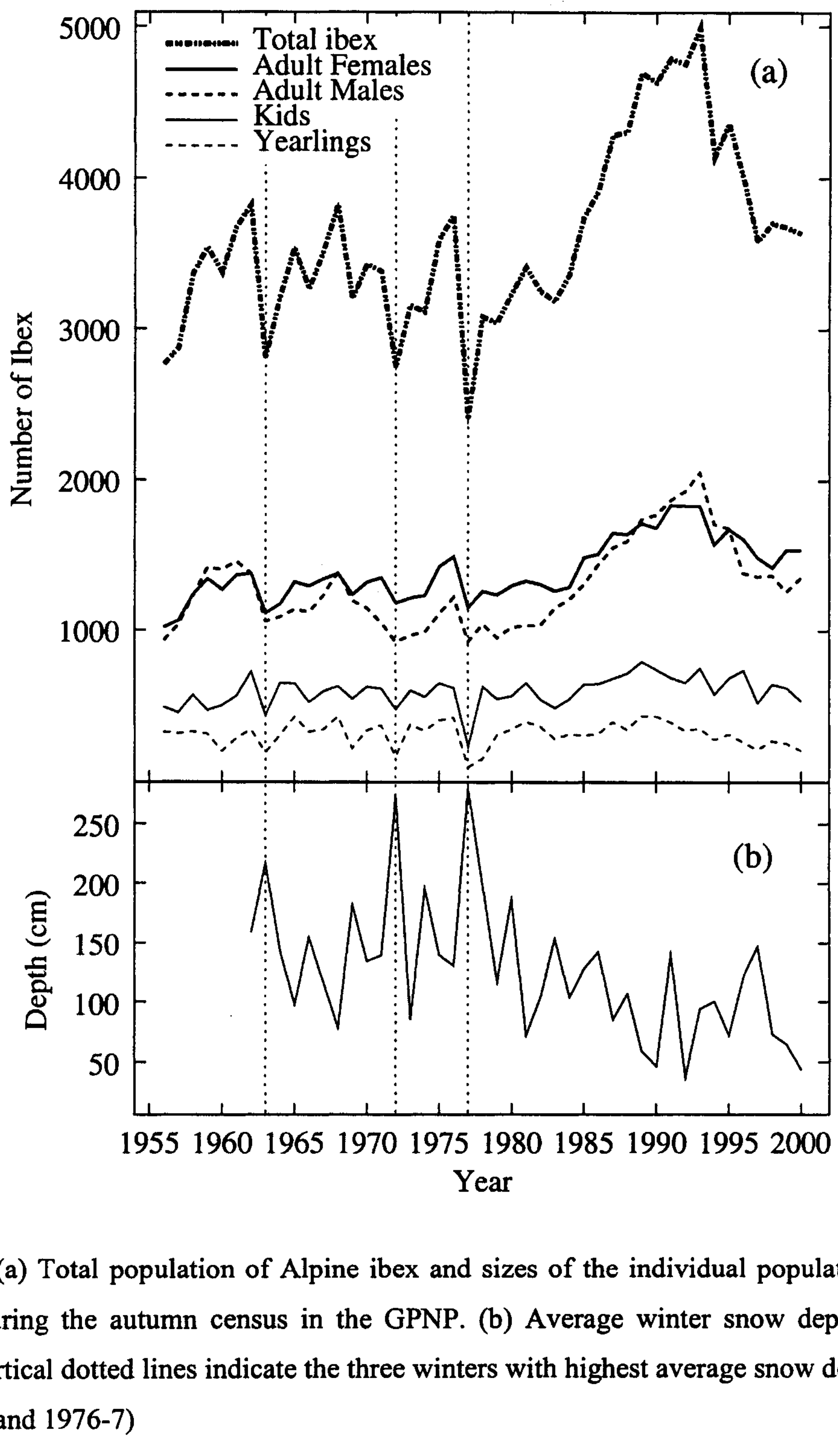

Figure 1: (a) Total population of Alpine ibex and sizes of the individual population classes counted during the autumn census in the GPNP. (b) Average winter snow depth at Serrù station. Vertical dotted lines indicate the three winters with highest average snow depth (1962-3, 1971-2, and 1976-7)

There is a clear univariate correlation ($r = -0.48$) between year-to-year changes in population size and average winter snow depth at the Serrù station (Fig. 2a). A plot of population change versus density (Fig. 2b) suggests that there was a stronger dependence on density in years with deep snow. In particular, three deep-snow winters between 1962 and 1977 were associated with population declines of more than 15%, and the steady increase from 1982 to 1993 (Fig. 1) occurred during a period in which the average winter snow depth was lower than in the previous period ($p < 0.001$, Wilcoxon rank sum test): between 1962 and 1982 the average snow depth was 152±58 cm, whereas between 1983 and 1999 it was 92±36 cm. These observations suggest a climatic amplification of density dependence, an effect which is investigated in the following sections. One possible way that this amplification may manifest itself is by a threshold effect, in which b of (Eq. 3 or 5) would take on two different values, one in mild winters and one in harsh winters.

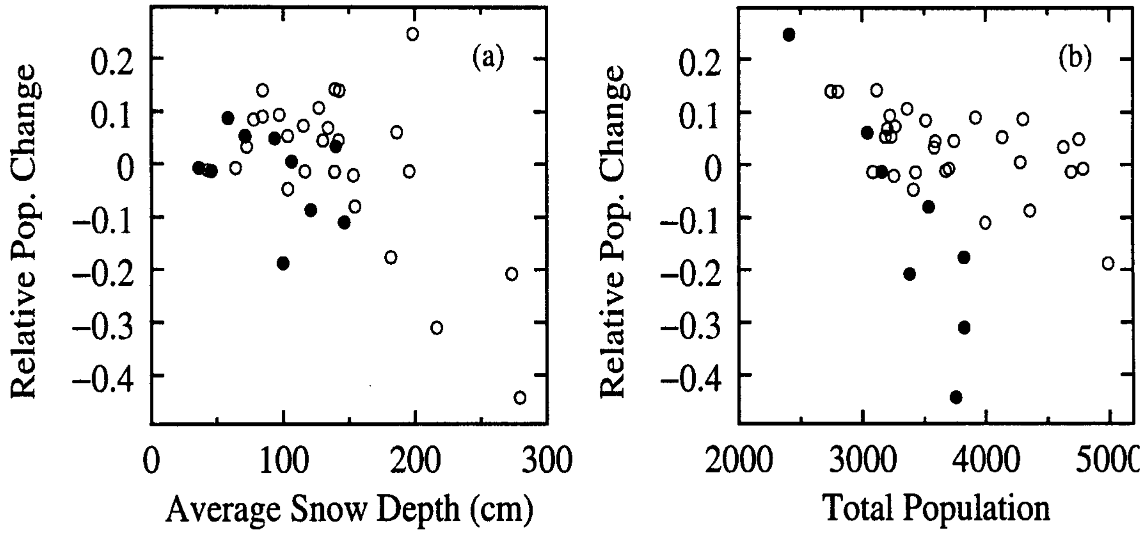

Figure 2: (a) Relative change in ibex population size, y of Eq. 1, plotted against average winter snow depth at Serrù station. Filled circles represent years for which the total ibex population, N, was large (greater than half a standard deviation above mean). (b) Relative population change plotted against population size. Points are shaded if the annual mean winter snow depth at Serrù is more than half a standard deviation above the average.

Modeling density dependence and the effects of snow depth

We first attempted to detect density dependence without the effects of climate using the stochastic Gompertz and Ricker models discussed in *Population models.*. To detect direct density dependence-i.e., a non-zero value of the parameter b in Eq. 3 or Eq. 5-we employed the Bulmer test, the randomization test and the parametric bootstrap.

These three tests were performed on our dataset of 44 year-to-year differences. The Bulmer tests do not suggest density dependence in this population, since the R and R^* values are above the estimated first and fifth percentiles (Table 1). The parametric bootstrap and shuffle results are inconclusive, however. Of the eight permutations of the tests, two indicate density dependence at the $\alpha = 0.05$ level (Table 2).

Table 1. Results of Bulmer's first (R) and second (R^*) tests (Bulmer, 1975) for ibex total population changes in the GPNP between 1956 and 1997. The R test is more powerful, but the R^* test is more conservative in the face of errors in the observations. Also given are the estimated first and fifth percentiles of the density-independent distribution.

Test Statistic	Value	First Percentile	Fifth Percentile	Density Dependence
R	2.105	1.316	1.824	not detected
R^*	-0.103	-0.333	-0.243	not detected

Table 2. Significance of density dependence tests for year-to-year changes in total autumn ibex counts based on a distribution of 100,000 surrogate datasets. The tabulated P value is the two-sided probability of incorrectly concluding that the population is density dependent. "LR" indicates that the test statistic is a likelihood ratio of the conditional probabilities of the test model and the null model (Dennis and Taper, 1994; Pollard *et al.*, 1987), whereas "*b*" is the model density dependence parameter. R^2 is the proportion of variance in the observed population changes which is explained by the model, and AIC is the Aikaike Information Criterion value. The results of the skewness, kurtosis, Lin-Mudholkar (LM), and Lilliefors Kolmogorov-Smirnov (LKS) normality tests are given as P values against a two-sided alternative hypothesis of non-normality, so a significant result indicates that the residuals are not plausibly Gaussian. P values significant at the $\alpha = 0.05$ level are underlined. In the last column, the percentage of residuals autocorrelation function (ACF) members above the expected level is listed (see *Methods: Model evaluation*).

		P *value*						Normality tests		
Model	Test statistic	Bootstrap	Shuffle	R^2	AIC	Skewness (P)	Kurtosis (P)	LM (P)	LKS (P)	ACF (%)
Gompertz Eq. 3	LR	0.0614	0.1055	0.175	62.1	<0.0001	<0.0001	0.0004	0.2827	2..3
	b	0.0579	*0.0047*							
Ricker Eq. 5	LR	0.1345	0.2486	0.15	60.7	<0.0001	<0.0001	0.0039	0.1676	2..3
	b	0.2010	*0.0131*							

While these results confirm that density plays a role in the dynamics of this ibex population, analysis of the model residuals (See *Model evaluation* above) reveal that density, whether in the form of Eq. 3 or Eq. 5, is not by itself a sufficient explanator of the observed population changes. The results of the residuals analysis for the stochastic Gompertz model (Eq. 3) are shown in Table 2 and in Appendix B. Neither model explains more than 18% of the variance in observed population changes, and some of the residuals in y are suspiciously large. While only 2.3% of the ACF elements (1 of 44, excluding the zero-lag correlation) exceed the level of ±0.3, an extreme value of the ACF at three year's lag, and the systematically high values at 14-20 years' lag indicate that there may be deterministic variations that are incompletely treated by this model. The residuals also do not pass the skewness, kurtosis, and LM tests for normality. Together, these results indicate that the residuals are neither Gaussian nor uncorrelated, and we therefore conclude that density dependence alone does not satisfactorily explain the observed population changes.

Delayed density dependence

We also explored the possibility that population changes depend on time-delayed dynamics. Density variables with lags l from one to 10 years were tested with adapted versions of Eq. 3 and Eq. 5. The P values for this test reveal that delayed density dependence is at best a second-order effect. The 3- and 8-year cycles found in earlier spectral analyses of this population (von Hardenberg *et al.*, 2000; Bassano *et al.*, 1992) are suggested by these results. Perhaps most importantly, the one-year lagged results are among the least significant, suggesting that the high autocorrelation of residuals at one year's lag (Appendix B) is probably not due to delayed density dependence.

Climate effects

To ascertain whether climate variations affect the abundance of Alpine ibex, we tested the dependence of relative population change on climate without the effects of density dependence. Because counts were conducted in autumn, total population changes are compared with climate from the previous summers and winters. For instance, the change in population between 1969 and 1970 is compared with climate in the winter of 1969-70 and the summer of 1970 (Appendix C).

The winter total precipitation and all the snow depth variables have significant negative correlations with changes in total ibex population. This relationship can be clearly seen in Fig. 1. At both stations, the winter precipitation variables are significantly correlated with year-to-year changes in total ibex population. Since these variables are not independent, however, we choose only one of them for further analysis. Building on the observation of Nievergelt (1966) that ibex avoid slopes with deep snow depth, we reason that the snow depth variables are likely to be more directly relevant to population dynamics than total precipitation. Although the number of days with above-average snow depth has a slightly more significant correlation with changes in ibex population, we have chosen annual average snow depth for further analysis. This quantity is simpler to compute, and does not depend on the definition of the climatological mean snow depth at a station. The interdependence of all the climate variables and the resulting power of average snow depth to represent climatic conditions was also confirmed by an exploratory principal components analysis of all the environmental indicators (not shown). Although both spring and summer total precipitation are negatively correlated with changes in the ibex population, we did not further explore the roles of these variables.

We also tested for lagged climate dependence, with the same procedures used for lagged density dependence (*Delayed density dependance,* above). Significant positive correlations with relative changes in ibex population were detected in all four winter precipitation variables at lags of both one and four years.

Lagged exogenous variables may act as a proxy for population density if extreme climatic conditions in one year hamper population growth, setting the stage for greater population growth in later years due to reduced competition. In analyses which include both climate and density dependence (next section), one- and four-year lagged snow depth is no more of an improvement to the population growth models than is unlagged snow depth. We thus conclude that lagged climate forcing does not make a significant contribution to the population dynamics.

For explaining changes in ibex population, a model with snow depth dependence alone,

$$y_i = a + cv_i + \sigma \epsilon_i \tag{9}$$

is a significant improvement over a simple random walk. This model displays normally-distributed residuals for data at either meteorological station, with no significant peaks in the ACF (Appendix B). However, it only explains between 16% and 25% of the observed population change variance.

Interaction of climate and density

Although snow depth plays a strong role in the dynamics of the Alpine ibex population, it leaves much of the deterministic variability in ibex abundance unexplained. Bootstrap and jackknife significance tests indicate that some mechanism for including both snow and density is warranted. We therefore analyzed models including density, snow depth, and a term representing the interaction of these two quantities (either $v_i n_i$ or $v_i x_i$). These complete models are

$$y_i = a + bn_i + cv_i + ev_i n_i + \sigma \epsilon_i\,, \quad \text{and} \tag{10}$$

$$y_i = a + bx_i + cv_i + ev_i x_i + \sigma \epsilon_i. \tag{11}$$

Other "subset" models formed by dropping individual terms from the complete models were also tested. However, as suggested by Fig. 2 the dynamics of this population might be well represented by a two-state system, or threshold model. In a threshold model there are two different sets of parameter values, one set for years with low snow depth and one set for years with high snow depth. Thus we also investigated a suite of threshold models, for which *b*, *c*, or *e* take on different values in different years (the *a* and *σ* do not depend on snow depth). The threshold value chosen is that of Fig. 2, namely the mean snow depth plus ½ the sample standard error, about 154 cm. The details of model performance clearly depend on the choice of threshold value. We conducted a limited sensitivity study on this matter, and this particular value yielded the best performance in that study. The complete list of climate-density models is given in Table 3 and in Appendix D.

Table 3. Performance of complete list of climate- and density-dependent models.

	Terms‡							Normality tests				
Model†	*b*	*c*	*e*	Dens. form§	No. par.‖	R^2	AIC	Skew. (*P*)	Kurt. (*P*)	LM (*P*)	LKS (*P*)	ACF (%)¶
Regular												
(D.1)	✓	✓	✓	*n*	4	0.62	**−78.3**	0.39	0.97	0.37	>0.2	0.0
(D.2)	✓	✓	✓	*x*	4	0.65	**−81.2**	0.46	0.87	0.43	>0.2	0.0
(D.3)		✓	✓	*n*	3	0.62	**−80.3**	0.39	0.98	0.37	>0.2	0.0
(D.4)		✓	✓	*x*	3	0.65	**−83.2**	0.46	0.87	0.43	>0.2	0.0
(D.5)	✓		✓	*n*	3	0.59	−77.3	0.61	0.67	0.57	>0.2	2.6
(D.6)	✓		✓	*x*	3	0.59	−77.0	0.92	0.49	0.91	>0.2	2.6
(D.7)	✓	✓		*n*	3	0.55	−73.5	0.76	0.79	0.75	>0.2	2.6
(D.8)	✓	✓		*x*	3	0.58	−76.4	0.95	0.51	0.94	>0.2	2.6
(D.9)			✓	*n*	2	0.46	−68.5	0.62	0.91	0.59	>0.2	0.0
(D.10)			✓	*x*	2	0.27	−56.8	0.79	0.28	0.79	>0.2	0.0
Threshold												
(D.11)	✓	✓	✓	*n*	7	0.8	**−97.6**	0.69	0.31	0.62	>0.2	2.6
(D.12)	✓	✓	✓	*x*	7	0.8	**−97.0**	0.66	0.31	0.58	>0.2	2.6
(D.13)		✓	✓	*n*	5	0.75	**−92.5**	0.38	0.96	0.36	>0.2	2.6
(D.14)		✓	✓	*x*	5	0.75	**−92.2**	0.49	0.95	0.47	>0.2	0.0
(D.15)	✓		✓	*n*	5	0.69	−83.6	0.38	0.45	0.29	>0.2	**5.1**
(D.16)	✓		✓	*x*	5	0.67	−81.1	0.55	0.29	0.44	>0.2	**5.1**
(D.17)	✓	✓		*n*	5	0.57	−71.2	0.47	1.00	0.47	>0.2	2.6
(D.18)	✓	✓		*x*	5	0.65	−78.8	0.48	0.40	0.38	>0.2	**5.1**
(D.19)			✓	*n*	3	0.47	−67.2	0.36	0.61	0.38	>0.2	2.6
(D.20)			✓	*x*	3	0.3	−56.5	0.34	0.03	0.44	>0.2	0.0

Notes: Normality tests, R^2, and AIC are as defined in Table 2. The four best AIC scores in the Regular and Threshold model groups are boldface.

† Model numbers correspond to equation numbers in Appendix D. Models D.1 and D.2 are the complete models given by Eqs. 10 and 11 in the text.

‡ A check mark in the *b*, *c*, or *e* column means that the corresponding term from the complete models, D.1 or D.2 (Eq. 10 or 11), was included.

§ The form of the density variable: *n* is the total ibex counts and *x* is log(*n*).

‖ The number of model parameters.

¶ The percentages in boldface differ significantly from the expected values of zero at $\alpha = 0.05$.

Models fit to Serrù climate data consistently outperform the same models fit to Teleccio data, possibly because the Serrù station is at a higher altitude and thus reflects more accurately the snow depth in ibex habitat. Only results for the Serrú climate data are presented here.

Four threshold models using Serrù data (Eqs. D.11-D.14 in Appendix D) , stand out from the others. They account for about three-quarters of the input variance, and their residuals pass the battery of normality tests (Table 3, Appendix B). These candidate models were retained for further analysis.

Testing model predictive ability

A powerful test of a model's performance is whether it can predict population changes in a later period when its parameter values are determined only from data limited to an earlier period ("out-of-sample" prediction). Model predictions of y are perturbed with random fluctuations ϵ as in (10), with standard deviation σ determined from the residuals of the trained model fit. Since this is a stochastic prediction, this procedure is carried over an ensemble of 1,000 trials to get stable statistics on the prediction.

We tested how well the candidate threshold models (Eqs. D.11-D.14 in Appendix D) predicted the population variability during the evaluation period of 1981-2000 with parameter values determined from the training period 1961-1980. We present two measures of out-of-sample performance: a bias parameter and a confidence interval width. The bias parameter is the average over the evaluation period and over the ensemble trials of the deviations of the model predictions from observations:

$$\beta = \frac{1}{1000}\sum_{j=1}^{1000}\left[\frac{1}{19}\sum_{i=1}^{19}(\hat{n}_i^j - n_i)\right], \tag{12}$$

where j is the index of the ensemble trial, and $\hat{n}_i^j$ is the jth ensemble prediction of n_i. The confidence interval width is the mean over the evaluation period of the difference between the 95th and 5th percentiles of the 1,000 member ensemble (Table 4). While these evaluation criteria can be computed for both y and n, the differences between models are most evident in n space.

Two models, the 5-parameter model (D.13) and the 7-parameter (D.11), outperform the other models in this out-of-sample test (Fig.3). Both of these models do an adequate job of reproducing the mid-1980s population eruption. Due to its better out-of-sample performance, we regard the 5-parameter model as more robust than the 7-parameter model. In particular, its parsimony may make it less susceptible to the danger of overfitting.

Table 4. Performance of candidate models on the out-of-sample test. The bias parameter and confidence interval are defined in *Results: Testing model predictive ability*. Both parameters have dimension of number of ibex.

Model	Bias, β	90% CI width
(D.11)	56.1	1040
(D.12)	275	1290
(D.13)	9.7	979
(D.14)	143	1100

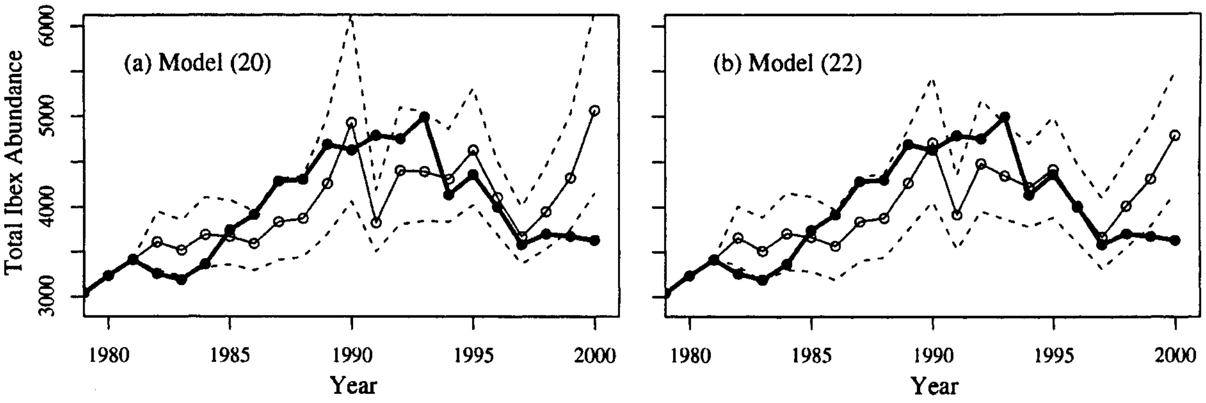

Figure 3. Out-of-sample prediction: predictions of two of the candidate models trained using only the first half of the census and Serrù climate data (19 years, from 1962-1980). Panel (a) is for model D.11, and panel (b) is for model D.13. (models are defined in Table 3, and their expressions are given in Appendix D). The model predictions of the relative population change, *y*, have been translated into *n*, the resultant total population size, so that model performance in reproducing the eruption is more evident. In both plots, observations are shown with a thick line, the thin solid line is the mean of 1000 stochastic predictions, and the dashed lines are the 5th and 95th percentiles of those predictions.

Equilibrium Density

The final two candidate models admit equilibrium solutions of no growth for $y_i = 0$. The resulting equilibrium density (ED) for model D.11 is

$$n_i^* = \begin{cases} \exp\left(-\dfrac{a + c_l v_i}{b_l + e_l v_i}\right) & \text{if } v_i < v_{\text{thresh}} \\ \exp\left(-\dfrac{a + c_h v_i}{b_h + e_h v_i}\right) & \text{if } v_i \geq v_{\text{thresh}} \end{cases} \tag{13}$$

where the l and h indices represent parameters for low and high snow-depth years respectively . Similarly, the model D.13 has an ED of

$$n_i^* = \begin{cases} \exp\left(-\dfrac{a + c_l v_i}{e_l v_i}\right) & \text{if } v_i < v_{\text{thresh}} \\ \exp\left(-\dfrac{a + c_h v_i}{e_h v_i}\right) & \text{if } v_i \geq v_{\text{thresh}}. \end{cases} \tag{14}$$

The ED is analogous to a dynamic carrying capacity which changes as a function of snow depth v_i. It is interesting to consider this quantity for management purposes, as it estimates the instantaneous number of animals which the habitat could support.

Historical n_i^* values compare favorably with observed total ibex counts (Fig. 4). Changes in the ED largely mirror the population variations before the eruption, and increase dramatically in the 1980s. Mismatches between the ED and the actual ibex population, both in the few years around 1975 and in the years of the eruption, serve as a reminder that although these simple models perform remarkably well at qualitatively reproducing the gross features of the population variability, they are unaware of the more complex dynamics of this population (See *Discussion*).

Because of the long life expectancy of ibex (Toïgo *et al.*, 1997), a population's reaction to a sudden change in the environment would not be instantaneous, but would take some time to manifest itself in full (e.g., Murray, 1993). The inherent delay in the reaction of a population to changes in its environment is functionally equivalent to a low-pass filter, which attenuates higher frequencies while leaving longer-term variability untouched (Oppenheim and Schafer, 1989). In Fig. 4, the ED has been smoothed by low-pass filtering the snow depth data used in (32) and (33). Estimates of the ED obtained in this fashion should be considered at most suggestive of the real carrying capacity of the system.

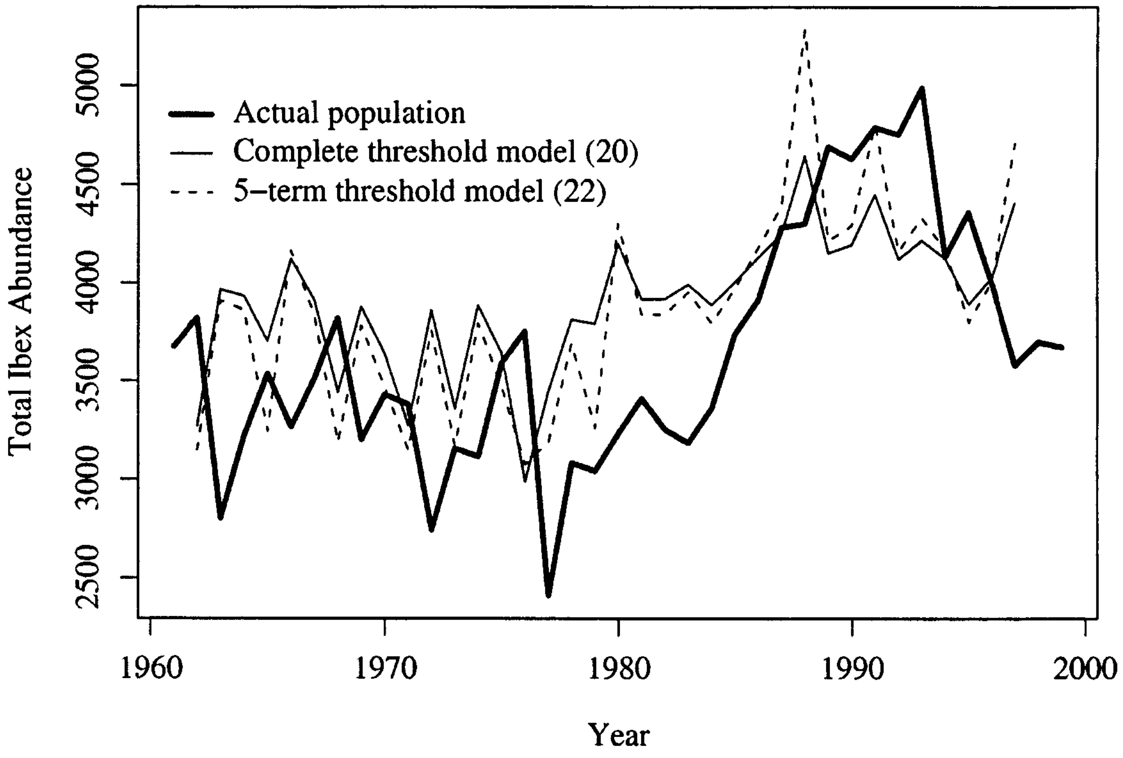

Fig. 4. Equilibrium density (ED) population level, n_i^*, for the two final candidate models (D.11 and D.13). Also shown is the actual total ibex count (thick solid line) during the study period. The ED values were computed using Eqs. 13 and 14 with a low-pass filtered series of average snow depths at Serru` in order to suppress high-frequency variability (see *Results: Testing model predictive ability*). A six-point Hann window was used for the low-pass filtering of snow depths.

Discussion

Changes in the total Alpine ibex population in the GPNP are negatively affected by population density, by winter snow depth, and by the interaction between these two variables. Our results suggest that the population growth in the 1980s was mainly due to increased adult survival, although the series of mild winters may have also increased recruitment (Appendix E). We will first discuss the implications of these results, then consider the limitations of our study.

Models in ecology usually describe historical changes in population size rather than predict them, but this study tests the predictive power of population models including density-dependent and climate effects over a period of about 20 years (about three ibex generations). Information on total counts and snow depth collected over the first part of the study successfully predicts the population increase of the 1980s and the subsequent decline in the 1990s, but fails to reproduce the apparent stability of the population after 1997. It is striking that the models can predict this dramatic population change, since their parameters were determined only by total population size and snow depth in the first half of the study period, when the population remained well below the levels attained in the second half.

Variability of vital rates affecting recruitment could explain some of the observed population changes. Reviews of ungulate population dynamics (Gaillard *et al.*, 1998; Sæther, 1997; Gaillard *et al.*, 2000) suggest that juvenile survival, and to a lesser extent fecundity, especially of young females, can vary considerably from year to year. Recently, Gaillard *et al.* (2000) detailed a framework of incremental responses in vital rates as population density increases. This pattern begins with an increase in the age of primiparity, followed by increased juvenile mortality, then increased yearling mortality and in extreme cases, decreases in adult fecundity and survival. Because climatic extremes tend to have a stronger impact on population growth at high rather than at low population density (Forchhammer *et al.*, 1998b; Sæther, 1997; Post *et al.*, 1999; Portier *et al.*, 1998; Post and Stenseth, 1999; Coulson *et al.*, 2000), we expected sharply reduced juvenile survival during high density periods associated with harsh winters. However, this does not appear to be the case.

Indeed, changes in juvenile survival cannot explain the substantial increase in the ibex population after 1985. Although the number of kids counted increased with the number of adult females, the number of yearlings was only slightly higher during the population increase than before (Fig. 1). During the eruption, from about 1983 to about 1990, adult survival increased while weaning success and kid survival remained stable or decreased (Appendix E). Consequently, the increase in population following years of low snow depth could be attributed mostly to increased adult survival. This conclusion is at odds with evidence from other ungulate studies suggesting that adult mortality is relatively insensitive to changes in climatic conditions because adults tend to be in better body condition than juveniles and have a lower surface to mass ratio (Gaillard *et al.*, 2000; Jorgenson *et al.*, 1997, but see Gunn *et al.*, 1989; Fryxell, 1987; Owen-Smith, 1990). As Gaillard *et al.* (1998) pointed out, adult survival has a very high elasticity. Therefore, a small increase in this vital rate over a short run of years could lead to a substantial increase in population growth.

Deep snow may cause direct mortality of all age classes in the guise of avalanches. Alternatively, snow depth may have had a relatively stronger effect on the survival of senescent ibex, those older than about 10-12 (Toïgo *et al.*, 1997). The absence of predation on adults and the high survival of prime-aged ibex likely meant that a substantial proportion of the adult ibex reach senescence. It is possible that a series of years with low snow depth increased the average longevity of adults and thus the average age of the population. Very little is known about the effects of density and climate on survival of senescent adult ungulates, partly because sample sizes of known-age old individuals accumulate very slowly (Gaillard *et al.*, 2000). Some studies, however, do suggest that older ungulates are sensitive to unfavorable weather: in greater kudu (*Tragelaphus strepsiceros*) the survival of older females is affected by drought more than the survival of prime-aged females (Owen-Smith, 1990) and in Dall sheep (*Ovis dalli*), severe winter weather affects the survival of old but not prime-aged ewes (Burles and Hoefs, 1984). More recently, Garrott *et al.* (2003) showed that in an unhunted population of elk, the survival of females older than 9 years declined with increasing snow depth, while the survival of younger females was only affected by an exceptionally severe winter. In ibex, the adult male aggregate survival rate was substantially more variable than that of adult females (Appendix E), indicating that survival of adult males may be more sensitive to environmental conditions.

The rapid population decline after 1993 despite the lack of an accompanying resurgence in snow depth is a potential signature of density-dependent dynamics. Both kid survival and weaning success may have begun to decline in the late 1980s, and therefore the decline may have been partly due to lower recruitment. As indicated by Festa-Bianchet *et al.* (2003), an increase in average ibex age would likely cause an increase in average adult mortality, which could lead to the appearance of density-dependent mortality when none may exist. Without data on known-age animals, we cannot properly assess the level of density-dependence in adult survival.

The inability of the threshold models' out-of-sample predictions (See *testing model predictive ability,* above and Fig.3) to fully reproduce observed population variability in the 1997-2000 period is consistent with the attribution of the eruption to enhanced adult survival. Since the simple models described in this paper do not take the age structure of the population into account, they cannot reproduce the effects of increased mortality due to the aging of the population. The apparent density-dependent mortality suggested by Festa-Bianchet *et al.* (2003) could explain much of the decrease in population size from 1993-1997. The predicted sharp decline in population growth in 1991 (Fig. 3b) is caused by a moderately high winter snow depth which has a disproportionately strong effect because of its nonlinear interaction with the high population level in 1991 (Fig. 1). This failure could be attributed to several causes, but a likely candidate is the model's inability to consider the cumulative effects of successive years of low snow depth (Garrott *et al.*, 2003), which could render the population more able to withstand a single year of high snowfall. The nonlinear model also predicts a strong rebound of the population in the period 1997-2000, due to continued low snow levels during a period of low density. Recent censuses indicate, however, that while the adult portions of the population may be recovering, kid survival remains depressed (Appendix E). This may be due to a lagged effect of high density in the 1990s on kid survival or on age of primiparity. If lag effects only occur following years of very high density, models like the ones discussed here should be expected to fail.

Other studies of sexually dimorphic ungulates typically report that adult sex ratio becomes increasingly female-biased as density increases (Jorgenson *et al.*, 1998; Clutton-Brock *et al.*, 1997), although adult male mortality is generally not density-dependent (Clutton-Brock *et al.*, 1997; Jorgenson *et al.*, 1997). In the ibex population observed by Toïgo *et al.* (1997), no significant sexual differences in survival of prime-aged adults was found, a result which suggests an even sex ratio. Population counts of ibex in the GPNP suggest a weak positive relationship between population size and adult sex ratio (Fig. 5).

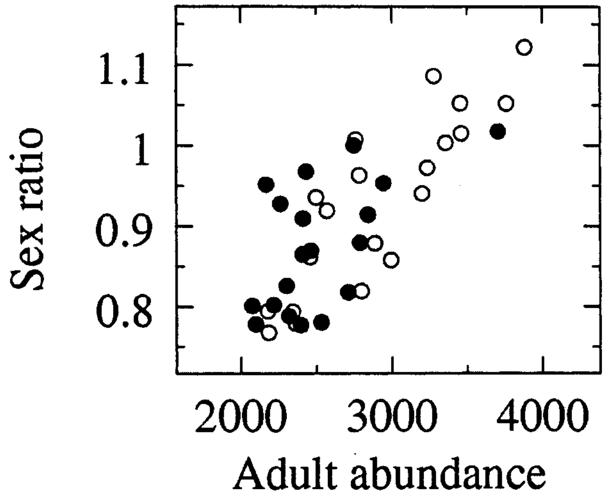

Figure 5. Adult sex ratio (ratio of males to females) plotted against total adult ibex ($r = 0.79$). Empty circles represent years with average snow depth at Serrù below the mean of 126 cm; filled circles represent years with average snow depth above the mean. The correlation between sex ratio and adult ibex abundance in years of low snow depth (empty circles) is stronger ($r = 0.86$) than that for years of high snow depth (filled circles, $r = 0.58$).

A bias in the sex ratio toward males during the eruption would be consistent with a scenario in which adult male ibex react more strongly than adult females to changes in climatic conditions. Ibex males likely enter the winter in worse body condition than females because of strenuous activity during the rut. A similar situation was reported for red deer in Norway, where the survival of adult stags was affected by spring weather, while the survival of adult hinds was not (Loison and Langvatn, 1998). A series of mild winters could therefore have a greater positive effect on male survival than on female survival. A detailed long-term study of marked individuals initiated in 1999 will address the hypothesis that adult males, and possibly older males in particular, are more sensitive to climatic conditions than adult females.

A serious limitation of our data set is the low level of resolution of age classes in the census data. Age structure plays an important role in ungulate population dynamics, because of strong age-dependent schedules of reproduction and survival (Loison *et al.*, 1999; Bérubé *et al.*, 1999; Gaillard *et al.*, 2000). As discussed above, the shortcomings of the models presented in this paper may be in part due to the fact that they have no means of considering the age dependence of important processes (Coulson *et al.*, 2001a).

In conclusion, our analysis of the time series of ibex counts in the GPNP reveals that an interaction of density and climatic conditions was responsible for most of the interannual change in total population. A simple model did a satisfactory job of reproducing observations, and predicted a major change in the total population size over a period longer than two ibex generations. Changes in ibex density during the population eruption were apparently due to changes in adult ibex survival and may indicate an overall aging of the population. The rapid decline in ibex counts from 1993-7 may be due to density-dependent mortality of senescent adults. Unlike other species of sexually dimorphic ungulates, this ibex population does not show an increasingly female-biased adult sex ratio as density increases.

It appears that for about 25 years, the GPNP ibex population was mostly limited by the episodic occurrence of harsh winters characterized by large amounts of snowfall. When snow accumulations decreased after 1982, the population increased substantially. Other long-term studies of ungulate population dynamics recognize a strong role of climate (Solberg *et al.*, 1999; Portier *et al.*, 1998; Coulson *et al.*, 2000; Messier, 1994,1991). Density dependence may not be detected if extrinsic factors prevent populations from reaching densities where food limitation affects vital rates (Crête and Courtois, 1997). The Alpine ibex population at GPNP may have entered a dynamic regime in which density dependence, possibly including new lag effects, will play a different role over the coming decade than it has in the past.

The results of our study imply that changes in the amount of snow falling on the Alps could have drastic effects on ibex populations. At this time, predictions about regional effects of global climate change are limited, and it is generally agreed that more accurate predictions for high-altitude ecosystems will depend on future generations of climate models and data analysis systems (See e.g. `http://www.clivar.org`). Ibex and other Alpine ungulates may provide sensitive indicators to the advance effects of any such changes (Forchhammer *et al.*, 1998b; Post *et al.*, 1999), and thus populations such as this one deserve careful monitoring over the coming decade. In addition, dramatic population changes of such a large herbivore would have profound long-term consequences for several other components of the alpine ecosystem.

Acknowledgements

We thank the two generations of Park Wardens who conducted the ibex counts that provided the basis for this paper. Our research is financed by the Gran Paradiso National Park and by the ISI Foundation. MF-B's research program is supported by the Natural Sciences and Engineering Research Council of Canada and by the Italy-Québec Scientific Exchange Program. L. Prezioso, M. E. Picollo, and J. von Hardenberg assisted with processing the meteorological observations. We thank J. Bucci, T. Coulson, J.-M. Gaillard, M. Gatto and J. Spiesberger for helpful comments on the manuscript. We are also thankful to A.E.M Torino for kindly providing us with the climatic data from the Teleccio and Serrú meteorological stations.

Appendixes A-E can be found in ESA's Electronic Data Archive: *Ecological Archives* E085-043. http://www.esapubs.org/archive/ecol/E085/043/default.htm

CHAPTER III

Methodological notes

SECTION IIIA

A method to weigh free-ranging ungulates without handling

Bruno Bassano, Achaz von Hardenberg, Fanie Pelletier, Giacomo Gobbi

Wildlife Society Bulletin (2003) *31(4),* 1205-1209

Body mass is an extremely important phenotypic trait in the life history of ungulates. The measurement of this variable in free-ranging populations is, however, not trivial. This paper presents an innovative method to weight free-ranging ungulates without handling them. This method was used extensively to obtain the body mass data used in the papers presented in Sections Ia end Ic of this thesis. Although B.B. invented and developed the proposed method, my contribution to this study was central. I tested the method in Alpine ibex, planned and performed statistical analyses, organized the outline of the manuscript and wrote extensive parts of it.

RH: A method to weigh ungulates • Bassano *et al.*

A method to weigh free-ranging ungulates without handling

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Fanie Pelletier, Département de biologie, Université de Sherbrooke, J1K 2R1 Sherbrooke, PQ Canada.

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Abstract

There are a limited number of studies able to document individual body mass changes in free-ranging ungulates due to difficulties and risks associated with repeated captures of wild animals. We tested a remotely controlled platform scale on Alpine ibex (*Capra ibex*) and bighorn sheep (*Ovis canadensis*). Animals were baited onto the platform scale with salt, and weights were recorded by observers with binoculars and spotting scopes. We found this method was both accurate and effective and suggest it has application for other studies on ecology and management of wild ungulates.

Key words: bighorn, body mass, *Capra ibex*, *Ovis canadensis*, scale, ungulates, weight

Introduction

Individual body mass plays an important role in the life history and population dynamics of ungulates (Festa-Bianchet *et al.* 1997, Réale and Festa-Bianchet 2000). Low body mass has been associated with decreased survival (Festa-Bianchet *et al.* 1997, Loison *et al.* 1999) and decreased fertility (Albon *et al.* 1983). Consequently, many fundamental and applied studies of ungulates would benefit from accurate, multiple measurements of individual weights over 1 season and in different years. Repeated measurement of body mass of wild ungulates, however, is difficult, normally requiring repeated capture and handling. Capture operations are expensive, time consuming, and often involve some risks to animals. Capture-related mortality rates can be high. For example, in white-tailed deer (*Odocoileus virginianus*) mortality ranged from 2.0–20.7% (Haulton *et al.* 2001). Repeated handling of individuals can result in acute mortality from injury or long term mortality from stress-related disease including capture myopathy (Chalmer and Barrett 1982, Fowler 1986, Beringer *et al.* 1996).

Because wild ungulates are difficult to capture, few studies document seasonal and long-term trends in body mass for a population. One exception was the long-term study of bighorn sheep (*Ovis canadensis*) at Ram Mountain, Alberta, Canada, where animals were repeatedly captured in a corral trap (Festa-Bianchet *et al.* 1998). A method to repeatedly weigh wild ungulates without handling would be of great interest to researchers and managers. Here we present our experience using a remotely controlled electronic platform scale baited with salt.

Study areas

We developed the method in Gran Paradiso National Park (GPNP) in northwestern Italy (45°26' N, 7°08'E) and tested it on Alpine ibex (*Capra ibex*) in GPNP and on Bighorn sheep in the Sheep River Provincial Park, southwestern Alberta (50°40'N, 114°35'W).

Elevation in the GPNP Alpine ibex study area (Levionaz) ranged from 2,300–3,820 m and included alpine meadows, rock cliffs, and glaciers. During the ibex census of September

2001, 94 males and 119 females were counted. Males aged 3–16 years were tagged with ear-tags and radiocollars (23 in 2000 and 47 in 2001) as part of a long term study of ibex in GPNP. Ibex were weighed in Levionaz from June – mid-September.

Elevation in Sheep River Provincial park ranged from 1,450–1,700 m and was characterized by grassy slopes, cliffs, and aspen (*Populus tremuloides*) forest. A long-term study has monitored the bighorn population since 1981 (Festa-Bianchet 1986, Jorgenson *et al.* 1997, Loison *et al.* 1999). Over 20 years more than 600 individuals were marked with ear tags, but little information on body mass was available (Festa-Bianchet *et al.* 1997) until 2000 when we began using the platform scale.

Materials and methods

At GPNP we used a Weigh-tronix model "Diamond series" electronic scale (Weigh-Tronix, Fairmont, Minn.), with an advertised precision of 0.1 kg. We modified the scale substituting a wooden platform (60 x 120 cm) fixed on a metal frame for the original metal platform (61 x 61 cm). Modification was necessary because initial trials suggested ibex were frightened by the metallic sound when stepping on the original platform. The scale was connected by a 30-m cable to an electronic display suitable for outdoor use. A sealed rechargeable lead battery connected to the display powered the system (Figure 1). Cost of the entire apparatus was about $ 2,500. We positioned the scale against rock walls in traditional saltlicks so individual ibex had to step on the scale with all 4 legs to access salt. Access to salt was limited to 1 individual at a time by stones and logs (Figure 1). We placed a log on one side of the scale so the observer could clearly see whether ibex stepped on the platform with all 4 legs (Figure 2). Observers used either binoculars or spotting scopes from 50–60 m to avoid interference with animal activities.

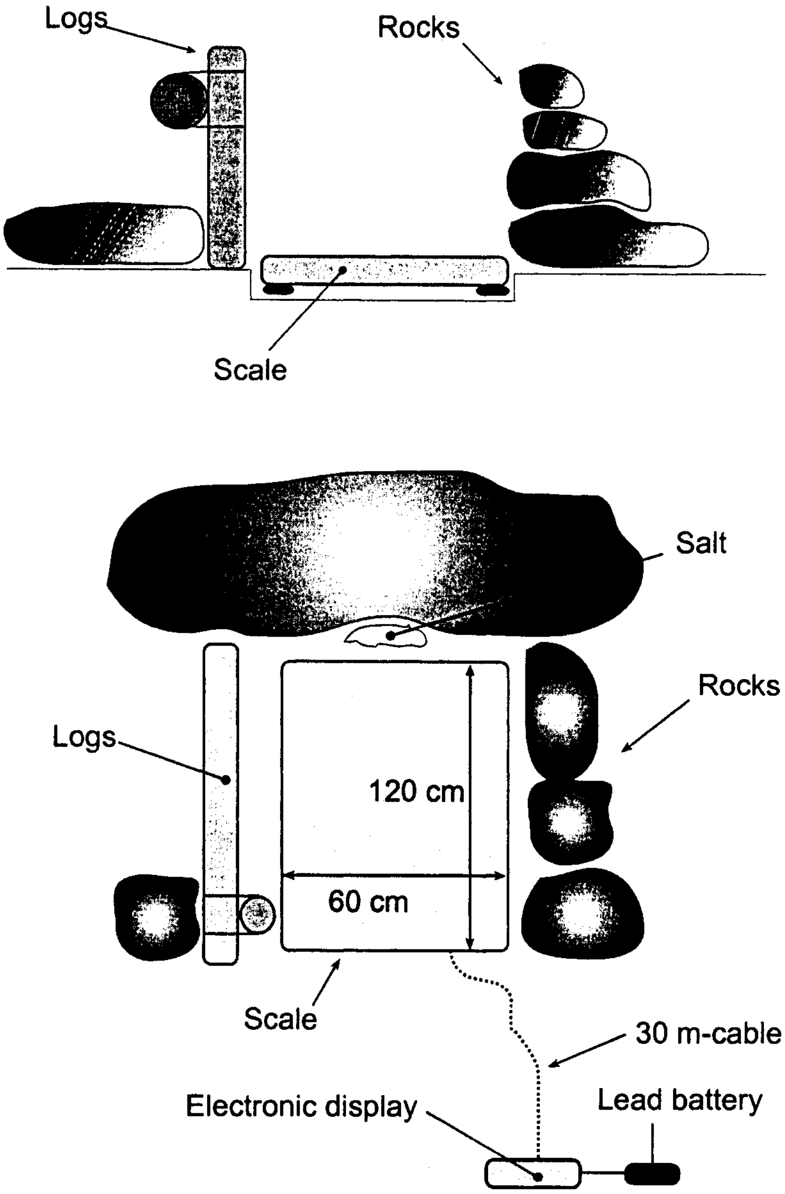

Figure 1. Schematic diagram illustrating the remotely controlled electronic scale used in Levionaz (GPNP, IT). Art by B. Bassano.

Figure 2. Weighing adult Alpine ibex males at the scale in Levionaz (GPNP, IT). Note the log on 1 side of the platform preventing more than 1 ibex at the time from reaching the salt. A log was preferred to a rock wall because it permitted observers to see if the subject had stepped on the platform with all 4 legs. Photo by A. von Hardenberg.

At Sheep River we used a Weigh-tronix model "AlloyWeigh" scale (Weigh-tronix, Fairmont, Minn., 50 cm x 120 cm, maximum load 455 kg, accuracy 0.5 kg), with an extra 70 meters of cable for the display which allowed us to run the cable to the monitor which was usually kept in a vehicle. Cost of the entire apparatus was about $ 1,400 in 2000. Because bighorns could be found at a number of sites along a road, we used a mobile rather than a fixed platform. Unlike ibex, bighorns were tolerant of each other and readily licked salt while in contact with other bighorns. Consequently, we required a system to only allow 1 bighorn at a time on the scale. Using plywood and 5 cm deep and 10 cm wide wood boards (2 x 4), we built side panels 60 cm high and 120 cm long and placed large rocks on the base of the side panels so bighorns could not push them out of the way (Figure 3). The entire apparatus could be loaded in a truck and set up at a new location in less than 5 minutes. We placed small pieces of 2 x 4 under each corner of the scale, to level it. Bighorns were not afraid to step on the metal platform; therefore, we did not modify the scale and used it as provided by the manufacturer. We installed the scale close to a group of bighorns and placed a salt block in front of it. In most cases we installed the scale along the road. When a bighorn stepped on the scale, an observer in a vehicle or on the ground read its tag number with binoculars, verified all 4 legs were on the platform, and read the weight on the remote display. Side panels limited scale access to only 1 bighorn at a time and forced the animal to put all 4 feet on the scale. Because bighorns are highly gregarious, we did not place salt against an obstacle or several bighorns would have climbed on the platform together despite the side panels. Consequently, several bighorns could lick the salt with only 1 standing on the scale.

During summer 2000 some marked ibex were weighed $\leq$ 4 times in less than an hour. We used those repeated measures to assess the repeatability of weight estimations following the formula by Lessels and Boag (1987).

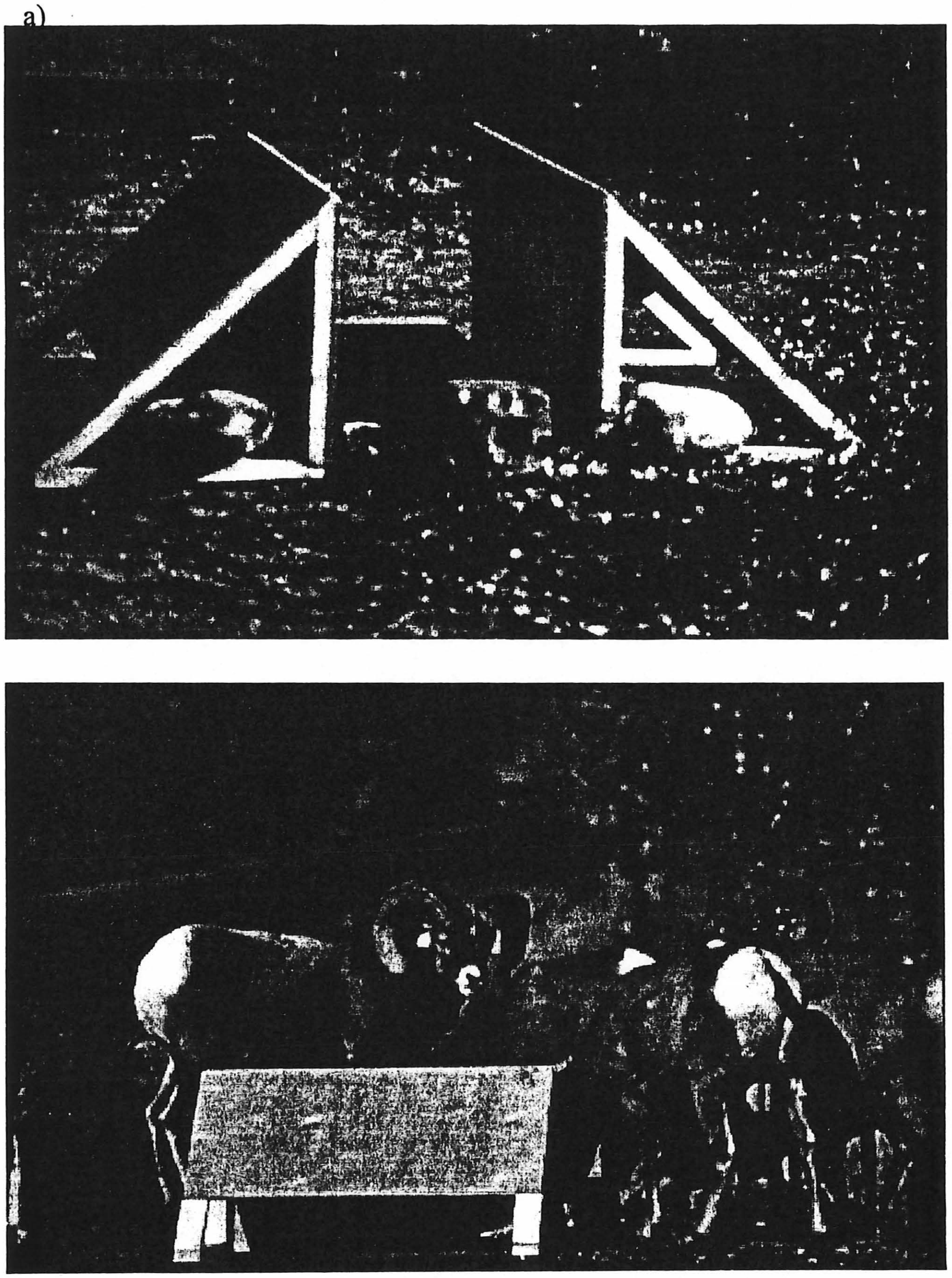

Figure 3. a) The scale used in Sheep River Provincial Park, AB and b) Weighing bighorn sheep at Sheep River Provincial Park, AB. Photos by F. Pelletier.

Results

The method provided accurate, repeated weight measurements of free-ranging ungulates without handling. During summer 2000, 181 ibex weight measurements were collected at GPNP. Of these, 140 were of unmarked individuals and 41 were of 14 different marked individuals weighed ≤ 6 times each (Figure 4). Of the marked ibex present in the study area during summer 2000, 78% were weighed at least once. During summer 2001 we collected only 97 weight measurements because the ibex spent much of their time at higher elevations (up to 3,200 m) away from the saltlick. Of 97 measurements taken in 2001, 76 were of 36 marked ibex representing 88% of all marked individuals present. Nineteen marked ibex were weighed at least twice (Figure 4).

Repeatability, estimated for ibex weighed repeatedly within less than an hour (2 times: 23 cases; 3 times: 7 cases; 4 times: 1 case) was extremely high ($F_{30,40} = 12\ 748$; $r = 0.999$). Measurement error was 0.01% of total within-individual variance ($MS_w/MS_a \times 100$).

At Sheep River success of the weighing system increased substantially over the years of study. In the first year, we obtained 24 weights from 18 marked bighorns representing 20% of the total number of marked animals. In 2001 we weighed 72% of the marked bighorns obtaining 227 weights from 68 individuals (Table 1). In 2001 we collected at least 2 weights for 71% of weighed bighorns ($n = 68$), and we weighed some bighorns up to 9 times (Figure 4). We weighed up to 30 individual bighorns in 1 session. At GPNP ibex came to the scale mostly in the evening from approximately 17:30 until darkness (around 21:30). We, therefore, intensively monitored the scale for about 4 hours every evening from the beginning of June until mid September. We also collected weights at other times of the day whenever we saw animals near the scale, which was visible and within < 1 hour walk from most of the study area. At Sheep River, we started weighing sessions only when we found animals near the road. We spent about 4 hours a day from mid-September to mid-December attempting to weigh bighorn sheep.

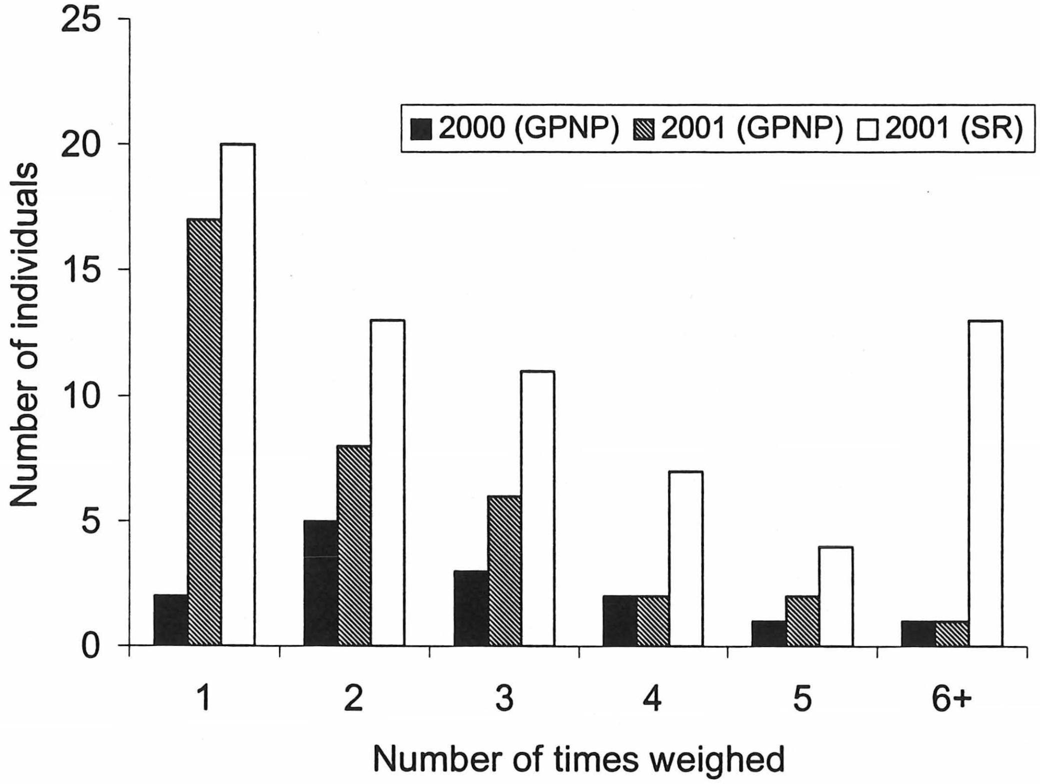

Figure 4. Number of times individual animals were weighed during summers 2000 and 2001 for marked Alpine ibex in Levionaz (GPNP, IT) and for bighorn sheep during 2001 in Sheep River (SR) Provincial Park, AB. "6+" refers to animals weighed more than 6 times.

Table 1. Percentage of marked bighorn sheep weighed in Sheep River Provincial Park, AB, 2000–2001. Total number of marked bighorns was 89 in 2000 and 95 in 2001.

Age-class	2000	2001
Ewes	29.4	74.3
Rams	0.0	70.8
Yearling females	0.0	100.0
Yearling males	100.0	100.0
Lambs	28.6	54.2

Discussion

Scales on which animals step voluntarily have been developed for wild birds. For example, king penguins (*Aptenodytes patagonicus*; Lemaho *et al.* 1993) and dark–eyed Juncos (*Junco hyemalis*; Vézina *et al.* 2001) have been successfully weighed this way. Dittus (1998) enticed toque macaques (*Macaca sinica*) to sit in a baited weighing pan hung in a jungle tree. Platform scales are commonly used to weigh domestic cattle and sheep. Wild ungulates kept in captivity are sometimes trained to step on scales in research enclosures. For example, captive moose (*Alces alces*) have been weighed in this way in Alaska (Schwartz and Hundertmark 1993). To the best of our knowledge, however, this is the first time this approach has been used to weigh free-ranging wild ungulates. Our field tests indicate the method is both accurate and effective. Cost of the apparatus was reasonable for the data obtained and instruments were strong and should last many years. We feel the time and effort involved is justified by the quality and quantity of data obtainable.

This method is potentially applicable to many other species that could be attracted with baits. Besides Alpine ibex and bighorns, this method is currently being used to weigh mountain goats

(*Oreamnos americanus)* at Caw Ridge, Alberta, Canada, and preliminary results are encouraging: 69% of 96 marked goats were weighed this way in 2001(Y. Gendreau, Département de biologie, Université de Sherbrooke, personal communication).

We suggest this method has potential to provide useful information for a wide range of studies of wild ungulate populations. The technique, however, would be of limited use in the absence of marked or otherwise recognizable individuals and we advise against trying to use it to determine mean weights of different sex-age classes in unmarked populations because of the risks of pseudo-replication (Machlis *et al.* 1985). We found some individuals were repeatedly weighed and appeared "salt-happy". Had they not been marked, this would have biased our results. Our experience is limited to gregarious species. Applicability of this method to territorial ungulates such as male Alpine chamois (*Rupicapra rupicapra*; von Hardenberg *et al.* 2000) is still to be investigated.

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SECTION IIIB

Low genotyping error rates when analysing microsatellite DNA from wild ungulate faeces sampled in winter

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The development of non-invasive methods for sampling DNA is of great interest for conservation biology and behavioural ecology. This paper asseses the accuracy of DNA analyses from faeces of two ungulate species, Alpine ibex and Corsican mouflon, collected in different seasons. My contribution to this paper included ideas for collecting faeces in the field, the collection of all Alpine ibex faeces, and statistical advice.

Low Genotyping Error Rates When Analysing Microsatellite DNA from Wild Ungulate Faeces Sampled in Winter

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Running title: Genotyping errors using ungulate faeces

Abstract

We show that Alpine ibex (*Capra ibex*) and Corsican mouflon (*Ovis musimon*) faeces yield useful DNA for microsatellite analysis. However, we detected a higher genotyping error rates for spring faeces than for winter faeces. We quantified the genotyping error rate by repeatedly genotyping (100-200 per species and per season) four microsatellite loci. Respectively, 99 and 95% of mouflon and ibex genotyping repetitions provided a correct genotype using winter samples. Whereas spring samples provided only 52% and 59% correct genotypes. We recommend that before starting a project involving non-invasive sampling, researchers should conduct a pilot study to quantify genotyping error rates for each season, population and species to be studied.

Introduction

The potential usefulness of non-invasive sampling methods for wildlife molecular studies has been widely reported (see review by Kohn & Wayne 1997). Faeces sampling has been shown to be an effective means of acquiring DNA from numerous free-ranging mammal species (e.g. Woodruff 1993; Gerloff *et al.* 1995; Taberlet *et al.* 1997; Kohn *et al.* 1999; Ernest *et al.* 2000; Goossens *et al.* 2000; Kohn & Wayne 1997). Intestinal lining cells in faeces provide DNA for molecular analysis of individuals. Faeces abundance, its relatively ease of collection, and the fact that faecal samples are not subject to CITES constraints facilitate extensive sampling from endanger species.

Many wild ungulates are endangered and subject to CITES regulations (Shackleton 1997). However, only a few studies on the accuracy of non-invasive sampling methods for ungulates have been performed (Flagstad *et al.* 1999; Yamauchi *et al.* 2000). Although more and more studies have reported relatively good DNA yields from faeces samples, numerous problems associated with DNA extraction and PCR amplification have been described. For example, faeces samples might fail to provide useful DNA for molecular analysis due to the low quantity and quality of DNA in faeces (Gerloff *et al.* 1995; Wasser *et al.* 1997; Flagstad *et al.* 1999; Goossens *et al.* 2000). The low quantity and quality of nuclear DNA extracted from faeces can also cause false alleles and/or allelic dropout that lead to erroneous genotyping (e.g. Gagneux *et al.*1997; Taberlet *et al.* 1997). Numerous factors influencing the frequencies of these errors have been reported. Technical features such as sample preservation and extraction procedures can cause great variations in genotyping error rates (Wasser *et al.* 1997; Frantzen *et al.* 1998; Flagstad *et al.* 1999; Murphy *et al.* 2000). Intrinsic individual or species variations of faecal DNA content might also affect genotyping accuracy (Gerloff *et al.* 1995; Gagneux *et al.*1997; Goossens *et al.* 2000). Diet impact on faecal DNA extraction and amplification was recently reported for captive brown bears (Murphy A., Waits L. and Kendall C. *submitted*).

In this paper we report another factor that can affect genotyping error rates from ungulates faeces: seasonal variations perhaps resulting from variations in vegetation and diet quality. We consider the impact of faeces sampling season for two wild ungulates: Alpine ibex (*Capra ibex [ibex]*) and Corsican mouflon (*Ovis gmelini [musimon]*). Using repeated genotyping of four microsatellite loci from faecal DNA, we quantify the types and rates of genotyping errors encountered from winter and spring faeces sampling.

Material and Methods

Fresh faeces (0-12h post-defecation based on moisture aspect for mouflon or collected immediately after defecating for ibex) were collected from wild populations: Gran Paradiso (Italy) and Belledonne (France) populations for ibex samples and Asco (Corsica-France) population for mouflon samples. For each season and each species, only one sample collection was conducted. Faeces were collected from isolated groups of droppings during April-May for spring samples and during November-February for winter samples. For ibex and mouflon, 8 and 11 faeces samples were collected respectively in spring, and 20 and 24 in winter (depending of availability of the animals). Only one dropping from each faeces group was collected using a disposable tooth pick or flame-sterilized metal tweezers. Crushing of the dropping was carefully avoided. Each dropping was stored in a 25-ml leak-proof vial containing 15-ml of 95% ethanol. Spring and winter samples were stored for only 2-4 weeks at room temperature before DNA extraction.

Mucus on the dropping surface containing intestinal cells was washed using a lysis washing buffer (Tris-HCl-0.1M, EDTA-0.1M, NaCl-0.01M, N-lauroyl sarcosine-1 %, pH 7.5-8). One dropping from each individual was washed in 350 µL of washing buffer during 15 min in a 25 ml tube on rotary agitator. The supernatant (approximately 300µL) from surface-washed faeces was used for DNA extraction using the DNeasy^{TM} Blood Kit (QIAGEN GmbH, Germany) following the manufacturer's procedures. Unlike Wasser *et al.* (1997) and Flagstad *et al.* (1999) only one spin column was used for each extraction. The success of the DNA extraction was verified by amplifying a microsatellite locus (that is known to be clearly visible on agarose gel: BM4505 for ibex, and ILSTS011 for mouflon), by running 5 µL of this amplification on a 1.6% agarose gel containing ethidium bromide, and by visualizing under UV light.

Four polymorphic microsatellite loci with a relatively large amplified DNA fragment (180 to 300-bp) were chosen for each species (see Table 1). PCR amplifications were performed in a total reaction volume of 15 µL containing 4 µL of 2-fold dilution of the extracted DNA, 0.5 µ*M* of each primer, 100 µ*M* dNTP, 2,5 m*M* $MgCl_2$, PCR buffer (10 mM Tris-HCl (pH 8.3), 50 m*M* KCl), 2 ng of BSA, 0.5 U of AmpliTaq Gold^{TM}-Polymerase (PE Applied Biosystems). PCR amplifications were conducted in a PE Applied Biosystems 9700 thermal cycler using the following conditions: an initial denaturation step at 95°C for 10 min followed by 10 cycles of 30 s at 95°C, 30 s at 60 or 65°C (depending on the locus; see Table 1) with one degree reduction at each cycle and 60 s at 72°C, followed by 40 cycles of 30 s at 95°C, 30 s at 50 or 55°C, and 60 s at 72°C. PCR products were electrophoresed on a 6% denaturing polyacrylamide gel on an ABI Prism 377 automated sequencer (PE Applied Biosystems).

Our objective was to precisely measure genotyping error rates (cause by allelic dropout and false alleles). This objective is different from studies to identify a correct "consensus genotype" in which only 3 to 8 genotyping replicates are necessary (Taberlet *et al.* 1996; Gagneux *et al.* 1997; Goosens *et al.* 2000). Many replicates (e.g. > 20) are necessary to quantify the error rates because genotyping error rates are often very low. The genotyping error rate was estimated by repeatedly genotyping heterozygous individuals for several loci in independent PCR reactions. We used only heterozygous individuals to facilitate the detection of allelic dropout (false homozygotes) and false alleles (e.g. three alleles/individual). For the mouflon, the genotyping of one heterozygous individual for each of the four loci was repeated 25 times for each season. For the ibex, the genotyping of two different heterozygous individuals for each locus and each season was repeated 25 times. Thus, 4 and 8 individuals (giving 100 and 200 genotyping repetitions) were genotyped respectively for mouflon and ibex, and for both winter and spring sampling faeces. In order to detect genotyping errors due to individual or sample variations, it would be preferable to conduct the genotyping repetition using one individual heterozygous for the four loci. However, considering the limited DNA extract quantities and the low polymorphism observed in the two studied species (Maudet *et al.* 2002), we were obligated to repeat the typing of different individuals for each locus.

The results were scored using the following terminology: (i) "failed DNA extraction" indicating that no amplification products were visualized on an agarose gel after one extraction, (ii) "allelic dropout" or false homozygote, (iii) "failed amplification" designating an unsuccessful genotyping repetition, (iv) "ambiguous profile" resulting in a microsatellite pattern difficult to score (see Figure 1, D and E), and (v) "correct genotype" or consensus genotype.

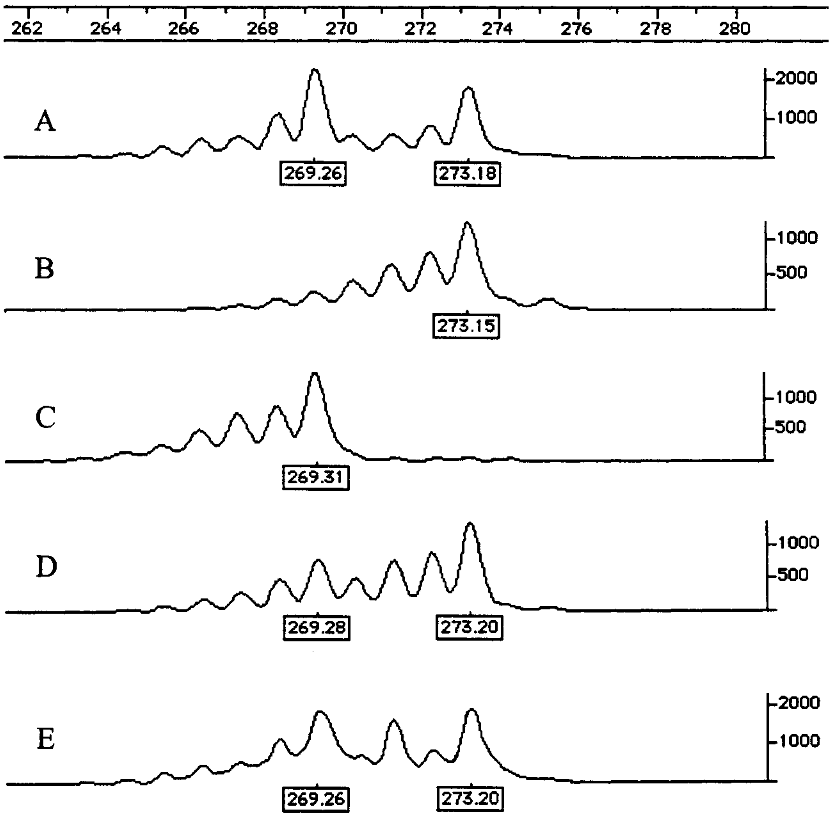

Figure 1. Example of results from five independent genotypings using the same DNA extraction from a heterozygous individual at the INRABERN185 locus. A: correct genotyping (alleles 269-bp and 273-bp), B: Allelic dropout with only the 273-bp allele detected, C: Allelic dropout with only the 269-bp allele detected, D and E exhibit ambiguous microsatellite patterns that might lead to erroneous typing.

Results and Discussion

Overall, the extraction and amplification failures occurred more frequently (Wilcoxon signed-ranks test, P = 0.018 for ibex and P = 0.008 for mouflon) using DNA extracted from spring faeces than from winter faeces for both the ibex and mouflon samples (Table 1). Comparing genotyping from spring and winter samples, we showed that genotyping repetitions were achieved without ambiguity for (respectively for spring and winter) 71 and 95% of ibex, and 83 and 99% of mouflon of (Table 2). The relatively low error rate, especially for the mouflon, is encouraging and is of similar magnitude to the error rates found for some tissues yelding good quality DNA such as blood (Jeffery *et al.* 2001, Slate *et al.* 2000).

Consistent with Flagstad *et al.* (1999) who used an initial surface wash (on domestic sheep and reindeer faeces), DNA extraction from wild ungulates faeces provides a reasonably high yield of DNA. The advantage of the wash is that it can recover the DNA from the exterior of the faeces while leaving behind most of the PCR inhibitors on the inside of the faeces. All of the winter sample extractions yielded visible amplification products on agarose gel, while 27 and 29% (respectively for ibex and mouflon) of the spring samples failed to provide an amplffiable DNA (Table 2). Furthermore, more variations of PCR product quantities (fluorescence intensities on agarose gel) were observed among spring samples compared to winter samples (data not shown). The unsuccessful DNA extractions might be due to the low quantity or quality of DNA in the faeces or the presence of dietary inhibitors (Deuter *et al.* 1995). If we take into account the samples that failed to provide usable DNA (about 30% in spring but 0% in winter), the percentages of correct genotypings from spring faeces were even lower. When including the failed extractions, the total success rates for spring faeces were only 52% and 59% respectively for ibex and mouflon (compared to 71% and 83% when not counting failed extractions).

Repeating heterozygote typing, three types of genotyping errors were occasionally observed. First, as reported in non-invasive studies (e.g. Taberlet *et al.* 1996; Gagneux *et al.* 1997), we observed some allelic dropout due to a spurious amplification of only one of the two alleles present in the template. Second, we encountered some ambiguous microsatellite profiles that could lead to a misinterpreted typing (see Figure 1, D and E). This latter problem has never been reported in previous studies, but can be explained by the poor quality of DNA extracted from faeces and/or the presence of PCR inhibitors in the extract, which disturbs the amplification processes (Deuter *et al.* 1995). Third, we encountered non-amplification of some genotyping repetitions (no PCR product on sequencer gels). This also could be explained by the low DNA quantity in the sample, but also by experimental errors like slight DNA volume variations added in the PCR repetitions. The frequency of these genotyping failures was also always higher when using spring faeces than winter faeces (Table 1 and 2). Similar to Gagneux *et al.* (1997), we did not observe much difference in genotyping error rates between microsatellite loci for either the ibex or mouflon loci (Table 1). Moreover, the number of genotyping errors was similar for all ibex and mouflon samples (8 ibex and 4 mouflon samples) in spring and winter. This suggests that differences in genotyping error rates are due to seasonal differences.

Although the genotyping error rate from spring samples is relatively high, it is still comparable (indeed lower) than of those observed in non-ungulates species (e.g. brown bear, Gagneux *et al.*1997; arboreal ape, Goossens *et al.* 2000). Thus, DNA extracted from spring faeces can be used with a multi-tube approach (Taberlet *et al.* 1996) in order to detect genotyping errors from allelic dropout and ambiguous typing. One way to increase DNA yield might be to use more than one dropping per individual in the DNA wash and extraction. However this could increase PCR inhibitors or increase the risk of mixing DNA from two different individuals, if pellets from different individuals were mistakenly collected together. Another way is to add more of the DNA extract into the PCR reaction, but this would decrease the total number of possible PCR's and perhaps increase PCR inhibitor concentration.

As reported by Flagstad *et al.* (1999) the nature of faeces from many ruminant species (compact pellets) allows washing of surface mucus containing intestinal cells, which facilitates the DNA extraction from faeces (concentrating the DNA with fewer PCR inhibitors from inside the faecal pellet). Nonetheless, we observed a substantial number of extraction/amplification problems using spring faecal samples. During the spring period, both ibex and mouflon eat essentially buds and young shoots; while during summer and winter they eat more ligneous plants (twigs, dry grass, lichens, ferns) (Gauthier *et al.* 1991; Demeautis, 1991). Higher quality of spring diet reduces the digestion time (Lechner-Doll *et al.* 1995), and thus decreases the time of contact between faecal matter and intestinal membranes. In the same way, lower diet fibre contents during spring increases the digestibility of the diet and thus, reduces abrasion of the intestinal membrane by the undigested fibres (Doreau *et al.* 2000). Consequently, we speculate that DNA contents of spring faeces is probably lower than during other seasons. Indeed, Murphy A., Waits L. and Kendall C. (*submitted*) have shown that diet can significantly influence the success of faecal DNA extraction and amplification in captive brown bears.

Another factor that could explain the seasonal DNA yield difference is degradation of the DNA. Some winter samples were collected from snow (or at low temperature) while spring sample were collected from grass or rocks. However, faeces were collected soon after defecation (0-1h for ibex and 0-12h for mouflon), thus DNA degradation differences between spring and winter samples are probably limited. In addition, great care was taken not to sample after a bad weather period (precipitation). Finally, the slightly better DNA yield observed in mouflon compared to ibex (both in spring and winter) might be due to diet differences (mouflon are known to eat more abrasive ligneous plants than ibex) or to morphological factors (a 20% longer intestine in mouflon; Demeautis, 1991).

In summary, we have shown that nuclear DNA extracted from faces of wild goats (Alpine ibex) and wild sheep (Corsican mouflon) is useful for microsatellite analysis. Winter faeces can potentially be used without extensive repeation of genotyping, as for normal tissue samples. However, the quantity and/or quality of the DNA extracted from ungulate faces is apparently influenced by the sampling season. Future studies should be conducted to clarify the effect of season and to resolve the causes of seasonal and species variation in faecal DNA quality (e.g. using quantitative PCR, estimation on intestinal cell quantity). Although spring faeces can be useful for DNA analysis, great caution should be taken, and multiple samples and multiple repeated PCR amplifications will often be necessary. We recommend that, before starting a study using faeces sampling, researchers should conduct a pilot study to quantify genotyping error rates for each season, each species, and perhaps even each population to be studied.

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Table 1. Results of genotyping repetitions for each microsatellite locus. Twenty-five genotypings were performed using each of four loci on one or two heterozygous individuals (respectively for mouflon and ibex). That is to say that 1 or 2 different individuals were genotyped for each locus (respectively for mouflon and ibex). Thus a total of 4 and 8 individuals were studied. In total, 100 and 200 genotypings were conducted for mouflon and ibex, respectively, for each season (winter and spring).

Locus (Allele lengths, bp)	Result type	Winter	Spring	Annealing temp. (°C)	Reference
Capra ibex [ibex]		N=50	N=50		
BM1818	Correct GT	49	40	60-50	Bishop *et al.* 1994
(262-266)	Allelic dropout	0	5		
	Other problem*	1	5		
BM4505	Correct GT	48	35	60-50	Bishop *et al.* 1994
(280-286)	Allelic dropout	0	4		
	Other problem	2	11		
INRABERN185	Correct GT	46	39	60-50	Kappes *et al.* 1997
(268-274)	Allelic dropout	0	5		
	Other problem	4	6		
SR-CRSP-9	Correct GT	47	29	60-50	Bhebhe *et al.* 1994
(231-233)	Allelic dropout	0	6		
	Other problem	3	15		
Total	Correct GT	190	143		
Ovis gmelini [musimon]		N=25	N=25		
BM415	Correct GT	25	24	65-55	Bishop *et al.* 1994
(180-184)	Allelic dropout	0	1		
	Other problem	0	0		
ILSTS011	Correct GT	24	18	65-55	Brezinsky *et al.* 1993
(282-284)	Allelic dropout	0	3		
	Other problem	1	4		
MAF214	Correct GT	25	20	65-55	Buchanan & Crawford 1992
(188-194)	Allelic dropout	0	3		
	Other problem	0	2		
SR-CRSP-8	Correct GT	25	21	65-55	Bhebhe *et al.* 1994
(215-245)	Allelic dropout	0	0		
	Other problem	0	4		
Total	Correct GT	99	83		

N is the number of genotyping repetitions per locus (independent PCR amplifications).
* "Other problems" are ambiguous microsatellite profiles or failed amplifications.
Correct GT is the number of correct genotypes.

Table 2. Percent of failed extractions and genotyping errors for genotyping repetitions of heterozygous individuals.

	% Failed extractions	% Failed amplif.	% Allelic dropout	% Ambiguous profile
Capra ibex [ibex]				
Winter	0.0 (0 / 8)*	2.5	0.0	2.5
Spring	27.3 (3 / 11)	6.5	10.0	12.5
Ovis gmelini [musimon]				
Winter	0.0 (0 / 20)	0.0	0.0	1.0
Spring	29.2 (7 / 24)	2.0	7.0	8.0

* In parentheses is the ratio of failed extraction to attempted extraction (see details in Material and Methods).

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

Alpine ibex numbers declined sharply between the 16th and the 19th century, eventually disappearing completely from all Alpine countries except Italy. At the beginning of 19th century the ibex was on the verge of extinction, with possibly fewer than 100 individuals surviving on the Grivola massif, within today's Gran Paradiso National Park, and including the area of Levionaz, where most of the studies included in this thesis have been conducted. The first edict for the complete protection of Alpine ibex was issued in 1821. Protection was enforced in 1856 with the institution of a royal hunting reserve by king Vittorio Emanuele II. To prevent poaching, a unit of royal gamekeepers was soon created, all coming from local villages and chosen "...fra il fior fior di bracconieri" ("among the very best of the poachers"; Leopoldo Guala, Commander of the Aosta hunting district in about 1900, cited in Passerin d'Entréves, 2000). Despite much hunting by the royal family and their guests, thanks to the strict control of poaching by the royal gamekeepers, the population of Alpine ibex in the Gran Paradiso area recovered rapidly. The yearly censuses, started in 1877 by the royal gamekeepers, recorded a constant increase in the total numbers of Alpine ibex, from 790 individuals in 1879, to 2673 in 1905 (Passerin d'Entréves, 2000). In 1922, the Gran Paradiso National Park - the first Italian national park - was instituted, after the donation of the royal hunting reserve to the Italian government by king Vittorio Emanuele III. Nowadays, about 40,000 Alpine ibex can again be seen in hundreds of populations all over the Alps after the great reintroduction efforts started in all Alpine countries after the Second World War. All founder animals came, directly or indirectly, from the Gran Paradiso National Park population. The studies presented in this thesis were therefore conducted in the only truly autoctonous population of Alpine ibex in the world, which has not been legally hunted since 1922. Moreover, motivated park wardens, have collected over the years an incredible amount of invaluable data and materials, including over 40 years of reliable ibex censuses, and an impressive collection of over 2000 ibex skulls, systematically collected from animals found dead. Great human efforts, have then permitted, five years ago when I started this Ph.D., to individually tag many ibex, starting the long-term study on the ecology of Alpine ibex in the study area of Levionaz. Long term studies on individually tagged animals are essential to fully understand the biotic and abiotic factors affecting the life history of individuals and their long-term effects on population dynamics (Gaillard *et al.*, 2000). Unfortunately, only few studies of this kind have been conducted in free-ranging populations, and most of them have been conducted in hunted (Coltman *et al.*, 2003) or insular or fenced populations (Byers *et al.*,1990; Hogg *et al.*1997; Rose *et al.*, 1998; Coltman *et al.*, 1999). Studies on unhunted, open, populations, such as the Gran Paradiso Alpine ibex study, are therefore urgently needed to test if the results previously found can be generalized. The Gran Paradiso National Park appears therefore to be an ideal natural laboratory to study life-history strategies and population dynamics in free-ranging mountain ungulates, although the absence of large predators must be taken into account.

The studies presented in Chapter II strongly suggest that the population dynamics of Alpine ibex are driven mostly by changes in adult winter survival, mediated through the depth of snow cover, rather than by changes in recruitment. In this, Alpine ibex seem different from most other ungulates (Gaillard *et al.*, 2000). The out-of-sample prediction presented in Section IIa showed that a simple deterministic model including both density dependence and snow depth provided an acceptably close prediction of the population trajectory of the following 20 years. In particular, the model forecast the eruption which happened from 1985 to 1995 even though it was based on data collected in years when the population never reached that level. The model was, however, unable to predict the relative stagnation that followed the subsequent decline. To better understand ibex population dynamics, future research should analyze the Gran Paradiso ibex population taking into account also age structure, because it is likely that prime-aged and senescent adults will react differently to varying levels of density and snow depth. The ongoing accumulation of survival data on marked individuals, will permit such an analysis in a few years. A good understanding of the factors influencing the Gran Paradiso Alpine ibex population and thus the capacity to predict its future dynamics, is vital for the long term conservation of this historic population. Moreover, the Gran Paradiso Alpine ibex population will likely become in the future a good study model to investigate the effects of global warming on the dynamics of large ungulates. The effects of global change are likely to be strong at high altitude where ibex live.

Ibex appear to have very high adult survival, which declines for both sexes after about 10 years of age (Toigo, 1997). This high longevity, make Alpine ibex a good model to study the phenotipic effects of senescence. In section Ia and Ib, I documented evidence of senecence in parasite resistance and in the length of yearly horn growth segments, but not in their asymmetry. Studies on fluctuating asymmetry of secondary sexual traits, and the claim that the asymmetry of these traits was a indicator of developmental instability in their bearers, inflated the scientific litterature in the last decade. More recently, however, the role of fluctuating asymmetry in sexual selection has been very much resized (Bjorksten *et al.*, 2000). In Section Ic, I show evidence that horn size is also correlated with multilocus heterozygosity. Until 2004 such a correlation would have been interpreted as evidence for inbreeding depression. Recent papers, however, showed the intrinsic fallancies in this view (reviewed in Pemberton, 2004). Associative overdominance of some microsatellite loci with loci directly affecting fitness, is currently viewed as the most plausible interpretation of this correlation. The study of genetic effects on fitness related traits is important for Alpine ibex conservation, and could have strong implications, especially for reintroduced populations, which recently underwent genetic bottlenecks, and in which thus loss of genetic variation and the negative effects of inbreeding depression can be expected to be strongest (Maudet *et al.* 2002). Future research should compare individual genetic variability with its effects on fitness-related traits between the Gran Paradiso population and reintroduced populations testing the hypothesis of a stronger effect of inbreeding depression in the reintroduced populations. In Section Ia I also describe an anthelmintic treatment experiment, aimed to test the hypotheis of a direct detrimental effect of gastrointestinal parasites on body mass gain. My results, unfortunately, are inconclusive, because of small sample size. The effect of the treatment, however, was in the expected direction. New investigations, with a larger number of treated individuals, will therefore be necessary to test fully the hypothesis of a direct effect of gastrointyestinal parasites on body mass gain in Alpine ibex. Section Ia also analyzes the age specific body mass gain in ibex males, showing that in this population, maximum body mass is reached very late, at 12 years of age. This result, in accordance with life history theory, suggests prolonged investment in future reproduction. Future research should aim at investigating male reproductive success in Alpine ibex, although such a study appears very challenging because of the difficulties in sampling kids and their mothers, and thus perform a paternity analysis. In conclusion, as often happens, the studies presented in this thesis open the way to further interesting researches, which promise to give a great contribution to our knowledge of the life history and population dynamics of mountain ungulates and will likely be pivotal in the future conservation of Alpine ibex.

ANNEX I

Another one bites the dust:
Does incisor arcade size affect mass gain and survival in grazing ungulates?

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Abstract

Incisor arcade size affects foraging efficiency in grazing ungulates and should be under strong selective pressure. We investigated individual variation in incisor arcade size and its relationship with body mass and survival in bighorn sheep (*Ovis canadensis*) at Ram Mountain, Alberta (Canada) during nine years. In adult ewes, incisor arcade breadth and depth decreased with age, probably due to tooth wear. We found no effects of incisor arcade size on survival for lambs or adult ewes. In adult ewes, an apparent positive effects of incisor arcade size on survival disappeared when age was accounted for. Incisor arcade breadth and depth had no effect on summer mass gain in lambs or adult ewes. Although linear models suggested that arcade breadth in lambs was correlated with summer mass gain, a latent variable path analysis model revealed that the correlation was due to an allometric relationship of arcade breadth with body size. Variation in incisor arcade size in bighorn sheep appears due to individual variation in body size and age rather that to directional selection.

Introduction

Individual variation in traits directly affecting foraging efficiency should have considerable fitness consequences. For example, beak morphology determines foraging efficiency in granivorous birds and variability in this trait can be maintained through oscillating selection following recurrent changes in environmental conditions (Gibbs and Grant 1987; Smith 1987; Smith 1990). Few studies, however, have investigated individual variation in mouth morphology in mammals.

Illius and Gordon (Illius and Gordon 1987; Gordon and Illius 1988; Illius *et al.* 1995; Gordon *et al.* 1996) suggested that incisor arcade breadth (the distance between the outer edges of the last incisors on the left and right ramus) is under strong selective pressure in grazing ungulates. They argued that individuals with wider incisor arcades are able to grasp more forage per bite, and thus have higher fitness than those with smaller muzzles. A wide mouth should be advantageous especially when forage is scarce at high herbivore density. In an island population of feral sheep, Illius *et al.* (1995) claimed that during a winter population crash due to starvation, survival increased with incisor arcade breadth. Despite an apparent strong selection gradient against narrow arcades, however, arcade breadth retained considerable variability in the population. Illius *et al.* (1995) speculated that narrow-mouthed sheep may be advantaged at low population density, being able to forage selectively on swards dominated by mature and senescent grass. Coarse grassland of low digestibility, requiring selective grazing, dominates swards when grazing pressure is low. Based on those results, Fryxell *et al.* (1999) presented a simple Mendelian genetic model showing how variability in incisor arcade size could be maintained in populations characterised by dynamic instability, when the fitness advantage of arcade size varies with time among different genotypes.

Later, however, the same research group (Milner *et al.* 1999) recognised that some individuals had been erroneously assigned in the survival analysis, invalidating the results of the Illius *et al.* (1995) study. The hypothesis of a direct selection on incisor arcade size in grazing ungulates is thus poorly tested.

The idea of a selection gradient on a morphological trait directly involved in foraging efficiency is intuitively appealing but presents a major methodological problem. Both incisor arcade size and body mass depend on the overall body size of an animal: larger individuals weigh more and have bigger muzzles. Body size, particularly of young ungulates, is often positively correlated with survival (Festa-Bianchet *et al.* 1997; Milner *et al.* 1999; Gaillard *et al.* 2000). Selection on body size may thus appear to act on incisor arcade size even in the absence of selective pressure on arcade size. This is a classic example of confusing "selection of" a trait (a trait and survival are correlated) and "selection for" a trait (survival is affected by the trait) (Sober 1984). Even if there is a selection gradient, incisor breadth and survival may simply share a common cause (large size) which is itself the target of selection (Kruuk *et al.* 2002).

If selection acted directly on incisor arcade size, individuals with larger arcades for a given body size should have greater foraging efficiency at high population density, and thus gain more mass than individuals with relatively smaller arcades. That assumption has never been tested.

To test this idea, one could experimentally manipulate incisor arcade size and compare the mass gain of individuals of the same body size. Such an experiment cannot be performed in a wild ungulate population. Traditional statistical methods, like multiple regression, are also of limited usefulness because they cannot distinguish between a correlation due to a third unmeasured variable and one due to a causal relationship. A series of recently developed statistical methods called "Structural equation modelling" are appropriate to solve this problem, because they permit testing of hypotheses involving explicit causal relationships (Shipley 2000). The rationale behind these methods is that while correlation does not necessarily involve a cause-effect relationship, causation necessarily implies a series of partial correlations and constraints on the pattern of covariation between variables (Shipley 1999). It is thus possible to test whether the matrix of covariation between test variables fits the predicted covariation matrix of a specific causal model.

Here we investigate individual variation in incisor arcade size, body mass and survival in a free-ranging population of bighorn sheep (*Ovis canadensis*) over nine years. We first analyse the variation in incisor arcade size with age in adult ewes and test whether such variation might be ascribed to natural selection. We then explore the relationship between incisor arcade size, mass gain and survival of lambs and adult ewes. Finally, we use a structural equation model to test the hypothesis of a direct causal relationship between incisor arcade size and mass gain in lambs.

Materials and methods

We collected data at Ram Mountain (52°N 115°W; elevation 1082 - 2173 m) Alberta, Canada, from 1994 to 2000. Bighorns were captured in a corral trap from late May to early October. Adult ewes were marked with canvas collars, lambs were tagged with coloured Safeflag plastic strips attached to numbered metal Ketchum ear-tags. All adult ewes and lambs were marked, and were weighed at each capture to the nearest 250 g using a Detecto spring scale. Spring mass (SW) and autumn mass (AW) were respectively the mass of each sheep adjusted to June 5 (June 15 for lambs) and September 15 using repeated mass measurements of each individual each year (for more details about weight adjustment, see Festa-Bianchet *et al.* 1996).

Impressions of the incisor arcade of captured sheep were collected on dental wax. Incisor arcade breadth (IAB) was the distance between the outer edges of the last incisors on the left and right ramus, measured with a precision calliper (Fig. 1) (Gordon and Illius 1988). Following Gordon and Illius (1988), incisor arcade depth (IAD) was defined as the perpendicular distance between a line connecting the two outermost incisors and the front of the first incisors (Fig. 1).

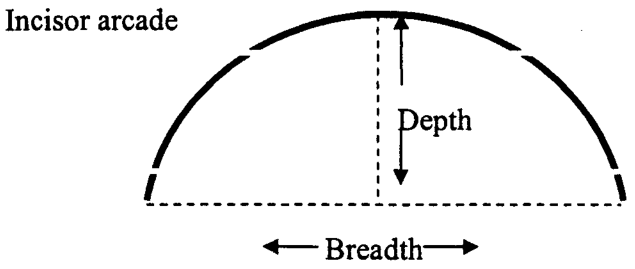

Fig. 1. Variables measured on Incisor arcade of bighorn sheep ewes and lambs. The figure represents the incisor impressions left by the sheep on the dental wax.

IAB of lambs increases substantially during summer. Therefore, IAB of lambs that were captured at least twice was adjusted to August 10 (midsummer) using each lamb's own incisor breadth growth rate. For lambs with only one IAB measurement during summer, arcade breadth was adjusted to midsummer using the mean IAB growth rate of lambs for which we had at least two measurements in that year. Because IAD was not significantly correlated with capture date, no adjustment was applied for that variable. For lambs with more than one IAD measurement in the same year, we used the mean of those measurements. IAB and IAD were not adjusted for capture date for adult ewes, and all incisor arcade measurements were collected between May 25 and June 30.

Data analysis

To assess measurement error, we took two dental-wax impressions for each individual during the first capture in 1999, and each impression was measured once. This procedure estimated not only the measurement error due to the use of the calliper, but also potential inaccuracies in the dental-wax impressions. Repeatability of measurements was estimated with the intraclass correlation coefficient (Lessels and Boag 1987; Sokal and Rohlf 1995). Because of trait dimension differences among age/sex classes, we performed the analysis separately for lambs and adult females (>4 years old). We analyzed incisor arcades of 122 lambs and 75 adult ewes.

Incisor arcade breadth and height increase until 4 years of age. Two new incisors (one for each side of the symmetry plane) replace the corresponding milk teeth each year until the full set of adult teeth is completed at age 4. Because the relationship of age with the incisor size variables is not linear, and because tooth replacement often left gaps in the wax impressions, we only considered ewes older than 4 years. We included in the analysis 75 ewes, for which we had at least two years of data. We excluded ewes with broken or missing incisors.

We used Linear Mixed Effects models (LME) (Pinheiro and Bates 2001) to examine the effects of age on incisor arcade size and to explore the relationship between arcade size and summer mass gain in adult ewes. Fitting individual identity as a random grouping factor, LME models estimate the effects of age on an individual scale and avoid pseudo-replication due to repeated measurements of the same individual at different ages. All the LME models were fitted by maximum likelihood and the significance of fixed terms was assessed with conditional F tests. Goodness of fit and distributional assumptions were checked by examining the residuals graphically (Pinheiro and Bates 2001).

As lambs were considered only in the year of birth, no repeated individual measures were used, therefore, generalized linear models (GLM) were fitted to explore the relationship between incisor arcade size and summer mass gain in lambs. GLM, with a binomial distribution and a logit link function, were also fitted to explore the effects of incisor arcade size, population density and body mass on lamb survival. We modelled survival in adult ewes with Generalized Linear Mixed Models (GLMM) using Penalized Quasi-Likelihood (Venables and Ripley 2002; Wolfinger and O'Connell 1993), to account for repeated measurements of the same individuals in different years. All models were fitted using S-PLUS 2000 (MathSoft Inc.).

To test the hypothesis of a direct causal link between incisor arcade size and summer mass gain we built a latent variable path analysis model using a structural equation model (SEM) approach (Shipley, 2000). Briefly, the qualitative causal relationships between the variables, as specified in our hypothesis, was specified as a system of linear equations with free and fixed parameters following this multivariate hypothesis. We then derived the constraints on the predicted covariance matrix that must exist in the data if the qualitative multivariate hypothesis is true, fit the free parameters of the structural equations to the data by maximizing the likelihood, and then compared the observed and predicted covariance matrices using the maximum likelihood chi-square statistic. This statistic is distributed as a chi-square distribution with appropriate degrees of freedom if the observed and predicted covariance matrices are equivalent up to random sampling variation. Path models were built using EQS 5.7 for Windows.

To avoid problems with non normally distributed data, we tested the goodness-of-fit of the SEM models, testing for the significance of the Santorra-Bentler scaled chi-square value rather than the normal chi-square (Shipley, 2000). When the chi-square is not significant, the null hypothesis cannot be rejected and the corresponding model is considered to fit the data. Nested models, including direct causal links between IAB or IAD and autumn mass were tested using the chi-square difference testing procedure proposed by Santorra and Bentler (1999). The resulting Santorra-Bentler scaled chi-square difference test follows a chi-square distribution, and a significant *P* value indicates a better fit of the nested model against the basic model. More details on model fitting procedures are provided with the results.

Results

Measurement error

Within-individual variability (measurement error), estimated on the repeated incisor impressions taken in 1999, was significantly smaller than variability among individuals. Repeatability was high for both ewes and lambs and for both incisor arcade breadth and height (Table 1). Variability in incisor arcade breadth and depth among individuals was thus not due to measurement error.

Table 1. Repeatability analysis for incisor arcade measurements of bighorn sheep taken in 1999 at Ram Mountain, Alberta. A significant F ratio indicates that within-individual variability (measurement error) is less than between-individual variability.

Trait	Age class	*d.f.*	*F* ratio	Repeatability *r*
Incisor arcade breadth				
	Lambs	15	7.68***	0.77
	Adult ewes	31	34.50***	0.94
Incisor arcade depth				
	Lambs	15	7.78***	0.77
	Adult ewes	31	23.16***	0.92

*** = $P < 0.0001$

Variation of incisor arcade size with age

In adult ewes, incisor arcade breadth and height appeared to decrease with age (Fig 2). The apparent effect of age on incisor arcade size could be due to either selective mortality (if ewes with small arcades were more likely to survive than ewes with large arcades) or to tooth wear. To explore the relationship between IAB and IAD and age, we used a LME model fitting individual identity as the random grouping factor. This design corresponds to an analysis of covariance (ANCOVA) with individual identity as a random factor, incisor breadth or height as the dependent variable, and age as a covariate. If the decrease in incisor arcade size occurs within individuals (and thus is probably due to tooth wear), there should be a significant within-individual negative regression with age. Indeed, both incisor arcade breadth and depth decreased with age within individuals (IAB: $\beta = -0.031$, $F_{1,201}=82.29$, $p<0.0001$; IAD: $\beta = -0.037$, $F_{1,201}=71.1$, $p<0.0001$).

Effects of incisor arcade size on summer mass gain

We explored the effects of IAB, IAD, density and age on summer mass gain in adult ewes using LME models with individual identity as a random factor. We fitted autumn mass as the dependent variable, and spring mass as a fixed covariate. Consequently, any effects of arcade size on autumn mass would have been due to effects on summer mass gain. Age was fitted as a quadratic function (Bérubé *et al.* 1999). As expected, age and spring mass had positive effects on autumn mass (Age: $\beta = 2.27$, $F_{1,196} = 15.08$, $p = 0.0001$; Age^2:$\beta = -0.1$, $F_{1,196} = 11.21$, $p = 0.001$; Spring mass: $\beta = 0.31$, $F_{1,197} = 47.67$, $p<0.0001$). Density had no effect on mass gain ($F_{1,196}= 0.30$, 0.58) and neither did incisor arcade breadth ($F_{1,196} = 2.19$, 0.14) nor incisor arcade depth ($F_{1,196} = 2.19$, $p = 0.14$).

The effects of IAB, IAD, sex and density on summer mass gain of lambs were explored with GLMs. Autumn mass in lambs was a function of spring mass ($\beta = 0.71$, $F_{1,71}= 1.70$, $p = 0.02$), population density ($\beta = -0.06$, $F_{1,71}= 12.79$, $p = 0.0006$), and IAB ($\beta = 13.64$, $F_{1,71}= 12.79$, $p < 0.0001$). IAD ($F_{1,72} = 1.03$, $p = 0.36$) and sex ($F_{1,71}=0.02$, $p = 0.89$) did not contribute significantly to the model although when a larger sample was considered, male lambs were about 10% heavier than female lambs (Festa-Bianchet *et al.* 1996). Incisor arcade breadth thus appeared to have a strong positive effect on mass gain in lambs.

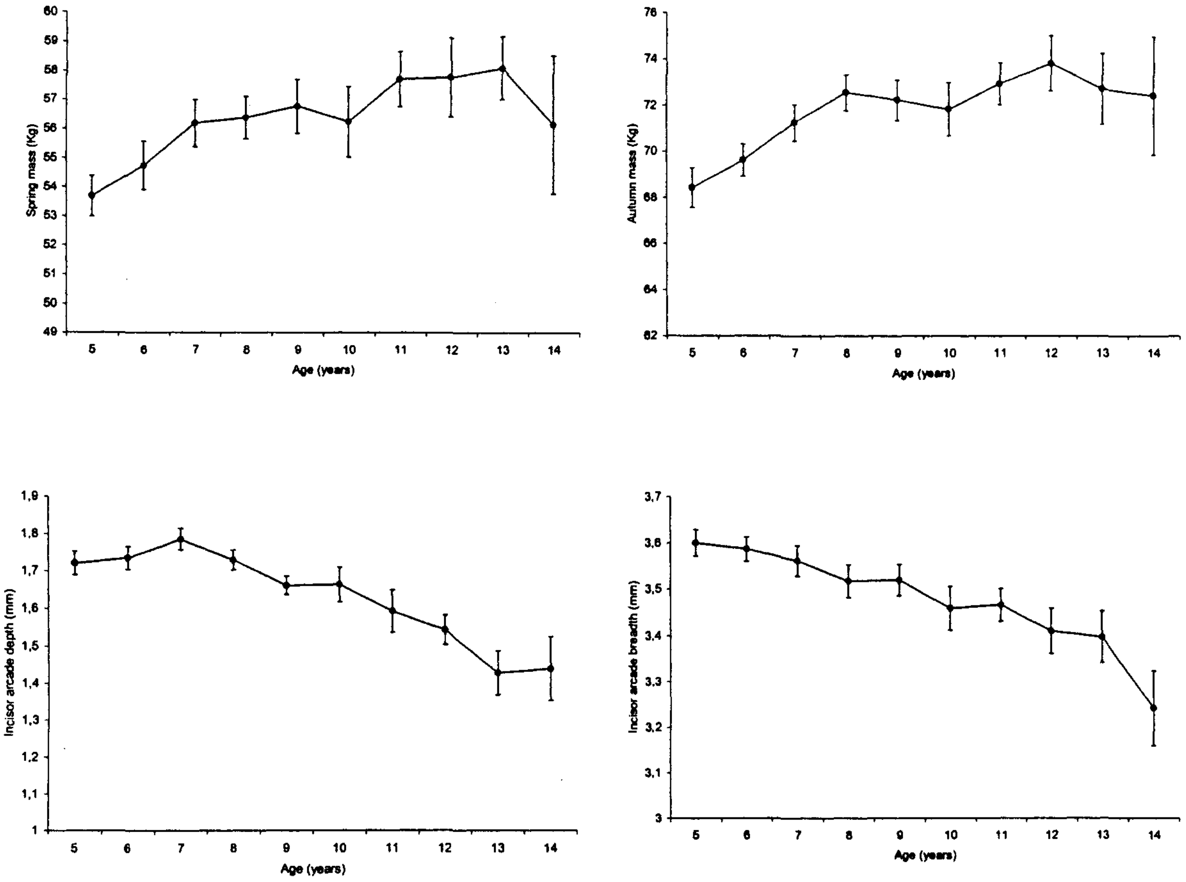

Fig. 2. Variation with age in the four morphological traits analyzed in this paper for adult bighorn ewes in Ram Mountain, Alberta.

Effects of incisor arcade size on survival

The GLM showed no evidence of an effect of IAB and IAD on lamb survival. Sex had an effect on survival while autumn mass was almost significant (Final GLM: Survival ~ sex + autumn mass; sex: $\beta = -0.2$, deviance = 5.84, d.f. = 1,120, p = 0.02; autumn mass: $\beta = 0.11$, deviance = 3.3, d.f.= 1,118, p = 0.07; rejected terms: IAB: deviance = 0.13, d.f. = 1,117, p = 0.71; IAD: deviance = 0.14, d.f. = 1,116, p = 0.71). Within this sample, male lamb survival to one year of age was 29.9 %, while female lamb survival was 45.2 %.

For adult ewes, when age was ignored, IAB and IAD appeared to have an effect on survival, while spring mass and autumn mass did not (Final GLMM: Survival ~ IAB + IAD; IAB: $\beta = 2.44$, d.f. = 201, t = 2.99, p = 0.003; IAD: $\beta = 2.38$, d.f. = 201, t = 3.21, p = 0.001; rejected terms: autumn mass: d.f. = 200, t = -0.48, p = 0.63; spring mass: d.f. = 198, t = -0.98, p = 0.33). When we included age, however, IAB and IAD dropped out from the final model (Final GLMM: survival ~ age; age: $\beta = -0.61$, d.f. = 200, t = -8.89, $p < 0.0001$; rejected terms: IAB: d.f. = 200, t = 1.54, p = 0.12; IAD: d.f. = 200, t = 1.33, p = 0.18), suggesting that IAB and IAD were just a proxy for age.

Testing for a causal relationship between incisor arcade size and mass gain in lambs

We first tested a basic measurement model, with a SEM approach, including body size as a latent variable and incisor arcade breadth, incisor arcade depth, autumn mass and spring mass of lambs as dependent variables (Fig. 3). The basic model adequately describes the relationships among these variables (goodness-of-fit test: Santorra Bentler $\chi^2 = 0.404$, $d.f.= 2$, $P = 0.82$). The path coefficients of the accepted measurement model are reported in Fig. 3. Autumn mass showed the highest correlation with latent body size ($r^2 = 0.905$). The other observable variables had the following determination coefficients with the latent variable body size: spring mass, $r^2 = 0.216$; incisor arcade breadth, $r^2 = 0.348$; incisor arcade depth, $r^2 = 0.016$.

We then tested for the significance of two nested models, relaxing the constraints between incisor arcade breadth or depth and autumn mass, testing therefore for possible direct causal links between incisor arcade size and mass gain, independent of body size (Fig. 3). If there was an effect of Incisor arcade breath or depth and autumn mass that was not already explained by the common allometric of effect of body, then the addition of these direct effects would significantly improve the fit of the model. Neither the nested model including a causal link between IAB and autumn mass nor the one with a causal link between IAD and autumn mass significantly improved the basic measurement model (Table 2). There is therefore no evidence for such direct effects and supports the hypothesis that the association between incisor arcade breadth or depth and autumn mass is due simply to a general allometric relationship with body size.

Table 2. Chi-squared difference test of a possible direct causal links between incisor arcade size measurements and mass gain in bighorn sheep lambs. Following Santorra and Bentler (1999) *c* is the scaling correction factor, *cd* is the difference test scaling correction, and *TRd* is the Satorra-Bentler scaled chi-square difference test. A significant *P* value would indicate that the nested model provides a better fit than the basic measurement model.

Model	*C*	*cd*	*TRd*	*P*
Basic model	0.658			
+ IAD->AW	0.564	0.752	0.270	0.60
+ IAB->AW	0.625	0.691	0.003	0.96

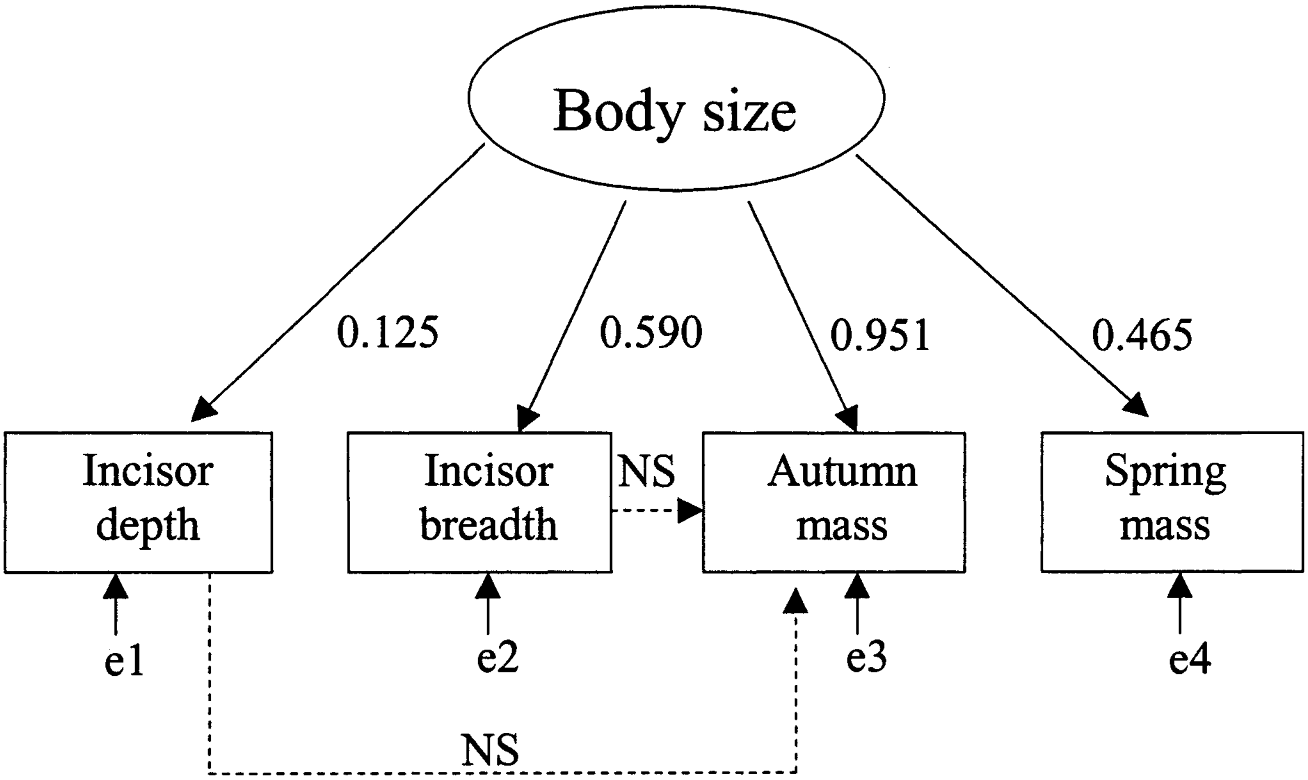

Fig. 3. Path analysis diagram for body size components in bighorn sheep lambs. Solid arrows represent the basic measurement model with Body size as a latent variable. Numbers accompanying the arrows are path coefficients. Dashed arrows represent nested models testing for the hypothesised causal links between Incisor breadth or depth and Autumn mass. Significance of the nested models is provided above the arrows.

Discussion

Both in our and in other studies (Illius *et al.* 1995; Gordon *et al.* 1996; Milner *et al.* 1999), dental-wax impressions were taken on live animals, in field conditions that are not optimal. The accuracy of measurements made on those impressions may thus be questioned. Estimation of measurement error is very important for traits hypothesised to be under direct selective pressure, because biologically significant patterns could be masked by measurement error. For bighorn sheep, within-individual variability (measurement error) in arcade size was smaller than variability among individuals, and repeatability was high for both incisor breadth and depth. Variability in incisor breadth and depth among individuals was thus not simply due to measurement error. Dental-wax impressions, therefore, reliably measure incisor breadth and depth in wild ungulates captured and processed under field conditions.

We found no evidence of a direct effect of natural selection on incisor arcade size in bighorn sheep. Arcade size decreased with age in adult ewes. Such a decrease might be due to a selection gradient against larger incisor arcades. A directional selection gradient against large incisor arcade size could in theory develop at low density, when swards are high and sheep with narrow muzzles may be able to better select the most nutritious plant parts (Illius *et al.* 1995). Indeed, density declined dramatically in Ram Mountain, the number of adult ewes decreasing by half during this study. The age-related decrease in incisor arcade size, however, occurred only within individuals, and thus is most probably due to tooth wear. Tooth wear with age is well known in ruminants, and it is even used to estimate age (Hewison *et al.* 1999). Virtually all studies to date in free-ranging ungulates have analysed tooth wear in molars, but tooth wear in incisors should also be expected. Veterinarians use incisors to estimate age in domestic sheep and cattle (Fraser *et al.* 1991). The outer incisors (actually incisiform canines) of sheep have a typical conic form, with the cone pointing towards the palate. A reduction in height of the incisors due to tooth wear, would consequently reduce the dimensions of the incisor arcade, as documented in this study. Our results emphasise how tooth wear with age should be considered in studies on selection on incisor arcade size. Ignoring the age effect may lead to spurious interpretations.

There was no effect of incisor arcade breadth or depth on lamb survival, which was affected by autumn mass, in accordance with previous studies on lamb survival (Festa-Bianchet *et al.* 1997). In this sample we also found a lower survival of male than of female lambs.

In adult ewes, also in accordance with previous studies (Festa-Bianchet *et al.* 1997), only age influenced survival, survival decreasing with age. Incisor breadth and depth did not appear to affect survival. Interestingly, when we fit incisor breadth and incisor depth without including age in the model, both variables appeared to affect survival while body mass did not. When age was included in the model, the effect of incisor arcade size disappeared. Because age is negatively related to incisor arcade size, ewes with larger incisors survive better simply because they are younger and survival decreases with age (Loison *et al.* 1999). Illius *et al.* (1995) showed an effect of incisor arcade breadth on survival of adult feral sheep, but they did not consider age in their analyses, not accounting thus for possible tooth wear with age. In food-limited populations such as the Soay sheep studied by Illius *et al.* (1995), tooth wear with age could be particularly strong. Skogland (1988) found that in wild reindeer (Rangifer tarandus) tooth wear with age in food-limited females was twice as fast as among well-fed females. Moreover, Illius *et al.* (1995) pooled together in the same analysis yearlings and adults, despite reporting that yearlings have a lower survival and smaller incisor breadths than adults.

One assumption of the hypothesis that incisor arcade is under a selective gradient, is that it influences foraging efficiency (Gordon *et al.* 1996). Using linear models we showed how incisor arcade breadth is related to summer weight gain in lambs but not in adult ewes. That result, however, must be interpreted with caution because of the collinearity among fixed effects. When we explored these relationships further using a latent variable path analysis model with a Structural Equation Modelling approach, the best fit was given by a basic measurement model with body size as a latent variable that was the single common cause of all observed variables. Autumn mass was the variable most correlated to body size. Adding explicit causal links between arcade breadth or depth and autumn mass, independent of body size, did not improve the model. We therefore conclude that the relationships between incisor arcade size and body mass are just due to an allometric relationship with body size. We found no causal relationship between incisor arcade size and mass gain. Big sheep survive better than small ones, but incisor arcade appears not to be under directional selection in this population. Possibly the morphology of the incisor arcade in our study population reflects adaptation to highly variable environmental conditions. Bighorn ewes on Ram Mountain experience much annual variability in food availability over their lifespan of 8-15 years. In addition to changes in density that affect forage availability, ewes switch seasonally from browsing to grazing and feed on swards of different heights. Our results suggest that variability in incisor arcade size among individual bighorn sheep is mostly due to variation in body size and age.

In conclusion, researchers studying natural selection on morphological traits should be cautious in inferring directional selection on a trait from patterns of covariance with survival. A third variable, such as body size or age in this study, could be the common cause of the variation both in survival and in the size of the trait, leading to a spurious correlation between the trait and survival. Mixed models, which account for intra-individual variability, and structural equation models, which explicitly model causal links among variables, are powerful tools to identify real selection patterns acting on correlated traits.

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