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Thinhorn Sheep Evolution
and Behaviour







ABSTRACT

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Diss.

The major results of this study of thinhorn sheep evolution and behaviour have come from investigations of (i) the impact of glaciation on evolutionary history, (ii) the potential for MC1R to determine coat colour darkness, (iii) the role of colour as an indicator of quality, (iv) the relationship between horn growth rate and longevity and (v) behavioural reactions of sheep to human disturbance. I have found that hybridisation is a more important factor in mountain sheep evolutionary processes than previously thought. Contact between bighorn (*O. canadensis*) and thinhorn sheep (*O. dalli*) occurred prior to the last ice age which appears to have resulted in a zone of unique phenotypic colour variation. I attempted to determine whether colour variation is determined by the melanocortin 1 receptor (MC1R) gene. However, there was no evidence to suggest a relationship between individual darkness and mutations in MC1R. Behavioural evidence from sheep in central Yukon demonstrated that facial darkness is a good indicator of individual quality in rams, although I did not verify its use as a signal. Horn growth rate was negatively correlated with longevity indicating that a trade-off exists between these two traits that affect individual mating success. Finally, human generated disturbance resulted in differing reactions in thinhorn sheep genders and age classes.

Key words: Colour morphology; human disturbance; ice age; growth rate; *Ovis*; quality indicator; refugia.

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LIST OF ORIGINAL PUBLICATIONS

My thesis is based on the following five papers which will be referred to in the text by their Roman Numerals I-V. In each of the five papers I have been the primary person responsible for the planning of the research, fieldwork (when applicable), data analysis and writing. Contributions to data analysis were made by A. Grapputo (I), H. Högmander (V) and S. Kärkkäinen (V). Sequencing of DNA was performed by K. Worley (I & II) and J. Moe (II).

- I Loehr, J., Worley, K., Grapputo, A., Carey, J., Veitch, A. & Coltman, D. W. 2006. Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. *Journal of Evolutionary Biology* 19: 419-430.
- II Loehr, J., Worley, K., Moe, J., Carey, J. & Coltman, D. W. MC1R correlates with thinhorn sheep colour cline but not individual colour. Manuscript.
- III Loehr, J., Carey, J., Ylönen, H. & Suhonen, J. Coat darkness is associated with individual quality in a mountain sheep hybrid zone. Manuscript.
- IV Loehr, J., Carey, J., Hoefs, M., Suhonen, J. & Ylönen, H. Horn growth and mortality: Implications for natural and artificial selection in thinhorn sheep (*Ovis dalli*). Manuscript.
- V Loehr, J., Kovanen, M., Carey, J., Högmander, H., Jurasz, C., Kärkkäinen, S., Suhonen, J. & Ylönen, H. 2005. Gender- and age-class-specific reactions to human disturbance in a sexually dimorphic ungulate. *Canadian Journal of Zoology* 83: 1602-1607.

1 INTRODUCTION

1.1 Overview

In this study of behaviour and evolution I address three major themes: To give a foundation for later work I investigated thornhorn sheep (*Ovis dalli*) evolutionary history and genetics. This gave insight into past evolutionary events (I) and allowed for the development of hypotheses as work continued (III). The degree of colour variation in this species is striking and a major focus of my work (III) and I assessed the potential for genetic determination of the colour trait (II). The second major theme looks at the role of sexual and natural selection in thornhorn sheep ecology. I focus on the potential for darkness of coat colour as an indicator of individual quality (III) and the cost of rapid horn growth (IV). The third theme investigates the effect of human activity on thornhorn sheep evolution and behaviour. For many species knowledge of evolutionary ecology and behaviour is not complete without investigating the influence of humans (e.g., Palumbi 2001). I assess the mechanism proposed by Coltman et al. (2003, 2005) for human harvesting to have a selective effect on horn growth (IV) and the effect of human disturbance on thornhorn sheep behaviour (V).

1.2 Evolutionary history

Isolation in differing glacial refugia has been found to attribute for much of current morphologic and genetic variation in species (Pielou 1991, Hewitt 1996, 2000, Willis & Whittaker 2000). Research thus far has concentrated on the identification of major glacial refugia, however, the evolutionary importance of small glacial refugia is not as well known.

It is thought that during the last ice age thinhorn sheep occupied ice free regions in Yukon and Alaska (known as Beringia) while bighorn occupied southern ice free areas in what is now western United States. Thus morphological differentiation has been thought to be a result of isolation in differing glacial refugia during the Pleistocene (Cowan 1940, Pielou 1991, Geist 1999). However, recent knowledge of glacial limits demonstrates that sheep currently inhabit four regions known to have been ice-free during the last ice age. The two major refugia of Beringia and southern North America are well documented. However, portions of the Mackenzie Mountains of Canada's Northwest Territories (Dyke & Prest 1987, Duk-Rodkin & Hughes 1991), and a region further south in northeastern British Columbia (Catto et al. 1996) also remained ice-free. I test whether sheep survived glaciation only in the two major refugia or if the two smaller ice free areas served as glacial refugia as well. I also test the hypothesis that long term isolation of bighorn sheep and thinhorn sheep has occurred.

1.3 Colour morphology and MC1R

In many wildlife species there is a very strong correlation between melanocortin 1 receptor (MC1R) genotype and the degree of individual darkness (Mundy 2005, Ritland et al. 2001, Eizirik et al. 2003, Nachman et al. 2003). Typically alleles for functionally active MC1R result in a darker pelage, while alleles for non-functional MC1R are recessive and result in a lighter pelage. The intergradation of white and dark colour morphs in thinhorn sheep is very similar to that of the lesser snow goose which is one of the best examples of MC1R determining individual darkness (Mundy 2004). To attempt to establish the genetic component of thinhorn sheep coat colour I test whether MC1R is correlated with individual phenotype. Genetic analysis of MC1R also allows for further investigation of phylogeographic patterns and contributes to the overall picture gained from my work on evolutionary history.

1.4 Coat darkness and sexual selection

Hybridisation can instantaneously introduce variation in phenotypic marker traits that can be used for mate choice or conflict assessment (Seehausen 2004). Hybridisation between bighorn and thinhorn sheep has introduced colour variation across the length of the hybrid zone. This has resulted in populations with great variation in individual darkness. Variation in mammalian coat darkness can indicate individual social dominance (West & Packer 2002). At the physiological level a link between darkness of hair colour and aggressive behavior exists because testosterone regulates hair growth (Thornton et al. 2001)

and melanin production (Wilson 1983). Hair darkness and seasonal variation in testosterone levels has also been demonstrated in white-tailed deer (*Odocoileus virginia*) (Bubenik & Bubenik 1985). Given the high degree of colour polymorphism and intrasexual selection mountain sheep of the hybrid zone are ideal organisms to test the effect of ancestral hybridisation on current sexual selection.

1.5 Life history trade-offs and implications for natural and artificial selection

Do male characteristics that aid in mating success also result in improved viability? This is a central question in the study of sexual selection (Kokko et al. 2003, Hunt et al. 2004) and has direct implications for life history theory (Stearns & Koella 1986). Many aspects of thornhorn sheep ram morphology and life history have undoubtedly evolved through intrasexual selection, and previous research suggested that a trait (horn growth) that is an advantage in male dominance interactions is balanced by a cost of longevity (e.g., Geist 1966, 1971). However, small sample size and the lack of control of confounding variables in these earlier studies has meant that subsequent studies have assumed that rapid horn growth provides a survival advantage (Coltman et al. 2003, 2005).

The evolutionary consequences of human induced selection on harvested populations have recently gained great attention (Palumbi 2001, Ashley et al. 2003, Ernande et al. 2004, Olsen et al. 2004). An interesting example of a phenotypic based harvest can be found in the trophy hunting activity placed on mountain sheep. This type of selective harvesting is especially a concern because it focuses on a highly heritable trait (horn growth rate) (Coltman et al. 2003, 2005). Thornhorn sheep harvest regulations are such that a ram can only be shot once its horns grow past a certain degree of horn curl. This creates a situation in which rams that have slow horn growth can avoid being shot until later in life, whereas rams with fast horn growth are at a higher risk of being shot early in life. This would apparently place an artificial selection pressure on rams that selects against rams with rapid growth (Coltman et al. 2003, 2005).

1.6 Human disturbance and behaviour

There is ample evidence that individual characteristics affect antipredator behaviour (Elgar 1989, Berger 1991, Magurran and Nowak 1991, Fitzgibbon & Lazarus 1995, Sinclair & Arcese 1995, Bleich 1999, Shine et al. 2000). If behavioural reactions to human disturbance stimuli correspond to antipredator behaviour (Sutherland 1996, Frid & Dill 2002), individual variation in reactions

to a human stimulus should also be observable. However, evidence to support individual variation in reactions this is limited to a few studies (Walther 1969, Steidl and Anthony 1996, 2000). In other studies, which have attempted to test individual variation in behavioural reactions to disturbance no significant differences have been found in bighorn sheep (Stockwell et al. 1991, Bleich et al. 1994), or woodland caribou (*Rangifer tarandus caribou*) (Duchesne et al. 2000). In this study I test whether reactions to a human disturbance stimulus depends on age and gender in thinhorn sheep.

2 STUDY SPECIES

Mountain sheep in North America belong to a widely distributed genus (*Ovis*) of ungulates that stretches from Europe (where many populations of wild living sheep have been introduced) across southwestern Asia and then northeast through central Asia to Siberia and into North America. The genus is a relatively recent arrival to North America. Fossil and mtDNA evidence suggest that sheep first colonised the continent about 500,000 to 1,000,000 years ago by crossing the Bering land bridge (Ramey 1993, Bunch et al. 2006). Currently two species of sheep are recognised in North America, bighorn sheep (*O. canadensis*) and thinhorn sheep (*O. dalli*). This distinction is largely based on morphological differences measured in skulls (Cowan 1940) although a reanalysis of Cowan's data with modern statistical techniques revealed fewer differences (Ramey 1993). Currently the species status of *O. dalli* and *O. canadensis* is questionable due to the discovery of hybridisation between them (Loehr et al. 2006).

My research has primarily focussed on thinhorn sheep which inhabit mountain ranges of British Columbia, Yukon, and Northwest Territories, Canada, and Alaska, USA. Two subspecies are currently recognised Dall's (*O. d. dalli*) and Stone's sheep (*O. d. stonei*). The most obvious character to differentiate these subspecies is coat colour. Dall's sheep generally have coats that are all white, while Stone's sheep coats are darker. Complete intergradation between dark and white morphs exists on a geographic scale (Sheldon 1911). Analysis of microsatellite nuclear DNA and mitochondrial DNA has revealed substantial hybridisation between subspecies (Worley et al. 2004, Loehr et al. 2006).

Thinhorn sheep require habitat which has large grass and sedge meadows for forage and steep cliffs and rock outcrops which are used as escape terrain for predator avoidance. Importantly, winter range is found in areas of relatively low snowfall and high winds that clear snow from foraging areas. In summer sheep spend most of their time in alpine areas, while winter range can be found in both alpine and subalpine areas (Geist 1971, Nichols & Bunnell 1999).

The primary predators of thinhorn sheep are wolves (*Canis lupus*), coyotes (*Canis latrans*), and black bears (*Ursus americanus*) while golden eagles (*Aquila chrysaetos*) prey on lambs (Nichols & Bunnell 1999). Typically, mountain sheep

populations are not the primary source of food for any predator species, however, individual predators have been known to specialise on a sheep population. Such a case has been recorded in a bighorn sheep population in Alberta, Canada where a cougar (*Puma concolor*) learned to prey on sheep and was a major contributor to a population decline (Ross et al. 1997).

Mating in thornhorn sheep occurs between mid November and mid December. The primary mating tactic involves a dominant ram defending one estrous ewe. Estrous can last 1-3 days, during which a defending ram typically stays in close proximity to the ewe and wards off challengers. Rams that do not have access to a ewe can attain matings by using the coursing tactic. A coursing ram can be successful by chasing an estrous ewe and forcing copulation. Coursing is a tactic adopted by subordinate rams and a coursing ram may succeed in chasing a ewe away from a defending ram (Geist 1971, Nichols & Bunnell 1999).

Ram dominance is settled by display behaviour and physical conflict (Geist 1971). Physical conflict to resolve dominance status involves repeated horn clashing. Injury and death can result either from collisions or from falls from precipitous terrain during conflicts. Ram horns, however, are not used for piercing opponents, and injuries of this kind are not sustained in conflicts. Endurance is probably an important aspect of dominance battles as dominance fights can last many hours and one has been recorded to have lasted 25 hours (Geist 1971).

3 RESULTS AND DISCUSSION

3.1 Evolutionary history (I)

I investigated the evolutionary history of North American mountain sheep (*O. dalli* and *O. canadensis*) with the aid of mitochondrial DNA. I used 223 sequences from a 604 base pair portion of the control region. The refugial origins of mountain sheep populations differed from the expected pattern of refugia in Beringia and southern North America. In addition to evidence supporting the major refugia I found that a population in British Columbia appears to have survived the last ice age in a small ice free area in British Columbia, and that a population of sheep also survived in the Mackenzie Mountains of Northwest Territories. Habitation of these ice free areas was inferred by using mtDNA as a molecular clock. This showed that divergence between refugial populations and their neighbouring populations all predated the receding of ice which occurred about 10,000 years ago. Evidence from glacial limits supports the idea that small areas in British Columbia and Northwest Territories were ice free throughout glaciation, and that they were probably suitable to sheep habitation (Dyke & Prest 1987, Duk-Rodkin & Hughes 1991, Catto et al. 1996). This is the first evidence to suggest that any organism survived glaciation in these refugia.

Sheep in British Columbia showed evidence of a genetic bottleneck, which was probably caused by survival of only a few individuals in a small ice free area. This evidence is supported by nDNA microsatellite analysis which also showed low genetic diversity (Worley et al. 2004). In contrast the sheep in the Northwest Territories had relatively high haplotype and nucleotide diversity, which may be explained by larger refugial size.

The survival of the populations in small glacial refugia has preserved the genetic signature of hybridisation between bighorn and thornhorn sheep. Most haplotypes in the British Columbian population were more similar to bighorn sheep than other thornhorn sheep populations. This may be explained by assuming that this population was originally a thornhorn sheep population and

the near fixation of bighorn haplotypes occurred during the population bottleneck. However, it is also possible that this population is an ancestral form of bighorn sheep, which has hybridised with thinhorn sheep from the Northwest Territories.

3.2 Colour morphology and MC1R (II)

I analysed 40 sequences of the entire melanocortin-1 receptor (MC1R) from across the range of thinhorn sheep. Sequenced genotypes were compared to colour morphology at the population and individual level to assess whether there was a relationship between mutations in MC1R and colour morphology. A mutation in MC1R at base pair position 921 was discovered which coincided fairly well with the geographic cline. Previous studies on other species have shown that a mutation in MC1R can cause loss of function of the gene, and result in white phenotypes (Ritland et al. 2001, Mundy 2005). However, in the case of thinhorn sheep the mutation was not directly associated with white coats. Furthermore, analysis at the individual demonstrated no discernable association between coat darkness and MC1R mutations.

This result is perhaps surprising because the melanin based coat colour variation in thinhorn sheep appears to be a likely candidate to be affected by MC1R. The negative result in the case of thinhorn sheep underlines the fact that our knowledge of genes that affect vertebrate colour is still very limited (Mundy 2005), and that MC1R cannot be expected to always have an effect on melanin based variation in colour. It is important to note that the mutation at bp 921 is outside of the region in MC1R that has been repeatedly shown to affect melanin production.

In thinhorn sheep MC1R is useful in that it lends further support to evidence from nDNA (Worley et al. 2004) and mtDNA (II) which demonstrates a zone of introgression between Stone's and Dall's sheep populations.

3.3 Coat darkness and sexual selection (III)

I analysed colour morphology of 30 adult rams and 26 adult ewes in the same population where growth rate and darkness were correlated. Rams were darker than ewes in the flank and face regions of the body, but did not differ in neck darkness. Ram flank and face darkness increased with age, however, this was not the case in ewes.

I recorded agonistic interactions of rams and ewes November-December 2003 and 2004. Ram facial darkness was clearly associated with dominance rank and mating opportunities. More conflicting results were found in neck and flank darkness, a trend likely due to a higher heritable component to darkness

in these regions of the body. In ewes no single colour trait reliably predicted dominance outcome, however, when all colour traits were combined darker ewes were more often victorious in dominance dyads.

I compared horn growth rate of three populations that differed in darkness and found that darker populations did not have faster growing horns than lighter populations. However, at the individual level darkness was positively correlated with horn growth rate. Thus there is no difference in growth rate between light and dark populations; however, within a population with variable individual colour morphology, darkness has become linked with horn growth rate.

According to the criteria given by Dale (2000) male facial darkness was found to be the most reliable predictor of individual quality in sheep from the Pelly Mountains. The usefulness of neck and flank darkness to indicate quality is probably limited by higher heritable components in these regions of the body. The result that individual darkness reliably indicates ram quality may be unique to the hybrid zone. In one of the parent populations (Dall's sheep) there is a complete absence of colour variation. It would be interesting to test if the colour signal is available in bighorn sheep. Colour variation is evident in this species, however, its usefulness as an indicator of quality may be limited if variation is highly heritable.

Facial darkness may function as a low cost signal to advertise individual quality to other rams (Rohwer & Rohwer 1978, Evans & Hatchwell 1992, Hurd 1997). Because horn and body size are also very likely to be indicators of quality, facial darkness could function as one signal in a multicomponent signal system. When more signals are available to the receiver information can become more accurate (Johnstone 1995) and the signaler can benefit from improved signal detection, recognition, discrimination and memorability by the receiver (Rowe 1999).

Sheep of the Pelly Mountains are a very good example of evolution by hybridisation. Hybridisation has resulted in a population with unique colour morphology that distinguishes it from both parent populations. Interestingly, a trait that was completely absent in one of the parent populations has become a good indicator of individual quality in the hybrid population.

3.4 Life history trade-offs and implications for natural and artificial selection (IV)

I found that, on average, rams that grew horns rapidly in the first five growing seasons of life, tended to die of natural causes at a younger age than slower growing sheep.

I used data from a collection of horns from 91 individuals gathered 1969-2005, from un hunted populations or populations with very little hunting. In this sample rams with faster growth died younger than those with slower

growth. For each individual sample I knew the population of origin, however, I did not know the birth year of the sample. Horn growth can also vary depending on year of birth (Hik & Carey 2000) or population of origin. To determine that the result was not due to a population or cohort effect I constructed a Monte Carlo model that accounted for population and cohort fluctuation. The model demonstrated that population and cohort alone could not account for the result, and therefore, it could be verified that rapid growth does come at a cost of decreased lifespan.

The reasons for the longevity cost of rapid growth are poorly understood (Metcalf & Monaghan 2001, Olsson & Shine 2002). The cost may be physiologically imposed in the form of greater tumour production, increased incidence of coronary lesions, and higher susceptibility to external parasites (Eklund & Bradford 1977, Saunders et al. 1992). The cost of rapid growth may also be due to aspects of the thinhorn sheep mating system. In mammals with polygynous mating systems that involve male male competition a cost of early reproduction may be present (Clinton & Le Boeuf 1993) Although little evidence is available on rut associated mortality it is thought that faster maturing rams are more likely to be fully active in the rut at an early age, and exhaustion and injuries incurred during the rut are considered the greatest indirect or direct cause of mortality for mature rams (Geist 1971).

This finding has broad implications for the evolution of mountain sheep life history. Previously, it has been assumed that rapid growth is only advantageous to the individual. It was thought that rapid horn growth provides an advantage of greater longevity as well as greater horn size and thus greater dominance rank and annual and lifetime mating success (Coltman et al. 2003, 2005). Our results demonstrate that at least in thinhorn sheep a trade-off is present. The mating and dominance advantage incurred through large horn size can be achieved in two ways. Relatively large horns can be achieved either by growing rapidly or by greater longevity. Of these two independent variables growth rate is probably a better predictor of horn size than age. Previous research on bighorn sheep has shown that annual mating success is affected by an interaction between age and horn size (Coltman et al. 2002). Therefore, although sheep with slower growth will probably have slightly smaller horns on average they will also be relatively old, and receive a mating advantage through older age and longevity. The effect of the trade-off may be that it limits the evolution of larger horn size. Because there is an advantage to both rapid horn growth and slower horn growth genes for both rapid and slow growth will be passed on.

Furthermore, our results have implications for the study of artificial selection on horn size. The harvest system of most sheep populations is based on growth rate. It has been thought that this system results in sheep with rapid growth becoming shot long before they would have died naturally (Coltman et al. 2003, 2005). Our results indicate that the mechanism to facilitate artificial selection may not be as strong as previously thought.

3.5 Human disturbance and behaviour (V)

I analysed the reactions of 35 sheep to a human disturbance stimulus. Reactions of rams, ewes, adults and juveniles were recorded during disturbance trials where one or two photographers approached the sheep at close proximity. Behaviour during disturbance was compared with behaviour of sheep when no humans were in close proximity. Ewes decreased bedding and increased foraging. There was a trend for a greater increase in vigilance among adults than juveniles during disturbance.

Because of smaller body size ewes probably have adopted an antipredator strategy that relies on mobility to escape predation. This may explain why bedding was less frequent during disturbance trials. The trend for greater vigilance among adults during disturbance may be associated with young inexperienced individuals not recognising a threat even though it is visible to them. Parents may also exhibit greater vigilance if they are motivated by warning descendent kin (e.g., Clark 1994, Blumstein et al. 1997).

On the whole reactions to this type of disturbance were quite mild and similar to that found in caribou (*Rangifer tarandus*) (Duchesne et al. 2000). With proper precautions and continued monitoring disturbance of this type does not present a danger to this sheep population.

I found differences in the ways sex and age classes reacted to disturbance and future disturbance research should carefully consider how potential individual differences are taken into account.

4 CONCLUSIONS

In the course of my research a number of general trends have emerged that are important to mountain sheep evolution and behaviour. First, it is evident that hybridisation has been a very important aspect of mountain sheep evolution. The verification of hybridisation between mountain sheep species and subspecies through molecular evidence (I, II) has been an important step in understanding other aspects of their evolution (III). Previously, diversity in mountain sheep has been thought to have primarily originated as a result of geographic isolation (Pielou 1991, Geist 1999), however, it is clear that diversity can also be created when isolated populations make contact. This realisation is also an emerging trend in vertebrate research in general (e.g. Seehausen 2004).

In the past the study of mountain sheep has proven to be very fruitful in the field of sexual selection (e.g., Geist 1966, 1971, Hogg 1984), and my own work is no exception. It is evident that in addition to horn and body size another signal is probably involved in indicating individual quality (III). Sheep in the hybrid zone appear to be a rare example of a mammal that indicates individual quality through colour (also see West & Packer 2002). I have also found that conflicting selection occurs between natural and sexual selection on horn growth rate (IV). The discovery that rapid horn growth comes at a cost of decreased longevity has broad consequences for mountain sheep life history evolution. Mountain sheep have also provided one of the first examples demonstrating a cost of rapid growth in a wild population (also see Olsson & Shine 2002).

Finally, awareness of how human behaviour affects mountain sheep behaviour and evolution is of current importance, and my work underlines the value of behavioural and evolutionary ecology as contributors to wildlife management and conservation. In a study of thinhorn behaviour I found that reactions to human disturbance can be quite complex and differ according to age and gender (V). I also addressed the potential effect of hunting practices on horn growth (IV). My work contributes to the growing body of research on the selective effects of human harvesting (Palumbi 2001, Ashley et al. 2003, Coltman

et al. 2003, Ernande et al. 2004, Olsen et al. 2004). In the study of artificial selection a basic knowledge of life history and selective forces that affect species cannot be underestimated and in some cases artificial selection can be very similar to the trend seen in natural selection (IV).

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YHTEENVETO

Ohutsarvilampaiden evoluutio ja käyttäytyminen

Miksi kaikki ohutsarvilampaat Yukonjoen itä-puolella ovat tummia, mutta joen toisella puolella valkoisia? Minkä takia lampaiden väri vaihtelee niin paljon? Näillä kiehtovilla kysymyksillä aloitin tutkimukseni, ja väitöskirjan aikana on tullut monia muita mielenkiintoisia kysymyksiä vastaan. Ohutsarvilamma on hyvin mielenkiintoinen laji pelkän evolutiivisen historiansa takia, mutta laji on hyvin antoisa tutkimuskohde myös ajankohtaisille kysymyksille kuten: Miten ihminen vaikuttaa lajin evoluutioon metsästyksellään?

Aloitin tutkimukseni ohutsarvilampaiden evoluutiosta DNA-tutkimuksella. DNA-tutkimukset antavat näkökulman lajin evolutiivisista tapahtumista. Aikaisemmin on oletettu, että kaikki ohutsarvilampaat (*Ovis dalli*) asuttivat jääkauden aikana Beringian jäättömiä alueita Alaskassa ja Länsi-Yukonissa. Lampaiden DNA:ssa on merkkejä siitä, että jääkauden aikana pienillä populaatioilla oli mahdollista säilyä hengissä myös Northwest Territorioiden Mackenzie -vuoristossa sekä Brittiläisen-Kolumbian Kalliovuorten pohjoisosissa. Tämä on ensimmäinen biologinen todiste siitä, että laji pystyi säilymään näillä jäättömillä alueilla. Mitokondrio-DNA tutkimukset osoittivat myös, että ohutsarvilamma ja paksusarvilamma (*O. canadensis*) hybridisoituivat ennen jääkautta, ja että geenivirtaa on tapahtunut Dallin (*O. d. dalli*) ja Stonen lampaiden (*O. d. stonei*) välillä. Dallin ja Stonen lampaiden välisen risteymän mahdollisuus sai lisää todisteita melanokortiini 1 reseptorigeenin (MC1R) kautta. MC1R on osoittautunut hyödylliseksi myös lajien sisäisen väri vaihtelun tutkimuksessa, sillä MC1R on pystynyt selittämään yksilöiden tummuusasteen vaihtelun geneettisen perustan. Ohutsarvilampaan väri vaihtelee valkoisesta lähes täysin tummaan. Tämän perusteella odotin, että sama yhteys MC1R:n ja väri vaihtelun välillä olisi havaittavissa ohutsarvilampailla. Tulokset kuitenkin osoittivat, ettei MC1R selitä ohutsarvilampaiden yksilöllistä väri vaihtelua.

Tieto lajin evolutiivisesta historiasta ja lajien ja alalajien risteytymisestä antoivat kehysten tutkia lajin morfologiaa ja käyttäytymistä. DNA-tutkimukset todistivat, että valkoisten ja tummien lajien ja alalajien risteytymisen tulos on populaatio, jossa lampaiden väri vaihtelee runsaasti yksilöittäin. Tutkimuksessani selvitin onko risteytymisen tuottamalla väri vaihtelulla evolutiivista merkitystä. Tulokset osoittivat, että naaraiden ja koiraiden väri poikkesi toisistaan erityisesti kyljissä ja naamassa. Koiraat ovat usein naaraita tummempia, ja tämä antoi vihjeen, että värillä voisi olla merkitystä seksuaalivalinnassa. Koiraiden naaman väri osoittautui korreloivan hyvin sen laatua kuvaavien morfologisten ja käyttäytymisominaisuuksien kanssa. Naaman tummuusaste kasvoi iän myötä ja tummemmilla koirilla sarvenkasvu oli nopeampaa kuin vaaleammilla yksilöillä. Tummemmat urokset dominoivat vaaleampia yksilöitä ja saivat

paremmat mahdollisuudet paritella naaraiden kanssa. Naaman tummuus luultavasti toimii signaalina ja osana monisignaalisysteemiä tällä lajilla.

Tutkin myös ihmisten mahdollista vaikutusta ohutsarvilampaiden evoluutioon ja käyttäytymiseen. Metsästyssäännökset on suunniteltu niin, että metsästäjät saavat ampua koiraslampaan vasta silloin, kun sen sarvet ovat kasvaneet täyskierroksen. Tämän säännön seurauksena nopeakasvuiset koiraat voivat tulla metsästetyksi nuorempina kuin hidaskasvuiset. Tällä metsästyskäytännöllä saattaa olla evolutiivisia seurauksia, koska sarven kasvunopeus on periytyvä ominaisuus. Tutkimuksessani selvitin myös kasvunopeuden ja iän mukaisen kuolleisuuden suhdetta, eli eroavatko metsästetyt ja ei-metsästetyt populaatiot toisistaan? Tulos osoitti, että nopeasti kasvattavilla uroksilla on korkeampi kuolleisuusriski kuin hitaasti kasvattavilla uroksilla myös niissä populaatioissa, joita ei metsästetä. Suhde kuolleisuuden ja kasvun nopeuden välillä on hyvin samanlainen molemmissa populaatioissa. Tämä tulos poikkeaa aiemmista tutkimuksista, joissa on oletettu, että populaatioissa, joita ei metsästetä, nopeakasvuisat urokset elävät kauemmin kuin hidaskasvuiset.

Ihmisten aiheuttamat häiriöt voivat lisätä stressiä ja aiheuttaa muutoksia eläinten käyttäytymisessä. Tutkimukseni ihmisen läsnäolon vaikutuksista lampaiden käyttäytymiseen osoitti, että lampaat olivat valppaampia ihmisen läsnäollessa. Vanhempien lampaiden reaktio ihmiseen oli voimakkaampi kuin nuorempien, ja naaraat eivät maanneet yhtä paljon ihmisen läsnäollessa. Verrattaessa lampaiden näitä reaktioita petojen vaikutukseen, totesin niiden olevat varsin lieviä. Ihmisten aiheuttama häiriö ei siten todennäköisesti juuri vaaranna populaatioiden säilymistä.

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