7th International
Deer Biology Congress
Huilo Huilo - Chile

ADVANCES AND CHALLENGES IN DEER BIOLOGY

Werner Flueck - Jo Anne Smith - Andres Charrier
Editors

August 1-6, 2010

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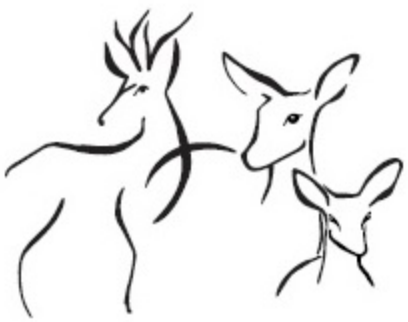
EDITORS



7th International
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Abstracts of the

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1-6 August 2010

PREFACE

Ruminants have played an important role for ecosystems and the development of human societies. The northern hemisphere was the principal region for the cervid radiation, whereas Africa was the center for bovids. Although South America never contained any native bovids, it was the only place in the southern hemisphere where cervid radiation took place. Today this continent holds a high diversity of native deer species. Moreover, with the introduction of several exotic deer species to Australia and New Zealand in recent times, the presence of cervids has been substantially increased in the southern hemisphere. The distribution of cervids, nearly worldwide today, has hence resulted in broad research and management programs on a global scale.

This is the first time that the International Deer Biology Congress (IDBC) takes place in South America. Holding the congress in Chile provides many neotropical deer biologists and managers a first-time opportunity to participate in an IDBC. This is reflected in the many presentations, seminars, workshops and activities related to the neotropical region. At the same time it will be the opportunity for many northern hemisphere deer scientists to visit and learn about the reality of deer research and management in this very unique continent with its many threatened deer, for which little is known. Additionally, colleagues from Australia and New Zealand can take a comparative look at the same exotic deer species introduced here, with the difference that South America has native cervids, camelids and large predators. By coming together from various corners of the world, we can share our research findings and interpretations to improve upon future endeavors related to deer, be it for research, conservation, management, medicine, production or recreation. One critical workshop, for instance, will look at the situation of overabundant deer with respect to protected areas, an issue of global relevance.

The 7th IDBC brings together a broad diversity of professionals working with deer from 21 different countries, including scientists, conservation biologists, wildlife managers, production specialists and graduate students. We would like to thank all the participants for their sincere efforts to make it to this congress and present their latest insights on the fascinating field of deer biology to share with the global community of deer experts and enthusiasts.

We welcome all of you and hope you will have a memorable visit to the Huilo Huilo area in Chile.

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PLENARIES

Reintroducing the Persian Fallow deer - a chronology of ups and downs

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1. Reintroductions are expensive high-risk conservation oriented procedures. The Persian fallow deer (*Dama mesopotamica*) reintroduction by the Nature and Parks Authority (NPA) in Israel is based on a permanent breeding core (Hai-Bar Carmel). The core was established in 1976, before IUCN guidelines for reintroduction were composed, and existed for nearly 20 years with little thought devoted to the reason for its establishment. By the early 90s the breeding core had nearly 40 adult females and it became evident that a reintroduction program is necessary.
2. The program. The permanent breeding core allowed a new long-term adaptive management approach based on multiple releases. Associated with this approach were several questions that had to be addressed in the program, such as: How often should releases be carried out and how many individuals should be included in each release? Should releases be in one location or more? How to construct the habituation enclosure to allow for repeated releases? When to end the project? How long will it take? In addition, there were ecological questions that could only be addressed by post-release monitoring, such as: What are the ecological consequences of repeated releases in the same area using animals that were raised in captivity for many generations? Based on a maximum sustained yield approach, IUCN criteria, and simulations of population performance, the program detailed a release strategy of 12 females/year aged 3-6, within a time frame of ~9 years (the estimated time required for the population to reach the 'vulnerable' status according to IUCN criteria). Releases were to be carried out sequentially in three connected reserves, with ca. 3 annual releases in each. The program was approved by the NPA but was delayed due to 'lack of funds'.
3. Implementation. In 1996, a newly appointed NPA director (then called the Nature Reserves Authority) considered the project worthwhile and funding was secured. The project began in 1996 with releases in the Kziv reserve (the site we evaluated as best) using a specifically designed 11-hectare release enclosure. Releases were carried out twice a year (6 females each time). Less than 50% of released females were within the specified age groups. Due to agricultural damage by the deer, the northern region of the NPA objected to expanding the reintroduction into the other two reserves and the project eventually was forced to select a new, less favorable, site in the Judean hills (central Israel) with no linkage to the former location. Release in this area was based on individuals from Hai Bar Carmel and from a second breeding core established in the nearby Jerusalem Zoo. The less favorable site and behavioral problems of the zoo animals hampered the success of this reintroduction. Personnel changes in the NPA have recently re-opened the opportunity for a new release site in the northern region.
4. We re-assessed the original sites and other possibilities using an individual-based spatially

realistic population growth model based on empirical data from the Kziv reserve. We considered options of releases in 5 sites carried out in parallel or sequentially. The simulations indicate that the best strategy, in terms of numerical growth and spatial expansion, is obtained by repeated releases in two sites carried out in a sequential manner.

5. Scientific merit. Pre-release models proved to be useful and reliable tools. In the Kziv reserve, survival was better and recruitment was as projected in the release program in the original release site, but were poor once the project was forced into a new location not originally considered. The repeated release strategy proved beneficial as animals from later releases used formerly released animals as cues and established home ranges faster. Annual home range dynamics and social structure were comparable to other, similar deer species. The spatially realistic individual-based population growth model mentioned above was based on empirical data from the first 2.5 years of the project, and reliably projected the numerical and spatial growth of the population over a 5-year period. This model was then used to assess future risks imposed by regional development plans and expected human sprawl. Reintroduced deer transported viable seeds of many species by ingestions (endozoochory) and for some plant species germination was enhanced by passage through the digestive system. This suggests that reintroductions, in addition to enhancing the survival of endangered species, should be viewed also as an important tool for restoring ecosystem processes.

6. Although the project was carefully planned, stochastic events within the NPA organization played an important role in its progression. Conservation projects are commonly subject to disturbances due to unexpected changes within the implementing organization. The use of an adaptive-management repeated-releases approach in reintroduction enables overcoming unexpected difficulties and enhances the eventual success.

Keywords: reintroduction, captive breeding, ex-situ, conservation, Persian fallow deer, *Dama mesopotamica*

Deer overabundance revisited: Recent advances, challenges, and opportunities

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During the 20th century, deer (*Odocoileus* spp.) populations in many parts of North America changed from locally extirpated to locally overabundant. In 1997, two comprehensive, edited books were published on the topic of deer overabundance to help wildlife professionals understand the complex biological, ecological, biopolitical, public, and legal issues associated with managing overabundant deer, especially in protected areas and urban/suburban areas. Since then, there have been several recent publications, advances, challenges, and opportunities develop in dealing with deer overabundance, which are briefly summarized in this review article. Most notably, since 1997 wildlife scientists have conducted research that more definitively characterized the ecological consequences of chronically overabundant deer populations, not merely in terms of direct impacts on plant communities but also in terms of cascading effects on animal communities, ranging from invertebrates to carnivores. In some situations, adaptive responses by predators have helped to reduce locally overabundant deer populations, especially via increased predation on fawns. On the wildlife policy front, some natural resource agencies have enacted new programs to manage overabundant deer populations in protected areas and urban/suburban areas that they had not considered appropriate 10-20 years ago. Examples of these programs include special urban deer management permits approved by state legislatures and issued by state wildlife agencies, as well as public hunts and sharpshooting programs to control deer in state parks. Continued conflicts between humans and overabundant deer (e.g., threat of deer-vehicle collisions, damage to vegetation) motivated many of these agencies to implement programs they previously would not have considered because of perceived public opposition. A better understanding of the human dimensions associated with deer overabundance has helped many agencies define appropriate public education programs. However, despite these efforts to educate stakeholders about deer overabundance, there have been legal challenges associated with programs designed to control deer populations in some suburban communities. Some nongovernmental organizations (NGO), such as the Quality Deer Management Association (QDMA), have developed award-winning, classroom-based educational modules to help children learn how to live with deer. Some NGOs also have encouraged public hunters to realize the important role they can play when hunting is viewed as an ecological service to help control deer populations. Still other NGOs have worked to facilitate linkages and cover liability concerns to enable bowhunters to help control deer in some suburban communities. There also have been major advances in the potential use of innovative methods for managing overabundant deer with fertility control, including the recent registration by the U.S. Environmental Protection Agency of an immunocontraceptive vaccine for use in deer (Gonacon®). In sum, wildlife biologist now understand the complex issues associated with deer overabundance better, and there are more "tools in the chest", both in terms of agency policy and actual methodology than in 1997. Yet, human dimensions, public education, and stakeholder concerns continue to be the most challenging aspects of managing deer overabundance. Therefore, this issue

will continue to confront wildlife professionals for many decades to come, whether in North America or other parts of the world.

Keywords: deer overabundance, plant community diversity, predation, hunting, sharpshooting, fertility control, deer-human conflicts

Stem cells, stem cell niche and antler development

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Introduction

Antlers are deer cranial appendages that are cast and fully regenerate each year. Antlers do not renew directly from a deer's head; but instead from the apices of permanent protuberances, known as pedicles. Deer are not born with pedicles, which begin to develop from frontal crests (behind the eye sockets) as male deer approach puberty. When a pedicle reaches a species-specific height (5-6 cm for red deer), shiny skin which is sparsely populated with hair starts to emerge from its apex, indicating the commencement of antler transformation. Growing antlers are then enveloped by this newly differentiated skin that has a velvet-like appearance, and is hence called velvet skin. When the breeding season approaches, these first antlers become fully calcified and the blood supply is occluded, which causes the demise of velvet skin. The dead velvet skin is subsequently shed to expose the bare bone of hard antlers. Hard antlers are cast in the following spring, and regeneration of the second set antlers on their living pedicle stumps follows immediately. From then on annual renewal of subsequent antlers enters a well-defined cycle: previous hard antler casting and new soft antler regeneration take place in spring, rapid antler growth and maturation in summer, full antler calcification and velvet skin shedding in autumn, and the bare bony antler phase in winter. Both antler generation and regeneration are unique zoological phenomena; understanding the mechanism underlying these processes may help to unlock the secrets of organogenesis as well as mammalian organ regeneration.

Antler stem cells

The era of antler stem cell (ASC) research started when Hartwig and Schrudde (1974) discovered the uniqueness of the antlerogenic periosteum (AP), a bone membrane that overlies the frontal crests of pre-pubertal deer. Removal of AP abrogates future pedicle and antler development from the original place; whereas, transplantation of AP elsewhere on deer body induces ectopic antler formation. Subsequently, Goss (1985; 1990) greatly extended this discovery by defining the threshold mass and orientation of AP, and regional skin competency for ectopic antler induction. Li et al (1999) successfully isolated and cultured AP cells, which laid the foundation for subsequent characterisation of AP cells.

Although the tissue type, AP, that gives rise to first antlers was successfully identified through tissue deletion and transplantation, a similar approach failed to pinpoint the tissue type for antler regeneration (Goss, 1995). Both Wislocki (1942) and Goss (1983) believed that pedicle skin provided the cell source for antler regeneration. Based on the "double-head" formation (an unusual antler growth phenomenon), Kierdorf et al (1992) suggested that it is the pedicle periosteum (PP) that is likely to give rise to regenerating antlers. Successful identification of the cell source for antler regeneration was not forthcoming until the time when a breakthrough was made via serial histological examination (Li et al, 2005). In this examination, establishment of the initial growth centres for antler regeneration were found to be solely derived from the distal periosteal cells of a pedicle stump. To functionally confirm this histological finding, Li et al (2007) deleted the total PP prior to antler regeneration, and found that properly timed PP removal can effectively inhibit

pedicle stumps from initiating antler regeneration. Therefore, PP is shown convincingly to be the precise tissue that gives rise to regenerating antlers.

The unique attributes of AP and PP promoted us to think that the cells resident in them must be ASCs (Li et al, 2004). In order to provide evidence for this claim, we characterised these cells, and found that both of them express key embryonic stem cell markers Oct4, Sox2, Nanog, CD9, telomerase and nucleostemin; and could be induced to differentiate into chondroblasts, osteoblasts, adipocytes, myoblasts and neuronal-like cells (Li et al, 2009; Harper et al, 2010). Consequently, we concluded that AP and PP cells are ASCs.

Advancement of the ASC niche hypothesis

1. Pre-existing experimental results implicate skin as the niche candidate

As stem cells, ASCs must reside in and interact with their niche. While carrying out tissue transplantation experiments, Goss (1990) noticed that AP could induce ectopic antler formation only when AP-derived tissue came in close contact with the overlying skin, which lead him to think that interactions between AP-derived tissue and skin were indispensible for the initiation of antler formation, and this close association seemed to facilitate the establishment of these interactions. Detailed histological examination of skin transformation from scalp to antler velvet during initial antler generation strongly supported Goss's claim (Li and Suttie, 2000). This was because velvet skin transformation does not take place until the AP-derived perichondrium and the overlying skin become intimately bound together through total compression of the interposing subcutaneous connective tissue (SLCT) layer. Likewise, antler regeneration may also rely on the interactions between PP and the enveloping skin, since antler regeneration only happens when PP and the skin at the distal end of a pedicle stump have fused with each other (Li et al, 2004). All these observations suggest that the closely associated skin is likely to be the primary element of ASC niche for both antler generation and regeneration.

2. ASC niche hypothesis

Based on the aforementioned observations, we put forward an ASC niche hypothesis: antler development including generation and regeneration is triggered by the interactions between ASCs and the niche, i.e. the closely associated skin cell populations. ASCs transform the skin from scalp type into antler velvet; instructive feedback from the transformed skin triggers the proliferation and differentiation of ASCs to initiate antler formation.

Testing of the ASC niche hypothesis

1. Dependence on interactions between ASCs and the niche for antler development

To functionally test whether the interactions between ASCs and niche cell populations is indispensible for antler development, we carried out two experiments: one was for antler generation (that is, the first antler) (Li et al, 2008) and the other for antler regeneration (subsequent antlers) (Li et al, 2007). In the generation experiment, either impermeable or semi-permeable (0.45 μm pore size) membranes were inserted between ectopically grafted AP and the overlying skin. Interposition of the impermeable membrane completely inhibited antler formation from the grafted AP, whereas the semi-permeable membrane only significantly delayed this (for a year), but did not stop velvet skin transformation. In the regeneration experiment, two types of pedicle stumps were firstly created: full-length and 2/3-length stumps. PP and the enveloping skin were in tight contact at the distal end of a full-length stump, whereas they were only loosely associated in 2/3-length stumps. Insertion of the impermeable membrane into PP and the enveloping skin of full-length stumps did not prevent antler regeneration, although skin failed to participate the process; whereas,

for 2/3-length stumps it completely stopped antler regeneration. These results indicate that when PP is separated from skin before the interactions (loosely associated) occur, antler regeneration cannot take place; whereas, after the establishment of interactions, (closely contacted) skin is no longer required for antler regeneration. Taken together, interaction with the closely associated skin is indispensible for ASCs to initiate antler generation or regeneration, and these interactions are achieved through diffusible molecules.

2. Identification of niche cell types

ASC niche, i.e. deer skin, consists of epidermis and dermis. Epidermal cells must participate in interactions with antler stem cells, as during the initiation of antler formation, scalp epidermis is transformed into antler velvet epidermis. It is not known whether the interactions between ASCs and the epidermal cells are accomplished through directly exchanging diffusible molecules or via dermal cell mediation. In his serial AP transplantation experiments, Goss (1987) found that all areas of deer skin, except those that cover the snout of the nose and the tail ventral surface, are competent to interact with the grafted AP for the initiation of antler formation. The common feature of snout and ventral tail skin is that it is devoid of hair follicles. Therefore dermal papilla cells (DPCs), the only dermal component of hair follicles, may participate in these interactions by relaying the signals between ASCs and epidermal cells.

To clarify the DPC involvement, we (Li et al, 2008) transplanted AP and partial deer skin which had been sutured together onto a nude mouse head. The partial skin was only composed of epidermis and the dermal portion which contained hair follicles. Interestingly, the epidermis of the partial deer skin was fully transformed into antler velvet epidermis by the sutured AP. However, this experiment did not provide evidence for the direct participation of DPCs in these interactions, although it demonstrated that the interposing SLCT and non-hair-follicle-containing dermal tissue are not necessary for the establishment of interactions. Therefore, we conducted another experiment, within which AP tissue was directly delivered underneath the hair follicles (DPCs) through an intradermal transplantation approach, and at the same time used subcutaneous transplantation as a control (Li et al, in press). The intradermal approach showed that for 1/8 of AP tissue mass or more there was a 100% success rate of antler induction at the grafted sites; whereas for the subcutaneous approach, 1/4 of AP tissue mass or less did not induce ectopic antler formation. Because removal of the interposing tissue barrier between AP and hair follicles greatly stimulated antler formation, DPCs of hair follicles must have been involved in the interactions between ASCs and epidermal cells.

3. Origin of initial inductive molecules

Just like deer skin, AP tissue also consists of two layers: a fibrous layer (closest to the skin) and a cellular layer (abuts the bone). It is not known whether the initial inductive molecules are derived from the fibrous, the cellular or both layers. To clarify this, we conducted the following experiment (Gao et al, in press). In the study, a piece of AP that was sampled from one antler growth region was inverted before being put back, i.e. making the cellular layer face the overlying skin; and from the other region was directly put back without inversion. The results showed that in the AP inverted region, antler generation took place without passing through a distinguishable pedicle stage; whereas, in the AP non-inverted region antler formation occurred only when its pedicle grew up to the species-specific height. These results indicate that the AP cellular layer cells are the origin of inductive molecules that initiate the interactions between ASCs and the niche cell populations and subsequent antler development.

4. Effects of niche manipulation

Very recently, we experimentally manipulated the ASC niche and found antler development was

profoundly affected. When permeability of the interposing tissue layers between ASCs and essential skin cell populations were physically increased, antler transformation was greatly advanced. Normally antler transformation takes place when its pedicle reaches 5-6 cm in height in red deer; after the increase in permeability of the interposing tissue barrier, antler transformation took place from precocious pedicles (which were fully grown at around 2 cm in height). Destruction of AP cells in the central region, from which a pedicle develops, with or without damaging the interposing tissue layers resulted in completely opposite outcomes: antler formation from the marginal AP was inhibited when the interposing tissue layers were kept intact; whereas, antler formation from the marginal AP was promoted when the layers were interrupted. Consequently, we conclude that we have identified the ASC niche.

Summary and future work

Antler generation and regeneration are triggered by the interactions between ASCs and the niche cell populations (DPCs and epidermal cells) through exchanging diffusible molecules. In another words, the putative diffusible molecules play a key role in antler generation and regeneration. To help identifying these molecules, we recently established a co-culture system within which all the essential cell types were placed together *in vitro* in a way that can maximally mimic the *in vivo* situation. Eventual identification and isolation of these molecules will not only greatly enhance our knowledge of antler development, but will also have significant impacts on regenerative medicine in general.

Keywords: antler, pedicle, antlerogenic periosteum, pedicle periosteum, stem cells

Intraspecific variation in biology and ecology of deer: magnitude and causation

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Barash has noted that the search for patterns in biology to assist our understanding, often leads to over-simplification (*Barash 1997. In search of behavioral individuality. Human Nature 8:153-169*). That is, we are satisfied with statements that "the species as a rule does this" or, "males of this species do that". But within such generalisations are masked what are often important variations from that supposed norm and in practice there is tremendous variation in morphology, physiology, social organisation and behaviour of any one species.

The focus on a supposedly *mean optimal phenotype* has diverted attention away from variation around that mean, which is regularly regarded as a kind of 'noise' stemming merely from stochastic effects, and thus irrelevant to evolution. Yet it is becoming increasingly clear that this variation is by converse extremely significant and of tremendous importance both to evolutionary biologists and to managers.
Such intraspecific variation (IV) may be directly due to underlying genetic differences between individuals or populations within a species, but equally may include a degree of phenotypic plasticity whether as 'non-labile' traits which are expressed once in an individual's lifetime, as fixed characteristics inherited from the parents or as more labile traits which are expressed repeatedly and reversibly in a mature individual according to prevailing conditions.
Recognition of the extraordinary degree of IV which may be recorded within species has important consequences for management of cervids and conservation of threatened species.
In the written version of this paper we review the extent of intraspecific variation in diet, in morphology, mature body weight, reproductive physiology, in population demography and structure (sex-ratio, fecundity, frequency of reproduction) before also reviewing the striking variation to be observed in behaviour: differences between individuals or populations in ranging behaviour, migratory tendency, differences in social and sexual organisation. In each case we explore the factors which may underlie the variation observed, considering the extent to which variation described has a primarily genetic basis or is a more plastic response to more immediate social and ecological cues.
Because of the necessary constraints on time in a spoken presentation, the oral paper will concentrate on exploration of IV in social behaviour, reviewing across a range of species, the variation expressed in sexual segregation, in group composition, the size or plasticity of social groups- and finally, the marked variation in breeding system and reproductive behaviours.

Nutrition of deer - a quantitative approach

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The quantitative requirements of deer for energy, protein and water are reviewed, using where possible a factorial approach to defining requirements. Deer are slightly more efficient than cattle and sheep in metabolising digested energy and in converting metabolisable energy into net energy. The overall mean metabolisable energy requirement for maintenance (MEm) is 0.46 MJ/W(kg)0.75 per day. The MEm of reindeer and caribou is about 25 % less than for cervine or rusine deer. Temperate species have a higher requirement in summer, and tropical species in a subtropical environment have a greater requirement in winter. The MEm of males is less than for females, and it reduces with age. ME requirements and efficiencies of use for production are reviewed.

Diet protein contents that are adequate for maintenance and production are approximately 4 to 9% and 16 to 22%, respectively. Actual values vary with feed intake and animal age. It is difficult to use a factorial approach to describing deer protein requirements because of a lack of information on basal endogenous N excretion rates, the outflow of metabolisable protein from the rumen and the efficiencies of utilisation of metabolisable protein. Differences in digestive function between deer and other ruminants mean that data for cattle and sheep may not be applicable to deer.

Guides to drinking water consumption by deer are a water:dry matter intake ratio of about 3.5:1 and 139 g drinking water/W(kg)0.75 per day; but ambient temperature, physiological state and diet composition greatly affect water requirements. The salinity tolerance of cervine and rusine deer is approximately 8000 mg/kg drinking water.

Relationships between rank related behavior and antler growth in deer

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Recently it has been shown, at least in red deer that antler size has the potential to serve as a signal of a male's quality to females. Therefore, achieving reproductive success is mainly dependent on increasing antler size and enhanced antler branching. In our research we worked on the assumption that if antler size and structure reflect biological quality of the male then antler development should be related to social dominance activities during the period of antler growth.
This plenary is dealing with a thirty-year-lasting investigation focused on the relationship between dominance rank related behavior and antler cycle timing and antler growth in deer studied on red (*Cervus* elaphus), fallow (*Dama dama*) and White-tailed deer (*Odocoileus virginianus*), reindeer (*Rangifer tarandus*), and southern pudu (*Pudu puda*).
The initial study conducted on red deer indicated that the antler casting time of an individual male was dependent on his social status. The males of higher rank cast their antlers first and also tended to shed velvet earlier. Several factors modified this dependency, however. First, in seasons with linear hierarchy, correlations between rank and date of antler casting reached high, significant values, while in the season with nonlinear hierarchy it did not show such a close relationship. Thus, the linearity of a hierarchy seems to be one of the important factors that allow a close relationship between social position of a male and his antler casting time. Second, the strength of the relationship between male rank and casting and cleaning order under the situation of stabilized social hierarchy was significantly correlated with most of the indicators of general aggression (such as the number of killed males, incidence of broken antlers, etc.). The higher the level of aggression within the herd of males, the closer the relationship between rank and the timing of the antler cycle indicating that the process of antler cycle timing can be modified by aggressive behavior of a male related to his rank. Thus, both antler casting and cleaning should be regulated by hormones modulated by agonistic behavior.
In a following series of studies we found that social position and related agonistic activity of males during the velvet period influence the antler weight, length and number of points, and therefore the size of grown antlers are a consequence of previous social position and not vice versa. Antler development seems to be a dynamic multi-factorial process reflecting changes in the environment. For example, using fallow deer we examined how strongly any change in rank of a male during antler growth affected the pattern of development of his antlers. We tested whether the size attained by various parts of the antler is more closely related to dominance success of the buck during their development than to average success over the whole velvet period. Fallow deer bucks were observed throughout the velvet period. Most of the measured characteristics of the antlers reflected dominance success during the time that they were developing rather than the average rank over the whole period of antler growth. Thus, changes in behavior related to rank modified antler growth. Bucks gaining higher rank through fighting more bucks exhibited enhanced growth of that part of the antler that was just developing. This changed if the buck lost his position.
Originally we presumed that the relationships between rank related behavior and antler cycle timing

and antler growth is primarily testosterone dependent. Nevertheless, in the eighties Suttie et al. (1985 Endocrinology 116 846-848) compared seasonal variations of hormones with the progress of antler growth in red deer and concluded that insulin-like growth factor 1 (IGF-1) is an antler-stimulating hormone (see also their subsequent papers). This hypothesis was supposed to replace an earlier notion suggesting that the antler-stimulating hormones are androgens, particularly testosterone or its derivatives. Using the southern pudu, we obtained initially data indicating the possible link between dominance and blood levels of IGF-1. Our following investigation on other cervids disproved these expectations, however. We studied the role of androgens and IGF-1 in antler growth. In particular, we investigated whether the onset of antler regrowth is triggered by a short-term pulse of testosterone and if low levels of androgens are required for antler growth. The study was conducted on surgically castrated fallow deer bucks. Half of the animals (CA group) were given regularly the antiandrogen, cyproterone acetate (CA); the others were given vehicle solution only (control). All animals cast their antlers, followed by antler regrowth in all control bucks, but in only 67% of the CA-treated castrates. Plasma testosterone concentrations were extremely low in all animals (between 0.01 and 0.20 ng/ml), but were significantly greater in the controls. In both groups, a temporary increase in testosterone values was recorded around the time of antler regrowth, the peak being significantly higher in the controls. In contrast, plasma IGF-1 concentrations increased sharply during the antler growth spurt and did not differ significantly between the two groups throughout the study period. Antlers produced by the control bucks were significantly larger than those in the CA group. It was concluded that a plasma androgen concentration at least above a minimal threshold level is a necessary prerequisite for normal antler regrowth, and that this androgen effect is not mediated via circulating IGF-1. In combination with other studies on castrated fallow deer and intact red deer, reindeer, and White-tailed deer males, the combined results lead us to conclude that it is not IGF-1 but testosterone which is the antler stimulating hormone. Therefore we focused on the relationship between dominance rank related behavior, possible androgen/glucocorticoid feedback, and antler growth.

In the studies with red and fallow deer all agonistic interactions of top-ranking males were typically of dominant nature, whereas bottom-ranking males were always submissive. On the contrary, in a more recent study performed on a group of White-tailed deer, bucks developed an unstable hierarchy with relatively frequent changes in dominance ranking, particularly in the upper half of the hierarchy. Bucks of all ranks were attacked by others including some subordinates and in comparison with previous studies on red and fallow deer, the whitetails displayed fewer agonistic interactions and more triangular relationships in hierarchy. Under these social conditions, our results describing the relationship between rank position and antler cycle timing of White-tailed deer were in apparent contrast with previous studies on red deer. Therefore, we concentrated on elucidating the social situation and the link between rank related behavior and hormone concentrations.

Adding much younger and weaker red deer males into the experimental group of adult males altered the agonistic behaviour of the adults. Their general involvement in agonistic encounters did not change because the number of attacks remained the same. Also in other species the rate of aggression was not higher during unstable periods than during stable ones, thus suggesting that frequencies of aggression per se may have little effect on the rank. On the contrary, having available young partners, our experimental adult males targeted their attacks on individuals much lower in the hierarchy. Among adult deer maintaining rank position was a risky venture causing a stress reaction comparable to that seen in subordinate young males, who were otherwise exposed to permanent attacks from the adults. Social stress in adult males was the cost of dominance, not a consequence

of subordination. Our high-ranked individuals had the greatest physiological signs of stress when living in a competitive social situation with equally sized adult conspecifics. As a result, changing the social environment of adult red deer males resulted in change of the relationship between rank and testosterone and cortisol concentrations.
Understanding the relationship between rank and hormone levels is crucial for the interpretation of our previous results that showed a link between dominance rank and antler growth in deer. This is the field where the next investigation should be directed.

Keywords: rank, behavior, antler, antler growth, antler cycle timing

EVOLUTION AND CONSERVATION GENETICS

The phylogeny of the Colombian brockets: Preliminary results

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Brockets are small to medium-sized simple antlered deer from Central and South America, grouped together as the same genus *Mazama.* Recent cytogenetic and molecular studies have revealed that brockets are not a single monophyletic group but likely a morphotype adopted by several different species as the result of adaptation to dense forests and other closed habitats. In this context, the taxonomy of the genus *Mazama* should be thoroughly reviewed. Almost all the genetic studies have been made on Brazilian species, but no genetic studies have been developed with the species from Colombia, despite its high biodiversity and variety of ecosystems. Given this fact together with its position as the "gateway to South America" during the third "Great Migration" from North America during the Pliocene 5 to 3 MYA, Colombia potentially may hold an unsuspected diversity of brockets. The aim of this work was to describe the phylogeny of some species of brockets from Colombia, through morphological and molecular analyses. We sampled 15 captive specimens from different localities of Colombia, representing the two recognized groups: the brown and the red brockets. Only one specimen was completely identified as *Mazama bricenii* through its morphology and geographic origin. From the other 14 deer, six were morphologically classified as red brockets and eight as brown brockets. Preliminary morphometrics by principal components analysis using three measures (weight, body length and ear length) grouped red brockets from Colombia apart of those from Brazil and the brown brockets into three different groups. A 339 bp fragment of mitochondrial Cytochrome b was also analyzed. DNA was extracted from hair samples through the phenol chloroform method and the sequence was amplified using the primers L14724 and H15149. The sequences were aligned using ClustalW in MEGA4 and compared to those previously published of *Mazama americana, M. temama, M. nana, M. bororo, M. gouazoubira, M. nemorivaga, Odocoileus virginianus, O. hemionus, Blastocerus dichotomus, Ozotocerus bezoarticus, Hippocamelus bisulcus, H. antisiensis* and *Pudu puda*, using *Rangifer tarandus* as the outgroup. Preliminary phylogenetic analyses using the Neighbor-Joining method with bootstrap consensus trees from 1000 replicates, segregated Colombian red brockets and *Mazama temama* from México in a single clade that merged earlier than the Brazilian red brockets – *Odocoileus* clade. The Andean red dwarf brocket *Mazama bricenii* grouped in a clade apart that merged early of the red brockets clades. Regarding brown brockets they were segregated into two different clades, one grouping those from Colombian Andes together with *Mazama gouazoubira* and the other grouping those from the Colombian Caribbean coast. These results suggest almost one new species of brown brocket in Colombia and the possible occurrence of *M. temama* in South America. Further molecular and cytogenetic analyses are in course in order to clarify these results.

Keywords: molecular phylogenetics, morphometrics, brocket deer, Mazama, Cytochrome b, genetics

Challenges in the study of a group of cryptic species from South America: The *Mazama americana* complex

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The red brocket deer has been considered a single species with a wide geographic range from México to the north of Argentina. Taxonomy of red brocket has been controversial and recently two species were described based on karyotipical differences: *Mazama temama* from Mexico and *Mazama bororo* from Brazil. Moreover the cytogenetic differences among animals from western Amazonia and eastern Amazonia and south of Brazil suggested the existence of several cytotypes with geographic coherence. In order to understand the gene flow among populations and genetic variation and to propose red brocket deer genetic units for conservation, we initiated a cytogenetic and molecular genetic study based on representative samples from throughout their current Brazilian geographic range. We examined karyotypes (G-banding) and DNA sequences from the mitochondrial control region and cytochrome b partial gene of 18 individuals from 6 localities. We found 6 cytotypes (Rondônia, Juína, Jarí, Carajás, Santarém and Paraná) with a 2n ranging from 42–53 and FN ranging from 48–56. The 'Paraná cytotype' was recognized as the more ancestral karyotype for having the higher diploid and fundamental number (2n = 52/53; FN = 56) and was used to perform comparisons among the other cytotypes. The 'Paraná cytotype' originated from the inferred ancestral cytotype with a pericentric inversion in one acrocentric chromosome. The basic karyotype from Carajás evolved from fixation of a tandem fusion between 2 acrocentric chromosomes of the Paraná cytotype. The Santarém cytotype also originated from the Paraná cytotype by fixation of a first centric fusion, and a second centric fusion evolved into the Jarí cytotype. Another lineage with a low diploid and fundamental number may be due to the accumulation of a great number of tandem fusions that evolved into Juína and Rondônia cytotypes which are very close chromosomally. The molecular phylogenetic relationships among individuals of red brocket deer revealed clearly two separated lineages that are well correlated with the cytogenetic findings. The phylogenetic analysis using the cytochrome b and D loop concatenated fragment sequences also showed 2 independent clusters with high bootstrap value support: A (Rondônia, Juína and one haplotype from Santarém cytotypes) and B (Paraná, Carajás and another haplotype from the Santarém cytotypes). The pairwise computations of Fst using AMOVA indicate that both clades are significantly differentiated relative to a random collection of genotypes. A comparative genetic analysis including the closest phylogenetic species, *M. bororo* and *M. nana,* showed that the highest differentiation values and genetic distances were obtained among both clades (A and B). These data represent the first cytotaxonomical and molecular systematics, and although sample sizes are limited, our results clearly suggest that red brocket deer populations are significantly differentiated with respect to karyotypes and the mitochondrial sequences analyzed. We clearly recognized two independent species, with cytogenetics and mtDNA markers being

efficient tools to analyze, understand, and elucidate the evolution of the red brocket deer complex. However, for a suitable taxonomic description the sampling area needs to be extended in order to cover more thoroughly the geographic range, and moreover morphological studies are needed to determination of the holotypes.

Keywords: Red brocket deer, *Mazama*, chromosomal rearrangements,, molecular systematics, evolution, genetic

Genetic and genomic structure of Newfoundland Caribou (*Rangifer tarandus terranovae*) and its phylogeographic implications for the evolution of Caribou and Reindeer (*Rangifer* ssp.)

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Caribou on the island of Newfoundland on the eastern seaboard of Canada are a distinctive form of Woodland Caribou (*R. tarandus caribou*), whose range of post-glacial ecological adaptations spans that of other populations throughout the subspecies. Genetic analysis of 223 Newfoundland caribou from 14 herds over a 2,223bp region spanning the mtDNA Cytochrome *b* and Control Regions identified 32 haplotypes. Hierarchal Analysis of Molecular Variance partitions 0.03 ~ 0.07 of the genetic variance to geographically-defined regions or hunting compartments. Nested Clade Analysis identified significant phylogeographic associations due to restricted gene flow with isolation by distance, contiguous range expansion and long-distance colonization, coupled with fragmentation followed by range expansion. Clade diversity is greatest on the Northern Peninsula, whereas caribou on the Avalon Peninsula in the southeast are genetically depauperate, consistent with re-population across the northern Straight of Belle Isle rather than from southern coastal refugia. Analysis of complete mitogenomes (16Kbp) from 80 animals assigns individuals in the 32 haplotypes to four clades, three of which are endemic to the island, consistent with Newfoundland caribou as a distinct subspecies, *R. tarandus terranovae* (Bangs, 1896). The fourth, basal clade is more closely related to animals from Labrador as a persistent ancestral lineage rather than a recent migrant. Re-analysis of previous single-locus data in the genomic context suggests re-evaluation of the Flagstad & Roed (2003) biogeographic hypothesis of Reindeer / Caribou evolution: we suggest the Beringean-Eurasian reindeer clade (including Svalbard) is basal and the Nearctic caribou clade derived. Implications for a pan-*Rangifer* genomic investigation are discussed.

Keywords: rangifer, Newfoundland, phylogeography, mtDNA genomics, genetic, evolution of caribou, reindeer

Genomics solutions on developing reference genomes in BGI

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Next-generation DNA sequencing technologies provide ultra-high throughput at a substantially lower cost; however, the data is presented in very short read-length sequences, making de novo assembly extremely challenging. BGI has developed a novel method for de novo assembly of large genomes from short-read sequences and successfully assembled the panda (2.7Gb), cucumber (367Mb), Chinese cabbage (500Mb), and potato (830Mb) genomes. The development of this de novo short-read assembly method creates new opportunities for building reference sequences and carrying out accurate analyses of unexplored genomes in a cost effective way.
Empowered by the capacities, BGI announced the "1000 Plant and Animal Reference Genomes Project" (ldl.genomics.org.cn) in January and calls for collaborations from all over the world with the aim to generate reference genomes of a thousand economical and scientific important plant/animal species in two years.
Unraveling whole genome sequences of a species will tremendously accelerate basic research, increase knowledge on the functions of important genes, and facilitate their applications and manipulations. Genetic variations and evolutionary process can be identified through comparative analysis of population and individuals, and finally lead to huge impacts on scientific discoveries and society development.
In this talk, I will report the strategy and progress in developing reference genomes in BGI.

Keywords: DNA, genomics, evolution, conservation, production

Genetic characterization in Pampas deer (*Ozotoceros bezoarticus bezoarticus*) population from Emas National Park, Brazil

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Pampas deer (*Ozotoceros bezoarticus bezoarticus*) have faced a huge loss and fragmentation of their native habitat, leading to small and isolated populations. Currently this specie is considered as near-threatened by the IUCN red list. The present study was developed in Emas National Park, in Brazil, where the largest protected *O. b. bezoarticus* population remains. The ongoing habitat fragmentation caused the isolation of this population. Hair samples were collected from 22 individuals and five microsatellite primers were tested to determine the genetic diversity of this population. These primers, previously developed for another species, were successfully amplified and were polymorphic: CA71 (*Cervus axis* – 3 alleles); BM737 (*Bos taurus* – 13 alleles); RT01, RT09, RT30 (*Rangifer tarandus* – 12, 7, 5 alleles; respectively). The average allelic richness was 7.75 and no linkage disequilibrium was detected among them. The observed heterozygosities ranged from 0.62 to 0.89 and the only locus with deviations from Hardy-Weinberg equilibrium was the CA71 (P=0.0024), which showed a significant excess of heterozygote. The inbreeding coefficient was also low (F_{IS} = 0.017), indicating that this population still maintains an efficient mating system to avoid the loss of genetic variability. Despite the isolation of this population, the results obtained in the present study indicated the importance of the Emas National Park as a conservation means to ensure the protection of one of the last remaining populations and genetic stock of this subspecies of Pampas deer.

Keywords: conservation genetics, genetics, Ozotoceros, microsatellite, Brazil

Conservation genetics of the Pampas deer using the *COI* gene

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The Pampas deer, *Ozotoceros bezoarticus*, a neotropical deer that inhabits open grasslands, had a wide distributional range from to 5ºS to 41ºS in the recent past. However, it is classified as near threatened (IUCN red list 2010), though in a more precarious state in the southern zone. Molecular markers, particularly the mitochondrial DNA, are widely used to perform evolutive and phylogenetic analysis and to determine conservation genetic units. The *Cytochrome oxidase I* gene, known as the barcode of life, is being extensibly applied in vertebrates (e.g.birds) for taxonomic purposes to discriminate species and conservation genetic units. The objective of our work was to analyze a representative sample of Pampas deer from South American populations to determine the behavior of the *Cytochrome Oxidase I* gene for discriminate conservation genetic units. We amplified with a universal primer and sequenced a sample of 36 individuals belonging to populations from Argentina (Samborombom and San Luis), Brazil (Emas, Paraná and Pantanal), and Uruguay (El Tapado and Los Ajos). We also included an individual gray brocket deer, *Mazama gouazoubira*, as the outgroup. We analyzed 553 bp and identify 28 haplotypes, finding 106 variable sites, 30 informative sites and 0,191 segregate sites. The AMOVA was useful to analyze the hierarchical levels of genetic variation in the samples belonging to the different South American locations. The main source of variation was found within the populations, being less than the inter-population variation. A significant value of 0.1164 ($p < 0.01$), corresponding to the ΦST statistic using the Kimura2 Parameter, indicates high haplotypic diversity within populations. The analysis showed that the most diverse haplotypic populations were: Los Ajos, San Luis, El Tapado and Pantanal. These genetic data also showed that the species still retains high levels of genetic diversity, suggesting the possibility to recover if habitat is secured and protected.

Keywords: Pampas deer, cytochrome oxidase I, conservation genetic units, genetics

Transferability and characterization of five polymorphic microsatellite markers for Pampas deer, *Ozotocerus bezoarticus*

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The Pampas deer (*Ozotocerus bezoarticus*) is one of the eight deer species known in Brazil. Effects of hunting activities associated with the introduction of domestic animals, destruction, fragmentation and change in habitat quality are potential causes of threats to populations of this species, and the existing population in the Pantanal is the most significant in South America. Genetic studies with Pampas deer are relevant for knowledge about issues concerning the basic biology of the species and also about genetic erosion processes that are occurring in different populations, supporting conservation activities. To conduct such studies, microsatellite loci have been primarily used. The limiting factor for large-scale use is the labor and costly development of primers for each species of interest. Transferability of microsatellite loci between related species is possible due to the conservation of flanking regions of simple repetitive sequences. The Cervidae family is one of the most studied families in the order Artiodactyla, using microsatellite markers. The main factor for this large volume of studies is the extensive number of primers described. With this background, this study aimed to select heterologous primers for *O. bezoarticus*. For this purpose, five microsatellite loci of high frequency amplification among Artiodactyla species were selected from the literature: *Rangifer tarandus* (RT01, RT09, RT30, NVHRT16) and *Bos taurus* (BM757), in order to verify the transferability as well as the polymorphism of each of the initiators of the studied population. We used blood sample DNA of thirteen females from the Brazilian Pantanal, Nhecolândia region. The PCR products were visualized on 2% agarose gel and then were applied to polyacrylamide gel. The amplified fragmentsizes visualized in agarose gel were similar with the microsatellite locus described in the literature for the six primers. After sequencing the amplified fragments, the conservation of the microsatellite region confirmed in *O. bezoarticus*. The polymorphism of the tested markers was evaluated in polyacrylamide gel and found that RT01, RT09 and RT30 have five alleles each and BM757 and NVHRT16 seven alleles each. Thus, we obtained a set of five polymorphic microsatellite loci with potential application for characterization of genetic variability and population studies in Pampas deer, which will certainly contribute to the elucidation of questions about the biology of these animals, and also the generation of public policies for conservation of Brazilian deer.

Keywords: Pampas deer, *Ozotoceros bezoarticus* , microsatellite, primers heterologous, polymorphism, genetic

Phylogeography of the Gray Brocket deer *Mazama gouazoubira*

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The Neotropical gray brocket deer *Mazama gouazoubira* (Fischer, 1814) occupies a wide geographic range from Bolivia and Brazil to northern Argentina and Uruguay. Gray brockets also have a wide range of morphological variation throughout their geographic distribution. It is considered to be in the category of "Least Concern" by the IUCN (International Union for Conservation of Nature) and the species is undergoing extensive poaching in several South American locations. In order to assess the genetic patterns of variability within this species and to elucidate phylogeographic patterns throughout their range we analyzed a 260 bp fragment of the cytochrome b (Cyt b) gene of the mitochondrial DNA from 44 samples collected in Argentina, Bolivia, Brazil, Paraguay and Uruguay and Peru. In addition, in this study we compared the genetic variability of 16 individuals from Uruguay with those specimens from different localities in South America. In this fragment we identified 200 conserved sites and 11 informative polymorphic sites. Seventeen haplotypes were diagnosed, while only two are shared between different localities in South America. We found high levels of genetic variability the haplotype diversity index of 0.625 in the Uruguayan populations. Despite the small sample size, there is a trend of discrete population substructuring. The high levels of genetic diversity and moderates population structure patterns would suggest large population sizes and moderate levels of gene flow between localities.

Keywords: *Mazama gouazoubira*, Brocket deer, cytochrome b, haplotypes, mtDNA, phylogeography

Assessment of frozen-thawed red deer spermatozoa obtained post-mortem in relation to time after death

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Some studies have shown that is possible to cryopreserve viable post-mortem spermatozoa from red deer and the subsequent utilization in artificial insemination (Zomborsky et al 2005, Animal Reproduction Science 90, 185-190 and Soler et al 2003, Journal of Andrology 24, 746-756). However there is not enough information about the semen quality in relation with the time passed after death. The objective of this study was the in vitro assessment of frozen-thawed semen obtained from epididymides at different periods between death and semen freezing. Spermatozoa were collected post-mortem from twelve mature stags hunted during the rutting season. Samples were collected from the caudae epididymides which were washed with the media used for further processing and freezing (Triladyl®, Minitüb, Germany). The sperm mass was diluted to a concentration of 200×10^6 sperm/ml, cooled down with a refrigerator to 5ºC in about 1 h and equilibrated for 2 - 4 h. Samples were filled into 0.25 ml straws and frozen by holding in liquid N_2 vapor for 10 min and then plunged into and stored in liquid N_2. Thawing was performed by placing the straws in a 37º C water bath for 60 sec. The parameters evaluated were % of sperm motility (SM), % of individual motility (PIM), quality of individual motility (QIM) using a scale of 1 (lowest) to 5 (highest), sperm motility index (SMI= [SM + (QIM × 20)] × 0.5), percentage of live spermatozoa by staining with nigrosin-eosin (NE) and functional integrity of plasma membrane through the hypo-osmotic swelling test (HOST). Assessment of sperm quality was made immediately (0 h) after thawing (SM, PIM, QIM, SMI, NE, HOST), and after 2 h incubation at 37º C (SM, PIM, QIM, SMI, NE). Stags were analyzed in 2 categories of 6 animals each according to the interval elapsed between death and semen freezing (G1, short interval = 5 – 9.5 h; G2, large interval = 17 – 42 h). The results obtained with the semen assessment at 0 h were SM= 72% - 56%, PIM= 70% - 52%, QIM= 3.6 – 3.3, SMI= 72 - 61, NE= 73% - 61%, HOST= 58% - 58% in G1 and G2 respectively. At 2 h the results were SM= 57% - 46%, PIM= 49% - 39%, QIM= 3.1 – 2.3, SMI= 59 - 45, NE= 53% - 43% in G1 and G2 respectively. All the analyses except the HOST (similar results in both groups), resulted in higher values for G1 than G2 at 0 h and 2 h after thawing. This study shows a better quality of semen for stags with shorter interval between death and semen freezing, showing higher number of live spermatozoa and motility. These results agree with those reported by Soler et al 2003, Journal of Andrology 24, 393-400 and An et al 1999, Criobiology 38, 27-34. In previous studies carried out in other species it has been observed that the spermatozoa retain their fertilizing capacity for less time than motility (Jishage et al 1997, J Mamm Ova Res 14, 45-48 and Kikuchi et al 1998, Theriogenology 50, 615-623), being convenient to complete this study in the future with tests of fertility involving semen samples from stags with large interval between death and semen freezing. This is the first report of red deer semen quality from samples obtained post-mortem in Argentina.

Keywords: Red deer, *Cervus elaphus*, spermatozoa, post-mortem, artificial insemination,

production, semen, reproduction

Genetic resources and evaluation of Chinese deer species

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Deer domestication in China has a long history and can be traced back to year 1733. China is the earliest country to use different deer parts as folk medicines. Currently, sika deer (*Cervus nippon*), wapiti (*Cervus canadensis*), white-lipped deer (*Gervus albirostris*) and sambar (*Cervus unicolor*), etc. are farmed in China for harvesting velvet antlers. Based on the Chinese Pharmacopeia, velvet antlers from sika deer and wapiti can be used as medicine. Therefore, sika deer and wapiti farming are called the "velvet deer" industry in China.

1. Chinese deer genetic resources

China is very rich in deer genetic resources. Deer species were thoroughly surveyed throughout China in 1980's. The project, lead by Ministry of Agriculture of China, reported a total of nine genera and 15 species. Among these, white-lipped deer and tufted deer are rare genetic resources in the world and only native to China. Based on morphology, anatomy, physiological characters and living environment, Jilin sika deer were classified into five elite breeds for velvet antler production: Shuangyang, Yitong, Longtan, Dongfeng, Fusong. Likewise, Chinese wapiti were divided into Northeast, Tianshan and Tahe, which are all velvet production breeds. These deer species and breeds constitute a very rich genetic resource for Chinese deer industry to breed elite deer for velvet production.

2. Chinese deer breeds

Velvet deer industry developed very rapidly since the establishment of New China in 1949. Utilizing the rich deer resources, seven velvet deer breeds were developed: Shuangyang, Xifeng, Siping, Audong, Xingkaihu, Dongfeng sika deer and Qingyuan wapiti, and one Changbaishan sika deer sub-breed. These names indicate the names of the places where they are farmed. The most obvious acquired attributes of these cultivated deer breeds are possessing large antlers, fat tender tips and bright color. These deer breeds have played important role in development of Chinese deer farm industry.

3. Evaluation of elite genetic resource of sika deer and wapiti

DNA polymorphism of 699 individuals from 9 sika deer and wapiti breeds was measured using 20 microsatellite marker, and statistically analyzed. Complete genome of the mitochondrial gene of sika deer and wafiti from Northeast and Talimu was sequenced. 425bp gene fragments from 441 individuals were investigated based on Mitochondrial Cytochrome b Gene. 44 haplotype, 56 variable sites, 18 transversion and 38 transition were identified. Myogenin gene and myostation of wapiti breeds was cloned, CDNA expression library of sika deer antler periosteum cells regeneration point was constructed, 11 EST sequence related to the antler periosteum development was screened. These results have precisely confirmed the genetic classification of the two main Chinese sika and wapiti populations, and manifested their genetic biodiversity, and laid the foundation for collection, integration and evaluation of germplasm resources of these deer breeds.

Deer species are widely distributed throughout whole China. Farmed sika deer are mainly dotted in Northeast, Huabei and Huazhong etc regions. The estimated number of farmed sika deer is around

1.2 million, which is concentrated in the Northeast region. About 420,000 sika deer are currently farmed in Jilin province. Farmed wapiti are mainly distributed in Xinjiang region, Inner Mongolia region and Liaoning Province, and estimated number is 400,000. These diversified deer species and breeds have laid the foundation for further development of the country's deer farm industry.

Keywords: genetic resources, evaluation, Chinese deer, Sika deer, *Cervus nippon*, wapiti, *Cervus canadensis*, White-lipped deer, *Gervus albirostris*, sambar, *Cervus unicolor*

Sika and Red deer populations in the Czech Republic: Is there any evidence of their crossbreeding in captive and/or in free-living populations?

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Sika deer from Japan as well as from continental Asia were introduced to the Czech Republic approximately 100 years ago. Introduced individuals were first bred in enclosures; however, after World War II several individuals escaped and formed free-living populations, which continue to increase in number. Expanding sika deer populations endanger the native red deer by crossbreeding. The aim of this study was to estimate genetic diversity and population structure of red and sika deer populations in the Czech Republic using microsatellite markers and to determine if hybridisation has occurred. To reduce the risk of misidentifying 'pure' individuals as hybrids due to ancestral polymorphism we also used samples of native Japanese sika and native Dybowski sika deer.
Up to date we have isolated DNA from 211 tissue samples from sika deer populations and from 61 tissue samples from red deer populations. Genotyping was performed using 13 microsatellite markers. Genetic structure and inter-specific hybridisation were assessed by STRUCTURE 2.3.3 and GENETIX 4.05.2.
Hybridisation and genetic structure of both populations were tested using a Bayesian admixture analysis approach implemented in STRUCTURE. This procedure detected the maximum likelihood for a model of three genetically distinct populations [Ln P(D)=-12187.3]. Samples were divided into clusters "elaphus", "nippon", and "hortulorum". The proportion of individual genotypes (Q) belonging to each of the three detected clusters corresponds to predefined populations based on the Factorial correspondence analysis plotting. Based on the membership to particular clusters we detected pure individuals (with $Q>0.985$) or hybrid individuals (with $0.010 <Q < 0.985$). Almost a half of captive Dybowski sika individuals in the Czech Republic (44.8%) were identified as hybrids with Japanese sika deer, red deer or with both Japanese sika and red deer. One individual previously determined as Dybowski sika was classified just a hybrid between red deer and Japanese sika deer. Hybrid individuals occur also in native population of Dybowski sika in Primoria: 16 of 50 samples were identified as hybrid individuals (32%). Among Japanese sika deer in the Czech Republic we detected 11 of 64 individuals (17.2%) as hybrids with red deer, Dybowski sika and with both red deer and Dybowski sika. One individual previously determined as Japanese sika was classified as pure red deer. Surprisingly, we detected crossbred individuals also among Japanese samples. 25 of 30 Japanese samples were verified as pure sika; however, 5 samples from Osaka seem to be crossbred with red deer and Dybowski sika. Proportion of crossbred individuals in the Czech red deer population was relatively low (7.5%) in comparison with Primorian population of Manchurian

wapiti (37.5%).
More crossbred individuals were detected in captivity than in free-living populations, and higher level of hybridisation was detected between sika deer subspecies than between sika deer and red deer. However, our results indicate that hybrid individuals between red deer, Japanese sika deer and/or Dybowski sika are present in free-living European red deer and sika deer populations and also in native Dybowski sika deer, Japanese sika deer, and Manchurian wapiti populations. The study was supported by grant 524/09/1569 of GACR.

Keywords: *Cervus nippon*, *Cervus elaphus*, hybridization, microsatellite loci, conservation, genetic

Cloning and characterization of the myostatin gene in Chinese Northeast Wapiti (*Cervus elaphus canadensis*)

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Myostatin (GDF-8, MSTN) is a member of the TGF-beta superfamily and functions as a negative regulator of skeletal muscle development and growth. A phenomenon known as double-muscling phenotype in cattle is caused by silencing of the myostatin gene. Like beef cattle, Chinese Northeast Wapiti has mainly been selected for meat production. The aim of this study was to carry out molecular cloning and characterization of the myostatin gene of Northeast Wapiti, thereby to dissect the molecular structure of the gene and provide basic data for the enhancement of wapiti meat production. Deer myostatin gene was studied based on the nucleotide sequences from cattle (GenBank: AB076403). Firstly, the 3 exons of the deer myostatin gene were amplified using polymerase chain reaction (PCR) and it was found that exons 1, 2 and 3 were made up of 373 bp, 374 bp and 381bp respectively. The boundaries of these exons were defined based on the 5'-GU-AG-3' rules. These 3 exons were comprised in total of 1128 bp, which encodes 375 amino acids.
Deer myostatin contained a putative amino terminal signal sequence (residues 1-21, MQKLQICVYIYLFMLIVA), one glycosylation site, a transforming growth factor-beta (TGF-beta) pro-peptide domain (residues 35-255), a TGF-beta domain (residues 263-375), and a RXXR proteolytic processing site (RSRR, residues 264-267, matching the RXXR consensus site). Out of 13 conserved cysteines residues in deer myostatin, nine were common to all of the TGF-beta superfamily. Deer myostatin amino acid sequence was highly conserved among the 19 species studied: 92-98% between mammals; 85-89% between birds; 60-67% between fish and other animals. The most conserved region of vertebrate myostatin was the TGF-beta domain, which was the mature bioactive domain of the myostatin protein. Analysis of the phylogenetic trees for the 19 species (myostatin coding sequences) demonstrated that the overall bootstrap values were high, hence provided strong statistical support for the main structure of the tree. This phylogenetic tree built based on myostatin coding sequences matches the known phylogenies of these represented species. Therefore, the myostatin coding sequence could be used as a phylogenetic marker. The six "loss of function" mutations that result in double muscling in cattle were not present in the deer myostatin molecule. Nevertheless, there were 39 nucleotide changes detected in deer myostatin gene compared to that of cattle. Among these changes, 23 were non-synonymous and 16 were synonymous. Two polymorphisms (SNPs) were confirmed to be the point of mutation in the 16 deer individuals. One was at 948/1128bp (G->A transition) that does not cause a change in amino acids (Val). The other was at 999/1128bp (T->A transversion) that gave rise to a change in amino acids (switching between Arg and Ser). Our study has provided valuable insights for understanding deer myostatin sequence variation and genomic information which will aid further investigation of the myostatin gene, and help for the evaluation of the role of Myostatin in myogenesis and meat quality in deer.

Keywords: deer, wapiti, *Cervus elaphus*, myostatin, cloning, myogenesis, production, meat quality

Studies on molecular genetic diversity of Chinese Sika deer and Wapiti

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China is one of the few countries in the world where most deer species reside. Amongst these species two, i.e. sika deer (*Cervus nippon*) and wapiti (*Cervus elaphus*), stand out as they are most abundant in China and successfully farmed for velvet antler production. In order to investigate the population genetic structure and evolution of these two species, we analyzed the genetic diversity of Dongbei, Gansu, Zuojia, Tianshan, Altai and Tarim wapiti, and Dongfeng, Xingkaihu and Zuojia sika deer using 20 microsatellite markers. Among these 20 microsatellite markers, 14 were derived from the red deer and the remaining from cattle and sheep. These markers consist of 4 monomorphic loci (TGLA10, BM757, BM5004 and IDVGA-29) and 16 polymorphic loci (BL42, BM203, MGTG7, TGLA127, BM2320, JAB1, ETH11, BM1225, BM3628, BMC1009, IDVGA-71, BM4107, BM6506, BOVIRBP, TGLA226 and BM848).

In total, 26 alleles were detected in wapiti and sika deer. The mean number of effective alleles was 1.43. Polymorphism information content of these loci was between 0.1187-0.9134. Mean heterozygosity value of these subspecies/breeds was between 0.0014-0.9180. Zuojia wapiti and sika deer had higher heterozygosity value than the other tested subspecies/breeds being 0.3342 and 0.2803, respectively; indicating extra genes were introduced into these two breeds during breeding.

The results of systemic hierarchical clustering are consistent with the geographical distribution of these deer sub-species/breeds. Based on our results, Chinese wapiti breeds/subspecies could be classified into four clusters. The first cluster includes Altai Wapiti and Tianshan Wapiti; the second cluster Northeast Wapiti and Zuojia Wapiti; the third cluster Gansu Wapiti; and the fourth cluster Tarim Wapiti. Chinese sika deer could be classified into two clusters. The first cluster consisted of Dongfeng and Zuojia sika deer; and the second cluster Xingkaihu sika deer. The time span of genetic divergence of Chinese wapiti and sika deer population was calculated from 86 to -2086 years. In sika deer, the time span of this divergence of Dongfeng and Zuojia Sika deer was the shortest at 110 years. In wapiti, the time span of this divergence of Tarim and Northeast Wapiti was the longest at 793 years; whereas, Altai and Tianshan Wapiti were the shortest at 86 years. Consequently, the time spans of genetic divergence of those sub-species/breeds that distribute in the same geographic region were relatively short, and vice versa.

The microsatellite genetic markers that are related to velvet production were identified through establishment of a general linear model. There was a significant difference between the AA type and the AB type at the BM4107 locus in Zuojia sika deer ($P<0.05$), and velvet antler yield of the AA type was significantly higher than that of the AB type ($P<0.05$). Likewise, a significant difference was detected between the AB type and the BB type at the TGLA226 locus in Xingkaihu deer ($P<0.05$), and velvet antler yield of the AB type was significantly higher than that of the BB type ($P<0.05$).

Overall, our results have provided useful information for an in depth understanding of molecular genetic diversity of Chinese sika deer and wapiti, and for molecular breeding of these deer breeds toward further increasing velvet antler production.

Keywords: Wapiti, Sika deer, *Cervus nippon*, microsatellite marker, genetics, diversity, *Cervus*

elaphus

Genetic diversity of native primorian and introduced Czech populations of Dybowski's Sika deer using microsatellite markers

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Native populations of Dybowski's sika deer (*Cervus nippon hortulorum*), originally distributed in Far East Russia, reportedly declined at the end of 19th and beginning of 20th and have started to increase since 1970s up to actual population numbers of more than 20.000 individuals. Thus, the genetic diversity of this population should be substantially affected by this bottleneck event. Before or during this decline, Dybowski's sika deer was introduced to the Czech Republic and its offspring is still living in captivity. Therefore, the Czech population is expected to be highly influenced by founder effect and inbreeding. The aim of this study was to estimate and to compare genetic diversity of both populations using microsatellite markers.
Up to date we have isolated DNA from 67 tissue samples from the Czech population and from 50 tissue samples from the Primorian population. Genotyping was performed using 13 microsatellite markers. The number of alleles (n_a), allelic richness (AR), number of private alleles (P_A), observed (H_O) and expected (H_E) heterozygosity were estimated for each locus in both populations using FSTAT 2.9.3.2 This programme was also used to estimate the inbreeding coefficient (F_{IS}) and the pairwise index of genetic differentiation (F_{ST}). Tests of the departure from Hardy–Weinberg equilibrium (HWE) were conducted using CERVUS 3.0.3. Recent bottleneck in the Primorian population was tested using the programme BOTTLENECK, and the genetic structure of both populations was assessed by STRUCTURE 2.3.3 and GENETIX 4.05.2.
All analysed microsatellite loci were polymorphic in both populations. In the Czech population the number of alleles varied from 4 to 17 (average 10.0, AR=9.32), with observed heterozygosities between 0.33 and 0.91 (overall H_O=0.61, H_E=0.75). Significant deviations from HWE after Bonferroni correction (adjusted p=0.0038) were observed at 7 of 13 loci. In Primorian population the number of alleles varied from 4 to 16 (average 9.3, AR=9.24), with observed heterozygosities between 0.12 and 0.82 (overall H_O=0.60, H_E=0.72). Significant deviations from HWE were observed only at 2 of 13 loci. We detected high number of private alleles within both populations (Czech: 0-8 per locus, Primorian 0-7 per locus). A moderate level of genetic differentiation was detected between the Czech and Primorian populations (F_{ST}=0.085, P<0.0001), supported by a perfect assignment in Bayesian cluster analysis and by Factorial correspondence analysis. Estimated values of Wright's fixation index (Czech F_{is}=0.188; Primorian F_{is}=0.167) indicates a certain level of heterozygote deficiency and high level of inbreeding in both populations. Significant heterozygosity excess was detected only using infinite allele model (Wilcoxon sign-rank tests, P<0.05). In addition, there was no significant deviation from the normal L-shaped distribution of allele frequencies.
High level of genetic differentiation between both populations, which was not detected previously using mtDNA markers, could be a result of long-time segregation, small number of founding individuals of the Czech population, and/or genetic drift in the Primorian population during its

population decline. High level of H_o, number of private alleles and unverified recent bottleneck could indicate that both populations are not completely isolated and contain genes from immigrants from other populations. Crossbreeding with Manchurian/European red deer or with other sika deer subspecies seems to be possible and has to be verified by analysing genetic structure and diversity of all these populations together.

Keywords: Sika deer, *Cervus nippon hortulorum*, genetics, mtDNA, inbreeding, bottleneck

Transferability of eight microsatellites loci from three Cervidae species to Marsh deer, (*Blastocerus dichotomus*)

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Marsh deer (*Blastocerus dichotomus*), an endangered species, is classified as "vulnerable" by IUCN. Nowadays, the distribution of this species in Brazil is rather small and fragmented, becoming residual populations in the states of MT, MS, TO, RO, PR and SP. This is due to an accentuated reduction of its area of occurrence, related to the drainage of marshes for agricultural purposes and the construction of large hydroelectric power plants. Thus, genetic studies with these populations are of utmost importance. The microsatellite markers are the most appropriate tools for such studies because they are the most polymorphic class of molecular markers existing currently. Although extremely efficient, the use of microsatellite molecular markers depends on a previous stage, which consists in the characterization and description of these loci in the target species. Due to the high cost and time for the development of species-specific primers for microsatellite loci, many studies have been using primers described for related species. Transferability of heterologous microsatellite is easily performed when carried out in taxonomically related species or genera. The family Cervidae is one of the most studied among Artiodactyla by means of heterologous microsatellite markers due to the immense quantity of primers described. Thus, eight primers selected and tested by Mantellatto (2010) in five species of the genus *Mazama* were tested in *Blastocerus dichotomus* to verify its transferability: CA71 described for *Cervus axis*, RT05, RT07, RT09, RT13 and RT30 described for *Rangifer tarandus* and Mber99B Mber710B and described for *Moschus berezovskii*. Blood samples from 5 marsh deer *(Blastocerus dichotomus)* housed at the Núcleo de Pesquisa e Conservação de Cervídeos (NUPECCE) of São Paulo State University (UNESP) were used for this experiment. The amplification reactions were performed as described in the literature for each primer. PCR products were applied on 2% agarose gel to verify if the size of the amplified product coincided with the size described for the primers. The confirmation of homology was achieved through the sequencing of the amplified products, confirming the conservation of the microsatellite region of the species for which it was described and the region obtained in *Blastocerus dichotomus*. Therefore, heterologous primers described in this study have potential application in population genetics studies for *Blastocerus dichotomus*. This genetic data is essential to guide decisions towards the policies in order to help Marsh deer conservation.

Keywords: transferability, microsatellites, Marsh deer, *Blastocerus dichotomus*, conservation, DNA, genetic

Intraspecific phenotypic variation in deer: The role of genetic and epigenetic processes

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Intraspecific phenotypic variation (PV) in deer is common, at times impressively diverse, and includes effects on morphology (size, shape, color), development, physiology, and behavior. These variations, of both genetic and non-genetic origin, may contribute to adaptability and micro- and macroevolution when heritable. Until recently considered a nuisance in evolutionary and taxonomic studies, PV has now become the primary target to study fossil and extant species. Phenotypes are traditionally interpreted to express primarily interactions of inherited genetic variants. PV certainly originates from different genotypes, but additional PV referred to as phenotypic plasticity (PP) results from gene expression which is responsive to environmental conditions and other epigenetic factors. During the not-so-long-ago "genomic era", the focus was on hard wiring, the primary DNA sequences and variability therein. Recently however, several higher order architectural genomic features were detected which all together affect PV. **Genes**: poli-genic traits with up to several hundred involved genes; pleiotropic genes affecting multiple traits; poli-allelic genes with the CFTR gene having >1500 alleles; gene dosage (copy number variants, CNVs); single nucleotide variance in coding and gene regulatory regions; mtDNA recombinating frequently in animals, and leakage resulting in paternal mtDNA inheritance. **Gene products:** pleiotropic gene products with multiple, independent effects on many different aspects of development, behavior, physiology and morphology; multiple protein structures through alternative splicing; variable gene product concentrations affecting chemical reactions due to gene dosage. **Gene expression:** a) epigenetic regulation at the DNA, nucleosomal and chromosomal levels (including DNA methylation, chromatin remodeling, non-coding RNA, histone tails modification, combinations of small RNAs, RNAbinding proteins involved in both RNA editing and microRNA access to their target mRNAs); b) large-scale genomic structural variation (i.e., CNVs imbalance, affecting 12% of the human genome); c) Transcription factor proteins (TFs) presenting 10% of all gene products, each regulating several dozens to some 500 target genes, with variation of TF activity (and thus gene expression) between 7.5-25% among individual humans, which exceeds variation in coding DNA by 300-1000 fold. TFs themselves are regulated (often by other TFs); d) non-protein-coding RNA (98.5% of genome) constituting maybe hundreds of thousands RNA signals; e) gene expression responsive to external and internal environmental variation (including maternal physiology, parent-offspring interactions affecting DNA chemistry of offspring, cultural interactions, diet resulting in inheritance of feeding behavior or affecting morphology); f) Transgenerational epigenetic inheritance, TEI (from ubiquitous non-gametic interactions, as genomic imprinting, from epistasis and genetic effects where genetic factors in one generation affect phenotypes in subsequent generation, from transgenerational gene-diet interactions); g) epigenetic stochasticity resulting in random phenotypic plasticity.
Thus, whereas highly complex assortments of genotypes lead to a spectrum of phenotypes, the same

spectrum can result if a single genotype generates highly complex assortments of epigenotypes. Although DNA is the template of hard-wired heredity, it is the coordination and regulation of gene expression that results in wide complexity and diversity seen among individual deer. In fact, per generation there will usually be a greater variety of phenotypes available for selection than available genotypes. One of the most unique examples of a labile trait in mammals is found among cervids: the yearly regrowth of a complete appendage, the antler. An impressive example of epigenetics is the memory of injury on live antler tissue which is still expressed in new regrowing antler for up to 10 years. In conclusion, epigenetic processes have fundamental influences on the great intraspecific phenotypic variation found in deer which is reflected in broad ranges of environmental conditions under which they can persist.

Keywords: cervids, genetics, epigenetics, adaption, intraspecific, phenotypic variation

Genetic aspects of introduced Red deer in Patagonia, Argentina: Origins and variability

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A small group of red deer (*Cervus elaphus*) was introduced to the foothills of the Andes in Patagonia in the early 1920s. This species adapted very well to the habitat and climatic conditions in the area and presently may number as many as 100,000 animals. Several indices commonly used to evaluate the fitness of a species in its environment indicate that red deer thrive under very favorable conditions in Patagonia. For instance, body size, antler development, reproductive rates, herd health, and longevity are near the maximum described for the species. Furthermore, some local populations occur at densities much higher than encountered in their native ranges. The introduction was based on few deer and raises the question how the genetic background and the apparent success of the population relate. The objective was to examine several biological enzyme systems to test for variance in protein polymorphism in comparison to other well known populations of red deer in other parts of the world. Blood was collected immediately after animals were culled. Plasma was separated and erythrocytes washed twice in physiological saline. All samples (n=41) were kept frozen in liquid nitrogen until processed in the laboratory. The protein systems examined by electrophoresis in the plasma included: post-transferrin, transferrin, vitamin D binding protein, plasminogen, and complement component; and in the erythrocytes: hemoglobin, superoxide dismutase, glucose phosphate isomerase, and diaphorase I. Variation in plasminogen was lower than is typical for red deer, and glucose phosphate isomerase showed absolutely no variation. Furthermore, some occurrence of alleles typical for wapitoid species indicate that the introduced deer originated from English or European deer parks which have had a history of introductions of wapitoid species in the past. In New Zealand, the superoxide dismutase allele typical for wapiti was found in 1% of red deer, whereas it occurred in 11% of animals in the present study. The low variations are likely the result of the introduction based on few individuals. However, the outstanding performance of the present population contradicts the existence of any overt impact from this founder effect. The observed large body sizes may not only be due to good environmental conditions, but also due to previous hybridization with wapitoid species. Several specimens were heterozygous and 1 specimen was homozygous for wapitoid hemoglobin.

Keywords: Red deer, *Cervus elaphus*, Patagonia, invasion, wapiti, genetic, electrophoresis, founder effect

REPRODUCTIVE PHYSIOLOGY

Enhancing the incidence and timing of puberty in red deer hinds with melatonin

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High levels of puberty failure amongst rising-two-year-old (R2) red deer hinds have become a significant productivity issue for the New Zealand deer farming industry. This is manifest as scan pregnancy rates ranging from 40-95+% across farms. Barrenness in R2 hinds largely represents ovulation failure around 16 months of age, with these hinds opting to delay puberty by one year. By far the most common reason for this is insufficient body mass, with hinds needing to attain ~70% of their ultimate mature body mass to enter puberty. Early studies on the use of melatonin implants to advance puberty by several weeks indicated the possibility that treatment also increased the incidence of puberty without influencing body mass. This putative effect was studied within two commercial herds with histories of poor R2 hind performance.

A subset of 700 hinds (350 per farm) from a total combined population of 2512 R2 hinds received single subcutaneous melatonin implants (Regulin) on two occasions, 11 and 12 months of age. All hinds were joined with yearling stags for mating from 13 to 18 months of age. At stag removal each hind was scanned by rectal ultrasonography to assess pregnancy status and conception date (based on fetal aging). Live-weight was obtained for each hind at 12 months of age and used as a proxy for body mass around the normal timing of puberty.

Farm A: Melatonin treatment resulted in a significant ($P < 0.05$) advancement in conception dates (9 days) and increased synchrony of conceptions. Notably, however, treatment was associated with a significantly higher pregnancy rate (90.3% vs. 78.0%). The principle effect of melatonin treatment was to increase the pregnancy rate of hinds of low body mass. At 60 kg live-weight a probability prediction model indicated a pregnancy rate of 52% for untreated hinds and 83% for treated hinds. At 105 kg the rate for both cohorts was 90%.

Farm B: Melatonin treatment resulted in a significant advancement in conception dates (13 days) and increased synchrony of conceptions, but was associated with only a marginal but non-significant increase of conception rate (68.5% vs. 61.1%; $P = 0.106$). However, the overall pregnancy rate of the herd was ~20 percentage points lower than that of previous years' cohorts of R2 hinds, despite generally higher mean 12-month live-weights. At 60 kg live-weight the probability prediction model indicated a pregnancy rate of 20% for both treated and control cohorts, rising to 80-90% pregnancy at 105 kg.

The study has demonstrated that factors influencing puberty attainment in R2 red deer hinds can vary between populations. On Farm A, in which body mass of hinds immediately prior to their first potential breeding season may have been the principle limiting factor, melatonin appears to have instigated the pubertal process in hinds that would otherwise be of insufficient body mass. This effect is unprecedented in the available literature on puberty in seasonally breeding ruminants. It likely relates to augmentation of photoperiodic entrainment, allowing hinds that are physiologically constrained by low body mass to 'jump the hurdle' that prevents them from responding to short-daylength signals that instigate ovulatory processes in autumn. On Farm B, while there was a

profound relationship between live-weight and pregnancy status, melatonin treatment had only a marginal effect on puberty attainment. The principle factors limiting puberty in this case are not immediately obvious but may relate to prior events in the life of the hinds, which had been subjected to unusually severe drought conditions when they were calves. It is hypothesised that this may have predisposed the population to a higher level of puberty delay through alteration of live-weight thresholds for puberty attainment. This hints at complex life strategies structured around using early nutritional variables and growth trajectories to predict the ensuing environment to determine the suitable timing of first breeding.

Keywords: Red deer, *Cervus elaphus*, reproduction, puberty, melatonin, farming, production

From wild to domesticated in 30 years: Lessons from the reproductive management of farmed Red deer (*Cervus elaphus*)

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Reproductive physiology of red deer, a recent domesticant, has generally been modelled conceptually on other seasonally-breeding domestic ruminants such as sheep. While numerous studies over the last 30 years of deer farming have demonstrated the broad alignment to the accepted ovine endocrine template for events such as ovulation, pregnancy establishment/maintenance, parturition, spermatogenesis and general reproductive behaviours, it is my contention that 'the devil is in the detail'. In other words, the subtleties of how cervids, and any other non-traditional farmed species for that matter, express particular reproductive outcomes in relation to endocrine signals differ in many ways from 'model' ruminants. It is these subtleties that often foil our repeated attempts to manipulate and optimise reproductive outputs within the farmed or ex situ environment. In this paper I will discuss various aspects of reproductive management of farmed red deer for which there have been unexpected outcomes, such as for attempts to manipulate birthing season, to increase pregnancy rates of pubertal hinds and to adapt artificial reproductive technologies for genetic management. My central thesis is that the red deer is a species superbly adapted to highly seasonal annual reproductive cycles for survival in regions of climatic extremes and exhibit remarkably high levels of reproductive success within these environments. As such, they have evolved complex physiological buffering mechanisms to ensure adherence to this pattern in the face of short-term vagaries, and unpredictable outcomes, of climate and feed supply. Examples of this include profound environmental (non-genetic) control over gestation length, the ability to cue body-mass thresholds for puberty attainment from early-life nutritional environments and the interactive effects of various environmental modifiers of conception date (a lesson in reductionism vs. holism). These examples beg the question….do traditional domestic ruminants possess similar buffering mechanisms or has the process of domestication dampened their effect?

Keywords: Red deer, farming, production, reproduction, body weight, puberty

Ovarian ultrasonography to analyze follicular dynamics during superovulatory treatment in Red deer (*Cervus elaphus*) hinds

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Inconsistency of the superovulatory responses of donor hinds has been a general feature of all red deer MOET programmes (Asher GW et al 2000 Anim. Reprod. Sci. 59, 61-70). The development of the techniques has been by trial and error as there is usually a lack of basic information on which to base MOET protocols (Fennessy PF et al 1994 Theriogenology 41, 133-138). The objective of this study was to understand follicle development during a superovulatory treatment in order to improve ovulation rates and quantity of transferable embryos produced. During the breeding season, ten mature (3-5 years old) red deer hinds were synchronized receiving an intravaginal sponge containing 100 mg of medroxiprogesterone acetate for 13 days, with device replacement on day 11. Four days prior to the beginning of the FSH treatment, 0.5 mg of estradiol benzoate (Syntex SA, Argentina) was given i.m. to synchronize the follicular wave. Superovulation was conducted with a total dose of 120 mg of NIH-FSH-P1 (Folltropin-V, Bioniche Animal Health, Belleville, ON, Canada) given i.m. in four equal doses of 30 mg every 24 h, from Day 11 to 14. Forty eight h after sponge withdrawal, 0,84 mg of buserelin acetate (Receptal, Intervet, The Netherlands) was inyected i.m. to stimulate and synchronize ovulations. Ovarian scanning was performed by transrectal ultrasonography using a multifrecuency linear transducer (ESAOTE Pie Medical, Tringa Linear) on Days -1, 0, 1, 2, 3, 4, 5 and 6, being 0 the day of sponge withdrawal. The diameters of all follicles ≥3 mm were measured, and their three dimensional position were recorded to determine growth and ovulation. The average ovulation rate was 10.8 ± 1.6. The distribution of ovulations was 9.3%, 31.5%, 24.1%, 22.2%, 9.2% and 3.7% at 24, 48, 72, 96, 120 and 144 h after sponge withdrawal, respectively. The proportion of follicles that did not ovulate during the period of this study was 16.9%. The proportion of ovulated follicles according to their diameter was 9.3%, 68.5% and 22.2% for 3 mm, 4-5 mm and ≥6 mm respectively and were during the 24 - 96 h period for the first two follicles categories and after 96 h for the last category. This study shows a great variability of ovulations in the superovulatory protocols routinely used in red deer donors hinds. Improvement of the hormone treatment to induce a greater ovulation synchrony (within 72 h after progesterone device withdrawal) would increase the fertilization rates and the quantity of transferable embryos produced in red deer MOET programs.

Keywords: Red deer, *Cervus elaphus*, ultrasonography, follicles, superovulation, reproduction, ex-situ, production

Testis, testosterone and semen variations during the breeding season of Southern pudu (*Pudu puda*)

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The southern pudu (*Pudu puda*) has been classified as a vulnerable animal by different Red Lists (Glade, 1988; IUCN, 2010) because the forest loss, habitat fragmentation, and predation by domestic dogs (Silva et al 2010). Research on pudu reproductive physiology has been limited mostly to male endocrinology. However, limited studies has been done to describe methods on semen extraction, semen profile and hormones concentration during the breeding season. The aim of this study was to describe semen characteristics, testosterone plasma concentration and testicules measurements during the breeding season in the southern pudu.
Samples were collected from five adult animals (2-7 years old, 9-10 kg) from a private collection and maintained in the Wildlife Rehabilitation Center at Universidad Austral de Chile (CEREFAS), Valdivia, Chile. Once weekly from March 1st to June 30th, animals were manually restrained and anesthetized. Previous to semen collection testicular measurements (ie. length, scrotal circumference (SC)), and a blood sample were taken. A mixed protocol of transrectal massage and electroejaculation allowed us to obtain successful ejaculation in a 95.4 % of total trials, without any signs of adverse reactions. Repeated measures in time of volume (0.073 ± 0.009 ml), concentration (5.35 x 10^{n} ± 642 x 10^{6} spermatozoid/ml), progressive motility (0.5 ± 0.087), viability (0.617 ± 0.0759), normality (0.81.3 ± 0.045), primary and secondary abnormalities (0.122 ± 0.012 and 0.062 ± 0.024) were recorded for each animal. Serum testosterone decreased during the sampling period; however we were unable to detect significant differences between weeks. Significant temporal variations in testicle measurements, testosterone serum concentration and sperm quality were found during the breeding season sampling period.
To the authors knowledge, this is the first report of quantitative and qualitative variations of pudu semen during its breeding season. Important fluctuations were observed in all parameters evaluated during the study related to the seasonality of the species. The information obtained from this study can be used as the first step in the development of reproductive biotechnologies in this species.

Keywords: reproduction, semen, pudu, *Pudu puda*, beeding season, spermatozoa

Effects of hypertonic diluent on epididymal spermatozoa stored at two different temperatures before freezing-thawing

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Red deer (*Cervus elaphus*) is a new farming species, which offers opportunities for some reproduction studies, those which can be applied to different contexts such as farming, hunting, and conservation (of endangered cervid species or populations).
As a first experimental model, epididymal spermatozoa from slaughtered livestock bulls were recovered at 30, 54, 78 and 102 hours after death. The scrotal contents were stored both at 5 and 20ºC. The sperm cells of each treatment (time+temperature combinations) were frozen with Triladyl (T) or Triladyl+trehalose (TT) diluents. In order to assess the sperm viability and integrity, post-thaw evaluation included motility, supravital stain, HOSTest, acrosome damage and chromatin structure assay (SCSA).
Both at raw and refrigerated states, the sperm motility rate was higher in the 5ºC stored group, compared with the 20ºC stored group for all times, maintaining 20% motility at 5ºC and 102 h (fresh group). When comparisons were carried out at post-thawing state, motility was higher in the 5ºC group, achieving best results at 102 h with TT (7,5%). However, when supravital stain and HOSTest were observed, viability and membrane integrity were well preserved even at 102 h post-mortem (30% and 36% with TT diluent at 5ºC, respectively). These results may confirm that low motility rate could be present in frozen-thawed epididymal spermatozoa while most of them remain alive. Acrosome status was not greatly affected by storage time, TT diluent being the best crioprotector for that parameter.
As a second step, epididymal spermatozoa from hunted red deer stags were recovered at 4 and 30 hours after death. The scrotal contents were stored at 20ºC, because that temperature is more close to field and shipment conditions of epididyms. The sperm cells were frozen with Triladyl+trehalose (TT) diluent. Post-thaw evaluation included the same parameters indicated for the bull spermatozoa. The assessment of 30 h frozen-thawed spermatozoa may confirm that in this time acceptable motility rate (35%) and viability (39,7%) were achieved. High plasma membrane integrity (47,9%) and acrosome integrity (59,3%) allows us to suppose that, in addition to the chromatin status, the TT diluent, combined with 30 h post-mortem epididymal spermatozoa recovery, is a useful method to obtain viable and fertile sperm cells to be applied in assisted reproduction programmes in endangered deer species.

Keywords: Red deer, *Cervus elaphus*, frozen-thawed sperm cells, hypertonic extenders, epididymal spermatozoa, reproduction, production, conservation, semen

The surprisingly characteristic of the Amazonian Brown brocket deer (*Mazama nemorivaga*) semen: The red semen

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Mazama nemorivaga (Amazonian Brown Brocket deer) lives in the region of the Amazon rainforest and it is considered a medium sized species. It is classified by IUCN as LC and, practically, there is no information on their reproductive traits. Thus, the objective of this paper was to make a preliminary description of the ejaculate of this species. In order to do it, we have used two individuals aged 6 and 5 years old, with body weight of 12.9 and 16.0 kg, respectively, with hard antlers; these individuals are kept in captivity at NUPECCE/Brazil. We have performed a collection of each animal (electro-ejaculation - Animal 1 and a rectal massage - Animal 2) during April 2010. The animals were anesthetized (7 mg/Kg Ketamine and 1 mg/kg Xylazine) and the collected semen was immediately evaluated for volume, viscosity, coloration, odor (subjectively by direct observation) and pH (reagent strip Merck®, Germany). It was submitted to smears stained with quick panoptic to evaluate the presence of erythrocytes and an aliquot was diluted (1:200) in buffered-saline formal for subsequent evaluation of the concentration (Neubauer chamber under optical microscope) and the sperm morphology (wet preparation under a phase contrast microscope). The collected semen was also evaluated for mass movement (scale 0-5) and, after the pre-dilution (1:2 in Botu-Bov® diluent, Brazil), it was evaluated on individual motility (%) and vigor (scale 0 - 5) under optical microscope with 400X magnification. The semen volume of Animal 1 was 0.145 mL, milky appearance, red-brick coloration, *sui generis* odor, pH=8.0, mass movement zero, 70 % of motility, vigor 3 and concentration of 0.32×10^9 sperm/mL. About the sperm morphology, we found 16.5% of major defects and 49.5% of minor defects (and of these, 73.73% was coiled tail). The semen volume of Animal 2 was 1.59 mL, with milky appearance, red-brick color, *sui generis* odor, pH=8.0, mass movement 2, 80% of motility, vigor 4 and concentration of 0.37×10^9 sperm/mL. As for sperm morphology, we found 46.5% of major defects (and of these, 78.49% of middle piece defects) and 23% of minor defects. The red coloration of the semen suggests the presence of red blood cells; however, we did not find red blood cells in the smear. These preliminary analyses may suggest that the species *M. nemorivaga* presents testicular volume and seminal patterns similar to other species of the same genus, which typically have large individual variation in testicular volume, sperm cell concentration between 0.1×10^9 and $3,9 \times 10^9$. However, the coloration of the semen of this species is a striking and distinctive feature in relation to other species of deer studied. The results presented here were not influenced by the collection method or by diet, since other species of NUPECCE receive the same management without production of red coloration semen. Likewise there is no evidence of contamination by urine or blood. So, as the coloration of the semen is a peculiar feature, it suggests further studies that may clarify the origin of the pigment and its role in the reproduction of the species.

Keywords: Amazonian Brown brocket deer, *Mazama nemorivaga*, semen, male, reproduction

Fecal progestins during pregnancy in free-living Pampas deer (*Ozotoceros bezoarticus*) from Pantanal, Brazil

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Pampas deer (*Ozotoceros bezoarticus*) occupies open habitats throughout the central region of South America. This species has experienced the most important population retractions of all neotropical deer. Little is known about the endocrine aspects of their reproductive cycle. This study aimed to characterize the profile of fecal progestins during pregnancy in free-living animals from the Brazilian Pantanal. We captured fifteen females using anesthetic darts. They were fitted with VHF radio collars and were monitored for sixteen months. Only seven females were visualized with their offspring confirming the pregnancy. The onset of pregnancy was considered 210 days prior to the first observation of the fawn. The deer were observed at monthly intervals and the fecal samples were collected immediately after defecation. The fecal samples were dried in an oven at 57^0C for 72 hours. Dried fecal samples were pulverized and steroids were extracted using 80% methanol and stored in a freezer at -20^0C for later analysis. The fecal progestin concentrations were measured by enzyme immunoassay (EIA) using the antibody CL425. Intra-assay coefficients of variation were <10%. Interassay coefficients of variation for two separate internal controls were 12.2% (~ 35% binding, n = 8) and 14.0% (~ 75% binding, n = 8). The comparison between the different physiological phases were performed using analysis of variance followed by Tukey's test with 5% significance level ($p < 0.05$) and all fecal data are expressed on a dry-weight basis. The mean fecal progestin concentrations were 3.30 ± 0.46 μg/g feces before pregnancy; 4.42 ± 0.61 μg/g feces at the beginning of pregnancy (1st and 2nd months); 6.65 ± 0.50 μg/g feces in the middle of pregnancy (3rd, 4th and 5th months); 9.50 ± 1.12 μg/g feces at the end of gestation (6th and 7th months); and 3.96 ± 0.88 μg/g feces in the postpartum period. It was possible to detect significant differences ($p < 0.05$) from the middle and late pregnancy compared to levels belonging to non pregnant periods. Four females died during the experiment, precluding their use for this study. Four of the eleven females who remained alive during the experiment were not observed with their calves, but the endocrine analysis of these animals showed a similar profile to pregnant animals, which may suggest abortion in late pregnancy or loss of the fawns soon after birth. Based on the hypothesis of loss of fawns, the survival of offspring would be 63%. These levels of mortality could significantly affect the sustainability of the population of Pampas deer in the Pantanal region.

Keywords: Pampas deer, pregnancy, fecal progestins, Pantanal, *Ozotoceros bezoarticus*, reproduction

Female effect on Pampas deer (*Ozotoceros bezoarticus*): Semen differences between males in permanent contact or isolated from females

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In deer, information regarding socio-sexual stimulation is scarce and mainly related to male effect. In male deer, seminal characteristics are dependent on testosterone concentrations. Antler cycle and antler characteristics are indirect indicators of testosterone secretion. Pampas deer is a southamerican species considered near threatened (IUCN), from which the largest semi-captive population in the world is located at the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (ECFA, Uruguay). It is suggested that differences in Pampas deer antler characteristics from males in contact or absence of females could be related with greater testosterone secretion stimulated by females (Ungerfeld et al., 2009; Can. J. Zool. 87:734-739). The aim of this work was to determine if in Pampas deer semen characteristics are improved by permanent contact with females. This work was done at the ECFA, where animals were organized in five breeding groups, composed by 1 adult male (5-6 years old) and 5-10 hinds, allocated in a 0,5-1 ha paddock, and another group composed by six adult males (similar age and characteristics) located in a different paddock separated from paddocks containing females (minimum distance=3m). During November (spring) 2009, semen was collected by electro-ejaculation under anesthesia from males in contact with hinds (n=5) or isolated from females (n=6). The following seminal parameters were evaluated: volume, quality (0-5 scale), sperm concentration (spermatozoas x 10^6/ml), total spermatozoas in the ejaculate (spermatozoas x 10^6), percentages of sperm motility, progressive motility, spermatozoa with morphological abnormalities, spermatozoa with normal acromosomes, and spermatozoa with normal membrane function (15 minutes after incubation, evaluated by HOST). Variables were analyzed by students' t test. Data from total spermatozoas in the ejaculate was previously normalized by log transformation. Greater volume (545.0 ± 64.9 vs 346.0 ± 84.4 µl; P=0.04), quality (4.0 ± 0.3 vs 3.1 ± 0.3; P= 0.03) and progressive motility spermatozoas (58.0 ± 9.0% vs 31.4 ± 6.8%; P=0.02) were collected from males in direct contact with females. Percentage of motile spermatozas tended to be greater in semen from males in contact with hinds than isolated males (72.0 ± 6.6% vs 57.7 ± 8.8%; P=0.10). There were no differences in total spermatozoas in the ejaculate (113.0 ± 45.7 spermatozoas x 10^6; P=0.40), spermatozoas with morphological abnormalities (78.2 ± 2.9%; P=0.36), with normal acrosoma (69.6 ± 3.2%; P=0.19), and with normal membrane function (72.9 ± 2.7; P=0.48). Direct contact with hinds stimulated some parameters of Pampas deer males semen, probably through an increase in testosterone secretion. As far as we know, this is the first report about a female stimulation of seminal characteristics in ruminants.

Keywords: Pampas deer, *Ozotoceros bezoarticus*, reproduction, semen, spermatozoa, quality, behavior

Pampas deer (*Ozotoceros bezoarticus*) reproductive seasonality: Changes in seminal parameters

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The Pampas deer (*Ozotoceros bezoarticus*) is a South American endangered deer species. Reproductive seasonality is a common strategy in deer in order to synchronize parturitions in spring. Therefore, if the breeding season occurs in autumn, considering that spermatogenesis length is approximately 60 days in deer, male reproductive changes must anticipate autumn at least that length of time. Most Pampas deer are born in spring, although births have been observed all through the year. The aim of this study was to describe seasonal semen changes in adult and young Pampas deer males under semicaptive management. The study was performed at the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (ECFA), Uruguay (33°47' S, 54°00' W). Two groups (6 males each), one of adults (5- 7 y-old) and another of yearlings (1.5- 2 y-old), were allocated in two separated paddocks (0.5 ha each), starting one year before the experiment began. There were no important variations in feeding during the year, as animals grazed native pastures, and received concentrate *ad libitum*. Once every season (winter: early July, spring: early October, summer: early January, and autumn: late March), semen was collected by electroejaculation under general anesthesia. Total sperm per ejaculate, semen quality (score: 1 to 5), and percentages of sperm motility, progressive motility, alive spermatozoa (eosin-nigrosin), and abnormal spermatozoa were determined. Data were compared using an ANOVA for repeated measurements. Total sperm per ejaculate was compared after a log transformation. There were no differences in relation to age, so results are presented grouped. Total sperm per ejaculate varied with seasons ($P = 0.046$), with greater quantities in summer and autumn ($299.3 \pm 81.3 \times 10^6$ spermatozoa, mean ± SEM) than in winter and spring ($96.6 \pm 28.0 \times 10^6$ spermatozoa). Both, semen quality score ($P = 0.002$) and percentage of spermatozoa with progressive motility ($P = 0.017$) were highest in autumn (3.7 ± 0.4 and, 60.7 ± 10.3 % respectively) and lowest in spring (2.2 ± 0.3 and 27.0 ± 5.8 % respectively). The percentage of alive spermatozoa also varied between seasons ($P = 0.05$), with maximum in summer (55.6 ± 5.5 %), and without any differences during the rest of the year (45.1 ± 4.4 %). The percentage of abnormal spermatozoa also had a seasonal pattern ($P = 0.009$), being highest in spring (82.5 ± 2.0 %) and without any differences during the rest of the year (65.6 ± 2.8 %). Percentages of motile sperm did not vary along the year (59.1 ± 3.0 %). We conclud that Pampas deer males present seasonal variations in the semen parameters, which are similar in adult and young males managed in semicaptivity. The best semen was obtained in summer and autumn, and the poorest in spring.

This characterization provides an important tool for future application of reproductive biotechnologies to Pampas deer conservation.

Keywords: Pampas deer, *Ozotocero bezoarticus*, reproduction, seasonality, semen, spermatozoa

Fecal glucocorticoid profiles during reproductive phases of female free-ranging Pampas deer (*Ozotoceros bezoarticus*)

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The Pampas deer (*Ozotoceros bezoarticus*) is a medium sized deer, occupying especially open environments, such as Argentinean and Uruguayan Pampas and Brazilian Cerrado. Due to direct and indirect man's interference, it is classified as near-threatened (IUCN 2010). The reproductive biology of neotropical deer, despite its importance to the knowledge of these species, is still poorly understood. The objective of this work was to correlate levels of fecal glucocorticoids with the reproductive periods (pre-pregnancy, pregnancy and postpartum) in females of Pampas deer (*Ozotoceros bezoarticus*) in Pantanal, Brasil. Seven females were monitored monthly (August/2008 to November/2009) with the aid of radio-telemetry to collect fresh feces that were immediately cooled in the field, then frozen once in the field lab until processing in the laboratory. The metabolites were extracted from 0.5 g of samples processed with the addition of 5.0 mL of 80% methanol left overnight in horizontal shaker, followed by centrifugation at 1500 rpm for 20 minutes, with the supernatant separated and stored in a freezer at – 20°C. The dosages were made by EIA, in the laboratory of Deer Research and Conservation Center (NUPECCE), at FCAV/UNESP Jaboticabal using monoclonal antibodies for cortisol CL425, coming from Dr. C. Munro, University of California, Davis, CA. Fecal cortisol levels are presented as monthly averages in ng/g feces ± the standard deviation, that were compared with Tukey test ($P < 0.05$). The females' level of fecal glucocorticoids in the three months prior to pregnancy were constant, with average values of 6451,05 ± 2018,38 ng/g, 7223,78 ± 1897,98 ng/g and 5333,24 ± 1143,16 ng/g. During the seven months of gestation, the average levels of fecal glucocorticoids were respectively 5262,45 ± 2594,61 ng/g; 7062,61 ± 3911,35 ng/g; 5436,11 ± 2979,05 ng/g; 4158,94 ± 1104,64 ng/g; 5563,37 ± 2635,48 ng/g; 4698,54 ± 1718,05 and 3818,65 ± 825,94 ng/g. In the month following birth, the level of fecal glucocorticoids was 4232,28 ± 1664,64 ng/g. The results demontrated one glucocorticoid peak in the second month of gestation, which would coincide with the initial period of implantation of the embryo to the maternal endometrium and placenta formation, suggesting a relationship of cortisol with this stage of gestation. Some authors have found high concentrations of cortisol in the end stages of pregnancy in females of some species. Low levels of cortisol found in this study at this phase may be related to the fact that fecal samples corresponding to the last month of pregnancy may have been collected until 30 days before parturition, due to punctual collect system. Although this study has shown trends of excretion of fecal glucocorticoids in different reproductive phases of female Pampas deer, further researches should be realized to understand the physiological processes related to reproduction of this species.

Keywords: reproductive physiology, glucocorticoid, Pampas deer, gestation, cortisol , non-invasive

techniques

Ultrasonographic image characterization of ovarian structures in the Southern pudu (*Pudu puda*)

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The southern pudu (*Pudu puda*) is an endemic deer that inhabit South American temperate rainforest of Chile and Argentina. Considered one of the smallest known deer species, the Southern pudu is classified as Vulnerable according to IUCN and is one of the less known neotropical deer. Physiological research on pudu has been limited mostly to endocrinology studies in males, reproductive-related studies are limited mostly to breeding programs under captivity systems.
Transrectal ultrasonographic technique has been used to study folicular and luteal dynamics in domestic and wild species in order to understand the function of different reproductive processes. This is a none invasive technique and can be performed in a serial manner without administration of anesthetics or sedative agents that could cause adverse effects.
The objective of this study was to characterize the ovarian structures present in the female pudu using real time ultrasonography.
Materials and methods: Two adult females (7-8 kg), maintened at the Wildlife Rehabilitation Center (Valdivia, Chile 39°43”S) from the Universidad Austral de Chile were used for this experiment. The study was conducted from March to May, during the breading season. Once females were habituated to the human presence and handling, they were introduced to an individual wooden black box for ultrasonographic examination of their reproductive tracts. To characterize and determine the stage of the estrous cycle, data from ovarian structures were correlated with changes in the vulva and vaginal citology. The procedure was done periodically 3 times a week during the whole breeding season. Ovaries were examined by transrectal ultrasonography using a real-time B-mode scanner (Aloka SSD 500, Tokyo, Japan) fitted to a 7.5 Mhz linear array prostatic transducer. In brief, observations were conducted with the pudu inside the wooden box and in standing position. After introducing a hydrosoluble contact gel, the probe was inserted into the rectum with the transducer orientated perpendicularly to the abdominal wall. When the urinary bladder was surpassed and the uterine horns were located, the probe was rotated laterally 90° clockwise and 180° counterclockwise to observe both ovaries and their structures. After ultrasonography examination, cotton-tipped swabs embebbed in saline solution was introduced in the vagina and a sample of vaginal cells were collected to characterize the stage of the cycle. Results: Twenty one ultrasonographic examinations were done per each animal from which 13 (61,9%) resulted successful to determine ovarian structures. It was possible to determine the number, size and echogenicity of each structure. Follicular diameter ranged from ≤2 to 3.5 mm and the maximun diameter of corpora lutea ranged from 2 to 5 mm. Edema, abundant mucus secretion and hyperemia in the vulvar mucosa was correlated with the presence of larger follicles and squamous and intermediate cells collected by vaginal cytology, whereas no vulvar signs or mucus secretion were observed under the presence of a corpora lutea.
Conclusions: Follicular and luteal dynamics is feasible to characterize using transrectal ultrasonography in the female pudu. However, high levels of training are necessary to achieve an

acceptable ultrasonographic image without compromise the good livestock practice and animal welfare. This is the first report of characterization of ovarian structures of female pudu during its breading season. The information obtained from this study can be used as a preliminary step to develop assisted reproductive technologies in this species.

Keywords: deer, pudu, ultrasonography, ovary, reproduction

Renin-angiotensin system-regulating aminopeptidases: Monitoring seasonal variations in Red deer and Fallow deer from a Mediterranean ecosystem

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The circulating renin-angiotensin system (RAS) is well known for its systemic role in the regulation of blood pressure, renal hemodynamics and fluid homeostasis. However, in mammals, several organs also contain a local RAS, including male and female reproductive tissues. Recently, the involvement of RAS in both male and female steroidogenesis regulation has been described as having a putative role for angiotensin III (AngIII) instead of angiotensin II (AngII, the main effector peptide of the RAS) in both sexes, according to the changes observed in their corresponding regulating aminopeptidase A (APA), aspartyl aminopeptidase (ASAP), aminopeptidase N (APN) and aminopeptidase B (APB). In fact, changes in several aminopeptidase activities have also been described concomitantly to the estrous cycle of female laboratory animals. In the present work we have analyzed in serum APA, ASAP, APN and APB RAS-regulating aminopeptidases in free-living populations of red deer (*Cervus elaphus hispanicus*) and fallow deer (*Dama dama*) as part of a study of annual cycles of growth and condition. Our aim is to detect seasonal variations in these specific activities and their relationship to the reproductive behaviour and health condition of both species in a Mediterranean environment. Our study was based on animals shot during sport hunting, herd management culls and programs for population control in 2008 in the eastern area of Sierra Morena, southern Spain. A total of 306 blood samples were collected from 172 red deer and 134 fallow deer shot at different times of the year covering the main features of the annual life cycle. Specific APA (E.C. 3.4.11.7), ASAP (3.4.11.21), APN (E.C. 3.4.11.14) and APB (E.C. 3.4.11.6) activities were assayed fluorometrically at a 412-nm emission wavelength with an excitation wavelength of 345 nm, using their corresponding aminoacyl-ß naphthylamides as substrates. Specific activities were expressed as picomoles of their corresponding aminoacyl-ß-naphthylamide hydrolyzed per minute and per mg of protein.
Our results show no changes in APA activity either in males or females of both species. However, ASAP and APB activities showed significant seasonal variations in both males and females. In both males and females there was a maximum peak of concentration in autumn but a second peak were detected in winter for males whereas in females there was high concentration also in summer. Furthermore, ASAP, APN and APB activities were higher in male red deer than in male fallow deer, although no differences were found in females of either species. These changes may be related to a different endocrine status according to their seasonal cycle, the decreased photoperiod in autumn and the normal timing of the seasonal growth cycle. Thus, RAS-regulating aminopeptidase activities modification could reflect the functional role of angiotensins through the annual cycle of both species, also suggesting an important role of these peptide hormones in these biological processes.
This study was supported by the P07-RNM-03087 and CGL-2008-00832 projects and Junta de

Andalucía through PAIDI BIO296 Experimental and Clinical Physiopathology Research Group, and was approved by the Bioethical Committee of University of Jaén.

Keywords: APA, ASAP, APN, APB, RAS-regulating aminopeptidases, Mediterranean ecosystem, *Cervus elaphus hispanicus*, *Dama dama*, Red deer, Fallow deer, seasonal

First report of Red deer (*Cervus elaphus*) embryos produced by MOET program using imported semen in Argentina

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Multiple ovulation and embryo transfer (MOET) programs for red deer (*Cervus elaphus*) have been developed and used commercially over the last years in Argentina for genetic improvement, however there have been no MOET programs yet applied to red deer herds using imported semen for the fertilization of the donor hinds. The objective of this study is to show the results obtained from the first MOET program applied in Argentina using semen imported from New Zealand. Five red deer adult hinds were used as donors while fifteen were used as recipients. Estrous synchronization treatment consisted of a 12 d placement of an intravaginal device containing 300 mg of natural progesterone. The device was inserted on d 0 (initial day of treatment) and replaced with a new one on d 10 in the donors. At the time of device removal (d 12), 250 iu of PMSG (Folligon, Intervet International, Boxmeer, Holland) was given i.m. to each recipient hind. Superovulation was conducted with a total dose of 180 mg of NIH-FSH-P1 (Folltropin®-V, Bioniche, Belleville, Ontario, Canada), given i.m. in eight decreasing doses every 12 h (40, 40; 27, 27; 15, 15; 8, 8 mg), beginning on the morning of day 10. Intravaginal devices were removed from donors at the time of the fifth injection of FSH. Twelve h after the last FSH injection, 2 ml of buserelin acetate (Receptal®, Intervet International GmbH, Germany) was injected i.m. into each donor and transcervical artificial insemination was performed using 1 straw per donor, by semen being placed into the uterine body. After the semen straws were thawed at 37 ºC in a water bath for 60 sec, a semen sample was examined using an optical microscope. Embryo recovery and transfer was performed by a surgical method 9 d after device removal. Embryos were identified by stereomicroscopical searching of the recovered medium. Embryos were washed, graded (Stringfellow and Seidel, 1998) and kept in holding medium (D-PBS + 20% cow serum) at 34 ºC until they were transferred to the recipients hinds. The period transcurring between the flushing of the donor and the transfer of every embryo was less than 3 h in all the embryos. Pregnancy of transferred hinds was assessed by ultrasonography 30 days after embryo transfer. The 5 straws analyzed after thawing showed a good quality of semen with a motility >30%. Donors showed varying degrees of superovulatory response with a range of 4 to 16 CL (9.0 ± 5.0 CL/donor). The total number of ova/embryos recovered from the five donor hinds was 40 out of 45 CL (88.9% recovery rate). These consisted of 15 transferable embryos (37.5%, 3.0 embryos/donor), 7 degenerated embryos (17.5%) and 18 unfertilized oocytes (45.5%). Of the transferable embryos, 3 were blastocysts (all of excellent quality), 2 early blastocysts (1 of excellent quality and 1 of very good quality), 1 expanded blastocyst of excellent quality and 9 morulae (2 of excellent quality, 2 of very good quality, 4 of regular quality and 1 of bad quality). The pregnancy rate in the recipients was 86.7% (13/15, 2.6 pregnancies per donor). The 2 non-pregnant recipients resulted from 2 morulae transferred (1 of excellent quality and 1 of very good quality). This study shows comparable results obtained by other authors (Asher GW et al 2000 Anim. Reprod. Sci. 59, 61-70

and Fennessy PF et al 1994 Theriogenology 41, 133-138), considering that this was the first MOET program carried out in Argentina using imported semen for the artificial insemination of donors. This opens new prospects for red deer genetic improvement in this country applying the mentioned reproduction biotechnology.

Keywords: embryo, semen, Red deer, *Cervus elaphus*, embryo transfer, genetic improvement, production

Effect of selecting thawed epididymal spermatozoa with different density gradients on sperm quality in males of Iberian Red deer clasiffied as "good" or "bad" freezers

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The aim of this work was to study the effect of selecting thawed epididymal sperm samples from Iberian red deer classified as "good" or "bad" freezers by means of different discontinuous density gradients (Percoll®, Bovipure® and Puresperm®) on sperm quality after selection procedure. The sperm samples were collected from epididymides of eight Iberian red deer hunted between 1995 and 1998, within 3 to 6 hrs of death. Epididymal spermatozoa were extended with Triladyl® (20% egg yolk) and cooled at 5 °C for 90 min, holding them at this temperature for 2 h more. Then, the extended sperm samples were frozen in nitrogen vapors for 10 min. After thawing, the subjective sperm motility (SM) was evaluated and the males with SM ≥ 70% were classified as "good" freezers and those with SM ≤ 69% as "bad" freezers. Sperm samples from males belong to the same freezing group were pooled. An aliquot from each pool was used in the following discontinuous density gradients: Percoll®, Bovipure® and Puresperm®. Sperm samples (0.8 mL) from "good" or "bad" freezers were layered over each discontinuous density gradients (2 layers of 1 mL each one) and centrifuged at 700 g for 15 min. After centrifugation, the supernatant above the sperm fraction was carefully removed. Bottom fraction (0.6 mL) was washed in 5 mL of Bovipure Wash® by centrifugation (300 g for 10 min) leaving 0.2 mL of the final pellet after removing the supernatant. Then, the percentage of recovered spermatozoa (RS) calculated as $[(\text{Volume x Concentration})_{\text{after centrifugation}}/(\text{Volume x Concentration})_{\text{before centrifugation}}]$, sperm motility (subjective), viability with nigrosin-eosin, VAP and LIN assessed by computer assisted semen analysis (SCA®), and mitochondrial membrane pontential (MT+/PI-: viable spermatozoa with active mitochondria) and DNA fragmentation index (DFI) assessed by means flow cytometry analysis, were evaluated for both freezing groups. The effects of density gradient and freezing group on sperm quality after selection procedure were studied by means of GLM-Anova. Differences were considered significant when $p \leq 0.05$. The percentage of recovered spermatozoa was similar between selection treatments (16.57%, 15.16% and 18.37% for Bovipure®, Percoll® and Puresperm®, respectively). The value of SM, viability and mitochondrial membrane potential were higher for the thawed epididymal sperm samples selecting with Percoll® and Puresperm® (72.50% and 66.04%; 81.50% and 87.46%; 64.27% and 71.78%, respectively) in relation to Bovipure® (55.83%, 78.92% and 55.91%). However, there were no differences in VAP, LIN and DFI between selection treatments. As expected, the values for most parameters assessed after selection procedures were higher for the group of males classified as "good" freezers in relation to "bad" freezers (RS: 23.32% *vs*. 10.08%; SM: 70.14% *vs.* 59.44%; Viability: 88.36% *vs.* 76.89% and MT+/PI-: 74.43% *vs.* 53.55%).There were no significant differences between both groups for VAP, LIN and DFI. In conclusion, our results can be useful for developing other assisted reproductive technologies, as *in vitro* fertilization or sexing sperm, in the cervid species. Supported by CDTI (2008/0478) Spain

Keywords: Red deer, spermatozoa, post-mortem, artificial insemination, *Cervus elaphus*, frozen-thawed sperm cells, reproduction, production, conservation

ANTLER BIOLOGY

Management of deer involving nutrition affects antler bone porosity as well as mechanics, architecture and mineral composition

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A series of studies by our group have shown that management and nutrition affects antler mineral composition, mechanical properties and structure. In fact, antler composition and other traits can be used to diagnose mineral deficiencies and management problems. It is well known that porosity bears an inverse relationship with intrinsic mechanical properties of bones. In this study, we examined porosity, mineral composition, cortical thickness and mechanical properties (Young's modulus of elasticity *E*, bending strength and work to fracture) in antlers of two deer populations: a captive population in a experimental deer farm kept with a high quality diet, and a free-ranging population feeding on wild plants of poorer nutritive quality in a game estate. As shown for minerals and mechanical properties in previous studies by our group, porosity increased from base to top of antlers reflecting physiological effort made to grow the antlers. Porosity also increased faster in deer with poorer quality diet. Despite porosity was inversely related to mechanical properties and positively with K, Zn and other minerals indicating physiological effort, it was these minerals and not porosity which better explained variability in mechanical properties. This suggest that ash, K, Zn and other minerals indicate reduction in mechanical performance before porosity starts to appear. In conclusion, porosity is related to physiological effort. Furthermore, applications may arise for human medicine if such effect occurred with internal bones: mineral changes in the bone may be an early indicator of decrease in mechanical properties and future osteoporosis.

Keywords: antler, nutrition, mineral, bone, porosity, quality, mechanical property

Factors promoting axon growth in the deer antler

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During its annual regeneration, antlers are innervated by trigeminal sensory axons growing at the highest rate recorded for any adult mammal. Analysis of this process can shed light on the mechanisms and molecules involved in the rapid axonal growth of adult neurons. Our research is centered on the identification and analysis of these factors and the evaluation of their potential role for nerve repair. Such knowledge could be of clinical importance, considering the restricted regeneration capability of adult nerves and its functional consequences for different pathologies. In the present contribution, we summarize the results that we so far have obtained by combining genomic and proteomic analyses with *in vitro* and histological studies. Histological studies allowed us to characterize and locate antler's innervation, confirming the presence of different types of sensory fibers (a, d and c-fibers) in the vascular layers of the deep velvet. Based on this finding, we have carried out *in vitro* assays using sensory neurons from adult and embryo rodents. These studies suggest that soluble proteins secreted by the velvet strongly promote neurite outgrowth. Using specific blocking antibodies, we demonstrated that NGF is partially responsible for these effects although other as yet unidentified proteins seemed also to be involved. We are now using proteomic techniques to identify these proteins. On the contrary, neither endocrine serum factors nor antler substrates seem to promote neurite outgrowth, although substrates from deep velvet layers cause neurite outgrowth orientation. We also followed a complementary approach, using a combination of several gene-expression techniques to identify and characterize the expression of axonal growth promoters not previously described in antler velvet. Microarray analyses of deer samples were used to build up a list of 90 extracellular or membrane molecules involved in axon growth that are potentially expressed in the antler. 16 of these genes were analysed using PCR and sequencing techniques to confirm their expression in the velvet and to compare it with the expression in other antler tissues as well as in pedicle and frontal skin. As a result, the expression of 8 growth promoters was confirmed in the antler velvet, 5 of them not previously described in the antler. However, most of these promoters, as well as others previously described in the literature, are also expressed in normal skin, questioning their relevance in determining the antler axon growth rate. In conclusion, our results point to the existence of an environment that promotes oriented axon growth in the deep velvet, in agreement with the distribution of the antler innervation. This environment is characterized by the expression of a variety of axonal growth promoters, although most of them are shared with normal deer skin.

Keywords: antler, antler regeneration, nerve growth rate, promoters

Establishment of an in vitro model using antler stem cells for study of the role of type X collagen in endochondral ossification through RNA interference

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Osteogenesis is not only an integral part of embryo development, but highly relevant to clinics. The main form of mammalian osteogenesis is called endochondral ossification. Endochondral ossification starts when condensed mesenchymal cells begin to differentiate into chondroblasts, then chondrocytes and finally hypertrophied chondrocytes before being replaced by osteoblasts to form bone tissue. Interestingly, type X collagen (Col X) is coincidently expressed in the terminally-differentiated chondrocytes, hence it has been considered that Col X may play an important role in the step of bone replacement. However, this assumption has not been experimentally confirmed. The current situation is at least partially due to the limitation of the currently available model, i.e. embryo long bone formation, as transition between each differentiation stage is very brief in the model and the hypertrophied chondrocytes are only made up of a narrow line (1-2 cells wide). Therefore, an alternative model that permits more detailed molecular analysis is required if the role of Col X in bone replacement is to be understood. Deer antlers are bony organs that are cast and fully regenerate each year. Antler regeneration is accomplished through endochondral ossification and comprises much more detailed cellular events than prenatal long bone formation. Therefore, antlers may offer an ideal model for the in-depth investigation of molecular mechanisms in endochondral ossification including the role of Col X in the step of bone replacement.

In the present study, we sought to investigate the role of Col X in endochondral ossification in vitro using antler as a model. We first constructed and characterized lentiviral vectors carrying small interfering RNAs (siRNA) targeting the Col X gene of sika deer. Ten high score siRNA sequences (S1-S10) were selected after removing the off target ones based on general design rules. The oligo DNA comprising both sense and antisense strands of each siRNA was ligated into pLVTHM-GFP (lentiviral carrier plasmid) using T4 DNA ligase. The newly recombined plasmids were subsequently transformed into DH5α (E coli). Positive clones were selected using PCR screening and then sequenced. The pLVTHM-GFP-siRNA (carrier plasmid + siRNA), pCMV-dr8.91 (packaging plasmid) and pMD2.G (enveloping plasmid) were co-transfected into a 293t cell line. The lentiviral particles produced from the co-transfected 293t cells were harvested and concentrated. Pedicle periosteum (PP), within which stem cells for antler regeneration reside, was sampled from a 2-year-old male sika deer and enzymatically digested to release cells for in vitro culture. PP cells were subsequently infected by the lentiviral particles either from 2 randomly selected high score siRNA constructs (S7, S9), or a negative control construct (absence of siRNA), and then seeded at a high-density (108/ml) to establish micromass culture.

The results showed that 3 cell nodules, 2 from S7 and S9 (siRNA containing constructs) and 1 from negative control (absence of siRNA), were formed around 70 hours after micromass seeding; these being 6, 5 and 4 mm in diameter respectively. These PP cell nodules strongly expressed GFP, implying that siRNA targeting Col X gene must also be expressed in these nodules.

Overall, in the study we partially established an in vitro model using antler stem cells for the

investigation of the role of Col X in endochondral ossification through RNA interference pathway. Further study is needed to determine to what extent Col X gene expression can be down-regulated after RNA interference and endochondral ossification affected using qPCR and Western blot analysis.

Keywords: Sika deer, *Cervus nippon*, Antler, Col X, Lentiviral vectors, RNAi, Micromass culture, growth

Assessing density of Red deer antlers with parametric volume modelling in CAD-3D

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The global density of antlers is a variable depending on growth, development, spongy bone percentage, mineral composition and the stag's condition and age. Therefore this study may be useful as an index of quality for monitoring stags and their environmental condition. However, in order to estimate global density it is necessary to know the exact volume of the antlers. In this study, we assessed two methods of volume measurement. Ten cast antlers of red deer were collected from the Sierra Morena Mountains, (Jaén, Spain) in the spring of different climatic years, so the casts belonged to stags of different ages, quality and condition. The cast antlers were dried and weighed. Volumes for reference were determined by water displacement. Previously, the bases and porous surface of antlers were varnished to exclude water from the internal spaces. Then we submerged the casts in an overflowing bath with a control tube and collected the water displaced by the antlers. As an alternative method, we determined the homologous parameters of each cast for volume modelling using CAD-3D (Computer Aided Design-3Dimensions). This method is based on the branched-tree shape of deer antlers. A set of homologous points are located at the junction points of each branch along the central beam of the antler. These points are the centers of a set of rings. A swept volume is modelled through all the rings to obtain the central stock. In a second stage tips are modelled in the same way, and only two more rings are necessary for each one. A third process smooths the surfaces and bevels the sharp edges. This method requires a measurement of XYZ coordinates and ring diameters at homologous points by means of a simple tape measure and a calliper, a task which can be performed outside the laboratory. The parameters are then saved in an Excel template file previously linked to a parametric 3d model generated in a commercial CAD 3D system (SolidWorks). Weights ranged between 219.93-1857.9 g, volumes between 207.38-1400.67 cc and 230.0-1412.0 with 3CAD or the hydrostatic method respectively. Finally, the global density (g dry matter –DM-/cc) of the antlers ranged between 0.94-1.33 and 0.91-1.34, respectively. There were differences among the global densities of the stags but not between the densities calculated from volume measurements determined by water displacement or by parametric modelling using CAD-3D. The computing process with the CAD 3D system calculates antler volume in a fast and accurate way. Here we propose this method as an alternative methodology because it avoids the problems of other methods which may damage the casts and is simpler and more robust than hydrostatic weighing, especially with antlers of lower densities and their subsequent problems of floatability.

Keywords: Red deer, *Cervus elaphus*, density, antler, volume, CAD-3D, material

Assessment of hard antler at one year old to predict hard antler variables at two years old in Red deer (*Cervus elaphus*) stags

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The genetic improvement in red deer breeding programs designed to increase trophy scores and hard antler production are based mainly in the selection of animals with superior antlers, with routine trophy measurements being the most important features to consider when males and females (through their sons) must be evaluated and chosen for a natural or artificial reproduction program. Usually the stag antler is evaluated in 2 or 3 year olds (y/o) because is at this time when the animal shows the real potential to produce hard antler. However it would be beneficial if this assessment could be improved by if selecting animals earlier. The objective of this study was to assess the hard antler of 1 y/o stags and its correlation with their hard antler produced at 2 years of age, (n = 465 red deer stags). Antlers were removed from stags after losing the velvet and 3 months later were evaluated by the same person. The antler measurements taken from the 1 y/o stags were the circumference of the coronet (CC), antler circumference taken halfway up the total length (HC), length of the antler (L), number of tine ends in the crown (TEC), number of tine ends situated below the crown (TEBC), length of the total tine ends in the crown (L-TEC), length of the total tine ends below the crown (L-TEBC), number of total tine ends (TTE), and weight (W). Meanwhile the antler measurements taken from 2 y/o stags were circumference of the coronet (CC), circumference of the antler as the average of the minimal circumferences taken below and above the tray tine (CH), length of the antler (L), length of the brow tine (LBT), length of the tray tine (LTT), inside span (IS), number of total tine ends (TTE), number of tine ends in the crown (TEC), and length of the total tine ends in the crown (L-TEC), weight (W). The International Council for Game and Wildlife Conservation score (CIC) was used for all specimens. The length and circumference measurements are expressed as average of both antlers. Pearson coefficients of correlation (*r*) were calculated to determine relationships between all the variables obtained for 1 and 2 y/o No negative value was obtained from 110 coefficients calculated in the statistical analysis, showing a positive correlation between 1 and 2 y/o antlers, with 26 values above 0.5. The variables for the 2 y/o that showed higher correlation values were CC, CH, W and CIC; while the 1 y/o variables with higher correlation was CC and W. The strongest correlations occurred with CC/CC (r=0.82), followed by W/W (r=0,77) and W of 1 y/o with CIC for 2 y/o (r=0,73). This study demonstrated that the antler weight of 1 y/o stags would be an interesting early variable used to select and evaluate animals for genetic improvement to increase hard antler production and trophy scores.

Keywords: Red deer, *Cervus elaphus*, antler, growth, measurements, predictability, genetic improvement

Synchrony /asynchrony in the casting of antlers of Reeves' muntjac (*Muntiacus reevesi*)

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For many species of deer it is reported that both antlers fall off on the same day. For 22 Reeves' muntjac in captivity in England (at latitude 52° 20') the exact casting dates of left and right antlers was recorded for a total of 109 antler cycles. The maturity of the bucks ranged from first heads to 15th heads.
The left antler was cast first on 39.5% of occasions, the right on 44.0% and both were cast on the same day in 16.5%. The intervals between casting of left and right antlers ranged from one to 13 days with the mean between five and six days, but 53% of castings had intervals of one, two or three days. Only 9% had intervals of nine or more days.
All casting occurred in May and June with the exception of one cast on July 1st and a pair on July 17. Median casting dates of May 27 (left) and May 26 (right) were reported for a small sub-set of this sample (Chapman & Chapman 1982, Acta Theriol **27**:107) and the current data set is in close agreement.

Keywords: antler, shedding, synchrony, behavior, Reeves' muntjac, *Muntiacus reevesi*

Examination of antlerogenic potential of different areas in an antlerogenic region in Sika deer (*Cervus Nippon*)

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Since the discovery by Hartwig and Schrudde (1974) of antlerogenic periosteum (AP), a membrane which overlies the deer frontal crest, antler biology research has entered a new era. Removal of AP prior to initiation of a pedicle (the permanent bony protuberance from which an antler forms and drops off) abrogates subsequent pedicle and antler formation, and transplantation of AP elsewhere on the deer body induces ectopic antler formation. Later, Goss (1985, 1987 and 1990) greatly extended this finding by defining the AP region and identifying the AP topographical potential in antler induction. In the latter study, Goss divided AP into halves: anterior, posterior, lateral and medial, before transplanting each half on a deer foreleg. The results showed that lateral halves induced the most ectopic antler formation on forelegs, from which Goss concluded that AP lateral half possesses the greatest antlerogenic potential compared to the other AP halves. However, it has been argued that this phenomenon may be caused by the traumatisation of major blood vessels and nerves when carrying out deletion of the AP lateral half where these blood vessels and nerves are located. In addition, in his study Goss only continued observation for 2 years, and most important of all, deer forelegs are not an ideal place for testing antlerogenic potential. In the present investigation, we selected deer forehead as the AP transplantation site, which has previously been demonstrated to be the best ectopic place for antler growth, and used 12 male sika deer calves (3 deer/AP half) that were observed for antler growth for 4 years. The results showed that in the AP anterior half transplantation group, 2 out of 3 formed antlers on both original and grafted sites. Antlers on the original sites did not grow brow tines and on the grafted sites were all spikes. Antler formation only took place on the grafted site but not in the original site in the last case, although the ectopic antler was branched and grew to a similar size as the one on the original intact antler. Therefore, AP anterior half is likely to control brow tine formation. In the AP posterior half group, 2 out of 3 formed antlers on both the original and the grafted sites. Antlers on the AP-deleted original sites formed brow tines and were smaller than the control intact antlers, but caught up in the 3rd year. Antlers formed on the grafted sites were all spikes. Antler formation only took place on the original site (AP posterior-half-less), but not on the ectopic site. Therefore, the absence of AP posterior half does not seem to have significant effects on subsequent antler formation. In the AP medial half group, 3 out of 3 formed antlers on both the original and the grafted sites. Antlers on the original sites were all spikes and smaller than the control intact side antlers, and were also spikes on the grafted sites. Therefore, AP medial half may control brow tine formation and antler size. In the AP lateral half group, out of 3 deer, 1 formed antlers on both the original and the grafted sites. Antlers on both the original and the grafted sites were spikes. Two only formed spike antlers on the original sites but these were smaller than the control intact antlers. Therefore, AP lateral half does not seem to play important role in antler formation. In conclusion, our study has convincingly demonstrated that antlerogenic potential is not evenly distributed in AP, and that the highest potential is held in the medial half. This finding provide essential guideline for antler stem

cell research and AP sampling.

Keywords: deer, antler, antlerogenic periosteum, transplantation, growth

Inhibitory effects of IGFBP-4 on antler mesenchymal cell proliferation in vitro

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Deer antlers are male secondary sexual characters and grow at a phenomenal rate (up to 2 cm/day). It is well established that the actual antler growth is under the control of growth factors, particularly insulin-like growth factor 1 (IGF1). During antler growth season circulating IGF1 level is positively and significantly correlated with antler growth rate, and growing antler tissue expresses abundant type 1 IGF receptor. Therefore, the role IGF1 plays in antler growth is mediated through a ligand-receptor pathway. In addition, growth regulation of IGF1 has another level of control, i.e. IGF binding proteins (IGFBPs), as under physiological condition the majority of circulating IGF1 (up to 99%) is bound to IGFBPs, and only free-IGF1 can exert its biological function. IGFBPs belong to a superfamily and consist of at least 6 members that have high-affinity to IGF1 (IGFBP1-6), among which IGFBP3 and IGFBP4 are mainly found in serum and hence most relevant to the regulation of IGF1 control of antler growth. Thus far, the involvement of IGFBP3 in antler growth regulation has been well studied, but the role of IGFBP4 in antler formation has not been reported. In general, IGFBP4 has a wide range of growth-inhibitory and apoptosis-inducing functions via IGF-dependent and IGF-independent mechanisms. It is assumed that a high apoptotic rate of antler cells in the growth zone is the mechanism that protects antlers from going cancerous. IGFBP4 may also be implicated in this apoptotic induction. In the present study, both growth inhibitory effects and apoptotic induction effects of IGFBP4 on antler cells in vitro were investigated using our human IGFBP4 produced in our laboratory.

To produce human IGFBP4, we cloned the gene into pGEX-6p-1 vector to form pGEX-6p-1-BP4, which was then transformed into E.coli. BL21 (E3) cells to express IGFBP4. After purification, the recombinant IGFBP4 was used in our antler cell study. Antler mesenchymal cells used in the experiment were collected from a sika deer antler tip after 60-days of growth and had reached the 3rd passage before use (viability over 90%). The experiment was allocated into 4 treatments and each treatment had triplicates using a 24-well-plate. Treatments 1, 2 and 3 contained 10, 20, and 40 μg/ml IGFBP4, and treatment 4 was the control D-Hank's solution containing no IGFBP4. Antler cells were seeded at 1 x 10^5/ml into each well in 1 ml DMEM medium, and IGFBP4 or D-Hank's solution was added into each well immediately following cell seeding.

The results showed that in the control group antler cells started to attach 24 hr after seeding, entered an exponential growth period 48 hr after seeding, and reached a plateau (2.4 x 10^6/ml) 96 hr after seeding. In contrast, in the IGFBP4 treated groups, both attachment and proliferation of antler cells were seriously affected right from the 1st day of seeding. The inhibitory rates of IGFBP4 on antler cell growth occurred in a dose-dependent-manner: 10%, 17%, and 26% in treatment 1, 2 and 3 respectively. In addition, apoptotic cells in the treatment groups were frequently encountered. Our preliminary study indicates that IGFBP4 may play a role in inhibiting antler cell proliferation through the IGF-dependent pathway; whereas by inducing apoptosis through IGF-independent pathway. Further studies are needed to confirm these deductions.

Key word: antler growth, insulin-like growth factor binding protein-4, antler stem cell, proliferation

Silencing of Cbfa1 gene by RNA interference inhibits osteogenesis in deer antler endochondral ossification in vitro

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Endochondral ossification is the main form of osteogenesis which occurs during embryo development and bone repair; hence research into endochondral ossification is relevant to both fundamental biology and clinics. The feature that sets endochondral ossification apart from other ossification types is that bone formation is accomplished through replacement of pre-formed cartilage. It is known that the process of endochondral ossification is regulated by numerous molecules, amongst which CBFA1 stands out, as its null-mutation results in total failure of replacement of pre-cartilage by bone during embryogenesis. Further research has demonstrated that CBFA1 is the master regulator of osteoblast differentiation. However, thus far the detailed molecular mechanism underlying CBFA1 regulation is not fully understood. This situation is undeniably caused at least partially by the limitation of currently available models. Deer antlers are bony organs and developed through endochondral ossification. Compared to embryo long bone formation, the transition between each cellular differentiation stage in antler endochondral ossification is much more comprehensive including mesenchyme, prechondroblasts, chondroblasts and chondrocytes before being replaced by bone cells. Therefore, antlers may offer an ideal model for dissecting the detailed molecular mechanisms underlying CBFA1 regulation of bone formation. In the present study, we sought to establish an in vitro model using antler stem cells to investigate the role of CBFA1 in endochondral ossification through RNAi pathway.

Sika deer pedicle periosteum (PP), within which stem cells for antler regeneration reside, was enzymatically digested to release cells. PP cells were then cultured in vitro. The full length deer Cbfa1 gene was cloned using RT-PCR and RACE. Six siRNA sequences targeting the cbfa1 gene were selected using general design rules. These six siRNAs (S1-S6) and a negative control (scrambled sequence) were chemically synthesized. Each siRNA was ligated into a lentiviral carrier plasmid (pLVTHM-GFP) using T4 DNA ligase. The siRNA-containing-carrier-plasmid, enveloping plasmid (PMD2.G) and packaging plasmid (pCMVdr8.91) were then co-transfected into a 293t cell line. The lentiviral particles produced from the transfected 293t cells were harvested and concentrated. The viral titer was tested and the virus was subsequently used to infect PP cells. The infected PP cells were seeded at a high density (10^8/ml) for a micromass culture. An additional control, within which PP cells for micromass culture were not infected by lentiviral particles, was also used. Whether siRNA was successfully expressed in each infected cell nodule was determined by whether GFP could be observed from the nodule under a fluorescent microscope. Inhibition rates of expression of both cbfa1 and type I collagen mRNA were examined using fluorescent quantitative PCR.

The results showed that the titer of recombinant lentivirus was reasonably high (10^6-10^8 TU/ml). PP cell nodules began to form 2-3 days after micromass culture seeding. Fluorescence could be observed from the infected nodules on day 7-9, whereas no fluorescence was detected from the uninfected PP cell nodules. Real time PCR results showed that expressions of both the cbfa1 gene

and the downstream type I collagen gene were significantly down-regulated in micromass-cultured PP cells from the S1-S6 groups compared to the negative control (scrambled RNA group). Among these siRNAs, S6 was the one that had most dramatic effects on down-regulating cbfa1 and type I collagen gene expression: 88% and 86.8%, respectively.
In conclusion, we have successfully established an in vitro micromass culture model using antler stem cells. This model has been successfully employed for the investigation of the role of cbfa1 in endochondral ossification through RNAi. We envisage that this model can also be effectively used for studying other factors regulating endochondral ossification other than CBFA1.

Keywords: CBFA1, ossification, RNAi, stem cell, antler

Experimental evidence that demonstrates self-renewal of antlerogenic periosteal cells in vivo in Sika deer

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Deer antlers are the only mammalian organ that once lost, can fully regenerate. It has been convincingly demonstrated that antler regeneration is a stem cell-based process, which is in contrast to the newt limb regeneration that is achieved through reversion of previously differentiated cells into embryonic-like cells. Stem cells for antler regeneration are resident in the periosteum (PP) of pedicles, the permanent bony protuberances from which antlers drop off and regenerate. PP is originally differentiated from the bone membrane that overlies a frontal crest in pre-pubertal deer. Removal of the membrane prior to pedicle initiation abrogates subsequent pedicle and antler formation; whereas transplantation of the membrane elsewhere on the deer body induces ectopic antler formation. Hence this bone membrane is called antlerogenic periosteum (AP). The cells of AP have been shown to express key embryonic stem cells markers, such as Oct4, Nanog and Sox2; and can be readily induced in vitro to differentiate into chondrocytes, osteocytes, adipocyts and myotubes. Therefore, AP cells are called antler stem cells. However, thus far no experimental evidence has been provided to attest that AP cells are self-renewing, an essential attribute for any cell type that can be claimed as stem cells. One way to find out this is to transplant a limited amount of AP tissue to an ectopic place where potential to form an antler is absent, then to track down how many sets of antlers can be produced from the limited antler stem cells. By so doing, it will remove any ambiguity of participation to antler formation from AP marginal periosteal cells at an original site, should AP cells per se become exhausted for antler formation. In the present study, we used 3 male red deer calves and autologously transplanted AP (2.5 cm in diameter) from one side of presumptive antler growth regions to the forehead region (non-antlerogenic region), and the other side AP was kept intact and used as control. Subsequent pedicle and antler formation were observed, photographed and followed for 6 years. The results showed that except for the pedicle and first-year-antlers that were spikes, all subsequent regenerated antlers were two-branched and weighed over 800 g. The rough stereology cell counting showed that around 5 million cells reside in a 2.5 cm in diameter AP. These 5 million AP cells so far have produced more than 4 kg antler tissue mass and no sign that antler formation from the grafted sites would cease in any case. Consequently, AP cells have to have the ability of self-renewal in order to form such quantity of tissue mass from only 5 million cells. In conclusion, our in vivo transplantation experiment has demonstrated that AP cells, the stem cells for antler generation, are capable of self-renewal, and together with their other attributes, i.e. expression of key stem cell markers and multi-potency, AP cells are true stem cells.

Keywords: deer, antler, antlerogenic periosteum, transplantation, stem cells

Unusual antler development in the first and second year of life in Red and Fallow deer

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There are some differences in the early body growth and development including sexual maturation between species of Telemetacarpalia (Tm) and Plesiometacarpalia (Pm) deer groups. In males, sexual maturation is believed to be completed when the first antler is finished/cleaned. In the case of some Tm species it happens around 7-8 months of age, with the first set commonly called buttons due to their very small size. It is well documented in roe deer (*Capreolus capreolus*) and reindeer/caribon (*Rangifer tarandus*), but occurs in White-tailed deer (*Odocoileus virginianus*) and moose (*Alces alces*) too. However in Pm species, according to our knowledge, the first antler is usually cleaned at the yearling age, around 15-16 months of life, which usually means September-October in the Northern Hemisphere . The only exception was published by Vogt (1936), when a red deer (*Cervus elaphus*) stag calf produced a pair of antlers, 40 cm in length in March and shed them in early May.The second antlers, as 8-pointers, were cleaned in mid August, at 15 months of age. In recent years we have experienced several similarly precocious/advanced antler development in red deer, as well as in fallow deer (*Dama dama*).

Red deer.

*First cas*e. In 2003 a stag calf was hand-reared in a hunting lodge yard in Somogy county of West-Hungary. It started to grow antler in the autumn. The 28-30 cm long spikes were cleaned in March 2004. After sheding the spikes in April, the second set, a 6-pointer yearling antler was formed.

Second case. A stag calf was shot in the same forest complex in the late January 2008. The length of spikes in velvet was about 20 cm, the clean carcass weigth was 37 kg. Two other stag calves weighed 44 and 48 kg on the same day.

Third case. On Deer Farm Bőszénfa (DFB, Somogy county) a hand-reared stag calf produced about 30 cm long antler during winter 2009/10. The cleaned spikes were shed in late April. Its body size (126 kg liveweight) was much above the average (89.32 ± 10.14 kg, n = 80) of the herdmates.

Fallow deer. Three buck fawns were hand-reared during the summer of 2009 on DFB. Two of them started to grow antlers in the autumn. Spikes about 7-8 cm in length were cleaned in April. In early June one of them shed the spikes and developed a pair of antlers with 3-3 points. The other fawn did not shed the spikes, but a double-head was formed with burrs below the bottom of those. Their ages were 16 months at the time of cleaning the velvet, late September.

With great probability the phenomenon is more frequent but has not been observed or reported. Compared to button-sized antlers in telemetacarpal deer, if antler growth does occur during the first year of plesiometacarpal deer, they can even grow to sizes normally found mainly in the second year of life.

Keywords: Red deer, *Cervus elaphus*, Fallow deer, *Dama dama*, antler, growth, calf antler, yearling antler

IGF-1 does not stimulate and testosterone does not inhibit antler cell proliferation in various types of *in vitro* experiments

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For several decades there have been some disputes about hormonal stimulation of antler growth. In this context the contradiction between the role of testosterone and insulin like growth factor-1 (IGF-1) on antler growth at various levels of experimentation was in full swing. Some of the *in vivo* results repeatedly indicated the importance of low concentrations of testosterone for the initiation of antler regrowth. In contrast, many *in vitro* studies suggested stimulatory effects of IGF-1 on growth of antler tissue. In this study we tested the effect of testosterone (1nM, 10nM), IGF-1 (6.5nM, 13nM), combinations of testosterone and IGF-1, estradiol (1nM, 10nM) as well as antiandrogens Cyproterone acetate (100nM), Flutamide (100nM.) and antiestrogen ICI (100nM) in various combinations on antler cell proliferation *in vitro*. Moreover, factors such as antler growth stages (15^{th}, 30^{th} and 60^{th} day after antler casting), animal individuality (5 three and 3 two year-old red deer stags), percentage of fetal bovine serum (FBS) (1% and 10%) and duration of the hormonal treatment (2x24 and 6x24 hours) were tested.
The proliferation potential of antler cells was measured by the DNA synthesis and incorporation of ^{3}H thymidine using the technique of TCA precipitation and liquid scintillation counting. For statistical analyses we used the General Linear Mixed Model.
Compared to some previous *in vitro* studies, which described a strong stimulatory effect of IGF-1 on the proliferation of antler cells, we were not able to record any significant stimulatory effect of IGF-1 in our study. IGF-1 mostly did not differ from the control, or it inhibited proliferation compared to the control (two experiments with significance $P<0.05$ and $P<0.01$). In two experiments, IGF-1 even had negative influence on the stimulating effect of testosterone ($P<0.05$ and $P<0.05$). Opposite to IGF-1, testosterone has never caused significant inhibition of the antler cell proliferation. Testosterone stimulated the proliferation in three experiments ($P<0.05$; $P<0.01$; $P<0.001$) and estradiol in two ($P<0.05$, $P<0.001$). Effects of the other hormonal treatments and their combination compared to the control and among them varied greatly with all factors. The intensity of cell proliferation was significantly influenced by the duration of the hormonal treatments ($P<0.0001$), percentage of FBS ($P<0.0001$) as well as by sampling day ($P<0.0001$). For instance, cells from the 15th day of antler growth reached the most intensive proliferative response.
Our results clearly revealed that none of the used hormonal treatments consistently stimulated or inhibited antler cell proliferation *in vitro*. The way we see it, the effect of hormonal treatment on antler cell proliferation *in vitro* depends on so many factors that it is very difficult to draw any general conclusions from a restricted experimental design.

Keywords: antler, antler cell, growth, in vitro cultivation, IGF - 1, testosterone, estradiol

Investigation of the STRO-1$^+$ cells derived from regenerating antler and pedicle cells of Red and Fallow deer stags

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Many new findings in antler biology have been published recently. Most of them deal with antlers as a biomedical model for bone regeneration including the proof of their stem cell based origin, establishment of a new stem cell line from antlerogenic cells and successful xenoimplantation of these cells. The stem cells were found in pedicle as well as in the primary and regenerating antler. Cells from regenerating antler and pedicle of red and fallow deer males (RD, FD) positive to surface antigen STRO-1 were sorted out of mixed and previously STRO-1 negative cell cultures. We used the method of magnetic cell separation (MACS®). STRO-1 positive cells (STRO-1$^+$) were visualized by immunofluorescence. Reverse transcription-polymerase chain reaction showed that under standard culture conditions [DMEM (Gibco) + 10% fetal calf serum (FCS)] STRO-1$^+$ cells do not express cbfa1, chondroadherin and osteocalcin, compared to the STRO-1 negative cells (Rolf et al. 2008, PLoS ONE 3:e2064).
In the present study we analyzed the factors potentially affecting the amount of STRO-1 positive cells obtained from MACS® separation, as these cells are used afterwards for *in vitro* experiments. The factors tested were the passage (primary culture and from 1st to 7th passage), part of the antler (antler growth zone, antler cartilage zone, antler bone and antler periosteum) or pedicle periosteum for FD and type of culture (mixed, STRO-1 negative) as well as culture medium [Dulbecco's Minimal Eagle Medium (DMEM), stem cell media and differentiation medium]. Since cell cultivation density cannot be specified in primary tissue cultures, we were not able to analyze the density as a factor.
For statistical comparison we used Mixed procedure (PROC MIXED, SAS, version 9.1) with least-squares-means (LSMEANs) and the Tukey-Kramer adjustment for multiple comparisons.
The statistical analysis showed significant differences between the various media $P<0.01$. For further analysis we used only culture medium DMEM with 10% FCS which was represented in the most cases. In both RD and FD cells the passage ($P<0.001$, $P<0.0001$) and the type of culture ($P<0.05$, $P<0.0001$) were significant factors. The highest amount of obtained STRO-1 positive cells was from the second passage both for RD (an average of 5.5%, 27 analyses) and FD (an average of 24.6%, 26 analyses). Reasons for that could be further differentiation or dedifferentiation of the cultivated antler and pedicle cells influenced by 10% FCS as part of the culture medium or by the type of cultivation as monolayer. We have not found any significant influence of the cell sampling part of antler or pedicle.
Our results suggest that the cultivation procedure affects the amount of obtained STRO-1$^+$ cells

markedly and hence this cultivation effect might override the anticipated effect of sampling location.

Keywords: antlers, antler cell, growth, pedicle cell, in vitro cultivation, growth, IGF - 1, testosterone, estradiored deer, *Dama dama*, Fallow deer, *Cervus elaphus*, Red deer, STRO-1+

NUTRITION

Recent advances in the nutritional ecology of Huemul

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Huemul (*Hippocamelus bisulcus*) numbers had already declined drastically by the 20th century. The first field studies date back to the 1970s (Chile) and 1980s (Argentina). Only 350-600 animals remain along 1800 km of Argentine Andes, without known cases of recolonization or numerical responses. In Chile, at least 2 populations have shown some recovery; the remaining have either decreased or are assumed to be stable. A list of factors hypothetically important for Huemul recovery was proposed in 1992, and some of these are still claimed dogmatically to cause declines and failure of recovery, though without supportive evidence. The main hypotheses (cattle, exotic trees, irrational forestry, exotic animals, illegal hunting, diseases, dogs, reduced numbers) can be rejected as key explanations for the *general* lack of recovery. Each factor may play an additive role alone or in combination in certain populations, but are rather unlikely primary causes. Our objective is to evaluate alternative factors which may explain the generalized lack of recovery. Several indications warrant us to postulate that nutritional ecology instead plays a central role in the general absence of Huemul recovery. A wide range of antler quality is encountered among Huemul, with well-developed specimens known primarily from historic times. If antler expression in Huemul is homologous to other cervids, it follows that most extant populations are under suboptimal conditions. Another important clue stems from one of the larger populations in Argentina, where a conservative prevalence of osteopathy of 52% was found among adults: 63% showing mandibular, 100% maxillary, and 78% appendicular lesions. We also have observed similar lesions in populations in southern Chile. Lesions were age-independent; also discarded as primary etiologic factors were gender, fulminating infections, congenital anomalies, disorders of the metabolic, endocrine, genetic, or neurologic systems, parasitism or marasmus, and fluorosis. Instead, we hypothesized that generalized secondary chronic alveolar osteomyelitis and osteoarthritis were related to nutritional ecology of Huemul. Meager antler development with high frequency of asymmetry, high prevalence of osteopathy, and low recruitment rates could all be related to common and limiting nutritional factors known to cause the described phenomena. Preliminary investigations point to several lines of evidence, including environmental features that support the hypothesis that deficiency in iodine and selenium (Se) might be involved. Among other things, such deficiencies impair bone growth, cause periodontitis in ruminants, and affect reproduction, neonatal development, and the immune and nervous systems. Se deficiency directly affects metabolism of iodine, which is regionally low. Only decades ago, overt iodine deficiency in humans living in these areas was very common. For free-ranging livestock, overt Se deficiency has been described in Chile: supported by geology, pedology, topography, and climatic patterns. It is well known that valley bottoms, flood plains, and habitats downwind from glacial areas provide higher provision of

iodine and Se. The nexus to the nutritional ecology of Huemul likely is the inaccessibility of most of the traditional winter ranges, elimination of migratory traditions, and concomitant elimination of source populations. Se and iodine provisions diminish with altitude, which at the same time increases physiological needs due to hypoxia, and intensified radiations and exercise. Most extant Huemul populations occur in remote high-altitude refuges, or inaccessible Pacific coastal areas. Migration, an acquired behavior, has been eliminated through past overhunting of this segment of the population; Huemul being very vulnerable to human predation were killed by the thousands to feed people, dogs, chicken and pigs, and their skins were used for shelters. Huemul currently dispersing from refuges are generally being killed when entering former source areas now occupied by settlers. Other ungulates driven into mountain refuge situations have been shown to be deficient in these trace minerals and responded well to mitigation of the deficiency.

Keywords: Huemul, *Hippocamelus bisulcus*, nutritional ecology, migration, winter range, trace minerals, iodine, selenium

Difference of purine derivatives from snow-urine of Sympatric moose (*Alces alces cameloides*) and Roe deer (*Capreolus pygargus bedfordi*) populations in Lesser Kingan Mountain, Northeastern China

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Population dynamics of ungulates are largely influenced by nutritional status. Especially in winter of the north, energy and protein requirements are high, while food quality and quantity are low. Indices related to purine derivatives (PD) in snow-urine is an effective and noninvasive method to discover the nutritional status of wild ungulates. Moose and roe deer are sympatric and suffering from a population decrease in Lesser Khingan Mountains of northeastern China and potential competition exists between the two populations. We collected snow-urine samples at the Erkehe forestry farm (48°39'30"-48°48'21"N, 127°59'05"-128°15'19"E), located in the northwestern slopes of the Lesser Khingan Mountains, northeastern China. Totally, 174 snow urine samples from moose and roe deer were collected, including 48 moose samples and 136 roe deer samples in January and February of 2007 and 2009. Two PD related indices (PD to creatinine ratio and purine derivatives to total PD proportion) were selected to discover nutritional status changes of moose and roe deer population using HPLC. Results showed that allantoin was absent from moose and 1/3 roe deer snow-urine samples, which suggested a constrained nutritional status for both of the moose and roe deer populations in 2007 and 2009. Indistinct changes of the two indices in January and February 2009 indicated a slight change of nutritional status for both moose and roe deer. Together with increased uric acid (U/C) and xanthine (X/C) to creatinine ratio (U/C, F=5.95, P=0.0164 X/C, F=37.85, P=0.0000 respectively), higher proportion of allantoin, xanthina and hypoxanthine (F=10.29, 26.67, 4.59 and P=0.0013, 0.0000, 0.0284 respectively) and lower proportion of uric acid (F=8.76, P=0.0038) in 2007 than in 2009 for roe deer indicated an increased endogenous PD metabolism rate and a more constrained nutritional status in 2009. Higher hypoxanthine-creatinine ratio (H/C, F=5.54, P=0.0229), xanthine-creatinine ratio (X/C F=4.23, P=0.0454) and uric acid to PD proportion (U/T, F=5.08 and P=0.0076) for moose also suggested an increased endogenous PD metabolism rate and a more constrained nutritional status in 2009. Results reflected an accordant change of moose and roe deer nutritional status with varied snow depth. Deeper snow lead to a worse and more constrained level of energetic metabolic condition and nutritional status.

Keywords: snow-urine, moose, *Alces alces*, Roe deer, *Capreolus capreolus*, purine derivatives, nutrition, body condition

Habitat enrichment effects on White-tailed deer foraging behavior in semi-arid rangelands

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Foraging by large herbivores can alter plant communities and thereby influence entire ecosystems. Humans, through agriculture, food plots, and supplemental feed can enrich habitat, which may influence herbivore foraging behavior. Herbivores may respond to habitat enrichment by reducing consumption of poor quality forage, reducing consumption of high quality forage, or reducing the amount of forage eaten without changing diet composition. We used tame White-tailed deer (*Odocoileus virginianus*) living permanently in 81-ha enclosures on semi-arid rangelands of southern Texas to test the effect of a pelleted supplement on composition and quality of the vegetation portion of deer diets.Diet was quantified using the bite-count technique seasonally over a 3-year period and pelleted feed consumption was measured seasonally using stable isotope ratios during 1.5 years. Pelleted feed composed 47 ± 7% (x±SE) to 80 ± 3% of deer diets, depending on season. The effect of supplement on forage selection varied by season and year. Supplemented deer increased consumption of browse in most seasons and decreased consumption of mast in spring and autumn and sub-shrubs during summer and autumn. The proportion of forbs in deer diets differed only during autumn of 1 year and was greater in supplemented deer. Digestible protein was greater in diets of supplemented deer in only a single season, whereas metabolizable energy was greater in supplemented deer diets in some seasons and lower in other seasons. Providing supplemental feed for White-tailed deer on semi-arid rangeland changes forage selection, but the threat of high-grading palatable forage classes appears low. Supplemented deer continued to eat moderate quality browse and rarely increased percent forbs in their diet. Changes in consumption of mast, specifically cactus and shrub fruit, could change vegetation communities in the long term. If dry matter consumption remains constant and 50% of the diet of supplemented deer is pelleted feed, a 50% reduction in forage consumption could reduce browse pressure on some forage classes.

Keywords: habitat enrichment, foraging behavior, nutrition, vegetation communities, White-tailed deer, *Odocoileus virginianus*

Effects of dietary protein level on Sika deer body weight and velvet production

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Introduction

In China, the main purpose of farming deer is to produce valuable medicinal material, the velvet antlers, and the main deer species for velvet production is sika deer. As China has long history of deer domestication, Chinese deer farmers and researchers have accumulated rich experience to maximize velvet antler yields. Among other factors, nutritional requirements are considered as paramount important for both deer body and velvet antler growth. In this study, the effects of the dietary protein level on these two parameters were investigated.

Materials and Methods

Twenty four 4-year-old sika deer stags were selected for this feeding trial during antler growth period (from 15 April to 7 August). The deer were randomly assigned to 3 groups (n=8/group). Each deer group was fed with one of the formulated diets containing 12%, 16% and 20% crude protein, respectively. These diets were made up of corn silage, corn meal, soybean cake, wheat bran, fish meal etc. Each deer was fed with 1.6kg concentrate containing 11.6MJ metabolic energy per kg dry matter. Animals were weighed before feeding at the commencement and termination the trial. Velvet antler weight of each deer was accurately recorded at velveting. Using conventional methods, protein level and crude fatty acid content and other materials in the antlers were determined.

Results and Discussion

The results showed that the average live weight gain of each group deer was 17.3 kg, 19.9 kg and 14.4kg in 12%, 16% and 20% dietary protein levels respectively during antler growth period; and velvet antler yield was 2.22 kg, 2.52 kg and 2.25kg for the respective treatments. The results of these experiments demonstrated that dietary protein level in the present study had no effect on the composition (protein, organic matter, crude fatty etc) of subsequent growing velvet antlers.

Therefore, in contrary to the currently held view, the present study showed that dietary protein level had no significant effect on body weight and velvet yield if given the routine level of nutrients, but there is the trend that 16% protein level diet group performed better than the rest. Consequently, we conclude that diet that contains 16% dietary protein was optimal for live weight gain and antler production in sika deer.

Keywords: dietary protein, weight, velvet production, Sika deer, *Cervus nippon*, antler, nutrition, production

Seasonal and specific diet variations in sympatric Red and Fallow deer of Southern Spain: A preliminary approach to feeding behaviour

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The Sierra Morena mountains have an important ecological value in Southern Spain where plant-communities typical of siliceous soil of Mediterranean ecosystems and savanna-like landscapes of the Iberian Peninsula known as "dehesas" are a dominant part of the agroforestry system. In this area red deer (*Cervus elaphus hispanicus*) are the most abundant and important game, but fallow deer (*Dama dama*) density has considerably increased since the 1950's when they were introduced in some game reserves. The negative ecological impact of overbrowsing and selective foraging have been described when there is an overabundance of deer. In this context, when wise management of herds is necessary, studies of feeding behaviour and use of food resources by sympatric herbivores are very important for understanding differences in resource exploitation, their impact on vegetation and their ecology. Therefore, as part of a research project in a Mediterranean environment, we studied their diet composition, analysing a collection of rumen contents samples from 81 red deer and 69 fallow deer shot monthly by research staff during 2008-2009 in Sierra de Andújar Natural Park, Spain. The rumen contents were mixed and samples of about 250 g were stored frozen until analyzed. These were later thawed and washed through sieves with 1cm and 0.5 cm meshes. The rumen contents were classified into three groups: 1. Browse (woody, tree foliage and shrub parts, including forbs, broad green leaves, stems and shoots), 2. Grasses (graminoids, monocots, aquatic plants, fungi and mushrooms) and 3. Fruits (acorns, olives, reproductive organs and seeds). The material was sorted, oven-dried to constant weight and weighed. The mean percentage dry weight composition of each of the three groups with respect to the total weight of the sample was then compared. Using a multivariate analysis of variance with a previous data transformation, we assessed differences of diet composition according to species, sex, ages and seasons. More specifically, we performed a bi-monthly categorisation. Differences in diet composition among grouping were tested using. Diet composition was found to vary throughout the year in both species and there was a statistically significant monthly and seasonal percentage difference of browses, grasses and fruits. Grasses were ingested more in Spring, and Browses were an important food resource in two periods: at the end of Winter and at the end of Summer. Both red and fallow deer behave like intermediate or mixed-feeders. However, red deer ingested a higher proportion of browse than fallow deer. Differences between the two sexes in red deer were also detected. Red deer are more browsers than fallow deer, and red deer stags more than hinds at the beginning of Spring and they have a second peak of browsing in September-October. Nevertheless, in July-August hind tend to be the more browsers than the other ones. There were also differences in relation to fruits. Fallow deer ingested a higher proportion of fruits, then during a longer time period (September to February) than red deer. Acorns from *Quercus* sp. and for olives were the most abundant fruits, being an interesting resource storage of energy reserves for nutritional constraint periods when damage due to browsing can be expected.

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Keywords: diet composition, *Cervus elaphus hispanicus*, *Dama dama*, feeding behaviour, mediterranean constraint, intraspecific

BEHAVIOR: FREE RANGING AND CAPTIVE DEER

Human disturbance trigger adaptation responses on circadian activity rhythms in Hainan Eld's deer

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The impact of human disturbance on wildlife is a major consideration in the management of many species. As human populations continue to expand and the populations of wildlife decline, understanding the interactions between people and species of concern is essential. Like natural factors such as resource dynamics and climate human activity can influence wildlife; however, the human disturbance element is often absent from ecological field research. The need to consider human disturbance becomes of even greater relevance in the management of highly endangered species, such as translocated populations of Eld's deer *Cervus eldi* inhabiting the Chinese island of Hainan. Here, using radio-telemetry we monitored two populations of Eld's deer, a source population within the bounds of a reserve (Datian National Nature Reserve) and a translocated free-living population near the reserve (Chihao locality), for twelve consecutive months. During regular periods of continuous sampling we were able to determine patterns of activity for three deer inside the reserve and eleven translocated deer and compare them over time. We also monitored the activity of ethnic Li villagers living amongst the translocated population. Our findings show that translocated deer broke away from a typical crepuscular activity adopted by deer inside the reserve and became increasingly nocturnal in the presence of humans. The activity patterns of the villagers were also strongly negatively correlated with periods of inactivity of the deer. This apparent flexibility of activity patterns in Eld's deer is discussed in the context of human disturbance and the need for further research to determine proximate impacts to this highly endangered animal.

Keywords: activity pattern, rhythm, anthropogenic habitat, *Cervus eldi*, Eld's deer, behavior

Comparative study of Pampas deer (*Ozotoceros bezoarticus celer*) behaviour under different degrees of interference with exotic ungulates (livestock, feral pigs) in the Bahía Samborombón Wildlife Refuge, Central Argentina

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Ozotoceros bezoarticus celer, the southernmost subspecies of Pampas deer, is endemic to the Argentine Pampas and seriously endangered. In Buenos Aires province, the last Pampas deer population is located in the Bahía Samborombón Wildlife Refuge. The dramatic decrease in Pampas deer population has been attributed to commercial and game overhunting, habitat alteration by agriculture and livestock, competition and diseases introduced by cattle and exotic species, such as feral pig and introduced deer. In the Wildlife Refuge hunting of native and nonnative species is forbidden all year round, which may have promoted an increase of feral pigs populations in the area. The refuge includes some protected areas, devoted to protect grassland and the Pampas deer, and ranches with extensive cattle ranching, being the main human activity (stocking rates of 0.5–1 animal ha^{-1}). The objective of this study was to compare the Pampas deer behaviour under different degrees of interference with exotic ungulates in the Bahía Samborombón Wildlife Refuge, and to evaluate the implication of this interference for its conservation. Data collection was carried out between winter 2008 and summer 2010 in seven seasonal samplings. An instantaneous scan sampling was used to collect behavioural data of Pampas deer. Sightings were made using a spotting scope. Behavioural observations were classified into three categories: maintenance (behaviours related to its own survival), social (intraspecific behaviours) and vigilance (alarm reactions). Twenty four scans were carried out from sunrise to sunset, during two consecutive days in each sampling site. Sampling sites were qualitatively distributed in an increasing gradient of interference to the Pampas deer: low interference (protected area Campos del Tuyú National Park): absence of cattle and low density of feral pigs; intermediate interference: presence of cattle and low density of feral pigs (cattle ranch); high interference: presence of cattle and high density of feral pigs (cattle ranch). Pampas deer showed differences in its behaviour among sites with different degree of interference (χ^2= 49.06; gl= 4; P<0.0001), with a lower frequency of social behaviours and a higher frequency of behaviours related to vigilance in cattle ranches than in the protected area. However, we found no significant differences in Pampas deer behaviour between sites of intermediate and potentially high interference (χ^2= 1.37; gl= 1; P>0.1). These results suggest Pampas deer changes its pattern of activities in presence of exotic ungulates. Social behaviours are related to reproduction, to spend less time on these behaviours may have long term effects such as

decreased reproductive success. The highest frequency in vigilance behaviours could be due to Pampas deer being exposed to a greater human disturbance in ranches than in the protected area, caused by cattle management and the presence of potential predators. This could cause physiological stress to the Pampas deer and thus affect population recovery. In the Bahía Samborombón Wildlife Refuge, where Pampas deer occur together mainly with livestock, these results contribute to propose recommendations for management practices for the benefit of livestock production and Pampas deer conservation.

Keywords: Pampas deer, *Ozotoceros bezoarticus*, interspecific, behaviour, livestock, feral pigs, Sus scrofa, conservation, Bahia Samborombon Wildlife Refuge

Sociomapping – new tool for analysis and visualization of social, spatial and hormonal links between members of a Red deer male group

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The presentation of results on social behavior in the animal communities may bring some difficulties due to many complications in a time dynamic system (e.g. formation of subgroups in the family or mutually benefiting alliances). Sociomapping is a new data visualization and analytical method (originally designed to exploit human subconscious cognitive subsystems) for easier data exploration by creating three-dimensional landscape-like graphs (Sociomaps). The Sociomaps are created on the basis of multiple Spearman correlation and it is able to localize sub-groups (coherences) of closely interrelated individuals (by the mutual distance on the Sociomap). It enables to visualize, explore and analyze the structure and dynamics of a system and to present it in a way easy to understand (http://www.qedgroup.cz/index.php?xSET=lang&xLANG=2). The Sociomapping method uses fuzzy theory, mathematical topology and pattern recognition to visualize complex structures.
The aim of this study was to analyze and visualize an influence of social instability on the social grouping in captive red deer (*Cervus elaphus*) males, on their plasma hormonal levels and on number and direction of agonistic encounters during the period between the antler casting and cleaning. We used two groups of red deer males. The first group (FG) consisted of ten adult males (3 and 4 years old) and the second group (SG) of ten male yearlings. Monthly, both groups were made socially unstable by adding one adult male from the FG into the SG. This male was replaced after one month by another one from the FG, while the replaced male returned back to FG. During the change of the group composition we collected blood from jugular vein for hormonal analysis. The agonistic encounters between individuals had been registered randomly 10 times each month during feeding of concentrates to monitor assumed dynamics. The fundamental data set in this study was based on inter individual distances between males calculated from geographical position recorded every hour for each animal by GPS-3300 Series neck collars (Lotec Wireless Inc., Canada). We also used plasma hormones (testosterone and cortisol) and agonistic encounters among the males as explanatory variables in the Sociomapping analysis. The Sociomapping method allowed us to visualize position of the individual on the Sociomap based on the testing between individuals and between subgroups of red deer males according to the above mentioned variables. The value of each variable for the male position in the Sociomap and the social relationship among group members will be presented visually.

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Keywords: Red deer, *Cervus elaphus*, social behavior, hormones, agonistic encounters, sociommapping, sociomap

Pre-orbital gland opening in Red deer (*Cervus elaphus*): Visual indicator of stress?

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Pre-orbital glands possessed in most cervids were found to serve an olfactory role, esp. in scent marking in adult males. Behavioural function of pre-orbital gland opening (POGO) has been also presumed. We are going to present results of our long-lasting investigations of POGO in stressful situation in red deer.
In our previous study, POGO in newborn red deer calves was invariably associated with stress; a newborn calf opened its pre-orbital gland when being exposed to a stressful procedure. POGO was suggested to be a simple and easily recognizable indicator of stress for farmers (Bartošová-Víchová et al. 2007, J. Anim. Sci. 85:494–496). Thus, we hypothesized this effect also in other age categories of red deer.
We observed pre-orbital gland status in red deer calves and adults on two experimental deer farms; in Prague (Czech Republic) and Albaceté (Spain). At both places, the animals were repeatedly subjected to similar stressful manipulations in handling facility (crush), including weighing, collar adjustment, blood sampling, etc. For all observations, openness of the pre-orbital gland was assessed visually if closed or opened in three periods: before the manipulation, during the manipulation and after the manipulation. All of the used stressful events and manipulations took part under farm routine practice and/or employed other projects running at the farms to minimize additional stress or discomfort in the animals. We observed altogether 46/13 (Prague/Albaceté) individual males (stags), 24/102 adult females (hinds), and 41/32 calves.
Pre-orbital glands were closed before manipulation. Compared to this, all of the tested age groups revealed significant increase of pre-orbital opening when manipulated ($P<0.0001$). In Prague, only 54.8 % of the 3-months-old calves opened their pre-orbitals (which was half of that what they did as newborns, $P<0.001$). Hinds and stags opened their pre-orbitals in 42.5 and 35.8 %, respectively. In comparison, only 37.5 % of the 3-months-old calves, 8.8 % of hinds and 53.8.0 % of stags opened their pre-orbitals in Albacete, which was significantly less often than in Prague for calves ($P < 0.05$) and hinds ($P < 0.001$). Lower incidence of POGO in Albacete was most likely caused by the effect of habituation resulting from higher frequency of manipulations performed in Albacete than in Prague. Comparison between both places in case of the stags should be taken with caution because of low sample size in Albacete.

In conclusion, the pre-orbital gland opening was clearly associated with stressful procedures in red

deer males and females of different categories. Nevertheless, our presumption of the POGO as a general stress indicator in red deer failed. As pre-orbital opening occurs in various situations associated with arousal, it is likely to be a side effect of general excitement, both positive and negative, of an organism (e.g. interest, forthcoming feeding or sexual arousal during courtship) as we proposed earlier (Bartoš et al. 2005, J. Anim. Sci. 83:124–129).
Grant support: GACR 523/08/P301.

Keywords: pre-orbital gland opening, stress, Red deer, *Cervus elaphus*, behavior, communication

Habitat selection of sympatric Blue Sheep (*Pseudois nayaur*) and Red Deer (*Cervus elaphus alxaicus*) during rutting periods in the Helan Mountains, China

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Habitat selection of sympatric blue sheep (*Pseudois nayaur*) and red deer (*Cervus elaphus alxaicus*) was studied in the Helan Mountains on the border between Ningxia and Inner Mongolia from September to December 2007 and 2008. We collected data by direct observation and examination of fresh sites used by blue sheep and red deer in 15 drainages distributed throughout the study area. Eighteen ecological factors from 177 sites used by sheep and 154 sites used by deer were measured on 21 transects in the study area. Habitat use of blue sheep and red deer differed in most ecological factors, i.e., vegetation type, landform type, dominant tree, aspect, slope location, shrub density, shrub height, coverage, slope, altitude, distance to water resource, distance to human disturbance, distance to bare rock, hiding cover, and tree height. Compared with habitat selected by deer, blue sheep selected montane steppe dominated by *Ulmus glaucescens*, steeper slope, taller trees, less shrubs, taller shrubs, less vegetation cover, lower altitude, sunnier aspects, lower hiding cover, closer to water resource and bare rock. Canonical scores indicated that habitats used by blue sheep were most separated from red deer during rutting periods (Wilk's $\lambda = 0.123$, $\chi^2 = 679.172$, $df = 10$, $P < 0.001$). The Fisher linear function discriminated sheep habitats is: 3.638×trees heigh, 0.242×distance to the nearest trees, 7.766×shrub height, 0.663×distance to the nearest shrubs, 0.232×vegetation coverage, 0.191×slope, 0.001×distance to water resource, 0.008×distance to bare rock, 0.307×hiding cover 31.078. The Fisher discriminant function of deer habitat is: 4.850×trees height, 0.321×distance to the nearest trees, 12.024×shrubs height, 0.929×distance to the nearest shrubs, 0.192×vegetation coverage, 0.482×slope, 0.002×distance to water resource, 0.001×distance to human disturbance, 0.003×distance to bare rock, 0.511×hiding cover 50.787. Discriminating variables that improved a stepwise discriminant model included (in order of importance) slope, distance to human disturbance, hiding cover, distance to bare rock, trees height, distance to the nearest trees, distance to water resource, shrubs height, distance to the nearest shrubs, and vegetation cover. Predicted accuracy of the model in classifying sheep and deer habitats was 99.7%.

Keywords: Blue sheep, *Pseudois nayaur*, Red deer, *Cervus elaphus alxaicus*, sympatry, habitat selection, behavior, stepwise discriminant analysis, canonical discriminant analysis, Helan Mountains.

Inter-individual variations in male and female mating bonds within a population of Eld's deer in Hainan Island, China

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The mating system of a semi-captive population of Hainan Eld's deer *Cervus eldi hainanus* was investigated in the Datian National Nature Reserve, China. Eighteen females and 11 males out of 61 semi-captive deer were involved in mating, and 43 copulations were observed in this study. The mating system of this population is polygynous, with an inter-individual variation in the type of mating bonds among males (i.e. polygyny, promiscuity) and among females (i.e. serial monogamy, polyandry, promiscuity) and an inter-individual variation in male mating tactics depending on the dominance rank of males. During entire rutting season, 72.2% estrous females copulated with only one male, and 27.8% estrous females copulated with two or more males. In four of 11 cases, males that involved in mating, mated with more than one female, and these same four males obtained 81.4% mating opportunities. Dominant males occupied the number of females in estrus significantly more than subordinate males. Mating dominance of a dominant male kept on at least 87 days during the rut, so that he copulated totally with 11 females. It was more possible for dominant males to be polygynous than did subordinate males. It was also more possible for polyestrous females to be polyandrous than did females with only one estrous period. Our results implicate that the inter-individual variations in male and female mating bonds largely depends on mating strategies of dominant males, operational sex ratio as well as multiple estrous periods in females. Our data clearly demonstrate that dominant males can monopolize copulations despite the long duration (over three months) of breeding season, and asynchronous estrus of females makes this possible without increasing energetic costs for dominant males.

Keywords: *Cervus eldi*, Hainan Eld's deer, mating system, polygyny, dominance rank, reproduction, behavior

Fecal 11-Ketoetiocholanolone measurement in Spanish Red deer: Validation of methodology using HPLC-MS/MS

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Fecal glucocorticoids metabolites (FGM) measurement is a valuable non-invasive tool for studying the physiological response of free-living animals to a variety of stressors and provides an useful monitoring technique in wildlife management and conservation. A GM derived from cortisol is 11-Ketoetiocholanolone (11-K) which has been widely determined by immunoassay in monitoring stress in several vertebrates. However, these assays have certain limitations with respect to specificity because of cross-reactivity with related hormones. Also, differences in the excretion of FGM among species and even sexes makes a validation in each case necessary. Therefore, our aims were: first, to develop and validate a HPLCMS/MS methodology for monitoring 11-K in feces of Spanish red deer; next, to investigate the increase of this FGM in a longitudinal study to detect adrenocortical responses to ACTH and other stressful stimuli like translocation or captivity; and finally, to assess the correspondence between fecal 11-K levels and circulating cortisol in plasma. The ACTH test was performed on 6 red deer stags and fecal samples were collected twice a day for a week. One blood and fecal sample each from another 7 stags were also collected after two weeks in captivity. This experimental study was performed before the mating season, over a short time period to avoid seasonal fluctuations. We extracted FGM from freeze-dried feces using methanol. Sample pre-treatment involved the use of one-step extraction with SPE cartridges (Waters TM Sep-Pak vac 5cc reversed phase C18). Chromatographic separation from a set of steroids known to be present in feces was achieved on a Waters ODS2 column (125×3 mm, 5μm), and 20 μL of the sample solution in methanol were injected, elution was carried out by gradient between mobile phase A (methanol–formic acid 1000:1, v/v) and B (water–formic acid 1000:1, v/v). The flow rate was 0.4 ml/min. The whole flow was directed into the mass spectrometer; and electrospray ionization in positive mode (ESI+) was used. Operating conditions were previously optimized by direct injection of the standard analyte into the ion source. MS/MS experiments were carried out by fragmenting precursor ion 287.0 and the most intense fragment ion 228.9 was used for quantification. The recovery of 11-K was assessed by eluting a standard solution at 1 ppm. Then a lineal calibration curve was generated with 5 points over a range of 10-500 ng/mL and the limits of detection and quantification were also calculated. A peak increase was detected in 11-K 36 hours after the adrenocorticotropine test and handling. A peak was previously detected in an individual, and in all individuals a second peak was reached at 120 hours of being kept indoors, before returning to the baseline. Maximal concentrations of 11-K ranged between 22.71-375.68 ng/g, which reflected a 9 to 45 fold increase over the basal levels. In the second stag group, levels of 25.09±20.53 ng/g (8.09-57.87) had a correlation of R_2=0.88 to the plasma cortisol, 5.46±5.51 μg/dl (0.2-15.4). This technique is capable of detecting changes in the levels of fecal 11-K, the values determined have a good correlation to the cortisol concentration in blood, and we also detected

difference in diffeent individuals' responses to the same stressors.
This study was supported by the P07-RNM-03087 and CGL-2008-00832 projects and was approved by the Bioethical Committee of University of Jaén.

Keywords: Red deer, *Cervus elaphus hispanicus*, monitoring, stress, feces, glucocorticoids metabolites

Temporal habitat partitioning between two species of Cervidae (*Mazama americana* and *Mazama nemorivaga*) in the Jamari National Forest, Rondonia State, Brazil

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The biological rhythm is an important characteristic to promote an internal temporal organization in physiology and behavior of the species and enable the synchronization with the external environment, helping the species to be prepared for periodic environmental changes. The activity pattern of species is considered one of the most important components of the niche. As a relevant fact in the natural history of species, knowledge of the activity period can be used as a tool to minimize efforts in fieldwork. In spite of that, information about activity period of the genus *Mazama* is still conflicting and scarce. The objective of the present study was the identification of activity patterns of two deer species in Amazonia western: *Mazama americana* (red brocket deer) and *Mazama nemorivaga* (Amazonian brown brocket deer). We used 20 automatic camera-traps connected to passive infrared sensor detection during 24 h to register the animal movement/activity. The monitoring was carried out in August, September, October and December of 2006 and January and February of 2007 in Jamari National Forest, Rondonia State, Brazil. The effort included 47,073 hours of monitoring. The camera-traps were set up at sites previously identified in the potential movement routes of the animals (trails and streams). We observed significant differences between activity patterns of *M. americana* and *M. nemorivaga* ($X2 = 44.96$; $P < 0,05$). *Mazama americana* demonstrated most activity in the period of 00:00h to 06:00h for 39.98% of the records, while *M. nemorivaga* presented only 2,27% photographic records for this interval. The period of most activity for *M. nemorivaga* was between 12:00h to 18:00h (61.35% of the records), while *M. americana* demonstrated less activity, only 8.32% of the photographic records in this same period. Between 06:00h and 12:00h it was apparent that there was no prominent temporal partitioning between these species as opposed to the other periods where partitioning was evident. The environmental factors like temperature, rain, interspecific competition, and tolerance/intolerance during extreme phases (less favorable) can explain those differences in the activity periods of the species studied. There is a clear tendency of *M. americana* to exhibit nocturnal activity, and *M. nemorivaga* diurnal activity.

Keywords: activity period, Camera-trap, Western Amazonia, *Mazama americana*, *Mazama nemorivaga*, temporal habitat partitioning, behavior

Circadian rhythms of female Pampas deer (*Ozotoceros bezoarticus*): Motor activity at different reproductive phases in Brazilian Pantanal

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The temporal organization of activities has implications for reproduction and survival of animals, and is a strong adaptive characteristic of the species. The time budget of activities during the course of 24 hours results from a compromise between natural behaviors of the species, always modulated by physiological, reproductive and environmental conditions. In the Brazilian Pantanal, it is known that the reproduction of the Pampas deer (*Ozotoceros bezoarticus*) is seasonal, and that their reproductive behavior is associated with more favorable seasons of the year to ensure the survival of the species. This study aimed to evaluate the circadian rhythm of motor activity of Pampas deer females at different reproductive phases in the central Brazilian Pantanal, known as Nhecolândia (18º59'15''S; 56º37'03''W). Five pregnant females, with normal reproductive functions, were captured using anesthetic darts and tagged with GPS collars ATS model G2110B. GPS collars were programmed to record animal location at intervals of 15 minutes over nine days, during periods of mating (February/2009), pregnancy (June/2009) and lactation (October/2008/2009). At the programming end, collars were recovered after release by a "drop off" system. The data stored in the equipment were analyzed by extension Animal Movement Analysis 2.04, the ArcView ® GIS 3.2. Motor activity and the pattern of displacement of the animals were assessed based on the distance traveled between successive location points. The average daily movement was equivalent to 3,847.85 m ± 928.35 m during the breeding season, 3,583.92 m ± 624.04 m during pregnancy and 4,143.27 m ± 937.24 m at the stage of lactation. The analysis of variance (ANOVA) followed by Tukey's test showed no significant difference in motor activity between the different reproductive phases, but showed great individual variation ($p < 0.001$). The t-test showed a difference of displacement between the light and dark phases of the day ($p < 0.0001$). The motor activity increased significantly after sunrise, peaking at 6:00h (254.80m ± 219.53m), 10:00h (235.01m ± 198.65m) and 17:00h (299.43m ± 227.20m) and decreased significantly after 18:00h, with the sunset. Nighttime also showed a peak activity at 21:00h (190.96m ± 241.85m). The results showed no different patterns of displacement between the reproductive stages, but showed well defined peaks of displacement along the circadian cycle. The analysis of the circadian rhythm of motor activity allowed defining diurnal and nocturnal for females of Pampas deer, with peak displacement significantly higher during the day.

Keywords: Pampas deer, Ozotoceros bezoarticus, activity pattern, rhythm, behavior, reproduction

Preliminary acoustic repertory in Brown brocket deer *Mazama gouazoubira* and first recordings from Amazonian Brown brocket *Mazama nemorivaga*

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South American solitary forest deer, such as the brown brocket *M. gouazoubira* and the Amazonian brown brocket *M. nemorivaga* do not seem to be exceptionally vocal compared to most species of deer, but they have a repertory of several calls and sounds that they use in their social and antipredator behavior. The brown brocket is widely distributed in Chacoan thorn scrub and Atlantic forest in Brazil, Bolivia, Paraguay, Uruguay and northern Argentina, while the Amazonian brown brocket is found throughout the Amazon basin. The objectives of this study were to record and describe the vocalizations and sounds that occur in these species, documenting the situations in which they occur, in order to infer possible functions.
Vocalizations from 12 individuals of *Mazama gouazoubira* (6 males, 3 females, 1 juvenile and 2 fawns) and 1 male adult of *Mazama nemorivaga,* were recorded in captivity and semi-captivity in 4 institutions in the province of Tucumán, Argentina, and 1 institution in Brazil. Recording equipment consisted of an Audio Technica directional microphone, a Sony TC-D5M cassette recorder and an ERIDOL digital recorder. Sounds were analyzed with the computer program Spectrogram 11.
We recorded 5 vocalizations from the deer and documented the use of one non-vocal sound, the foot stamp. 1) Distress cries were emitted by all deer when they were captured. These were high intensity tonal cries with many harmonics and some subharmonics. The fundamental frequency was lower in adults than in fawns while the average duration was longer in adults than fawns. Does respond with approach when their fawns produced this call, but attacks were not observed. 2) The alarm snort was recorded from females when their fawns were captured and from a male when disturbed by loud noises. Alarm snorts were short, noisy sounds given repeatedly. Bleats were produced in three different situations and are tentatively considered as different vocalizations because of slight differences in physical characteristics; for example, the average duration, fundamental frequency and maximal frequency were higher in fawns than adults. 3) Fawns bleat in response to their mother, in mild distress and when joining their mothers. 4) The bleats of females were produced by females with fawns. They call their fawn from hiding with this vocalization on their return, and the fawn comes out to nurse. This vocalization is also given in response to bleats from the fawn. 5) Male bleats were produced in courtship by males when following an estrous female. Bleats were produced by one male in aggressive interactions with other males. Frequencies and durations of bleats in males and females were very similar, but the small N of individuals recorded does not permit a conclusion as to whether they are the same or different vocalizations. The bleat was the only vocalization recorded from one male Amazonian brown brocket. The situation was difficult to analyze as it was produced by the deer when alone in captivity. The differences in physical characteristics between the males of the different species were in the fundamental and maximal frecuencies, which were higher in Amazonian brown brocket than in brown brocket. Foot stamp was a non-vocal sound, not recorded because of its low intensity. It is the most common alarm reaction and was also observed together with the alarm snort. All of these

vocalizations, except the distress cry, are low intensity calls designed for short range interactions. Vocalizations of this type are commonly observed in small, solitary monogamous or slightly polygynous deer, which include these species.

Keywords: vocalizations, communication, solitary deer, Mazama, behavior

Preliminary description of vocalizations in Red deer stags (*Cervus elaphus*) during the rut in Northwestern Patagonia (Argentina)

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The vocalizations of deer are notable for their diversity, with red deer (*Cervus elaphus*) being the most studied species in this aspect. The aim of this paper is to describe and characterize the acoustical properties of male vocalizations produced during the rutting period for a free-ranging red deer population located in the ecotone of the eastern Andean cordillera within the Nahuel Huapi National Reserve (Neuquen, Argentina).
The recordings of the vocalizations were made with a Sony TCD 5M portable recorder, a unidirectional microphone and cassette type II. The tapes were then digitalized and analyzed with Spectrogram 14 and Raven Lite. From these data, acoustic parameters were obtained. The recordings were conducted in 2007 during the peak of the rutting period, predominately during the height of the daily activity (approximately sunrise to 11:00, and 16:30 to sunset). Behavioral records were taken as well as photographs of recorded adult males (n=7), four of these being dominant breeders each holding territories with 7 or more females present.
From the spectrograms, three principal types of vocalizations were identified. These were mainly distinguished by their structure and total duration in seconds: 1) Common roar (or tonal roar), a harmonic structured sound with well-defined tones and formant frequencies (duration, 1.43 ± 0.19); 2) Harsh roar, a chaotic structured sound with some areas of greater intensity in its frequency range (duration, 0.65 ± 0.07); and 3) Cough, the shortest vocalization, showed an atonal structure, which was produced in a rapid series (duration 0.26 ± 0.02). This vocalization type was emitted only in conjunction with the harsh roar. The Mann-Whitman test showed significant differences in the comparison of these three vocalizations ($p < 0.05$). The most common vocalization was the common roar (48%), followed by the harsh roar (34%); the latter occurred in both isolated and serial emissions. The combination of harsh roar and cough was the most frequent combination. As for fundamental or lower frequencies, the common roar varied from 154.5 Hz to 161.5 Hz. The lowest frequency for the harsh roar and the cough was 60.5 hz and 292.5 Hz, respectively.
By comparing these vocalizations with those described in other studies we find that the fundamental frequency of the common roar in our records is a bit higher with a narrower range than that observed for red deer in England (64Hz to 140Hz). The common roar vocalization in the Nahuel Huapi population was the most variable of the three vocalization types observed, with mild to strong frequency modulation and non-linear phenomena, like subharmonics and deterministic chaos. The presence of these structures could be a useful indicator of body size or motivational state to other individuals.
Variation was observed in that the common roar was more common during the days building up to the peak of the rut, while the harsh roar was more frequently observed as the peak progressed. It has been suggested that these vocalizations provide information that allows females to select a mate. Our recordings of the four dominant breeding adult males − all with 11 to 14 point antlers and

holding territories containing females − supports this theory. Spectral characteristics that would enable individual identification can be detected in the common roar. Future studies of vocalization can further our knowledge regarding intra- and interspecific variation in deer.

Keywords: Red deer, *Cervus elaphus*, vocalization, acoustics, stags, communication, behavior, Patagonia, Argentina, rut, variation, mate selection, formant frequencies

Male vocal behavior and phylogeny in deer

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The phylogenetic relationships among 11 species of the Cervidae family were inferred from an analysis of male vocalizations. Eighteen characters, including call types (e.g. Antipredator barks, mating loudcalls) and acoustic characteristics (call composition, fundamental frequency and formant frequencies), were used for phylogeny inference. The resulting topology and the phylogenetic consistency of behavioral characters were compared with those of current molecular phylogenies of Cervidae and with separate and simultaneous parsimony analysis of molecular and behavioral data. Our results indicate that male vocalizations constitute a plausible phylogenetic character in this taxon. Evolutionary scenarios for the vocal characters are discussed in relation with associated behaviors.

Keywords: vocalization, phylogeny, cervidae, vocal behaviour

Allogrooming in Brown brocket deer (*Mazama gouazoubira*)

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Allogrooming is a common behavior in many species of ungulates where it is postulated to function in the removal of ectoparasites, especially ticks, from areas not accessible to self grooming, such as the head and neck. In impala (*Aepyceros melampus*), it is highly reciprocal, with both animals exchanging bouts of grooming in approximately equal numbers. Allogrooming is a fairly common behavior in the brown brocket deer, a small to medium sized, solitary South American deer found in Chacoan thorn scrub and the base of Yungas forest edges in Argentina, Bolivia, Brazil, Paraguay and Uruguay. Deer maintained in a large enclosure (14 ha) were observed for various periods in Tucumán, Argentina, from 1991-1998. A total of 146 allogrooming interactions were observed involving 8 males, 10 females and 20 fawns. Some animals were observed both as fawns and adults. Of these, 47% involved males and females and 39% involved females and fawns or juveniles. The rest involved males and fawns, related females and males with males, which usually ended in aggression. Most interactions involved an exchange of only 1-2 bouts, although a few involved more. (Interactions between males and females: mean 3.3±0.4; median 2; mode 1; N=97) Repeated interactions were observed between some males and females and between females and their fawns. The interactions were not highly reciprocal. Typically one animal gave more bouts than it received. In the case of mother-fawn interactions, the mother groomed the fawn exclusively in the first few weeks. Multiple interactions were observed between 5 male-female pairs involving 6 deer, and in 4 cases, one animal gave many more grooming bouts than it received (coefficient of reciprocity = 0.6-0.81). The tendency to allogroom more or less seemed to be an individual characteristic. The parts most often groomed were the neck, head and ears, parts inaccessible to self-grooming, suggesting the function of tick removal. Ingestion of ticks as a source of food is another possibility, since allogrooming of other species, such as tapirs (*Tapirus terrestris*) was observed. Allogrooming also seems to function in courtship and appeasement. Males commonly groomed females when approaching them and in the course of courtship, and females allogroomed males that approached and butted them. Since these deer are solitary and territorial, they probably interact with few other individuals, and the lack of reciprocity would be limited by individual recognition and tolerance.

Keywords: grooming, reciprocity, allogrooming, Brown brocket deer, *Mazama gouazoubira*, behavior

Chemical communication in Brown brocket deer

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Chemical communication is very important in small, solitary, forest dwelling deer, which are probably mostly territorial. The scent marks left in the territory or home range are a way of communicating with conspecifics that live in or visit the area. They persist in the absence of the owner, indicating his occupation of a given area. This form of communication is so important that it seems to be almost universal in territorial male mammals and thus appears to be essential for successful resource defense. Brown brocket deer (*Mazama gouazoubira*) are small to medium sized, solitary inhabitants of Chacoan thorn scrub and the base of Yungas forest edges in Argentina, Bolivia, Brazil, Paraguay and Uruguay. They were territorial in large enclosures, with males occupying large, non-overlapping areas and females, smaller areas with non-overlapping core areas. Home ranges are marked with numerous small latrines used by both sexes, and marks on trees made by scraping with the incisors and rubbing the forehead. Our studies have concentrated on the role of latrines in these deer. Most urination and defecation occurs in latrines in a sequence that includes usually sniffing, stepping, urination or defecation or linked urination and defecation. Latrines were located throughout the territories, especially on paths. One male showed a tendency to use latrines located on the border of his territory. In order to study the function of these latrines, we did experiments on the introduction of dung to the latrines of captive deer. The objective was to determine whether deer can distinguish their own dung from the dung of an unknown deer and whether they counter-mark dung from an intruder. Counter-marking occurs when animals respond to invaders' marks with a greater number of marks. Dung samples from unknown males or females and the experimental animal were introduced near the latrines of captive deer and their responses, including investigative (sniffing) and marking (urination and defecation) behaviors observed. Males investigated and counter-marked the dung from unknown males and their own latrine significantly more than their own. Females investigated unknown female dung significantly more than their own. The finding that males counter-mark most intensively dung from male "intruders" near their latrines may be related to intrasexual competition and resource defense. Again, if dung from an unknown male conspecific indicates a territorial intrusion, counter-marking by the male subject is an attempt to maintain the most and freshest marks in the territory. After detecting dung from unknown individuals near one of their latrines and counter-marking it, male deer walk to their latrine, investigate it thoroughly and then urinate and/or defecate in the latrine. Females also walked to their latrine and tended to urinate or defecate there after detecting dung from unknown females. This behavior suggests that the latrines could serve as centers of communication in that the deer go there to investigate and renew marks after the detection of a strange odor. The latrines could potentially announce an intruder, and dung renewal by the residents suggests that affirming dominant or resident status may be a motivation. Since feces deposited in a latrine are long-lasting and available to any individual, they are especially suitable for communication among various individuals living or visiting the same area, which would form a communication network. These data are consistent with the scent matching hypothesis: owners place marks where they are likely to be detected by

intruders since low quality intruders will usually withdraw from an identified owner who has demonstrated his potential to establish and maintain a territory. Intruders identify owners by detecting scent marks within the territory and match them to the odor of the owner when he appears.

Keywords: *Mazama gouazoubira*, scent marking, latrines, counter-marking, communication, Brocket deer

Vocal communication in deer: Application of the source-filter theory to the study of mammal sexual calls

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I will review recent studies of sexual communication in deer, to illustrate how the source-filter theory of vocal production can be applied to studying mammal vocal signals. Using a combination of anatomical investigations of the vocal apparatus, acoustic analyses, and playback experiments, we have shown that: (i) red deer and fallow deer males have a descended and mobile larynx, an anatomical innovation that enables callers to lower their formants during vocalising and exaggerate their apparent body size; (ii) minimum formant frequencies provide an honest indication of body size in red deer roars; (iii) stags use rivals' minimum formant frequencies in assessment during male-male contests, and adjust the formants of their own replies in relation to what they hear; (iv) oestrus hinds pay more attention to roars in which formant spacing indicates larger males, but prefer higher pitched roars; (v) harsh roars, a type of roar characterised by an a-periodical glottal wave, increase hinds responses to other call types and may have an "attention-grabbing" function. I will discuss these results in the context of the diversity of male deer vocal behaviour and reproductive strategies and suggest directions for future research.

Keywords: sexual communication, vocalizations, Red deer, *Cervus elaphus*, bioacoustics

Investigating the path to hybridization: Behavioral responses of male and female Red deer to male mating calls of Red and Sika deer

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Hybridization between species is an important but often overlooked threat to native species survival. Reproductive isolating mechanisms are integral to the process of speciation. These mechanisms often include species-specific signals in the context of mate attraction (to locate and identify appropriate mates) and mate competition (to identify and defend against potential mating competitors). When these isolating mechanisms are weak, hybridization may occur. Vocal communication in the context of sexual selection (mate choice/competition) is used extensively by many deer species and theoretically should present reproductive barriers to hybridization. However, in the UK, extensive hybridization and introgression between native red deer and introduced Japanese sika deer is currently threatening the genetic integrity of red deer. Our project examines two behavioral mechanisms behind this red x sika hybridization by testing species discrimination in male-male competition and mate choice in the context of reproductive calls using playback experiments. Differences in reactions to conspecific vs. heterospecific vocalizations between the sexes or species may point to weaknesses in reproductive barriers and potential sources of hybridization and introgression.

This talk presents the results of two playback experiment studies from the first stage of this project. During these playback experiments, reproductive vocalizations of red and sika males were broadcast to free-living and captive red deer while their behavioral reactions were recorded and evaluated. The first experiment involved playbacks to free-living red deer within the grounds of Bushy and Richmond Royal Parks in the UK. Target groups of females guarded by actively rutting males were presented with playbacks of red or sika calls (consisting of 3 calling bouts separated by 20 seconds each) while the behavioral reactions of the male and females were monitored. After 3 hours, the same group was found as soon as possible so that the other species exemplar could be played back to the same group. Behavioral responses evaluated include the duration and latency of vocal response, aggressive behaviors, and movement towards or away from the speaker. Preliminary results indicate that males were more aggressive towards conspecifics and females were more likely to approach heterospecifics.

The second experiment examined more fine-scale female choice decisions using playbacks of reproductive calls to females in states of induced estrus. This experiment was conducted on captive red deer at Redon Experimental Deer Farm in France managed by the Institut National de la Recherche Agronomique. Females individually entered an enclosure with speakers placed in opposite corners playing alternating call bouts of red and sika male deer (one species from each speaker). The behavioral responses monitored include the duration of movement toward particular speakers as well as time spent in proximity zones close to individual speakers. Significant movement towards one speaker or more time spent in the proximity zone of one speaker was

considered preference for that speaker. Preliminary results indicate that females overall prefer conspecific calls but not in all cases.

In summary, these playback studies highlight a possible role for vocal behavior in hybridization between red and sika deer through a potential weakened reproductive barrier between red females and sika males. However, further work is planned to investigate the physiological response of female red deer to conspecific vs. heterospecific calls as well as sika responses to similar vocalizations.

Keywords: *Cervus elaphus*, Red deer, *Cervus nippon*, Sika deer, hybridization, communication, sexual selection, playback experiments, behavior, reproduction

Social structure, interactions, reproduction and associated movements, of the endangered Huemul at Torres del Paine National Park, Chile

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There have been limited number of research studies done on the Chilean Huemul (*Hippocamelus bisulcus*) and all have been short-term. This is a 10-year study of the Huemul population at Torres del Paine National Park, in southern Patagonia, which has allowed us to cover a wide range of information regarding the socioecology of this endanger deer. In here, we present only the objectives related with topics of social structure, social interactions and some aspects of reproduction of this Huemul population, and their associated movements. Huemuls were observed in a central area of the sector Grey, within the park. When animals were located, natural marks or body scars, coloring of body and face, and shape and length of antlers in males were used to identify them. We also ear tagged 16 fawns (10 females and 6 males) from 2002 to 2008. Once a group was located we collected behavioral information of all members by using focal observations in periods of 15 minutes per each individual, and recorded the time spent on each activity during that period. Observations were done from 08:00 to 20:00 hrs, with an average of 5 hrs per day (range = 1 to 10 continuous hours). We identified six categories of Huemul groups: a) *family groups*, a male and a female, with or without yearlings and fawns; b) *solo females,* with or without fawn/yearlings, can be the female pre/post-parturient or a transient one; c) *solo male*, can be the family male in his home range or transient males looking for one of their own; d) *solo yearlings*, temporarily or definitively out of their family group; e) *pair of yearlings*, usually transients; and f) *mixed group*, usually groups of more than four individuals from both sexes and different ages during the fall/winter. The family groups were observed throughout the year, usually remaining in the same area. Females gave birth in late Oct or early Nov. It was usual for these females to become solitary when giving birth, remaining isolated for a couple of weeks following the birth of the fawn. We observed, in marked females, that the first mating was when they were about 16 months of age, and gave birth when they were 2-years old. We were not able to determine the age of the first mating for males. The group home range varied from 269 to 366 ha. The Huemul movements are associated with the seasons and reproduction cycles, which also implies group changes. During the rut, some solo males try to move into home ranges of resident males.

In fall-winter some family groups moves away from their home range (i.e., groups from higher grounds move to valleys). During the birthing season, pregnant females stay alone for short periods of time, while yearlings are temporarily expelled from their natal group, so the resident male is seen alone. All these changes during critical periods generate some antagonism among the Huemuls. In conclusion, the Huemul population at Torres del Paine National Park is structured in six categories of groups, which maintain agonistics interactions throughout the year, but particularly in the reproduction season (rut and parturition) when some antagonism is seen, especially among the males. There are four associated movements: within the group home range, seasonal, reproductive,

and social movements of the yearlings.

Keywords: Huemul, *Hippocamelus bisulcus*, social behavior, family groups, Torres del Paine, reproduction, movement

HEALTH AND DISEASE

Novel methods of spread of prion disease in deer: A preliminary investigation of the use of gut piles and carcasses by Wildlife. A summary of findings 2006-2008

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Remote cameras are used world-wide to address a variety of research and management objectives for wildlife species that are often difficult to find track, and capture. They are an effective tool for investigating wildlife behavior, as well as documenting species presence. There has been a growing awareness of the impacts of diseases on free ranging wildlife populations in North America. An awareness of various bacterial and viral wildlife diseases has always been present. Recent outbreaks of diseases such as Bovine TB and CWD in wild deer and elk populations, and the publicity associated with Avian Flu," Mad Cow Disease", and Lyme disease has heightened the public's awareness of wildlife diseases. The discovery and subsequent identification of Chronic Wasting Disease (CWD), a Transmissible Spongiform Encephalopathy (TSE) affecting cervids, in 1978 created a virtual panic among professional wildlife managers, hunters and the general public. The authors as well as other wildlife biologists, captive cervid owners and veterinarians have made casual observations of wild and captive deer associating with carcasses or gut piles of deer and other animals. A review of wildlife literature reveals scattered references of White-tailed deer feeding on meat protein. Michigan DNR records note observations of deer on South Fox Island in Lake Michigan feeding on dead alewives washing up on the beaches in the 1960's. Recent studies of predation of ground nesting birds, deer have been noted as casual predators. Whitetail deer have been reported as nest predators of Piping Plovers, as well as Clay colored and Vesper Sparrows. In their efforts to identify possible wild cougars on the Monongahela NF and the land- between- the-lakes, Kentucky, researchers from the Eastern Cougar Foundation noted whitetail deer were by far the most numerous species to visit camera traps baited with meat and scents.

In Mid August, 2006 volunteers were solicited to participate in a project to monitor wildlife activity around gut piles and carcasses of White-tailed deer and other wildlife left in the woods. In 2006-2007 58 volunteers participated and 42 volunteered for 2007-2008. Volunteers where located in 19 states including Florida, Georgia, Illinois, Indiana, Iowa, Kentucky, Massachusetts, Michigan, Minnesota, Missouri, Montana, New Jersey, New York, North Dakota, North Carolina, Oregon, Rhode Island, Texas, Virginia, Wisconsin, and Washington plus the provinces of Ontario and Saskatchewan.

During 2006-2007 and 2007-2008 approximately 70 volunteers actually had an opportunity to place cameras on gut piles or carcasses. Deer were photographed at (63%).of the sites. Observations and photos indicated four sites with deer actually feeding on carcasses during the study. It is also known that both TB and CWD produce organisms/ or transmittable material which is accumulated in the internal organs, connecting tissue and mesentery fat. It is also been shown that organisms and agents such as BTB and CWD are transmitted to the soil as a carcass decomposes transmission appears to be viable for a period of years. TB organisms have been shown to readily infect other deer feeding in an effected area 6 months after initial infection. Recent findings indicate the prions

causing CWD can persist in the environment for long periods of time and retain their infectious capabilities. Initial observations from this study seem to indicate gut piles and carcasses remaining in the woods could be a vector for Bovine TB and CWD. This may be especially important in relation to localized deer populations. We suggest further investigation is necessary to document the extent of risk these sites have as a potential transmission vector to other deer and other wildlife species.

Keywords: White-tailed deer, *Odocoileus virginianus*, chronic wasting disease, spongiform encephalopathy, prion, epidemiology, feeding behavior, scavenging, disease transmission, management

The role of Red deer, Fallow deer and Roe deer in the epidemiology of bovine TB and paratuberculosis in Southwestern Europe: Consequences for disease control

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The range and abundance of deer species is increasing throughout Europe, with consequences on conservation, agriculture and health. In the Iberian Peninsula in southwestern Europe, red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*) are abundant and generally widespread game species. In addition, red deer are also increasingly farmed for trophy production. Deer populations and deer health are receiving more attention in the last decade due to their potential implication in the maintenance of diseases such as bovine tuberculosis (bTB, caused by members of the *Mycobacterium tuberculosis* complex) and paratuberculosis (infection by *Mycobacterium avium paratuberculosis*, MAP). This presentation describes the current status and recent trends of deer populations and of deer mycobacterial diseases, and analyzes the consequences of these for disease control at the wildlife – livestock interface.

The current status of the three Iberian deer species is varied. Red deer are widespread, with generally higher densities in the southern half of the Peninsula. Fallow deer display a scattered distribution with locally high abundances, often in fenced hunting estates. Roe deer show an increasing abundance gradient from the south, with more scattered presence and generally low to moderate densities, to the north with higher densities and a more continuous presence. All three species are still expanding both in range and in population density. Bovine TB has been diagnosed in the three deer species. However, while high infection prevalences are recorded in red deer and fallow deer from southern Iberia, only sporadic cases are known in roe deer or in red deer from the northern third of the Peninsula. In red deer, data based on lesion monitoring suggests stability in the temporal prevalence trends. Paratuberculosis has received far less attention than bTB in Iberian deer populations. Apparent prevalence as inferred from serum antibody tests is higher in red and fallow deer than in roe deer. Contact with MAP is more uniform throughout the Peninsula, particularly where contact with domestic ruminants is high. Infection with *M. bovis* interferes in the use of serology to detect contact with MAP in red and in fallow deer. Clinical cases of paratuberculosis have been recorded only sporadically, in situations of overabundance and close contact with livestock, or in deer farms.

At least in areas with high deer density (>10 per sq km), red deer and fallow deer contribute to the maintenance of the *M. tuberculosis* complex and need to be integrated in the bTB control strategies. Regarding paratuberculosis, the limited control measures in domestic ruminants, along with the existence of other potential wildlife reservoirs such as the wild rabbit (*Oryctolagus cuniculus*), suggest that the relevance of deer species in MAP maintenance is less relevant, except for game farms and very specific wildlife settings. Ideas on disease surveillance and control in Iberian deer will be discussed. The authors acknowledge funding from EU grant TB-STEP and Plan Nacional research grant AGL2008-03875.

Keywords: tuberculosis, Red deer, *Cervus elaphus*, Fallow deer, *Dama dama*, Roe deer, *Capreolus capreolus*, paratuberculosis, Wildlife, pathology

Research progress in control of paratuberculosis of farmed deer

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Introduction

The prevalence of *Mycobacterium avium* subsp *paratuberculosis* (paratuberculosis (Ptb)) on New Zealand deer farms has increased over the past 20 years. Over 60% of deer farms and approximately 45% of farmed deer may be infected. In known infected herds the median clinical disease rate is 1.0%, outbreaks can have mortality rates of up to 21.5% and the highest disease incidence is in deer younger than two years of age. Reduction of carcass weight is 5 and 17.6% for young and adult deer, respectively. Thus, Ptb is significant economic risk for deer producers. While test (Elisa) and slaughter has been proposed, its application and effectiveness require validation. Management and environmental factors may contribute to expression of clinical disease, and with vaccination, may contribute cost-effectively to disease control. This paper summarises recent research into risk factors and vaccination as potential control measures for clinical Ptb in young farmed deer.

Risk factors for clinical disease

A case: control study of 174 farms included 107 Ptb culture positive. Environmental and management factors were screened for association with clinical disease incidence rate of above or below the median (0.4%) in the year of study, and multiple regression analysis performed on significantly associated variables. Variables significantly associated with a clinical Ptb incidence of 0.4% or more were: the presence of yearling beef cattle (OR = 4.55; $p<0.01$) and irrigation of the deer fenced area (OR = 5.18; $p = 0.01$). Variables significantly associated with a clinical Ptb incidence of less than 0.4% were: an increasing presence (i.e. proportion of total grazing days on deer pasture) of sheep relative to other livestock (OR = 0.94; $p = 0.03$), the purchase of yearling deer in 2005 (OR = 0.29; $p = 0.02$) and an increasing percentage of breeding hinds more than five years of age (OR = 0.06; $p <0.01$). Further research has replicated the apparent risk of cattle grazing and refined the apparent protective effect of sheep only to flocks without clinical disease.

Vaccination

A randomised controlled trial to assess the efficacy of Silirum® vaccine in the control of Ptb in deer involved six commercial deer herds in the South Island of New Zealand in 2008 that had a history of a high incidence of clinical Ptb. The study involved 1671 deer vaccinated at 3-4 months of age, and grazed together with 1664 unvaccinated control deer. Vaccine efficacy for reducing the incidence of clinical disease was 60% (95% CI: 5-83%, $p=0.04$). *Mycobacterium avium* subsp. *paratuberculosis* was isolated from 59/125 (47%) of vaccinated and 68/123 (55%) of control animals ($p=0.5$). Average daily live weight gain did not differ between vaccinates and controls.

At slaughter between 9 and 15 months of age, 1.4% of vaccinates and 4.5% of controls had enlarged mesenteric lymph nodes consistent with Ptb, a relative risk of 0.34 (95% CI: 0.19-0.54, $p<0.001$). There has been no negative impact on carcass quality or saleability. At approximately 12 months of age cross-reactivity to the mid-cervical test for bovine tuberculosis was 24% in vaccinates and 13% of controls of age. Ancillary tests were adequate for confirming cross-reactivity.

Conclusion

These studies have identified some biologically plausible management and environmental factors potentially contributing to expression of clinical Ptb and have demonstrated that a currently available vaccine reduces the incidence rate and economic impact. These factors need to be validated in a commercial farming environment.

Keywords: paratuberculosis, control, risk factors, vaccination, management, Johne's disease, pathology, production

The cost of subclinical diseases of farmed deer

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Introduction

Clinical disease is visible and its production and economic effects are transparent. By contrast, subclinical growth, reproduction, antler production effects, along with the economic cost of unnecessary treatment (e.g. anthelmintic) or supplementation (e.g. trace elements) are covert and remain widely unknown or misunderstood. This paper reviews existing and new data, largely from the Massey University Deer Research Group, on the subclinical effects of common diseases of farmed deer and cost-effectiveness of prevention.

Diseases and subclinical effects

Leptospirosis. Reduced liveweight at 12 months of 3.7kg (Study 1, individual animal) and up to 6.5kg (Study 2, herd mean), respectively, has been shown. This was preventable by vaccination, which was cost-effective at seroprevalence >19%, providing returns of ~260% and 500% at 50% and 100% seroprevalence, respectively. Weaning percentage in known pregnant hinds in vaccinated herds was up to 10% higher than in non-vaccinates, yielding a return on vaccination of up to ~700%, above a break-even reproduction gain of 1.3%. An estimated 67% of NZ deer farms could achieve a cost-effective response to vaccination.

Paratuberculosis (Ptb). National surveillance data showed a 2.4 (5%) and 17.6kg (25%) carcass weight reduction in young and adult deer, respectively, with enlarged mesenteric lymph nodes, a marker of high risk of Ptb infection. Economic cost was $20 and $140 per deer, respectively. While pregnancy and weaning rates were eight and 4.8 percentage points lower in herds with clinical Ptb, confounding due to management cannot be ruled out. The role of vaccination in reducing subclinical losses requires further investigation.

Trace elements. Risk of reduced growth of young deer occurs when serum copper (Cu) falls below approximately 3µmol/L. Up to 10kg live weight reduction was reported in spring when herd mean serum Cu was below 1 µmol/L. Supplementation would be cost-effective when live weight was reduced by ~0.7kg. Unnecessary supplementation causes economic loss. Iodine deficiency reduces reproductive efficiency and growth on some farms and supplementation is cost-effective if losses exceed 0.4%. There is inadequate data on Selenium to quantify subclinical effects.

Internal parasites. There is substantial evidence of the subclinical effect of parasites on growth, but not on other production outcomes. Reduction of daily weight gain (DWG) in field studies of clinically normal parasitized untreated young deer ranged from 59 – 90g/day (46-87%) before treatment criteria were reached, compared with treated controls. Data from one larval challenge study showed a dose-dependent DWG reduction of up to 66g/d (42%), in deer averaging 67 lungworm and 783 gastrointestinal parasites. A second challenge study showed a reduction of 60, 150 and 189g/d in deer given “low”, “medium” and “high” larval challenges resulting in a mean of 2438, 6899 and 11882 worms at slaughter after three, four and five weeks, respectively.

There is likely significant economic wastage due to common anthelmintic treatment of adults, and possibly over-use in of young animals. Better diagnostic data is needed for rational decision-making

about efficient and cost-effective internal parasite control.

Conclusion

Studies summarised demonstrate economically significant potential losses due to subclinical disease and its management or mis-management. Vaccination, supplementation and preventive treatment are potentially effective in reducing or eliminating production and economic loss, but only if used appropriately. Subclinical effects of other diseases remain to be investigated. Risk assessment, and application of sensitive diagnostic measures assist farmers in making rational decisions about management of subclinical disease.

Keywords: subclinical disease, paratuberculosis, leptospirosis, trace elements, copper, parasites, economic effects, production

National surveillance for paratuberculosis in New Zealand farmed deer herds: Development of a database

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Introduction. *Mycobacterium avium* subsp *paratuberculosis* (MAP) is prevalent in farmed deer globally. This paper describes an initiative undertaken in New Zealand to assist its understanding, control and prevention.
Aim. Develop a national database for paratuberculosis surveillance in New Zealand farmed deer.
Method. Meat inspectors characterise and electronically record lymph nodes (LN) as "normal" or "enlarged" (ELN) based on mesenteric LN (MLN) circumference (above or below 60mm) or gross lesion resembling tuberculosis (*M. bovis*). Validation involved physical and bacteriological investigation. Carcass origin, identification, age, weight and grade, and LN size are now recorded from every animal from deer slaughterhouses. Data are forwarded regularly to a central database for checking, collation and production of a quarterly descriptive monitoring report. Data are also available for research purposes.
Results. For validation, four meat inspectors recorded 1287 MLN demonstrating 99% specificity and 25% sensitivity for categorisation of ELN. Ongoing supervision is improving sensitivity. Screening showed 95% of 130 ELN were culture and/or histology positive. Of 240 randomly selected "normal" MLN from 57 farms, 45% (corrected for regional distribution) were MAP positive. Herd prevalence was estimated to be >60%.
Data from 1.5 million deer from 2,100 farms were recorded from January 2007 – December 2009. Enlarged LNs were recorded from 27% and 3.6% of farms in the South and North Islands, respectively during that time. Those farmers subsequently received advice, including availability of a network of specialised veterinarians. The prevalence of slaughtered deer with ELN was 1.08% and 0.01% in the South and North Islands, respectively, in 2009. Mean carcass weight of young (<2-years), and older deer with ELN was 2.4 (5%) and 17.6kg (25%), respectively, less than that with normal LN, confirming a significant ($p<0.001$) subclinical effect. There was no seasonal difference in prevalence of ELN in older deer but in younger deer, prevalence was higher in summer than winter ($p<0.001$).
Preliminary research of grazing associations and ELN farm prevalence was achieved by combining this data with a national livestock database. The prevalence ratios (PR) for ELN in deer when grazing with beef cattle alone or sheep and beef combined were 1.33 ($p=0.027$) and 1.41 ($p<0.001$), respectively, compared with grazing deer alone. By contrast, the PR of ELN on deer farms with sheep only was lower than for those with deer alone (PR 0.76, $p=0.064$).
Conclusion. This database is unique. It allows spatial and temporal tracking to quantify and monitor the disease, its effects and influence of potential control measures such as vaccination. Modelling is under way to further validate sensitivity of diagnosis. Data has been used to inform industry, heighten awareness of paratuberculosis and target high prevalence herds for intensive

control programmes. It provides a cost-effective means to identify herds of various disease and infection strata for case, case-control, longitudinal and intervention studies to better understand the disease and its control. The database is supported by the deer industry and is now owned and managed by an independent processor-funded agency (Johne's Management Limited) to guarantee access by industry and its secure and ongoing management.

Keywords: paratuberculosis, surveillance, database, slaughterhouse, lymph nodes, pathology, *Cervus elaphus*, production

Surgical amputation of a foreleg in Andean Deer (Huemul, *Hippocamelus bisulcus*) in Coyhaique, Chile

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Hippocamelus bisulcus is the southernmost distributed neotropical deer. Currently, in Chile separated populations exist between the regions of Bio-Bio and Magallanes and Antarctic Territory. Different, past and recent factors, like habitat loss and fragmentation, poaching, cattle sickness contagion, and domestic and feral dogs attacks have driven the species into the Endangered category.
Frequently, the Agriculture and Livestock Service (SAG) in Aysen of Region, offers veterinarian attention to Huemul individuals found with a variety of injuries of different origins. For these medical situations, a professional team from SAG and the private sector work together.
The first clinical case of surgery on a juvenile male Huemul found in the wild with an exposed fracture of the distal radius growth plate (Salter-Harris type I fracture) with concurrent fracture of the distal epiphysis of ulna, is presented in this work.
Anaesthesia, reduction of articular loosening and surgical technique protocols used in two surgeries are described. The first procedure was performed to reduce the fracture. Next, in order to save the individual's life, the foreleg was amputated.
Finally, the evolution and excellent recovery of this male in semi-captive conditions are described, as well as the possibility of him still serving well as a reproductive male.
This surgery represents a landmark amongst other surgeries conducted on this species. Hence the importance of presenting this work to scientific community, veterinarians and the institutions and people devoted to the species' conservation.

Keywords: surgery, anesthesia, amputation, limb, Huemul, *Hippocamelus bisulcus*, ex-situ

Tick-borne and infectious diseases in free-ranging White-tailed deer in Northeastern Mexico

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Tick-borne diseases as well as viral and bacterial diseases such as bovine viral diarrhea virus (BVDV), and brucellosis, may be linked to wildlife serving as reservoirs or vectors for spreading. Some results indicate that White-tailed deer (*Odocoileus virginianus*) are exposed to *Leptospira,* bovine viral diarrhea virus (BVDV), and infectious bovine rhinotracheitis (IBR) organisms; therefore, deer may play a significant role in the transmission of bovine diseases. *Rhipicephalus* ticks are the vectors of cattle fever, which are distributed worldwide. White-tailed deer (*Odocoileus virginianus*) are important secondary hosts for the cattle fever tick species *Rhipicephalus (B.) annulatus* and *Rhipicephalus (B.) microplus.* The objectives of this study were to 1) determine the frequency of *Babesia bovis* and *B. bigemina* and the prevalence of antibodies to them, 2) identify possible risk factors for bovine babesiosis in White-tailed deer, and 3) determine the prevalence of antibodies against brucellosis, leptospirosis, IBR, and BVDV in White-tailed deer in three northeastern states of México. Deer (n= 521) were captured from helicopter using a netgun on 15 ranches covering 62,114 ha in the states of Coahuila, Nuevo Leon, and Tamaulipas during the spring of 2004. Deer were physically restrained while collecting blood samples. Information about characteristics and management practices of ranches was also collected. Samples were tested for *B. bovis* and *B. bigemina* by nested polymerase chain reaction (n-PCR) (the primers for *B. bovis* identified the gene *Rap-1* and *B. bigemina* were specific primers) and by an indirect immunofluorescence antibody test (IFAT).
Logistic regression methods were used to test the association between management factors and the dependent variable of positive samples to nested-PCR or IFAT test. Chi square was used to measure the strength of association between seropositivity and management factors and to obtain odds ratios (OR) at 95% confidence intervals (CI). Nineteen samples (4.2%) were positive to *B. bigemina* and 6 (1.7%) to *B. bovis* by nested-PCR. Serological testing showed 59.9 % (n=274) of deer sampled were positive to *B. bovis* and 5.5% (n=25) to *B. bigemina* antibodies. The logistic model varied with different dependent variables. With positive nested-PCR and *B. bigemina* as the dependent variable, three factors were associated: habitat (presence of brush and exotic grasses; odds ratio (OR), 3.3;l confidence interval 95% (CI), 1.3-8.5), grazing system (continuous grazing OR 4.0; CI, 1.3-12.2) and tick treatment frequency (3-4 months; OR 7.0, CI 1.4-34.3; 5-6 months; OR, 11.0; CI, 1.9-62.7; >6 months; OR, 4.6; CI, 0.9-23.3). The prevalences of antibodies against leptospirosis, IBR, BVDV

and brucellosis were 5.6%, 41.1%, 63.5%, and 0.0%, respectively. Chi square ($P < 0.01$) analyses indicate that ranches with high fence had higher prevalence for IBR (45%) and rotational grazing systems (48%) increased the prevalence of IBR in White-tailed deer. Ranches that had high densities of deer (1 deer/10 ha.) had more positive cases (51%) of IBR, and ranches with low deer density (1 deer /15 ha.) had more positive cases to BVDV (70%). Ranches with cattle and deer had higher prevalence (66%) of BVDV in deer versus ranches with only deer. Ranches where brush and exotic grasses were abundant had higher prevalences of antibodies against IBR and BVDV. These findings suggest that White-tailed deer may act as a reservoir for the two bovine *Babesia* species and that White-tailed deer may be important in the epidemiology of babesiosis. However evidence is not available to support that White-tailed deer are or are not likely to be a host that could complete the transmission cycle of *Babesia*. White-tailed deer were exposed to *Leptospira,* IBR and BVDV organisms and deer and cattle may share disease agents when cohabiting northeastern of Mexico.

Keywords: diseases, White-tailed deer, cattle wildlife interactions, Mexico, parasites, pathology

Hypoderma larvae in Roe, Red and Fallow deer in Hungary: Prevalence, intensity and seasonality

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Hypoderma spp. are causing subcutaneous myiasis with serious economic losses in many domestic and wild Eurasian ruminants. *H. diana* can develop in species of the Cervidae family, such as roe deer (*Capreolus c.*) red deer (*Cervus elaphus*) and fallow deer (*Cervus /Dama/ dama*) in Central Europe. *H. actaeon* is known to be restricted to red deer.
The **aim** of this study was to examine the prevelance and intensity of *Hypoderma* larvae in these three deer species

Materials and methods

Deer shot in Western Hungary during the hunting season (mainly November to February) of 2007/08, 2008/09 and 2009/10 were examined. The carcasses were investigated in Öreglak Venison Processing Plant, SW-Hungary. Larvae were detected, counted and collected for laboratory examinations after the skinning process. In the course of the three seasons altogether 435 roe deer, 215 red deer and 197 fallow deer were inspected.

Results and discussion

In *roe deer* the overall prevalance was 52.6 % with 37.1 larva as mean intensity. Hypodermosis was very common (89.9 %) in does older than 1 year of age, but very rare (3,2 %) in kids. Prevalence was not significantly different among seasons.
In *red deer* we detected 67.9 % overall prevelence with 106.8 mean intensity. In regard of age and sex hypodermosis seems to be a general parasitosis in stags and yearling females contrary to adult hinds and calves. Intensity was lowest in hinds. About one-third of the hinds seemed to have aquired immunity. Both *H. actaeon* and *H. diana* larvae were often present in yearlings: 81.8 % and 59.1 %, and parallel infection was 45.5 %.
In *fallow deer* hypodermosis seems to be less common than in the other two deer species: 18.8 % overall prevalence with 6.5 larvae/infected host. Most of the larvae found were not alive, but died as young 2nd instar. None of the fawns was infected.
H. actaeon is believed to be strongly host-specific (stenoxen) to red deer. Contrary to it we found 25.6 % and 23.9 % prevalences, respectively in yearling and older *roe deer* during the examinations between mid-February and mid-March 2009. and 2010. Then in mid-March 2010 we found lavae of *H. actaeon* in 8 out of 9 *fallow deer* too. By our opinion the explanation of it is that 3rd stage larvae of this species can be found earliest in mid-February when most of the *H. diana* larvae have left the host. There is about 5-6 weeks difference between the phenology of the two species, including the adult female flies' oviposition.

Conclusions

Hypoderrmosis is very common in roe and red deer older than one year of age. In *fallow deer* probably most of the larvae die during their migration in the host and the remainder die as young 2nd instar under the skin.
The *oviposition* of the flies coincides differently with the birth periods of these three deer species.

Red deer calves are born earliest, mainly in early May, therefore 20-40 % of them will be infected. A much lower proportion of the roe kids (born in late May) will be exposed to oviposition, and no female flies survive by early June, when the fallow doer give birth.
Because of the lack or low level of infection in the first year of life, the prevalence and intensity is very high in yearling roe and red deer. In adult red deer stags the rut behaviour causes immundepression, resulting in high prevalence of *Hypoderma* spp as well as greater intensity.
Chemotherapy is contraindicated in the autumn in terms of venison-contamination. For the purpose of industrial quality deer leather only hides of the youngest age groups (calves, fawns and kids) are useful after careful inspection.

Keywords: hypodermosis, Roe deer, *Capreolus capreolus*, Red deer, *Cervus elaphus*, Fallow deer, *Dama dama*, prevalence, intensity, epidemiology

Genetic resistance and susceptibility to paratuberculosis in Red deer (*Cervus elaphus*)

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Objective: To investigate genetic resistance to paratuberculosis in red deer.
Introduction: Paratuberculosis (Johne's disease), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is common on many red deer farms in New Zealand and elsewhere. It causes deaths and subclinical production losses in 8-27 month old deer, as well as occasional deaths in older deer. Analysis of records of clinical disease and serological testing on a large New Zealand red deer farm showed evidence for a strong sire effect on resistance (R) and susceptibility (S) to paratuberculosis, with a heritability of 0.27.
Methods: Twenty four randomly selected paratuberculosis seronegative red hinds were inseminated with frozen semen from two stags showing R or S characteristics and they produced 18 offspring (9R and 9S). One wapiti x red (wapx) animal was also included. At four months of age these 19 deer each received an oral challenge dose daily for four days with 109 cfu MAP. The posterior jejunal lymph nodes of all the deer were surgically biopsied at 4 and 13 weeks post challenge (pc). Samples of small intestine and mesenteric lymph nodes were also collected at euthanasia from clinically affected animals 18-25 weeks pc and at slaughter from surviving animals 49 weeks pc. These samples were cultured for MAP and examined histopathologically. The deer were closely monitored, weighed and blood-sampled throughout the 49 week study.
Results: One animal died of misadventure. Four deer (2S, 1 R and wapx) developed typical clinical cases of paratuberculosis (loss of weight and condition, diarrhoea) and were euthanised 18-25 weeks pc.
The antibody levels in these four deer were climbing rapidly at the time of euthanasia, with titres of >100 ELISA units (EU). By contrast, four apparently resistant offspring were seronegative (titres <50 EU) throughout the study. The remaining animals fell into two distinct groups: four animals (3R, 1 S) that plateaued at 100-150 EU for 12-24 weeks pc and then declined to <50 EU by Week 47, and six animals (1R, 5S) that rose to a peak of 200-250 EU around Week 30 and remained high or declined to 100-150 EU by Week 47.
At Week 4, two S animals had mild histopathological lesions in the biopsied lymph node, but by Week 13 all 18 animals had mild to severe lesions. The four clinically affected deer had severe intestinal and lymph node lesions when euthanased.
At Week 49 the remaining animals, which were all apparently healthy, were euthanased. Their histopathological lesion status fell into two groups: eight animals (7R and 1S) that had no or very mild lesions, and six animals (1R and 5S) that had severe lesions. Lesions severity improved in R animals from Week 13 to Week 49, while it remained the same or got worse in S animals.
The nine R animals had significantly less severe lesions than the S animals (Chi-square P= 0.018).
At slaughter (Week 49), all the deer were MAP culture positive, except one R animal (which had been culture positive at Week 4 and 13).

Conclusion: These results confirm that resistance to paratuberculosis in red deer is highly heritable. Despite having randomly selected dams, the majority of the offspring of the S sire had significantly more severe disease than those of the R sire. At least one of the R offspring appears to have completely cured itself, and went from having obvious mild lesions of paratuberculosis affecting the mesenteric lymph node 12 weeks after challenge to having no signs of disease 9 months later.

Keywords: paratuberculosis, pathology, genetic resistance, Red deer, *Cervus elaphus*, production

Diseases in free-ranging Red deer introduced to Patagonia: Implications for native ungulates

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The red deer (*Cervus elaphus*) invasion in southern Latin America has been continuing for over a century. They are established in all mayor habitats between about 34-55°S, occupying >51,000 km² from sea level to > 2,450 m, and reaching densities of 100 deer/km² in ecotones and 40-50 deer/km² in steppes. Their distribution and daily and seasonal movement patterns raise concerns over their potential epidemiological role (e.g. aftosa, brucellosis and tuberculosis). Here we report diseases encountered in red deer and evaluate the potential impact on native ungulates. Females (>1 yr old) were randomly collected between 1991-2009. These together with radio collared deer that died naturally were necropsied in the field (n=522) to determine body condition, fat reserves, morphometry, reproductive state and gross pathology; nine of these females were examined exhaustively, additionally including the GI tract, in a national pathology laboratory. Disease prevalence is only indicated where a minimum number of individuals had been examined accordingly. No ectoparasites were present upon gross examination (n=21, 1993; n=9, 1995; n=43, 1998). *Fasciola hepatica* was encountered regularly with prevalences of 50% (n=20, 1995), 9% (n=43, 1998), and 13% (n=45, 1998-2009). *Taenia ovis krabbei* was identified, and taenid larval stages were found attached to liver, omentum and peritoneum, identified as *Cysticerus tenuicollis*, at a prevalence of 8% (n=12). *Ostertagia* sp., *Bunostomun* sp., and *Dyctiocaulus* sp. were found at a prevalence of 75%, 25% and 13%, respectively. Testing for aftosa in 1994/95 was negative (n=41). A likely case of tuberculosis was found in 1996, with substantial parietal pleural adhesions, granulomatous mediastinal lymph nodes, nodules covering the costal pleura, and additional lesions in the intestinal tract, including lymph nodes covering the rumen. Campylognathia was found in 2 females and 5 males, which were all otherwise healthy individuals.
In conclusion: general absence of ectoparasites is common for wild ruminants in cold temperate Patagonia, as reported for guanaco (*Lama guanicoe*), unless they are in contact with domestic livestock. Infection with cosmopolitan *F. hepatica, C. tenuicollis*, and *Ostertagia* sp. as well as exotic *T. o. krabbei,* are considered trivial for otherwise healthy cervid hosts. *Bunostomun* sp. and *Dyctiocaulus* sp. are also generally trivial, but can be a concern when deer are weak for other reasons. In Chile, *Ostertagia*, *Capillaria*, *Bunostomum*, *C. tenuicollis and Dictiocaulus* sp. have all been found at low levels in free-ranging exotic deer. These same parasites were lacking in an allopatric population of native Huemul (*Hippocamelus bisulcus*), where instead *Moniezia*, which is considered non-significant to its hosts, was found in 3 of 9 examined Huemul. Most of these parasites, generally considered commensals, are common in livestock. Considering that red deer have been in Patagonia coexisting with livestock for >100 years, both red deer and livestock play roles in the epidemiology of the various diseases they share. For Huemul, their sympatry with livestock is commonplace, whereas with red deer it is exceptionally rare, occurring in <2% of known populations. In one of these sympatric populations, livestock would generally be the

determining epidemiological factor, given that for each Huemul there were 1.2 red deer compared to 25.2 livestock. We can speculate that for other native ungulate populations, the primary factor regarding contagious diseases will also be feral and free-ranging livestock. Research, conservation and management efforts should be directed towards finding appropriate solutions, including livestock herd health programs or restriction of free movements, particularly if diseases are shown to impact recruitment of threatened natives. Livestock, being routinely researched and inspected at slaughtering time can provide a proxy for diseases afflicting co-existing ungulates.

Keywords: Red deer, *Cervus elaphus*, Huemul, *Hippocamelus bisulcus*, pathology, parasite ungulates, cattle, livestock, disease, parasites, Patagonia, Argentina, health

A review of paratuberculosis vaccination in Red deer (*Cervus elaphus*)

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Introduction: Paratuberculosis, (Johne's disease) caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), causes fatal disease and subclinical losses in all ruminants including deer. There are four main tools for Johne's control: management, test-and-slaughter, genetic selection and vaccination. This paper reviews vaccination as an option.

Review: Veterinary input is required to evaluate the cost/benefit value of vaccination against paratuberculosis for livestock and involves assessing risk factors, farm type, management factors, production losses, costs of vaccination and tuberculosis (Tb) history/status for cattle and deer. Ideally a paratuberculosis vaccine should prevent clinical disease, subclinical infection, shedding of MAP in faeces, and should not induce cross reactions with Tb tests or cause injection site abscesses. Unfortunately no current paratuberculosis vaccine can achieve all of these aims, largely due to the nature of MAP, its intracellular niche in macrophages and its ability to evade the immune system.

A number of commercial paratuberculosis vaccines have been developed for livestock, but are generally crude bacterins that contain killed MAP and mineral oil adjuvants. In sheep and cattle such vaccines are generally effective at reducing clinical disease, subclinical disease and shedding of MAP in faeces. However, they do not prevent infection, but they induce cross-reactivity with Tb tests in cattle, cause moderate injection site lesions and pose some risk to operators from self-injection.

Summary of studies: Over the last 10 years a number of commercial and experimental vaccines have been studied in red deer in New Zealand. Generally they caused moderate injection sites reactions that resolved to small nodules in 9-12 weeks, mixed antibody and cell-mediated responses and interference with Tb testing.

A recent efficacy study of Silirum™, a killed MAP and mineral oil adjuvanted vaccine, which involved experimental challenge of young red deer with MAP, showed non-significant reductions in clinical incidence in vaccinates (1/39) vs controls (4/40), fewer gross lesions in gut lymph nodes at slaughter (1/38 vaccinates vs 7/36 controls) and reduced severity of histopathological lesions in vaccinates.

A field randomised controlled trial of Silirum vaccine, involving 6 deer herds that had a history of clinical paratuberculosis, showed a significant reduction in confirmed clinical disease in vaccinates (7/1671 vaccinates vs 18/1664 controls) and less gross lymph node pathology at slaughter (18/1267 vaccinates vs 56/1249 controls), although there was no difference in mean slaughter weight between groups. MAP was isolated from 47% of faecal samples from vaccinates vs 55% of controls (p=0.5). In these MAP-infected herds, vaccination caused more false positive reactions to a single intradermal bovine Tb test in vaccinates (44%) compared to controls (23%), while a comparative Tb skin test (CCT) cleared almost all animals. Subsequent application of a Tb ELISA test cleared all vaccinates, while 2 controls remained positive.

A recent experimental infection study showed that vaccination with Silirum in deer that were subsequently infected with bovine Tb caused 64% false negative reactions to the CCT.

Vaccine use: Currently in New Zealand, Silirum is only used to vaccinate young deer destined for slaughter as yearlings, before they are eligible for Tb testing. It is not used in breeding replacement animals because it reduces Tb test specificity, and is contraindicated in Tb-infected or Tb-risk herds because it reduces Tb test sensitivity.
Conclusion: Silirum vaccination was shown to significantly reduce clinical disease and gross pathology in young deer and may be useful on deer farms where the clinical incidence is high enough. Vaccination appeared to have little impact on MAP transmission.
Further work is required to develop tests that can distinguish between MAP infection, MAP vaccination and Tb infection in deer.

Keywords: deer, paratuberculosis, vaccination, pathology, *Cervus elaphus*, production

High prevalence of *elaphostrongylus cervi* and *setaria cervi* in Pannonian Red deer populations

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Red deer (*Cervus elaphus L.*) is the most important game animal of Hungary due to the outstanding quality and distribution, furthermore its role in game parks has recently grown. Therefore, to collect information on the health status, important diseases and parasites of the stock is of basic importance. Among parasites, *Elaphostrongylus cervi* (CAMERON, 1931) is unique, since it migrates in the central nervous system (CNS) of the host during the postembrional development. To examine these biological features of this nematode, as well as its seasonal and host-age related characteristics were the main goals of this work.

Detailed sample and data collection was conducted in Pannonian red deer populations, especially in Somogy, Baranya, Tolna and Zala counties in four consequent hunting seasons, between 2005-2009. To detect the occurrence conditions of *E. cervi* in the cranial cavity, the heads of 370 shot red deer were examined. To check the worm migration, the vertebral column of 20 deer were opened. Visible alterations in the CNS were histologically sampled. The occurrence and position of the adult worms were determined in 32 deer carcasses during the venison processing. The host age was estimated by the dentition and teeth wear. For statistical analysis, the *Quantitative Parasitology 3.0* and *SPSS 11.5* programme was used at 5% significance level ($p \leq 0.05$). In special cases, Chi^2 and *Fischer tests*, *bootstrap test*, and *Pearson correlation analysis* were applied for the detection of possible interrelationship among data.

E. cervi was found in the ***cranial cavity*** (n=370) with a 29% prevalence and 1.18 mean intensity, respectively. These values were 42.9% and 2.26 in calves (n=238), 6.8% and 1.33 in yearling hinds (n=44) and 4.5% and 1.5 in adult hinds (n=88), respectively. The large-scale variations (16.7-100%) of prevalence in some samples during the winter season are worth mentioning. The worm burden of calves ranged between 1 and 12. The distributions of worms were strongly aggregated in the host samples. The discrepancy index was 0.749 in calves, 0.922 in yearlings, and 0.951 in adult deer. These values varied between 0.653 and 0.837 in the different winter seasons in calves.

In the examined ***vertebrate canals*** of calves, *E. cervi* occurred with 31.6% prevalence and 3.89 (1-11) intensity, whereas another nematode *Setaria cervi* (RUDOLPHI, 1819) occurred with 31.6% prevalence and 1.43 (1-2) intensity. In 3 calf vertebrate canals (15.8%), both nematodes were present simultaneously, however this was rare (1.9%) in the cranial cavity. The higher intensity values of both species and the high prevalence of *S. cervi* in vertebrate canals as compared to the values found in the cranial cavity needs attention. Presumably the worms occur in a higher number when migrating in the long vertebral canal as compared to their occurrence in the cranial cavity.

Adult *E. cervi* specimens were found in 11 out of 32 (34,4%) carcasses during the venison processing. Gross ***alterations*** were usually restricted to the inner surface of the *dura mater*. These were sometimes petechias, but more often yellow fibrinous tickenings and/or colorless, translucent pear-like protrusions. In hinds older than 2 years, wart-like growths could be often seen on the surface of the soft meninx (*pia mater*) at the joining lines of the brain hemispheres. Through histological examination of the surface of the *dura mater,* lympho-histiocytic infiltration was found. This was found usually diffused, and only rarely focal. In the first case, plenty of lymphoid follicles

were observed. In some cases worm cross sections were visible either singly, or in lines as a string of pearls in the subdural space, and rarely in the neighbouring brain tissue. Surrounding the worms, fresh or earlier petechias, focal necrosis (malacia), gliacell proliferation (gliosis) and lymphohistiocytic (lh) granulomas occurred. Gliosis and lh-granulomas indicate cellular immunity. Based on the lack of clinical symptoms and the effective acquired immunity we conclude that there is a well-balanced relationship between the red deer (final host) and *E. cervi*.

Keywords: *Elaphostrongylus cervi*, *Setaria cervi,* Red deer, cranial cavity, vertebral canal, prevalence, intensity, pathology

Pampas deer (*Ozotoceros bezoarticus*) parasitological assessment in Uruguayan populations

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The Pampas deer (*Ozotoceros bezoarticus*) is one of the most endangered neotropical mammal in Uruguay. The species decline was owed to human activities, currently two isolated populations survived. The parasitological load is a good indicator of the health of the species and the ecosystem. The objective of this study was to determine the parasitological composition fauna and estimate the parasite load in the two Uruguayan populations. We surveyed the copro-parasite load in both populations analyzing the effect of seasonality along the year, the micro ecosystem environment and the topography and also the livestock that inhabit in the same enclosures. The taxonomic assessment was based in adult individuals obtained from the necropsy post mortem examination in individuals found dead in the field. The genera of the endoparasite recorded through the morphological eggs, larvae and or adults were *Trichuris, Capillaria, Strongyloides, Fasciola, Paramphistomum, Moniezia, Haemonchus, Ostertagia, Trichostrongylus, Oesophagostomum* and Coccidias ooquiste. In a total of 829 fecal samples analyzed we estimated the load of grastrointestinal parasites which ranged from <100 to 2000 EPG (eggs per gram). Within this range, 74% of the samples contained <100 EPG, 11% had 100 to <200 EPG, 6% had 200 to <300 EPG and the remaining 9% had 400 to 2000 EPG. Our results showed that the parasitological community in the Pampas deer is strongly correlated with environmental conditions, topology, micro-ecosystem and the land use through the effect of the livestock load.

Keywords: Pampas deer, *Ozotoceros bezoarticus*, pathology, parasites, parasitic load

Gross anatomy of the heart of the Pampas deer (*Ozotoceros bezoarticus*, Linnaeus 1758)

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The Pampas deer (*Ozotoceros bezoarticus*, Linnaeus, 1758) was a widespread species, originally distributed in the open grasslands (Pampas) across eastern South America, from 5° to 41° S. However, habitat loss, unregulated hunting, competition with cattle, and transmission of cattle diseases have caused a drastic decrease in Pampas deer populations. The species is considered as Near Threatened by the IUCN, with small populations reported in Argentina, Brazil and Uruguay.
According to our knowledge, the macroscopic anatomy of the heart of the Pampas deer has not been described. To investigate this topic we dissected and described the hearts of fourteen Pampas deer of both sexes from a captive breeding station (Estación de Cría de Fauna Autóctona, Pan de Azúcar, Maldonado, Uruguay; ECFA; 34º3' S, 55º1' W; altitude: ~ 200 m). The cause of death of animals was not determined and the body condition was generally considered poor due to low fat reserves. The body mass (BM) of the males was 15.0 ± 3.4 kg (range 11.0-18.8) and that of the females 14.5 ± 2.4 kg (range 12.0-19.0). The hearts of the animals were dissected immediately after being found or frozen until dissection. The heart average weight was about 110 g (range 102 – 244 g, SD: 28.4). The pericardium partially covered the pulmonary trunk to 3.5 cm from its origin, and 3.5 cm of the aorta. The length from the origin of the pulmonary trunk to the apex was 11.0 cm. The circumference at the level of coronary groove was 15.1 to 23.0 cm (SD: 5.5). The craniocaudal length of the base was 4.8 cm. The paraconal interventricular sulcus ended by curving over the right ventricular border at 2.5 cm from the apex, and on the atrial face ended against the subsinuosal interventricular sulcus. The arrangement of the cusps and valves in the four major ostiums was similar to the sheep heart. In the right ventricle there were three papillary muscles. Septomarginal trabeculae were more complex than in sheep and observed in both ventricles. The thickness of the left ventricular wall was 1.8 cm and had two papillary muscles. The interventricular septum thickness was 1.4 cm at its thickest part. The main anatomical features of the heart of this deer were generally similar to small domestic ruminants. Our aim is to continue with studies based on measurements of different parts of the heart for comparison with other ruminants.

Keywords: anatomy, heart, dissection, circulatory system, Pampas deer, *Ozotoceros bezoarticus*

Identification and prevalence of rickesttsial agents (rickettsiales: anaplasmataceae) in Marsh deer (*Blastocerus dichotomus*), using serological and molecular methods

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Members of Anaplasmataceae Family (Order Rickettsiales) form a group of obligately intracellular gram-negative bacteria whose vectors are ticks and that can infect both animals and humans. The present work aimed to detect the presence of rickettsial agents DNA (*Ehrlichia chaffeensis*, *E. ewingii*, *E. canis*, *Anaplasma phagocytophilum*, *A. marginale* and *Neorickettsia risticii)*, by Polymerase Chain Reaction (PCR) in marsh deer (*B. dichotomus*) blood samples, as well as studying the prevalence of anti-*E. chaffeensis* and anti-*A. phagocytophilum* antibodies using Indirect Imunofluorescence Assay (IFA). The animals (n=143) were captured during the development of the Marsh Deer Project (1998 to 2002) in the flood area of the Porto Primavera Hydroelectric Power Plant, in Parana River, and divided in four sub-populations according to the local and moment of the capture, in areas denominated MS01, MS02 (Mato Grosso do Sul, before and after flood respectively), PX (Peixe's River, after flood) and AGUA (Aguapeí River, after flood). The frequency of seropositive animals for anti-*E. chaffeensis* and anti-*A. phagocytophilum* antibodies were 76,76% and 20,20% in MS01, 88,88% and 22,22% in PX, 88,88% and 5,55% in MS02, and 94,12% and 5,88% in AGUA, respectively. Sixty-one animals (42,65%) were positive for *E. chaffeensis PCR*, 38 (38,38%) from MS01, 4 (44,44%) from PX, 12 from MS02 (66,66%) and 7 (41,18%) from AGUA. Positive samples were sequenced, and showed 100% of similarity with samples of *Ehrlichia chaffeensis* from Argentina and United States (Genbank access EU826516.2 and AF416764.1, respectively). Seventy deer (48,95%) were positive for *A. phagocytophilum* genogroup PCR assay with 51 (51,51%) from MS01, 12 (66,66%) from MS02, 7 (41,18%) from AGUA, and 0 from PX. These sequenced fragments demonstrated 99% of similarity with samples of *Anaplasma platys* (Genbank access EU439943.1). Non –specific bands in *A. marginale* and *E. canis* PCR were observed, and conclusive results were not reached. Besides, sequence analysis didn´t reveal similarities with samples with these taxa. Sampled animals were negative for *E. ewingii* and *N.risticii* PCR. The results suggest that the Brazilian marsh deer has been exposed to *E. chaffeensis* and *Anaplasma platys*. Additionally, further studies should be done about ehrlichial infection epidemiology among Brazilian wildlife, vectors (ixodides) and the possibility of these agents to cause disease in humans.

Keywords: *Ehrlichia* sp., *Anaplasma* sp., *Neorickettsia* sp., Marsh deer, *Blastocerus dichotomus*, serology, PCR, parasite

Periodontal disease in relation to different fluoride levels in two separate Spanish Red deer populations

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The mandibles of 140 red deer (*Cervus elaphus hispanicus*) shot between 2001-2002 in the Sierra de Andújar Natural Park (n= 119) and National Park of Doñana (n=21), ranging in age from 5-8 years old, were studied in order to assess the occurrence of periodontal disease, its association with bone fluoride levels and the spatial variations in these separate populations of southern Spain. There were no significant differences in periodontal disease prevalences, (38.53 % and 33.33 %, respectively). Fluoride content, however was significantly lower ($P < 0.00001$) in animals from Sierra de Andújar Natural Park (249,54±170,884 mg F−/kg ash) than in deer from Doñana (691,523±507,996 mg F−/kg ash). A logistic regression model was carried out to test the influence of fluoride levels in each study area on the disease. There was a statistically significant relationship between both independent variables but the percentage of deviance in the disease explained by each model was of only 24,20%, indicating the presence of other influencing factors. In both populations the disease was associated with the higher levels of bone fluoride but these levels were much higher in Doñana (1,224.9±422.34 mg F−/kg ash in mandibles with periodontal disease, 423.83±291.39 mg F−/kg ash in healthy mandibles) than in the Sierra de Andújar Natural Park (353.48±201.84 mg F−/kg ash in mandibles with this disease, 188.56±112.69 mg F−/kg ash in mandibles without disease). Healthy levels of fluoride in one study area are the levels associated with disease in the other. These results show the necessity of research into the global mineral content balance and an improvement in knowledge of the possible deficiencies in certain minerals. Special care must also be taken in using fluoride as an indicator for monitoring diseases, the magnitude of environmental contamination or specific mineral imbalances.
This study was supported by the project P07-RNM-03087 (Consejería de Ciencia e Innovación de la Junta de Andalucía) and CGL-2008-00832 (Ministerio de Educación y Ciencia).

Keywords: Red deer, *Cervus elaphus hispanicus*, periodontal disease, fluoride, pathology

Blood chemistry values and hematologic characteristics of pudu (*Pudu puda*, Molina) in a semi-captivity population

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The southern pudu (*Pudu puda*) is one of the smallest deer in the world and is categorized as vulnerable on the IUCN Red List. It is an endemic species of the South American temperate rainforest of Chile and Argentina. The main threats to pudu are forest loss and fragmentation. In addition to habitat loss, pudus are apparently threatened by poaching and predation by domestic dogs. The aim of this study is to describe hematological values and plasma concentrations of biochemical variables in semi-captive pudus populations to create a database and generate reference limits that contribute to conservation.
This study was conducted in 29 pudus of different ages, 21 females and 8 males maintained under semi-captivity in province of Valdivia, Chile. Each animal was anesthetized (Xylacin® and Zoletil®) to obtain a blood sample that was collected into heparin to determine plasma concentrations (X ± SD) of total protein (78.1 ± 12.7 g/L), albumin (32 ± 4.2 g/L), urea (9.3 ± 3.2 mmol/L), creatinine (111 ± 23.9 mmol/L), cholesterol (1.7 ± 0.5 mmol/L), triglycerides (0.5 ± 0.2 mmol/L), glucose (10.3 ± 3.8 mmol/L), bilirubin (33.9 ± 19.9 mmol/L), lactate (6.5 ± 0.4 mmol/L), magnesium (1.1 ± 0.1 mmol/L), phosphorus (2.6 ± 0.7 mmol/L), calcium (2.3 ± 0.3 mmol/L), sodium (150 ± 5.9 mmol/L), potassium (4.0 ± 0.6 mmol/L) and chloride (120 ± 23.5 mmol/L). Also the plasma activity of amylase (***EC***. 3.2.1.1) (506 ± 242.1 U/L), lipase (***EC.***3.1.1.3)(23.3 ± 23.0 U/L), aspartate aminotransferase (AST, EC: 2.6.1.1) (151 ± 43.3 U/L), alanine aminotransferase (ALT, ***EC*** 2.6.1.2) (75.2 ± 21.6 U/L), gamma glutamyl transpeptidase (GGT, ***EC.*** 2.3.2.2) (67.8 ± 36.4 U/L), alkaline phosphatase (SAP, EC:3.1.3.1) (332 ± 297.6 U/L), creatine kinase (CK; EC:2.7.3.2)(386.3 ± 322 U/L) and glutathione peroxidase (GPx, ***EC*** 1.11.1.9) (330.6 ± 84.5 U/g Hb).
Another blood sample was collected into EDTA to determine the blood counts, hemoglobin, hematocrit (HCT), and protein and fibrinogen concentrations. The differential leukocyte count was performed on smears. The sickle shape was found in all samples. The erythrocyte count was 6,8 ± 1,7 x10^6µl in adults and 5.5 ± 1.1 10^6 µl in fawns, the HCT was 36.5 ± 3.7% in adults and 35.4 ± 2.8% in fawns, the hemoglobin was 139 ± 25 g/L in adults and 131 ± 11 g/L in fawns, protein was 69 ± 10 g/L in adults and 60 ± 3g/L in fawns. The leukocyte count was 8432 ± 2333 µl in adults and 9245 ± 2108 µl in fawns, absolute lymphocyte count was 6336 ± 1742 µl in adults and 7162 ± 2519 µl in fawns, the absolute neutrophil count 1815 ± 732 µl in adults and 1830 ± 905 µl in fawns, monocytes were 13 ± 42 µl in adults and 22 ± 49 µl in fawns, eosinophils were 265 ± 295 µl in adults and 118 ± 109 µl in fawns.
The values obtained will be form a database to establish reference limits that can be used in conservation programs for this specie.

Keywords: blood chemistry, clinical, hematology, *Pudu puda*

POPULATION DYNAMICS: REPRODUCTION AND MORTALITY FACTORS

Irruptive behavior of Sika deer: comparison of two contrasting populations

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A dominant paradigm of large herbivores is that following introduction to new range, or release from harvesting, the herbivore population at a low level will increase rapidly to a peak, followed by a crash, then recover to a lower density than peak abundance. Irruptive behavior is common in ungulate populations but is a complex and still poorly documented phenomenon. To test the irruptive paradigm, we have been monitoring two sika deer (*Cervus nippon*) populations and their habitats over 20 years; a deer population introduction to Nakanoshima Island (NKI) and a naturally colonizing deer population on Cape Shiretoko (CS) on Hokkaido, Japan. Two populations are contrasted as following points 1) origin: introduced population (NKI) vs naturally colonizing population (CS), 2) winter climate: warm and shallow snow (NK) vs cold, heavy snow, and floating ice (CS), 3) habitat: deciduous broad-leaved forest (NK) vs coniferous and broad leaved mixed forest and natural grassland. To monitor population changes, we used drive count method for NK herd and an air photographic count for CS herd. We monitored vegetation change to survey browse cover using fixed belt transect.

Both populations built to peak abundance, followed by a crash, which resulted in significant effects on the vegetation. However, there were marked differences in post-crash behavior between the two populations. Following the crash in 1984, the NKI herd increased again with a lower growth rate (rm = 0.15 for the period between 1964 and 1984 vs rm = 0.07 for the period between 1986 and 2000) and reached a higher peak of the population size in 2001 than the first irruption, while the CS herd showed oscillating behavior without decline in the peak abundance (1986 vs 2003).

On NKI, winter forages changed from dwarf bamboo, twigs and bark in the initial irruption to fallen leaves during post crash phase. On CS, dwarf bamboos disappeared in forest, however, remained on natural grassland, and were available for sika deer during winter. The NKI herd exhibited density-dependent changes in population parameters such as delayed sexual maturity, lower calf: female ratio, and lower body and antler growth as they exceeded carrying capacity in the initial irruption.

As a result of the irruption, there was a decline in both winter- and summer-range quality. Thus, competition for high-quality food among sika deer in the initial irruption could have been a limiting factor, whereas unlimited abundance of poor-quality forage (fallen leaves) permitted a slower growth to even higher density in the subsequent buildup. In contrast, the CS herd exhibited a high adult survival rate and calf: female ratio, and good antler growth, which indicated high quality of summer range. In addition, mortality patterns in crash years were also different between the

populations; for the NKI herd, mortality was co
Imposed of both sexes in all age classes throughout years, while for the CS herd, mortality was composed mainly of calves and adult males, but with few adult females. Although density-dependent resource limitation through interaction with winter climate was the important limiting factor of peak density for both populations, the carrying capacity differences, and lags between summer and winter might generate different fluctuations in numbers for the two populations.

Keywords: carrying capacity, irruptive paradigm, population dynamics, Sika deer, ungulate, *Cervus nippon*

Demographics of Pampas deer (*Ozotoceros bezoarticus*) populations in Uruguay

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The Pampas deer, *Ozotoceros bezoarticus* (L. 1758), is a medium size deer inhabiting open grasslands of South America from 5 to 41º latitude. In Uruguay, two isolated populations represent two newly described subspecies. *O. b. arerunguaensis* occurs in the Salto Department in the northwestern part of the country and *O. b. uruguayensis*, occurs in the Rocha Department in eastern Uruguay. .*O. b. uruguayensis* inhabits agricultural regions (primarily livestock and rice). Our objectives were to i) estimate population size, ii) assess demographic and social structure; iii) evaluate seasonally variations in the group composition and size, and iv) analyze population trends over the sample period. We conducted population censuses from 1996 to 2004. The number of animals, sex and age class per enclosure was estimated by road counts using a pick-up truck through fixed routes with an extension of 8 km with an average duration of 3 hours. In the sample period we conducted 44 censuses and there were counted a total of 2149 groups. Population size averaged 152 animals (54 min; 351 max; SD: 78) on the 8,000 ha study area and the sex ratio was 0.61 males per female. The demographic structure was 55% adult females; 34% adult males; 10% young; and 1% fawns. The average recruitment rate was 0.11. Group composition indicated that 19% occurred as individual animals, 19% occurred as pairs, 43% occurred as groups of 3 - 9 individuals, and groups larger than 10 individuals represented 17% of occurrences. A higher proportion of males (42%) than females (35%) conform mixed groups, 23% of females were in single sex groups, whereas only 15% of males occurred in single sex groups. Group size varied seasonally, on autumn and winter there was a decrease on small groups, when the basic units with two - four individuals (41% of individuals) was assembled in feeding groups. The trend in the population size was stable in the sample period. This population shows a typical structure of K-strategists, where the population number is near the carrying capacity of the habitat and that the population is characterized by having medium age individuals and a low recruitment rate.

Keywords: demography, group size, group composition, Pampas deer, Uruguay, *Ozotoceros bezoarticus*, behavior

Current distribution and threats for an endangered population of Pampas deer (*Ozotoceros bezoarticus*) in Santa Fe province, Argentina

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Pampas deer (*Ozotoceros bezoarticus*) is one of the eight species of Argentina's native cervids and it is considered the most endangered mammal in this South American country. Nowadays, only four populations remain, which are located in Corrientes, Buenos Aires, San Luis and Santa Fe. These populations are highly isolated from each other and they live in different ecological regions. In addition, it is remarkable that Santa Fe has the smallest and the most threatened Pampas deer population of Argentina.
According to the data that were collected from 1997 to 2009 in 36 field trips, we analyzed the current distribution of Pampas deer and assessed the importance of potential mortality sources using two analysis tools: 1) inspection of the areas to find indirect records, dead animal carcasses or skeletal remains and predator traces; and 2) information obtained by interviews conducted with qualified local people.
In accordance with the current distribution data, a map was carried out. Distribution is limited to 500 km^2 in an extent of occurrence and 225 km^2 occupancy area. Eight areas were identified with a higher occurrence of individuals whose environmental characteristics were similar (all of them were situated close to Palo Azul - *Cyclolepis genistoides* - groves).
We detected 30 cases of mortality, on the average 16 $\pm$ 1.8 individual per year (range from 0 to 6) attributed to: poaching (63.3 %), floods (20 %) and drought (16,6%). The results for mortality distinguished by sex were: female (53.3 %), male (40 %) and two unidentified specimens (6.6 %). According to ages the results were: 90% adults, 6.6 % under one year old and 3.3% fawns under three months. What is more, 19 individuals were hunted, of which 57.8% were adult females and 36.8% adult males, whereas 5.2% were males under one year of age.
The area where Pampas deer live in Santa Fe is one of the few natural areas in this province and the extensive cattle ranching is an economical activity that helps to preserve the Pampas deer's environment. However, the advance of the agricultural frontier is rounding up the deer population and could be considered as a potential threat for this kind of cervids due to the habitat change.
Besides, dwellers with more than 30 years living in the studied area mentioned diseases transmitted by cattle as another possible cause of mortality.
To conclude, field data suggests that hunting could be the main threat to Pampas deer in this region, whereas flooding and drought cycles affect this population in a smaller proportion. Poaching was

the main cause of death, althought it tends to decline over the period analyzed. Nevertheless, this practice has not been completely eradicated.
The distribution of mortality records shows more deaths due to flooding in poorly drained areas, especially in the north. Moreover, it is important to emphasize that 84.2% of hunting episodes are concentrated in the southern part of Santa Fe. This identification of critical areas could help direct efforts to fight against each one of the main threats that endanger Pampas deer population in Santa Fe province.

Keywords: Pampas deer, *Ozotoceros bezoarticus*, distribution, mortality causes, threats, conservation, Santa Fe Province

DEER REPRODUCTION

Suggestions for an adaptive management approach in reindeer husbandry

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Reindeer and caribou *Rangifer tarandus* (L) inhabit strongly seasonal habitats in the circumpolar north. Pasture quality is a major limiting factor for population density and herd productivity. Long-term climatic cycles, combined with unpredictable variations in weather and various external disturbances, change the quality and availability of the pasture and complicate the management of the grazing resource. Knowledge of how the grazing conditions vary over time is crucial in order to adapt the size and structure of the herd and the use of land to the available resource.
Adaptive management provides a supporting framework for management decisions in dynamic resource systems, where uncertainty has to be taken into account. A fundamental part is the frequent monitoring and interpretation of ongoing changes in the resources in relation to their use. This information, paired with knowledge and experience among resource users and decision makers, is used to develop and evaluate models of the system. Thereby, the theoretical understanding of the system is improved, enabling predictions of ongoing trends in the resource system and effects of management actions.
We have investigated possible indicators of changes in the resource bases for the winter and snow-free seasons, respectively.
In winter ranges, lichens are important food for reindeer, often constituting between 30 and 80% of the total diet. Mat-forming lichens are perennial and susceptible to grazing and trampling, and it can take decades for a lichen carpet to recover after intense grazing. Monitoring changes in the lichen resource could thus be a key part of an adaptive management approach. We suggest a simple method, based on repeated lichen height measurements in important habitats, for monitoring these changes.
Pasture conditions during the snow-free period vary substantially over space and time, and long-term changes are difficult to monitor. However, these pastures strongly affect growth and gain of body reserves of the reindeer and are reflected in their body condition in autumn. Autumn is the common time for reindeer slaughter. We have investigated the use of slaughter records (carcass mass and classifications based on the muscle and fat content of the carcass) as indicators of changes in the snow-free pasture. Yearly repeated monitoring of body mass of known individuals in the live herd is suggested as an additional indicator of general body condition in the reindeer herd.
Preliminary results from theoretical evaluations of the indicators are presented, with the aim to define critical levels of change in the indicators and the need of precision in their estimates. The evaluations were done using a dynamic model of the interaction between pasture resources and the reindeer.

Keywords: lichen, body condition, adaptive management, reindeer, Rangifer, production

Summer habitat utilisation and calving behaviour of Red deer hinds farmed in New Zealand high-country environments

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Until recently red deer were generally farmed in New Zealand under intensive lowland systems. However, competing land-use (mainly dairying) over the last decade has been associated with a general trend towards extensive farming of deer in high-country (sub-alpine) rangelands of the South Island. Extensive deer production systems have involved specialised fencing of large tracts of land of highly variable topography, soil structure, erosion risk and vegetation type. However, little is known about how farmed deer at high population densities (100-200 deer/km2) utilise and impact high-country range. Such knowledge is important for both the efficient production of livestock, and for the preservation and sustainability of these unique and often fragile systems.

Global Positioning Systems (GPS) technology was used to determine spatial distribution, resource utilisation and behaviour of farmed red deer hinds during the calving and immediate post-calving periods on an extensively managed high-country station (Haycocks Station, Landcorp Farming Ltd) in the Southland region of the South Island. The study site was three adjoining 200-250 ha paddocks that ranged in altitude from 420-700 m ASL and contained a complex vegetation mosaic of naturalised grasses and native tussocks/subalpine scrub. A vegetation map was constructed from a recent aerial photograph and validated through extensive ground-truthing. Over the course of two seasons the primary study paddock ('Rough Gully') of 250 ha was stocked with 700 pregnant hinds (2.8/ha) from late October and destocked post-calving in late May for calf weaning. In Year 1 seven hinds had been fitted with GPS collars programmed for hourly positional recording and in Year 2 eight hinds were fitted with collars programmed for half-hourly positional recording. Collars were recovered during yarding for calf weaning and positional data downloaded.

All GPS collars sustained considerable impact and water damage in Year 1, with only 5 logging positional data for variable durations from 3-7.5 weeks. In Year 2, following design changes, 7 units functioned for the full duration, logging 89-99% of all possible positioning recordings. Analysis was conducted on the data for 12 hinds in relation to vegetation and weather variables. Core Occupational Areas (COA's), representing 80, 50 and 20% of the occupancy for each deer, were derived from all positional data.

Within 3 weeks of collar fitting three hinds in Year 1 and one hind in Year 2 migrated permanently from the primary study paddock to adjoining paddocks of calving hinds. The 50% COA's for all hinds ranged from 7.7-27.7 ha, with hinds showing strong individualisation in their choice of topography and vegetation zone. Naturalised pasture was the preferred habitat for 9 hinds, with tussock preferred by 3 hinds. Heavier hinds selected lower, flatter zones dominated by pasture, while smaller hinds tended to select higher altitudinal zones dominated by tussock.

A simple Markov modulated regression model was used to identify time (and hence, site) of calving based on five pre-defined stages of peri-parturient movement behaviour previously described in the literature for red deer. Estimated or 'behavioural' birthing dates, characterised by a very abrupt reduction in hind movement, ranged from 8-30 November and 18 November-3 December for Years

1 and 2, respectively. Regression slopes within stages relating movement to an overall diurnal pattern suggested that movement within each stage except the actual birthing period was entrained to a pronounced diurnal pattern. A spline analysis of movement following the estimated birth events showed a gradual increase in mobility over the first 10 days of the calf's life that was consistent across hinds.
Selected birthing sites were mainly grass and tussock dominated areas, with only one hind selecting a scrub dominated zone.
Over the entire duration of positional recording there was clear evidence that diurnal patterns of distances travelled and altitudinal occupation were moderately influenced by weather variables such as solar irradiation, wind speed and rainfall. However, inclement weather avoidance over the summer/autumn months was relatively low-key, mostly involving subtle altitudinal changes rather than sheltering in scrub zones or lee slopes.
It is concluded that definable behavioural characteristics of red deer hinds over the calving period can be discerned from GPS positional data that may be useful in characterising habitat utilisation and birthing behaviours in environments in which direct observation of such events would not be possible.

Keywords: Red deer, *Cervus elaphus*, production, extensive farming, home range, GPS, reproduction

Does carcass suspension technique influence reindeer (*Rangifer tarandus tarandus*) meat quality attributes?

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Background

Variation in meat tenderness and techniques developed to minimise this variation, have been investigated in several animal species over a long time period. It is well known that the conditions during rigor development (*e.g.* muscle pH decline, temperature/pH relationship and carcass treatment) are very important in controlling meat tenderisation. Therefore, carcass suspension techniques have been studied for beef where the variation in tenderness is considered to be the main reason for consumer dissatisfaction. The purpose of this study was to evaluate the effects of two different carcass suspension techniques on tenderness, color and water-holding capacity (WHC) in reindeer meat.

Methods

A total of 8 reindeer steers (ages 3 – 6 years old) from the AFES Reindeer Research Program herd (University of Alaska Fairbanks, Alaska, USA) were used in the study. The reindeer were transported to Delta Meat and Sausage (Delta Junction, Alaska) and slaughtered under USDA inspection. Carcasses were split along the spine and sides randomly allocated to pelvic suspension (hanged in a butcher hook through the obturator foramen) or normal Achilles tendon suspension (control treatment). From all 16 carcass halves meat samples were collected from the loin (*M. longissimus*), inside (*M. semimembranosus*) and shoulder (*M. triceps brachii*) for sensory evaluation and measurements of shear force (tenderness). In addition, the loin samples were evaluated for meat color at 1 day post slaughter and for purge (WHC) after vacuum-packaged chilled storage (+2 °C) for 1week, 2 weeks and 3 weeks.

For tenderness measurements, loins were cooked in bags submerged in boiling water until the internal temperature of the sample reached 75°C; then samples were immediately cooled on ice. Ten 1 cm x 1 cm cross-section slices (bites) were prepared from the cooked sample with the muscle fibres running longitudinally along the slice. Each sample was then sheared with the long axis of the fibres running perpendicular to the blade, using a Meat color was registered; CIE L* (lightness), a* (redness) and b* (yellowness) values were measured (D65, 10º) in triplicate on a freshly cut 2.5 cm thick steak after 2 h of blooming at +2 °C using a Minolta Chroma meter (CR-300, Osaka, Japan). For purge measurements, loins were removed from their packages, dabbed dry with a paper towel and then weighed. Purge loss was calculated as the difference in the weight of the loins before and after storage expressed as a percentage of the original weight of the loins.

A descriptive test, conventional profiling, was carried out by a selected and trained sensory panel (University of Alaska Fairbanks) consisting of seven members. The meat was cooked in a conventional oven at 150°C to a core temperature of 70°C.T Samples were placed in plastic cups coded with three-digit numbers and were served to the panel members in randomised order, at room

temperature and in two replicates. The following attributes were evaluated; tenderness and juiciness. An unstructured continuous line scale from 0 (low intensity) to 10 (high intensity) was used.

Results

No significant effects of carcass suspension technique were found for reindeer meat color and WHC. However, shear force values for loin samples from pelvic suspended carcasses were lower ($p=0.001$) compared with Achilles tendon suspended carcasses, an effect that was not found in the other meat cuts (inside and shoulder). The trained panel judged loin and inside samples from pelvic suspended carcasses to be more tender ($p=0.001$) while no effect was found in the shoulder samples. Juiciness was not affected by carcass suspension.

Conclusions

Results from the pelvic suspension trial demonstrated that this technique indeed improved tenderness in the most valuable cuts from the reindeer carcass (striploin and inside).

Keywords: reindeer, Rangifer, meat, carcass suspension, tenderness, production

MANAGEMENT

Consevation status of deer species in India with special reference to aspects of ecology of musk deer in Uttrakhand Himalayas

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There are 8 true deer species and 5 species of Musk Deer and 1 species of Mouse Deer are identified after taxonomic changes and new report from India. They are spotted deer (*Axis axis),* Hog Deer *(Axis porcinus porcinus),* Sambar (*Rusa unicolor),* Eld Deer (*Rucervus eldi*), Swamp Deer (*Rucervus duvauceli*), Red deer (*Cervus elaphus*), Barking Deer (*Muntiacus vaginalis*), Leaf Deer (*Muntiacus putaoensis*), five species of Musk Deer (Moschus chrysogaster) belongs to family moschidae and Mouse Deer (*Moschiola indica*). Status, aspects of ecology and threat to conservation of Himalayan Musk deer (HMD) were studied at 13 sites in Uttarakhand Himalayas (2500-4500m at sea level) from October 2003 to December 2006. Morning trails were traversed for abundance estimation. Data on current status, and habitat use of HMD and associated species were collected by sampling of pellet groups. The relative abundance of HMD in terms of encounter rate (group/km) and pellet groups density was highest in Saukherk (1.3 /km), (58.8±8.9/ha). The Discriminant Function analysis (DFA) was performed to discriminate between the preference of habitat of musk deer and it's sympatric species. The DFA is able to discriminate between the musk deer and associated species by selecting the medium altitude, high shrub density, distance from human habitation and low herb density. Principal Component Analysis (PCA) and One way Analysis of variance were performed to determine the habitat use pattern of HMD in Uttarakhand Himalayas. The two PCs accounted for 23.7% of variation in data matrix. Conservation threats were investigated and management recommendations have been suggested.

Keywords: conservation, Himalayan Musk deer, census, pellet transect, habitat use

Multiple impacts of introduced deer on native forests in Northwestern Patagonia, Argentina

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Euro Asiatic deer were introduced in northwestern Patagonia ca. ninety years ago and among them red deer (*Cervus elaphus*) became invasive and currently inhabits forests, steppe, wet meadows and riparian habitats. I present the main results about direct and indirect effects of introduced deer as well as their synergistic effects together with other disturbances on forest communities. Also current knowledge gaps that should be filled for a better understanding of the relationship between introduced deer and native forests in northwestern Patagonia are identified. The studies were carried out on Isla Victoria, the largest island of Nahuel Huapi Lake, which is covered mainly by forests of southern beech *Nothofagus dombeyi* and conifer *Austrocedrus chilensis* with several subdominant tree species and dense understory vegetation. Additionally, Isla Victoria presents one of the oldest and largest histories of exotic plant and animal introductions in the area, which makes this island ideal for evaluating introduced deer and native forest interactions.

Deer in forest areas in NW Patagonia seem to be intermediate feeders, being woody species the bulk of year-round diet, with graminoids and herbs increasing during spring and summer. The overall effect is that introduced deer at high densities alter the structure, abundance and composition of native forest communities. Dominance of spiny and non-selected species and the decrease of highly selected species characterize the understory. Exotic deer strongly inhibit growth in height of the native dominant conifer, *Austrocedus chilensis*, facilitating the invasion of the exotic conifer, *Pseudotsuga mensiezii*. By contrast, the impact of exotic deer on the abundance of seedlings and saplings of the dominant tree species is unclear, with much variation observed. Many of these impacted species are of ethnobotanical importance to the native Mapuche people, being mostly used for medicinal and food purposes. At ecosystem level the available evidence shows that after seven years of deer exclusion, there were not differences in physical, chemical and biological soil properties, likely due to the buffer effects of volcanic soils. In addition, introduced deer interact with natural and anthropogenic disturbances such as wind-storms, forest decline and salvage logging modifying plant succession.

Much information about deer impacting forests in NW Patagonia comes from observational and comparative studies and to a minor extent from experimental studies. Furthermore, these studies were carried out in a few types of forest communities with high deer densities. Thus, further research requires more experimental studies involving a broader range of deer densities and diverse plant communities.

Keywords: introduced deer, *Cervus elaphus*, temperate forest, Patagonia, impact, invasive species

Physical differences among Mississippi deer populations: Genetics or environment?

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There has been considerable debate as to the relative contribution of genetic and environmental factors on the physical development (e.g., body mass, antler size) of White-tailed deer. Although answers to these questions would have important management implications, empirical studies are lacking. We took advantage of a natural experiment to examine the performance of similar genetic stocks in different geographical regions of Mississippi. During the restoration of deer in Mississippi (1931-1965), the Leaf River Refuge was a major source of stock for trapping and transplanting. The descendants of these deer are currently found statewide. We used genetic analysis based on 17 microsatellite DNA loci to confirm the genetic similarity of deer at 6 sampling sites to Leaf River deer. We then compared mean body mass and antler size of deer harvested at the 6 sites to Leaf River. We found clear differences among populations in body mass of yearling (1.5 yrs) and adult (>2.5 yrs) does (range: 2.3-6.4 kgs). Furthermore, antler size of mature (>2.5 yrs) bucks in 4 of 6 populations differed from Leaf River (range: 14-20 Boone & Crockett inches). Due to the nature of the data, we are not able to identify the precise source of the physical differences among populations. However, the presence of biologically meaningful differences among these populations 30-50 years after restoration suggests that factors other than genetic ancestry (e.g., habitat and soil quality), play an important role in the physical characteristics of White-tailed deer populations.

Keywords: White-tailed deer, *Odocoileus virginianus*, environment, nutrition, genetics, body mass, antler size, regional trends

The impact of cervid grazing on berry and mushroom production in Lapland

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Cervids eat both berry producing dwarf shrubs and mushrooms. How this affects the production of berries and mushrooms is less well known. In the Nordic countries picking these products have substantial recreational and commercial value to local people. We studied the impact of reindeer (*Rangifer tarandus*) and moose (*Alces alces*) on berry production of *Vaccinium* and *Empetrum* shrubs as well as on production of mushrooms in three different areas in Finnish Lapland in 2006, 2007 and 2009 by using exclosures and the reindeer fence on Finnish-Russian border.
Of the studied shrubs *Empetrum* (crowberry) was most common and also the one with most pronounced and consistently positive response to grazing. Both the number of berries (m-2) and the size of the berries were bigger in grazed plots in the most productive study area where cervids had a substantial impact on deciduous sapling canopy. At the oldest exclosures the biomass of *Empetrum* berries in grazed plots was 10-times as high as in the ungrazed exclosures in 2007. In this area also the other species (*Vaccinium* spp.) produced more berries in grazed conditions. On the other hand in the same area in unscarified stands, where there were no deciduous saplings on either side of the fence, there were no significant differences in berry production. On the Russian border (there are reindeer only on the Finnish side), where the main impact of reindeer is reduction of ground lichens in old unproductive pine forests, there were no statistically significant differences in berry production. In the mountain birch treeline conditions in the northernmost area *Empetrum* produced berries in grazed plots about twice as much as in ungrazed exclosures, but the other species did not respond. All shrub species grew higher in ungrazed plots in all sites, but the impact of cervids on their above ground biomass was mostly to same direction as the impact on berries. It seems that the positive indirect impacts of cervids via microclimate and competitive interactions are more important to the shrubs than the direct negative impact of deer feeding on them. Since *V. vitis-idaea* was just ripening at the time we collected data we could also observe that it was ripening earlier in grazed plots. Thus, cervids also affected plant phenology through the grazing induced changes in microclimate.
Also the impact of cervids on mushrooms differed depending on their impact on deciduous trees. In the pine heaths on Russian border all mushroom groups were more common in the grazed plots and the number of species producing fruiting bodies was higher in grazed plots. The biomass of fruiting bodies of all species combined was three times higher in grazed plots. In exclosure sites with deciduous saplings *Cortinarius* spp. and *Leccinum* spp. were more common in ungrazed plots. In these exclosures protection from grazing quadrupled the total mushroom biomass, mostly due to the large *Leccinum* fruiting bodies. It seems that the indirect impacts following the changes in tree or lichen layer due to grazing are the most important factors affecting the mushrooms. It's important to note that several species of these fungi are mycorrhiza of deciduous trees (including *Leccinum*). It's also known that disturbance to soil and litter layer may induce the fungi to produce fruiting bodies. Thus, the impact of trampling might be important, especially in the areas where reindeer has removed the protective lichen layer.

Keywords: reindeer, overabundance, fungi, vaccinium, Empetrum, impact, density

Attempts to reintroduce Chinese Water deer to Shanghai

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Chinese water deer *(Hydropotes inermis inermis)* is an endangered species native to China, and categorized as UV in IUCN red data book from 2009. The distribution of the water deer in China used to extend to Liaodong Peninsula, North China plains and the shores of the lower reaches of the Yangtze River and adjacent lake areas, with a region from latitude 28º to 42ºN and the eastern limit at longitude 110ºE. However, the population of the water deer has declined rapidly and its distribution is now limited and fragmented only to the coastal areas of Jiangsu Province, Zhoushan Archipelago of Zhejiang Province, and Poyang Lake areas in Jiangxi Province.

In order to know how to determine a conservation strategy, the cytb, D-loop and 12SrRNA of mtDNA were sequenced from samples collected noninvasively from Zhejiang, Jiangsu and Jiangxi province. The results show that Chinese water deer have high genetic diversity and the population in Jiangsu has the highest. The population from Jiangxi should be classified as an Evolutionarily Significant Unit and is worthy of conservation consideration. There is no clear divergence between the populations from Zhejiang and Jiangsu province. Another conservation suggestion is reintroducing this animal to the area where Chinese water deer existed before to enlarge and restore the population.

Assessing the feasibility for reintroduction of the Chinese water deer were based on literature research of deer populations in the past, survival condition, investigation of public attitude and local condition. The water deer disappeared in Shanghai only about 100 years ago, and most surveyed local people would like to invest towards the deer's reintroduction. We have tried to reintroduce the deer to suburb area of Shanghai and aim to recovery Chinese water deer in Shanghai.

The project selected an enclosure in Shanghai first to establish a breeding population for later releasing. Twenty-one Chinese water deer have been released into a Park on the round-the-city greenbelt area between rural and urban Shanghai in 2007, after breeding and monitoring of this population for 2 years, the deer have adapted to climate conditions of Shanghai and the population grew to more than 70. We have selected two typical habitats to try to release some cohorts with radio collar to more open areas around the city and trained them to adapt to the habitats condition in Shanghai in 2009. One place is the wood land near the Huangpu river, another is Shanghai Binjiang Forest Park, a Suburban Park in the estuary. In the present year, we planed to release some deer to the Nanhui wetland of the estuary in Shanghai with GPS collar. We hope the deer can reproduce without human intervention and recover natural populations step by step.

It is a test for reintroducing mammals, it need monitored continuously to evaluate the strategy.

Keywords: Chinese Water deer, *Hydropotes inermis inermis,* reintroduction, ex-situ, conservation, Shanghai

Do resource enhancement and increasing White-tailed deer (*Odocoileus virginianus*) densities interact to degrade habitat?

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Supplemental feeding is a common White-tailed deer (*Odocoileus virginianus*) management practice in North America; particularly in semiarid portions of the species' range. Availability of nutritious feeds may alter selection of natural forages by White-tailed deer; however, it is unclear how feeding and increasing deer densities impact vegetation. We hypothesized that increasing deer densities and consumption of nutritionally balanced pelleted feeds by deer result in increased foraging pressure on palatable plants, reducing their abundance. Our research was conducted on 2 ranches in southwestern Texas, USA. We constructed six 81 ha enclosures on each ranch (12 total enclosures). Each enclosure was surrounded by 2.5-m-tall woven wire fences. The experimental design was a randomized, complete block with 2 blocks and a factorial array of treatments consisting of 3 deer densities and 2 supplemental feeding treatments. White-tailed deer were added or removed twice per year so that enclosures supported deer densities that were low, moderate, or high relative to average densities in the region. During 2005-2009, densities based on biannual estimates using cameras averaged 14 ±1 deer/81 ha (mean ± SE), 27± 2 deer/81 ha, and 46 ± 2 deer/81 ha for low, medium, and high density enclosures, respectively. Nutritionally balanced pelleted feeds were provided *ad libitum* to one of each pair of similar densities on each ranch; deer in the other member of a pair of densities were not provided feed. Vegetation sampling was conducted during 2004-2009. Twenty 50 m transects were placed within each enclosure. Forb, grass, cacti, and browse biomass were estimated in forty 0.25 m^2 plots/enclosure during spring and summer. Biomass was harvested in 20 randomly selected plots/enclosure to correct estimates to wet mass; samples were then dried and estimates were corrected to dry mass. Percent canopy cover of forbs and sub-shrubs was estimated in sixty 20 x 50 cm quadrats/enclosure during June. Percent canopy cover of shrubs was estimated in twenty 30-m line intercepts/enclosure. Data were analyzed using repeated measures analysis. Vegetation biomass and canopy cover estimates were dependent variables in analyses; main effects in analyses were deer density (low, medium, and high), feed treatment, and sampling date. Sampling dates were analyzed separately when density or feed x sampling date interactions were significant ($P < 0.05$). Browse and cacti biomass during spring and summer were similar ($P > 0.05$) among deer densities and feeding treatments, although they varied among sampling dates. Deer density, feeding, and year of sampling had interacting ($P = 0.039$) effects on forb biomass during spring. During spring, forb biomass tended to be similar among deer densities except during 2009 when forb biomass tended to be greater ($P = 0.05$) in low density enclosures than in enclosures with higher deer densities. Averaged across feeding treatments and sampling dates during summer, forb biomass tended to be greatest ($P = 0.087$) in low density enclosures, with 61% greater biomass in low than in high density enclosures. Forb biomass was similar ($P > 0.05$) between feeding treatments during spring and summer. Percent

canopy cover of browse, cacti, and forbs was similar ($P > 0.05$) among deer densities and between feeding treatments, averaged across sampling dates. Our preliminary results indicate that high deer densities reduce biomass of forbs, which are more palatable to deer than browse, cacti, and sub-shrubs. Supplemental feeding neither ameliorates nor enhances impacts of White-tailed deer on vegetation.

Keywords: density, feeding, behavior, forage, habitat, nutrition, vegetation, White-tailed deer, *Odocoileus virginianus*, impact

The behavioural and physiological effects of narcotics-based anesthesia on reindeer (*Rangifer tarandus)*

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The objective of this study was to investigate the effects of narcotics-based anesthesia on the behaviour and physiology of reindeer. We hypothesized that anesthesia-induced hypoxemia would increase recovery times and return to normal behaviour, as evidenced by prolonged maze negotiation times. Six members of a captive herd of reindeer, 4 males and 2 females, underwent behavioural conditioning in a wildlife handling facility prior to immobilization. The design of the facility, with multiple pens and doors, allowed for a number of different routes to be set out through which the animals could navigate. Twice per week animals were contained within their "home" pen, and were required to navigate a novel maze to return to their home pen. In this way the navigational abilities of the reindeer, pre-anesthesia, were determined and subsequently contrasted with post-anesthetic navigation times both in the presence and absence of supplemental oxygen.
The reindeer were anesthetised on 2 separate occasions during mid to late summer, using 0.07 (±0.03) mg/kg etorphine and 0.68 (± 0.71) mg/kg xylazine delivered intramuscularly via remote delivery dart rifle. Each animal underwent one immobilization while receiving supplemental oxygen via intranasal catheter at a flow rate of 6 L/min, and one immobilization without oxygen. Each anesthetised animal was instrumented with an intra-arterial catheter to monitor blood pressure during the 30 minute capture. Arterial blood samples were drawn and analyzed using an i-STAT machine to determine pH, lactate, CO2 and O2 concentration. In addition, heart rate, respiratory rate, and rectal temperature were monitored every 5 minutes. Anesthesia was reversed using 2.84 (±0.26) mg/kg naltrexone hydrochloride and 3.03 (±0.21) mg/kg tolazoline hydrochloride.
Oxygen-supplemented animals had significantly higher PaO2 levels throughout the immobilization ($p<0.001$) and higher PaCO2 levels ($p<0.01$) at the end of immobilization as compared to non-supplemented animals. Unsupplemented animals demonstrated higher levels of lactate ($p<0.001$) as well as elevated heart rates ($p<0.001$) as compared to oxygen-supplemented.
Oxygen-supplemented animals recovered from anesthesia to standing in an average of 49.2 (±10.9) seconds, while the unsupplemented animals required 163.2 (±118.7) seconds. The substantial variability in animal behaviour during and after anesthetic recovery may be attributed to inherent variability in individual animal responses to anesthetic, the relatively small sample size of the study, as well the unexpectedly mild level of hypoxemia observed in a subset of the non-oxygen supplemented animals. Within the non-oxygen supplemented group, severely hypoxemic animals demonstrated longer recoveries than more normoxic animals. The oxygen-supplemented animals PaO2 range was 150.3-260.8 mmHg, with navigation times between 10 and 80 seconds. In the absence of oxygen PaO2 levels were 28.5-61.8mmHg, and 10-750 seconds navigation time was

required. Despite these findings, the average post-anesthetic navigation times were not significantly different between the oxygen and non-oxygen supplemented groups. We believe the onset of the rutting season and the proximity of opposite-sex animals in pens adjacent to the home pen played a major role in interfering with average navigation times in both groups of animals. Given the small sample size and lack of severe hypoxemia, we feel further studies are required to definitively comment on the post-anesthetic effects of hypoxemia on behaviour. We can conclude that narcotics-based anesthetic protocols may induce significant hypoxemia in reindeer, and that treatment of the hypoxemia with supplemental oxygen improves blood oxygenation, cardiovascular function, as well as recovery.

Keywords: anesthesia, reindeer, behaviour, physiology, capture, immobilization

Predicted distribution and management implications of Formosan sambar (*Cervus unicolor swinhoii*) in Taiwan

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Sambar (*Cervus unicolor*) is listed as vulnerable in IUCN red list due to sustained declines across its native range. In Taiwan, due to human overexploitation, it is also listed as a protected species. However, its population status remains unclear. In this study we used occurrence and absence data from line transect and camera trap survey in various parts of Taiwan to identify key habitat variables and to map sambar's potential habitats. Five species distribution models including logistic regression (LR), discriminant analysis (DA), ecological niche factor analysis (ENFA), genetic algorism for rule-set production (GARP), and maximum entropy (MAXENT) were applied. The results suggested that altitudes and distance to road best predicted sambar habitat suitability in ENFA and MAXENT, and these two variables were also two of the three most effective variables in LR. Sambar showed preference for areas which were over 1,900 m in elevation and away from the roads. The size of predicted habitats, which mainly located in Central Mountain Range and Xue Mountain Range, was about 1/6 (6,634 km2/36,000 km2) of the island. About 70.8% of predicted habitats were situated in national parks and wildlife refuges. However, the predicted habitats were divided into five separate regions by three major mountain highways which were Central Cross-Island Highway, Southern Cross-Island Highway, and Highway NO.7A (a highway which was along the border of Central Mountain Range and Xue Mountain Range). The roads brought habitat destruction, poaching, and human settlements which prohibited the dispersal of sambar populations. We recommend monitoring sambar hotspots which were closer to the highways for future establishing the corridors between different sambar populations.

Keywords: sambar, habitat suitability, management implications, population status, *Cervus unicolor*, Taiwan

Reversible anesthesia in captive pudu *(Pudu puda,* Molina 1782) with xylazine-tiletamine-zolazepam and yohimbine

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The southern pudu (*Pudu pudu*) is one of the smallest deer in the world and is a vulnerable endemic species of temperate rainforest in Chile and Argentina mainly due to the loss and fragmentation of their habitat. In addition to this, the pudú commonly has been hit by cars and attacked by dogs becoming a frequent visitor of Wildlife Rehabilitations Centers in Chile. The development and improvement of various methods of immobilization has become an indispensable resource for the proper management, monitoring and care of pudus, both in the wild and captivity. The purpose of this study was to evaluate the durability and stability of general anesthesia after intramuscular administration of a combination between xylazine, tiletamine-zolazepam and reversed with yohimbine on pudus.
Animals were held captive at "Parque Sendero Pudú" located in Callumapu, Valdivia, Chile. Between May and July of 2009 eighteen adults and middle age pudus of both sexes were anesthetized using a combination of xylazine (0.6 ± 0.1 mg/kg) and tiletamine-zolazepam (5.1 ± 0.9 mg/kg) administered intramuscularly. The drug combination produced 38 ± 3 minutes of effective anesthesia, with times of onset, latency and induction of 1.8 ± 1.3, 2.6 ± 1.8 and 3.5 ± 2.9 minutes, respectively. Physiological values were fluctuating between 118 ± 19 beats/min for heart rate, 38 ± 18 cycles/min for respiratory rate, 53.9 ± 13.6 mmHg for mean arterial pressure, 38.4 ± 1 ° C for rectal temperature and 90.5 ± 5.8 and 96.1 ± 3.6% O2 for hemoglobin oxygen saturation measured in tounge and ear, respectively. After the anesthetic procedure yohimbine (0.25±00.6 mg/kg) was administered intramuscular (n=9) and intramuscular/intravenous (n=9) to antagonize the effects of xylazine.Times to onset, latency and induction of anesthesia were lower than those reported for the same species using different anesthetic protocols. The heart and respiratory rates were slightly higher than baseline ranges described in unanesthetized pudus. The rectal temperature were lower than those referred for basal species and showed a statistically significant decrease ($P<0.05$) dependent on the dose administered xylazine. No adverse reactions were evident as described with the use of other anesthetic combinations in the same species and no gestation problems were found in pregnant females after the study. A long reversal period was observed that may have been affected by low temperature.
The information collected from this study may be used for clinical, conservation and research purposes for safe and adequate handling in both captive and wild southern pudu.

Keywords: anesthesia, pudu, chemical immobilization, capture

The future management of Chinese Water deer in the United Kingdom

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On a world scale, there is considerable conservation concern for the Chinese water deer *Hydropotes inermis*. The species was introduced to Woburn Park, Bedfordshire, England at the end of the nineteenth century. It bred well and was moved to other collections. Escapes and releases resulted in the current wild population, which occurs primarily in eastern England, but has the potential to spread over much of lowland southern Britain. However as a non-native species, following the principles of the Bern Convention, the UK Government is already considering risk management options to prevent further spread and deliberate introductions outside the current distributional range and what measure should be in place if the species enters protected areas. So far its impacts on native biodiversity and agriculture have been negligible though they are increasingly implicated in road traffic accidents.
On balance, social benefits occur from the presence of water deer. Members of the public generally enjoy seeing deer, and hunters are provided with an additional quarry and trophy species, and, in some cases, a source of income. In addition we must consider that the water deer is declining rapidly in its native range and is classified as "Vulnerable". The UK now hosts a significant and increasing proportion of the world population; if current trends continue, there will soon be more water deer in Britain than in China. Thus future management of the UK populations should be seen in a global, as well as a purely domestic context.
Discussion is now beginning as to the long term strategy for the population. This paper summarizes the current status of the UK population, the extent to which it might increase and the possible options for future management.

Keywords: Chinese Water deer, *Hydropotes inermis,* management, UK , invasion, conservation

Effects of different frequency of deer foraging on herbaceous communities in open site

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Deer populations have caused serious degradation of biodiversity though overgrazing in some regions in Japan. But the relationship between intensity of deer herbivory and vegetation diversity is not clear. This study aims to know effects of different habitat use intensity (HUI) on herbaceous communities. The study sites had been fully covered by Unpalatable species, *Juncus effuses*. Deer habitat use has been controlled by small fences, 6m by 6m from 2007. Fences were open periodically every month with different open days, 0, 2, 4, 8, 16 days and fully open plot (control). In the less opened plot, J. effuses became more decreasing and palatable species such as *Microstegium vimineum* became dominating. *Polygonum nipponense* was most dominated in 2 days open plot. This result showed some species can dominate under moderate deer herbivory. As Deer HUI go down, then biodiversity goes up. But some species will decrease according to HUI declining, even though they are palatable. To conserve plant community with higher biodiversity, it is better that various HUI sites exist in a mosaic.

Keywords: habitat use, intensity, herbaceous communities, periodically open fence, biodiversity, density, impact, overabundance

Space behavior and habitat use of partially managed Red deer (*Cervus elaphus*) population in Šumava National Park, Czech Republic

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Šumava National Park (690 km2) is montaneous NP (elevation up to 1400 m asl) covered by mixed and spruce forests, during winter time covered by snow. Forests are partially damaged by windstorms and bark beetles outbreaks, natural forest regeneration takes place in core areas of the National Park. Red deer (Cervus elaphus) play an important role in this ecosystem by browsing on regenerating trees. Without management, deer numbers will increase rapidly and natural regeneration of forests will be blocked by browsing. In the area, no important predators of red deer are available (wolves are missing, lynx may kill red deer but only during winter time). Two management rules apply to red deer populations: limiting numbers of deer by shooting (current deer density ca. 1,5 deer / km2), and a system of 14 winter enclosures which attract deer to overwinter inside the enclosures, where they are fed artificially and thus prevent them to influence the forests. There is no artificial feeding outside of winter enclosures. In these conditions, a GPS telemetry study is ongoing with the aim to study in detail the spacial behavior and habitat use of red deer (n=34, 20 males, 14 females, from 2005 until now, in some cases continuos 5 years of data sequence available). Home range sizes vary between individuals with and without seasonal shift, and mean values are 56,3 km2 for males and 27,6 km2 for females.A typical feature of behavior is a strong home range overlap between years (2005/2006 overlap was 73,6%). Concerning habitat selectivity, red deer prefer areas damaged by bark beetles (electivity 0,19; good source of food) and young regenerating forests (electivity 0,38; hiding cover), and all types of full grown forests are relatively avoided.

Keywords: Red deer, management, habitat selectivity, migration, behavior, density, culling, artificial feeding

Exotic Red deer (*Cervus elaphus*) in National Parks: A natural resource that harmonizes conservation and production

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Red deer (*Cervus elaphus*) arrived to Argentina in 1905 and now occupy over 50'000 km^2, including about half of 2 large National parks. Deer have locally reach high densities of 100/km^2 in ecotonal areas and 50/km^2 in steppe areas. Early attempts to control exotic deer numbers and geographical spread lead to public hunting since 1947 in Lanin National Park and since 1987 in Nahuel Huapi National Park. Being still practiced to the present, it has became the oldest such management program developed by the National Parks Administration. Several National parks include extensive National Reserves which are composed of private ranches, some small communities of settlers, and some fiscal lands. On these lands, some extractive uses including livestock production is allowed. More recently, the red deer management program has allowed a double effect for controlling exotic large herbivores by making the settlers of Nahuel Huapi National Park change or decrease their number of domestic livestock (which forms their livelihoods), in exchange for red deer hunting privileges by allowing them to manage all the economic benefits from this activity. Since 2001, several courses for hunting guides were held, and some 40 certified guides take hunters to the field. The emphasis has been on the harvest of large males, attracting hunters who result in enough economic benefits from deer hunting that it has generated the means to maintain the management program.

Keywords: protected area, policy, exotic deer, Red deer, *Cervus elaphus*, management, conservation, public hunting

Management of deer in Australia

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Six species of deer have been introduced to Australia and established wild populations (*Cervus elaphus, C. unicolor, C. timorensis, C. porcinus, C. axis*, and *Dama dama*). Importation of new species is controlled by the Federal Government who would be unlikely to allow more deer species to be introduced, and indeed recently halted a proposed introduction into farms of sika deer (*C. nippon*). However, Australia is a federal country of six States and two Territories each with its own laws on how to manage existing introduced species – State laws range from those that treat deer as game animals where the populations are managed as game (Tasmania and New South Wales), to those that have more mixed systems where the hunters or State agencies manage some populations as game and others as pests (Victoria), to those where all deer are considered pests and the State tries to keep them out (Northern Territory) or requires they be controlled (Western Australia, South Australia).

These differences have been debated in recent times after new laws in New South Wales gave significant management and regulatory rights to an agency representing game hunters. Hunters claim they can 'control' deer, pest management agencies are dubious, while conservation groups claim that deer damage native biodiversity and should be removed or controlled more rigorously than can be achieved by recreational hunters.

In this talk I will canvass one policy issue for the case where established deer populations cannot be eradicated, and describe one partial response from Victoria. The policy issue is "how should managers set management targets for deer populations?" In my opinion, the wider policy options depend on both the status of deer as native or exotic species, their impact on native biodiversity when they are exotic species, and the nature of the social values placed on them by hunters and their ability to harvest the deer. So, management targets for say threatened native deer species might be to maintain sustainable populations at carrying capacity. For abundant and non-threatened native deer the management target might be a take a sustainable harvest, or even a maximum sustained harvest. For introduced deer the management target should not be the harvest as above (zero or up to MSY), but should be a density of deer set to meet wider biodiversity goals. Since the assumption is that deer are 'out of place' and the reality is that most jurisdictions would either not now introduce deer or would prefer to limit their spread, this target densities usually range from zero (the deer is a pest and should be eradicated or extirpated) up to some modest density where their impact is tolerable. The density sets the harvest, and the harvest sets who can and should do it.

The State of Victoria has four deer species (sambar, red, fallow and hog) with established populations. They have the capacity to spread to suitable habitats over much more of the State. Hunting requires a licence and permission of the landowner, but is banned in national parks. Hunting seasons and bag limits depend on the species – open season and no limits for sambar, but a limited season and bag limit for hog deer. The State agencies and most hunters agree that further spread is undesirable but the question is how to stop this.

We are currently developing feasibility studies for one small outlier population of hog deer (and one each of feral goats and feral pigs) to see whether eradication is possible, what constraints would have to be overcome, and what it would cost, and who could achieve it.

Keywords: overabundance, *Cervus elaphus*, *Cervus unicolor*, *Cervus timorensis*, *Cervus porcinus*, *Axis axis*, *Dama dama*, exotics, invasion, management, policy

Pest or resource: Management of introduced wild deer in New Zealand

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Translocation of wild mammals to new countries can result in great benefit, great cost, or both. In some places, high economic, conservation, and human health costs have prompted extensive programmes to control or eradicate the invasive species as pests. The need for such programmes is seldom contested when the invasive species are viewed largely as pests (as for rats), but can be much more controversial when they are also valued as an economic or recreational resources (as for deer). In New Zealand, seven taxa of deer were introduced a little more than a century ago, and wild deer now occupy most of the country (particularly red deer Cervus elaphus scoticus). This includes most of the 8 million ha (31% of NZ) of public land managed by the Department of Conservation which has a primary goal of protecting native species. The deer are farmed as livestock, and are also valued as a commercial and recreational hunting resource, but are also widely seen as a conservation pest because there is a substantial body of research showing deer can cause major changes in the composition of native plant communities. As a result, the only legal status of wild deer is as pests. Despite that they are subject to active control programmes in only a few places. Instead an apparent 'laissez faire' management regime prevails over most conservation land, but that includes unrestricted access to the deer populations by recreational and commercial hunters alike. The consequence is that in many areas unrestricted commercial (helicopter-based) hunting keeps deer densities areas low, often obviating the need for additional control. In addition, hunter opposition to deer control can constrain conservation aspirations somewhat, particularly by requiring a strong and clear justification of the need for control (rather than allowing deer control to be undertaken as a precaution). The resulting long-standing conflict between hunting and conservation interests lead to governmental review in 2008. The findings of that review are summarized, along with some of the initiatives that flowed from it.

Keywords: invasion, protected area, management, policy, exotic deer, pest, hunting, overabundance

Policy on management of hyperabundant Wildlife populations in Canada's National Parks

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This paper discusses Parks Canada's policy on the management of hyperabundant wildlife populations in Canadian national parks. The development of the policy was triggered by the growing challenge of managing hyperabundant wildlife populations, particularly in Southern Canada where parks are small, and many fall considerably short of what is needed to conserve the full array of biological diversity and ecological processes necessary for their long-term persistence. Many of these parks exist in human-dominated landscapes where competing land-use activities have resulted in the disruption of some of the processes that have historically regulated wildlife populations.

The presence of hyperabundant wildlife populations has been shown to have adverse impacts on the ecological integrity of the affected parks, requiring intervention by Parks Canada to comply with the Agency's conservation mandate. At least seven species with hyperabundant wildlife populations have been identified in 10 of Canada's 42 national parks. In a few of these parks, hyperabundant populations of some large herbivores also pose significant threats to public safety, particularly due to collisions with motorists.

The policy provides a nationally consistent approach to the management of hyperabundant populations while offering sufficient flexibility for innovation and adaptation. It spells out the criteria and conditions under which a wildlife population may be declared hyperabundant, outlines broad ecosystem-based management principles that form the basis for active management, and provides implementation guidelines for management actions. The policy requires management decisions and actions to be based on the best available scientific information, and priority to be given to methods that best contribute to the maintenance or restoration of ecological integrity. It further requires hyperabundant wildlife populations to be managed under an adaptive management framework, where a combination of management, research, monitoring and evaluation provide more opportunities for improvement. Participation of Aboriginal peoples and other interest groups in the management of hyperabundant populations is encouraged, and public consultation and education are integral components of the entire planning and implementation processes. The policy was approved in 2007 and has been tested in the management of several hyperabundant populations in several parks.

Keywords: overabundance, policy, protected area, management

The concept and consequences of deer overabundance in the United States, with suggestions for management prescriptions

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Caughley listed four criteria for designating a population as overabundant and these include both biological and cultural components that are not always congruent. From an ecological perspective, high densities of deer make it difficult to reach management goals of biodiversity. The impact of deer browsing on plant communities is not a linear correlate of density, as feeding preferences result in widely divergent impacts on individual plant species at any one deer density. With the exception of introduced sika deer along the east coast of US, deer cannot be considered an invasive or exotic species in the US. Therefore the range of impacts through browsing is within the natural range of the system. In a forest community, a variable deer density over time would result in pulses of recruitment into a forest tree community. As opposed to a variable density, a chronic high density would invert the demographics of a forest toward older age classes and cause the eventual senescence of that system. Chronic high densities (as a result of loss of predators and hunting, and enhanced habitat productivity) are probably a unique condition for this system. Chronic high density deer populations in the US are almost exclusively reported in exurban landscapes or public areas where deer are protected from hunting. Whereas, the majority of focus has been on high density populations of White-tailed deer, there are also reports from elk and introduced sika deer populations. The effective response to these situations depends on the managing authority. Municipal communities have responded with either culling using both professionals and volunteers, or with contraception in conjunction with culling/removal. For large public lands the response has been generally one of inaction, with some exceptions in the east where regulations have been modified to allow culling. The effectiveness of culling in reducing deer density depends on accessibility of the deer herd, and its acceptance as a management tool rests on both safety concerns and bioethics. The loss of predators in some US forests, and the reluctance to reestablish these species, hampers effective long-term deer management. Restoration of wolves into Yellowstone ecosystem is a wonderful example of deer management through predation. Habitat manipulation could reduce deer densities through reduction in carry capacity, but the short-term response would be increased browsing pressure on remaining plants. As with designation of overabundance, the proper management response depends on both biological and cultural components. No management prescription, except exclosure fencing, removes deer entirely from a system, but management may restore variability to the annual population density. If survival of saplings is the criteria for reduced ecological impact, then deer densities would have to remain low for periods of 5-10 years depending on site growth conditions. Density reductions for this extended period will rely on culling through both volunteer and professional hunters. However, there are no tests of these ideas at a landscape scale in the US.

Keywords: overabundance, protected areas, management, policy

Monitoring deer population size in *Lugar Nuevo* and *Selladores-Contadero*, South Spain: Evaluating efficiency of management culls for reducing density

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The Sierra Morena mountains have an important ecological value in Southern Spain, with stable populations of several emblematic protected species coinciding with important populations of game that suppose an important economic resource**.** The herd management model from the 1970's on is characterized by the use of game fences to close estates perimeters, supplementary feeding and high population densities at times reaching levels of over 55-60 deer/Km^2, incompatible with the maintenance of vegetation and optimal animal conditions. This situation reached its extreme point with the death of thousands of deer due to the droughts of the early 1990's. In this area the estates *Lugar Nuevo* (LN) and *Selladores-Contadero* (SC) in Sierra Andújar Natural Park, Jaén Province, with over 10,000 hectares each, are official game estates of historical repute. In recent years they have become excellent areas for research, investment having been made to this end in order to obtain useful information for a wise game management and for the conservation of the Mediterranean ecosystem. From 1997 onwards, with the aim of solving the deer overabundance problem, changes were made in herd management, fundamentally a reduction in population density through the establishment of regular management culls, and the suppression of supplementary feeding. To evaluate the efficiency of culls for reducing density we monitor population size through a standardized programme for estimating abundance changes since 2002. Lineal transects for direct observation methods including all habitat types are surveyed at the end of September and/or first week of October in the same way and under the same conditions every year. Distance sampling software was used and also the average number of deer per kilometre surveyed was recorded as KAI. Population estimated in 2002 using Distance methods were 2,395 red deer and 450 fallow deer and 1,975 red deer and 36 fallow deer in LN and SC, respectively. Average fallow deer extracted in SC were 73.14±31.87 individuals/year. Cull levels of 373.57±127.14 red deer and 450.43±148.45 fallow deer in LN and 479.86±252.02 red deer in SC hunted at year since 2002, were not different statistically. We found that at least for fallow deer this method sub-estimates population because the estimated population is similar to the numbers of animals shot annually. However, these results allow changes to be monitored procedures applied like a multiplicative seasonal decomposition trend-cycle in the population studies. Population density varied in LN from 24.94±3.10 to 18.45±4.62 red deer /Km^2 (different, P-Value=0.05), and from 6.9±1.8 to 4±0.24 (different, P-Value=0.03) in 2002-06 and 2007-09, respectively. In SC from 19.25±2.19 to 12.8±2.36 red deer/Km^2 (different, P-Value=0.007), and from 0.78±0.71 to 0.75±0.52 (not different, P-Value=0.9) in 2002-05 and 2006-09, respectively. The levels of deer hunted were sufficient to reducing lightly the population in both estates, except for fallow deer in SC. The positive correlation found between Distance and the KAI results allow the use of this latter method for monitoring population as an alternate method for setting hunting quotas and to achieve specific management objectives. Because of the difficulty of estimating density precision and accuracy over

large areas and the expensive effort necessary, a set of indicators of animal and population performance is also being studied to test their utility for providing information on the population–habitat system and monitoring changes.
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Keywords: monitoring population, census, management, control, culls, reducing density, Red deer, *Cervus elaphus*, Fallow deer, *Dama dama*, mediterranean ecosystem

A review of introduced cervids in Chile

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In this paper we review the extent of exotic deer distributions in Chile. Red deer (*Cervus elaphus*) were introduced to central Argentina in 1906 and a few years later to the Andean foothills in Argentina. In 1928, a shipment of red deer from Europe arrived to the central valley of Chile. Since the 1940s, populations have expanded from Argentina into Chile, by way of easily accessible, low-mountain passes of the Andean range, accompanied by further direct shipments from Argentina. Fallow, axis and roe deer (*Dama dama, Axis axis, Capreolus capreolus*, respectively) also have been introduced to Chile. Negative ecological impact from red deer have been described since 1981, and rates of expansion were estimated for 8 populations in 1983. The area occupied by 1990 was estimated at 3,400 km², but this increased to 7,700 km² by 2002. The overall area invaded by 2002 was between 37°42'S-54°55'S and 73°36'W-69°50'W (Argentina and Chile combined, though not contiguous). Based on linear rates of expansion reported for Chile, a conservative rate of 1 km/year for the north-south and east-west dispersals can be assumed in Chile, but likely is frequently more rapid, particularly due to substantial presence of settlers with livestock, which have opened up forests through cattle use and intentional fires, thus allowing red deer to advance more efficiently. The pre-Columbian northern limit of the native cervid, the Huemul (*Hippocamelus bisulcus*), was 30°S, and as the red deer has invaded all other known habitat types used by Huemul, the potential northern limit for red deer could be >750 km further north of the present distribution. To the south, all areas are suitable for red deer. Overall invasion patterns will depend on additional local introduction on both side of the Andes. Already there are several major focal populations from which the invasion is radiating in various directions. Intentional introduction may still occur, but of more concern are new approved deer enclosures and the omnipresent risk of escapes. In Chile, exotic cervids are encountered in all provinces, including Fireland, except for possibly Region III; from limited information, many of the deer in these provinces live in captive herds, which total over 100. Fallow deer had been taken to an enclosure on Chiloe island, from where they escaped and have established themselves in the surrounding area. Red deer by far have the largest feral population of exotic cervids in southern South America, providing source animals that can easily cross the Andes, which implies that captive enclosures on both sides represent high risks for new source populations in case of escapees.

Keywords: *Cervus elaphus,* Red deer, Fallow deer, Axis deer, Roe deer, *Dama dama, Axis axis, Capreolus capreolus,* Chile, invasion, exotic

An optimum habitat model for the White-tailed deer (*Odocoileus virginianus*) in central Veracruz, Mexico

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The modeling of wildlife habitat availability for animal species has important implications for explaining the distribution of the organism in the wild, effective management and conservation. In this study we evaluated an Optimum Habitat Model based on estimation of the Habitat Suitability Index (HSI), for the White-tailed deer (*Odocoileus virginianus*) in central Veracruz, Mexico. We generated a GIS-based model from a combination of the main habitat requirements that influence the presence of White-tailed deer. Six variables were used for modeling the optimum habitat: mean annual temperature, slope, aspect, vegetation, water availability and anthropogenic pressure. With these data layers we calculated the HSI for deer in the study area. Vegetation cover and White-tailed deer presence data were obtained from field surveys. Two strip transects (500 x 2 m) were established in 18 localities, where all tracks and signs from White-tailed deer were recorded. Field data and data layers constitute the spatial database used for this project. The HSI estimated for the study area was from -0.667 to 0.905, where the lowest values were associated with poor habitat quality. Eighty six percent of total surface (5,167.71 km^2) included intermediate and low habitat quality (4,455.9 km^2) and only 13.52% was high habitat quality (711.81 km^2). We found 51.16% fecal groups in intermediate habitat quality. White-tailed deer frequently used oak forest, tropical deciduous forest and pastures. A Classification Tree Analysis indicated that temperature and aspect were the main habitat features influencing the White-tailed deer presence in the study area. Although there is high anthropogenic pressure in the zone, forest patches help to maintain some suitable habitat for small populations of this species. Conservation and restoration of the vegetation cover is necessary to promote deer populations recovery in Central Veracruz, Mexico.

Keywords: White-tailed deer, *Odocoileus hemionus*, management, habitat model

Effect of capture and electroejaculation on heart, pulse, and respiratory rates, and rectal temperature on adult and yearling Pampas deer (*Ozotoceros bezoarticus*) males

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As Pampas deer (*Ozotoceros bezoarticus*) is an endangered species, gamete preservation is a main strategy to improve its status. The objective of this experiment was to determine the changes in heart, pulse, and respiratory rates, and rectal temperature resulting from electroejaculation in adult and yearling Pampas deer males. The study was conducted during June and July, at the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar, Maldonado, Uruguay. Eleven males, 6 adults (age 4-7 years), and 5 yearlings (1-1.5 years) were allocated in two groups in 0.5 ha enclosures. The animals were captured with anesthetic darts containing 2mg/kg of xylazine, 1.6 mg/kg of ketamine, and 0.013 mg/kg of atropine. The induction time was considered from the moment of receiving the dart until lateral or sternal recumbency. Once asleep, electroejaculation were performed applying 1volt series during 4-5 s, with rest intervals of 2-3 sec, increasing 1V after 10 periods, until achieving ejaculation or a maximum of 8 volts. The parameters studied as a function of electroejaculation voltages were: heart rate (HR), pulse rate (PR) and respiratory rate (RR). Rectal temperature was recorded before and after the electroejaculation. After the electroejaculation process finished, yohimbine hydrochloride (0.26 mg/kg) was administered iv to reverse the anesthesia. All the animals survived, and semen was obtained from all of them. Induction time did not differ between categories: 16.7 ± 6.8 vs 16.8 ± 9.5 min in adults and yearlings respectively. Ejaculation began at 2.8 ± 1.6 and 3.8 ± 1.3 volts in adults and in yearlings respectively (P=0.3). The HR before electroejaculation did not differ between categories (50.0 ± 2.0 beats/min). When 1 volt was applied, HR increased to141.0 ± 29.0 and 92.3 ± 20.1 beats/min in adults and yearlings, respectively (P<0.01 in both categories), with differences between categories (P=0.01). The HR values remained higher in all the other voltages, being greater in adults at 2V (149.8 ± 9.0 vs 85.3 ± 9.0 beats/min, P=0.002) and 3V (152 ± 15.3 vs 100.0 ± 15.3 beats/min, P=0.001), and tending to be greater with 6V (153.0 ± 21.4 vs 116.7 ± 29.1 beats/min, P=0.07). Initially and before electroejaculation, the PR value was 59.5 ± 12.2 beats/min for both categories. With 1 volt, PR increased to 104.0 ± 5.7 and 86.8 ± 25.4 beats/min in adults and yearlings, respectively (P<0.01 in both categories). The PR values remained higher in all the other voltages, being greater in adults at 2V (118.3 ± 26.3 vs 90.0 ± 16.9 beats/min, P=0.04), 5V (104.8 ± 14.9 vs 74.0 ± 2.0 beats/min, P=0.006), and 6V (107.3 ± 18.9 vs 66.0 ± 18.9 beats/min, P=0.007), and tending to be greater with 3V (110.0 ± 22.9 vs 85.8 ± 18.6 beats/min, P=0.09) and 4V (106.3 ± 20.2 vs 87.8 ± 13.0 beats/min, P=0.06). The RR before and during electroejaculation did not differ between both groups. Initially the RR before electroejaculation was 25.0 ± 1.0 breaths/min, and did not vary during electroejaculation. Rectal temperature was similar between the groups and was not affected by electroejaculation (37.2 ± 0.4°C). The capture and anaesthesic methods allowed all animals to survive. The electroejaculation in Pampas deer caused an increase in HR and PR, which was greater in adult than yearling males. Respiratory rate and rectal temperature were not affected by electroejaculation.

Keywords: *Ozotoceros bezoarticus*, electroejaculation, anesthesia, male reproduction, ex-situ

¡Error! Marcador no definido.Distribution of White-tailed deer on South Texas rangeland

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In Texas, White-tailed deer (*Odocoileus virginianus*) have replaced cattle as the primary source of income from ranchland. This has lead to changes on the landscape. Ranches are frequently surrounded by high fencing, dirt roads are cleared to service hunting blinds and more water sources and supplemental feeding locations are developed for the deer. These features may influence the distribution of animals over the landscape.

To assess the effect of high fencing on home range size, GPS collars were placed for 1 year on 6 White-tailed deer (3 bucks, 3 does) inside, and 6 deer outside, a 200 ha high fenced enclosure. Annual home range sizes were calculated by the Kernel Home Range technique. On a second ranch, animal distributions were monitored over 1 year during 4 trials, each lasting 12 days. In each trial 6 white tailed deer and 9 cows were fitted with GPS collars programmed to take a position location every 5 minutes. Interspecies interactions were calculated from synchronized 5 min locations using Spatial Analyst in ArcView9. To assess animal distributions data was thinned to hourly locations to avoid problems of spatial autocorrelation. Habitat preference was calculated by chi-squared test of proportional use and availability of ecological sites. Effects of anthropogenic features were assessed by comparing animal locations and an equal number of random points on a distance surface grid.

The high fence failed to contain the deer, half the deer moved through the fence utilizing holes created by feral pigs. Home range sized of bucks and does were similar and there was no evidence that the fence constrained home range size. Deer and cattle showed extensive overlap in spatial distribution, but were separated in time. Close interactions were rare, but when they did occur individual deer did not show avoidance of cattle until inter-specific distances were < 50 m. The distribution of deer and cattle over-lapped most on productive ecological sites such as clay loam soils and riparian areas. However, deer had almost exclusive use of rocky slopes which were avoided by cattle. Cattle and bucks favored open clay loam sites with scattered trees and shrubs. Does favored sites with denser tree canopy including deep soil drainage areas within the rocky areas and along the riparian strip. Both deer and cattle were located closer to ranch roads than random distribution. Deer usually came to drink at dusk but did not linger near the water where the cattle congregated during the day. Bucks, but not does, were attracted to the supplemental feeding sites. Cattle were excluded from the feeders. We conclude that the current anthropogenic activities on rangeland had less effect on deer than expected. Deer populations within "deer proof" fences may not be constrained because the fences are not impermeable to deer. The idea that cattle limit access of deer to preferred ecological sites and water was not supported, instead there was short term temporal separation of species distributions.

Provision of supplemental feed had the most noticeable effect on the distribution of deer. Bucks, but not does, were attracted to the feeders, so strategic location of supplemental feeding sites may be useful in modifying the distribution of bucks. Ranch roads offer easy travel corridors for

animals in dense, thorny brush country so the additional roads may even assist in dispersing livestock grazing patterns throughout the pastures.

Keywords: animal interactions, habitat selection, GPS, *Odocoileus virginianus*, spatial distribution

Anatomical study of the gastrointestinal tract of the pudu (*Pudu puda*) by abdominal computed tomography

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The pudu (*Pudu puda*), which is the smallest deer in the world and inhabits central and southern Chile and Argentina, is a ruminant and a browsing herbivore. The aim of this study was to provide a reference for interpretation of the normal anatomy of the pudu's gastrointestinal tract as imaged by abdominal computed tomography (CT). For the study one adult female pudu was used. After a 24 hour fast, the pudu was anesthetized and positioned in sternal recumbency. Image acquisition began immediately after an intravascular injection of contrast media (MD-76® 370 mgI/ml) divided into two periods. Transverse images of 5 mm thickness and 5 mm interval were obtained with a fourth generation CT scanner, from the ninth thoracic vertebra (T9) until the first caudal vertebra (Cd1). CT images were labelled and compared with anatomical references for ruminants. Structures that were consistently identified in the abdominal cavity included the stomach with its 4 compartments (rumen, reticulum, omasum and abomasum), the small and large intestines, liver, spleen, kidneys and some major blood vessels. The distal loop of the ascending colon, the transverse colon, the pancreas and lymph nodes could not be identified. The resulting CT images provide a reference for normal cross-sectional abdominal anatomy of the adult pudu for use by veterinary radiologists, clinicians and students.

Keywords: pudu, abdominal anatomy, computed tomography

Comparability of the ecology among Odocoileini deer

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The tribe Odocoileini consists of 7 genera: Rangifer has maintained a holarctic distribution; Odocoileus historically occurred between at least 61°N and 18°S (spanning some 11'000 km); and the other 5 genera (*Blastocerus, Hippocamelus, Mazama, Ozotoceros*, and *Pudu*) have only been known in South America, with the exception of *Mazama americana*, which reaches as far north as Mexico. The Plio-Pleistocene *Odocoileus lucasi* of North America is considered ancestral to modern *Odocoileus*, but also is likely the ancestral line that took part in the Great American Interchange and resulted in some of today's South American species. We compare aspects of the ecology among Odocoileini to also provide indicative clues for species that still have limited information on their life histories and ecology. Among other things this paper considers extant and past distributions as influenced by pre- and post-Columbian human impact, body physiognomy, behavioral syndromes, sexual segregation, antipredator behavior, dietary behavior, group behavior, and population densities. The analyzed genera exhibit a wide range of habitat types that they can occupy, particularly when including the past distribution. This is accompanied by a large dietary breadth and variation in behaviors, densities and in body shape and size. Extant species, however, may occur mainly as remnant populations in only a fraction of the prior distribution due to pre- and post-Columbian human impacts. Once this plausible scenario is recognized, studies of these lesser known species need to be conducted taking this hypothesis into consideration, as rigidly adhering to assumptions that current habitat use and other modern day ecological attributes represent the norm for the species could lead to unwarranted conclusions.

Keywords: Odocoileini, ecology, habitat use, dietary breadth

Evaluation of an anesthetic protocol based on ketamine and xylazine and reverted by yohimbine in captive pudu (*Pudu puda*, Molina 1782)

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The pudu (*Pudu puda*) is one of the three native species of deer in Chile. It is restricted to the southern temperate rainforest of Chile and Argentina and it has increasing problems of conservation. The objective of the present study was to evaluate the anesthetic efficacy and safety of a combination of xylazine-ketamine reverted with yohimbine in pudu kept in captivity in order to be used in procedures of chemical immobilization and anesthesia.
Thirty anesthetic sessions were practiced with five animals every 7 days, based on a protocol of ketamine 10.20 SE± 0.7 mg/kg plus xylazine 0.61 SE± 0.04 mg/kg given intramuscularly and reverted by yohimbine 0.20 Â± 0.01mg/kg intravenously. Initial effects, period of latency, induction, recovery and incorporation times were recorded. Anesthetic (i.e. spontaneous position, lateral recumbence, sonorous stimulus response, mandibular relaxation, general attitude and pedal reflex) and physiological parameters (i.e. rectal temperature, heart rate, respiratory rate, oxygen saturation, and arterial pulse) were monitored every 5 minutes during the entire procedure.
The anesthetic induction was fast and smooth. Rectal temperature decreased significantly ($p<0.05$) up to 38 SE± 0.7ºC. The mean heart rate was 81 SE± 16 beats/min, and dropped significantly ($p<0.05$) towards the end of the procedure. In the same way, arterial pulse presented significant variations ($p<0.05$) between time with a mean of 80 SE±15 pulsations/min. The mean respiratory rate was 34 SE± 18 cycles/min, whereas the oxygen saturation was 95 SE± 3%. The anesthetic quality was catalogued as an excellent procedure. The mean duration of the entire procedure was 53.78 SE± 4.3 minutes from the injection of the anesthetic mixture to the animal incorporation. Side effects were not observed during or after the procedure.
In conclusion, Ketamine 10.20 mg/kg plus xylazine 0.61 mg/kg reverted with yohimbine 0.20 mg/kg is a safety anesthetic protocol combination recommended for short- and mid-duration procedures in adult males of pudus.

Keywords: *Pudu puda*, anesthesia, chemical immobilization, ketamine, xylazine, yohimbine, capture

Assessing the condition of Whitetail deer (*Odocoileus virginianus*) in an urban environment using serum leptin and traditional post mortem indices of body condition

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The intensive management of whitetail deer in urban environments is critical to promoting ecosystem health in urban parklands as well as addressing issues pertaining to public health. The ultimate success of these management programs is evaluated by monitoring deer densities and deer condition with respect to achieving sustainability within the urban forest environment. In conjunction with an ongoing deer management program in the Cleveland Metroparks (Cleveland, OH, USA) during the months of January and February 2008-2009, the body condition of whitetail deer was evaluated using serum leptin, Kistner subset score, Riney score, mandibular marrow fat and metatarsal marrow fat. Other parameters including age, sex, body weight, pregnancy status and gestational stage were also examined. A total of 298 animals were evaluated over a two year period (197 females, 101 males) representing animals from the fawn, yearling and adult age classes. Significant positive correlations were present between leptin, Riney score, Kistner subset score and fetal number ($p < 0.05$). There was a positive correlation between leptin and weight however this relationship was not significant. Leptin was also found to have a positive correlation with age ($p < 0.05$). Interestingly, leptin was negatively correlated with mandibular and metatarsal fat ($p < 0.05$). Overall, these data indicate that serum leptin is a useful marker of body condition in white tail deer from this urban environment.

Keywords: Whitetail deer, *Odocoileus virginianus*, leptin, condition, kidney fat, marrow fat, reproduction, management, urban park

Performance of *Hippocamelus* under captive conditions

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During the 1990 International Deer Biology Congress, a workshop determined that of the 39 globally endangered deer species, many have inadequate captive populations. South American cervids were considered the most vulnerable. Captive studies on reproduction and physiology were suggested as a priority for endangered species like Huemul (*Hippocamelus bisulcus*). Pressures causing extinction were judged unlikely to change in the near future, providing the participants with a strong argument in favor of establishing captive populations. In addition, IUCN recommends that captive programs be a component of conservation strategies for taxa with <1,000 individuals. Although 1,000–1,500 Huemul have >100 recognized herds, 60% of these contain only 10-20 individuals each: regardless, ex-situ strategies for Huemul in Argentina continue to be hindered by a popular concern that the species is unfit for captivity and manipulations are too risky. The objective of this paper is to demonstrate the feasibility of integrating Huemul in captive breeding programs as a tool towards recovery. Here we report on largely unknown details about successful historic cases keeping Huemul and its sister species, the taruca (*H. antisensis*), in captivity.

The precarious state of Huemul was recognized >8 decades ago leading to several early uses of ex-situ projects, however, these terminated prematurely for lack of support. Years later, attempts in 2001 to re-employ ex-situ strategies in Argentina were faced with strong opposition. The government decision to reject the proposed project was based on claims that Huemul cannot be raised in captivity since previous attempts had failed, that necessary manipulations were too risky, and that Huemul in semicaptivity were unnecessary in Argentina, despite only 350-600 individuals remaining in some 50 populations. Certainly, Huemul were often accidently killed from inadequate capture methods (e.g. dogs), inadequate transportation, or unsanitary holding facilities, failures which continue to be acknowledged, while the successful ones go unnoticed. In this report, we provide additional examples to further invalidate these misconceptions that continue to dominate decision making in Argentina.

As early as 1830, Huemul were found in captive centers. Individuals were tranlocated successully by ship over huge distances to London, New Zealand, and Paris. At the turn of the century, several landowners in northern and southern Patagonia successfully raised Huemul, and then in the 1930s, a pair were shipped from southern Patagonia to the Buenos Aires zoo where they bred and were kept for several years. Further successful efforts included Huemul births at an Argentine national park zoological station starting in the 1930s. In Chile, Huemul recovery in one southen national park can be traced to translocation projects; this would be one of 2 known recovering herds. Taruca, the only other member of this genus, has been successfully bred in European and North American zoos. Already in 1890, after three years in the Berlin zoo, taruca was found to thrive on the same food given to all other captive cervids and were considered by the director, Dr. Frädrich, to be one of the easier species to maintain in captivity. Records in captivity include one pair with at least 12

registered births (records are incomplete) until the animals were destroyed during the Second World War. The longest known recorded time in captivity for *Hippocamelus* was 10 years, 7 months and 18 days for a male. The Bronx Zoo also held taruca for 5½ years, as did at least two other German zoos. These accounts give credance to the feasibility of the proposal of 2001 to re-employ ex-situ strategies in Argentina. 2Corroborating this historical evidence, Chile stepped forward to approve a new captive herd in 2005, which has produced very positive results.

Keywords: Huemul, taruca, *Hippocamelus*, captive breeding, conservation, ex situ

CONSERVATION OF NEOTROPICAL DEER

Physical gaps in the biogeography of Andean dwarf montane deer

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The distribution of species along the Andean cordillera is affected by the continuity of habitats. Climate changes progressively from the tropics to temperate latitudes; however, other characteristics such as deep gorges and rain shadows affect distributions strongly, as unsuitable habitats of dry forests and lower altitudes are apparent for montane deer adapted to wet environments. Here, I analyze the distribution of the dwarf deer (genera *Mazama* and *Pudu*) that live along the Andes, in consideration to unsuitable habitat and competition among species. From north to south, the montane Andes are inhabited by *Mazama bricenii*, *Mazama rufina*, *Pudu mephistophiles*, *Mazama chunyi* and *Pudu puda*. We have to highlight that at least two *Mazama americana* subspecies, which also inhabit Andean montane forests, are small enough as to belong to this group: *M. americana carrikeri* and *M. americana gualea*.

The main physical gaps that could affect dwarf montane deer living in humid environments are listed. A lowland tract of dry forest between Santa Marta and the main Eastern Colombian Andes; the Tachira depression, a low altitude and drier forest gap between the Merida Cordillera and the Eastern Colombian Andes; the North Peru Low – NPL, or Huancabamba depression, a large tract of dry forest, deep valleys, and low altitudes encompassing the Huancabamba and Maranon dry valleys; four cuts in the east side of the Andean montane forests from central Peru to south Bolivia: the Huallaga dry valley, the Mantaro dry valley, the Apurimac dry valley, and the Tarija dry valley; and lastly, the huge tract of dry eastern Andes south of Tucuman, until the beginning of the Nothofagus forests in southern South America.

Mazama americana carrikeri is effectively isolated from the main Andean cordillera in the Santa Marta massif. *Mazama bricenii* occurs at both sides of the Tachira depression, while apparent suitable habitat exists between this species and the distribution of *Mazama rufina*. The distribution of *M. rufina* stops at the North Peru Low or Huancabamba depression, the same as the western *M. americana gualea*, while sympatric *Pudu mephistophiles* occurs at both sides of the gap. The southern distribution of *P. mephistophiles* seems to stop at the Mantaro dry forests. Despite a deeper and drier gap is the Apurimac dry valley to the south, *Mazama chunyi* occurs at both sides of it. To the south, the Tarija dry valley marks the southern distribution of this species in Bolivia. *Pudu puda* is totally isolated from the other species, explained by the large gap of dry areas that distributes throughout the southern eastern Andean chain until the beginning of the Patagonian/Valdivian humid forests, where this species inhabits. Some of the distribution gaps between species are well known; however, most are still unresolved, and the lack of data is a result of reduced sampling and research. The distribution of the montane dwarf species does not follow strictly the distribution of gaps, and the allopatry in the distributions of some species might be explained instead by competition or interference between them.

Keywords: Merida dwarf brocket, Northern pudu, Ecuadorian dwarf brocket, Peruvian dwarf

brocket, Southern pudu, Mazama, pudu, conservation, South America, distribution

Current status, main conservation background and prospects ahead for the Marsh deer (*Blastocerus dichotomus*) in Argentina

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The marsh deer is the largest native cervid of South America. Today, the species is categorized as globally vulnerable and endangered in Argentina. Despite being one of the largest and charismatic mammals of the country, knowledge both in science and by society about this deer is weak in all regions where its presence has been confirmed.
As marsh deer is an indicator of environmental quality of many wetlands, the conservation of these natural ecosystems is necessary for the survival of these populations. The actions associated with the creation and implementation of the Provincial Reserve Laguna Ibera in Corrientes province would have led to an increase of the population of this area. The finding in winter of many dead deer with high parasite loads could be interpreted to be a potential regulation through diseases where the deer have no natural predators and are being protected from poaching.
In the Parana River Delta, in the last decade, there have been surveys and conservation actions which generated valuable information of an area previously unknown. Habitat fragmentation of the area and the risk of consequent loss of connectivity between the population nuclei constitute an important threat in the long term. In large timber areas, the survival of these populations depends mainly on the control of poaching, safeguarding of areas with natural vegetation and retaining areas where deer can keep safe from flooding. In areas with less environmental modifications near the De la Plata River, the protection of floating marsh areas is essential. These areas are considered indispensable elements for this species, specially when big flooding occur. The conservation status of the population nucleus located in the Delta of Entre Ríos Province is rated as extremely vulnerable, due to its marginal status and lack of poaching control. If stronger protection measures are not implemented in this area in the short term, this population runs a serious risk of extinction in a short time.
In recent years, a survey was carried out in other areas of historical occurrence of the Paraguay-Parana river axis and areas of influence in order to clarify the presence and status of the species in the region. As a result of this research the existence of populations could be confirmed in several locations, giving a new shape to the distribution map for the species in Argentina. The results of this survey highlight the nuclei of eastern Formosa province, probably the marsh deer population second in importance for the country. Locations of previously unknown populations in Corrientes province were also important, as Riachuelo marshes, some sectors of the Paraná flood plain, and the Aguapey river basin are key sites for the conservation of the deer out of the Ibera marshes.
In August 2009 the First Workshop for the Diagnosis of the Status of Marsh Deer at a national level was held in Santa Fe province. In this meeting we could summarize the available information and obtained the commitment of organizations involved in moving towards a national conservation plan for the species. Over the last five years there was an increase of NGOs and states entities, committing support, research and conservation actions on the species. It will be necessary to keep and increase this trend in order to have an optimistic perspective on the future of this species in

Argentina.

Keywords: Marsh deer, *Blastocerus dichotomus*, conservation, Argentina, survey

Osteological comparisons of appendicular skeletons: A case study on Patagonian Huemul deer and its implications for conservation

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Early explorers described Huemul (*Hippocamelus bisulcus*) as stocky, massif and short-legged deer of mountains, comparing them to ibex (*Cabra ibex*), chamois (*Rupicapra rupicapra*), mountain sheep (*Ovis canadensis*) and mountain goats (*Oreamnos americanus*). Subsequent key paleontological work also claimed that Huemul are mountain deer, which however was unfounded. All these comparison of Huemul to other ungulates were done without any supporting data. The main result of these historic influences are twofold: a) the continued prevailing claim that Huemul are mountain deer; and b) that their natural range are the Andes mountains, as evidenced by the current distribution. We found that early writings about Huemul generally reported them to be rare, disappearing and going extinct. References to stocky and short-legged Huemul were casual remarks made on deer found mainly in refuge areas. Paleontological comparisons were based on a new fossil mountain deer which, however, has been shown to be a construct and declared a "nomen nudum". Some behavioral and physical factors of Huemul did simulate stockiness by distorting body shape. The alarm stance involves shifting of the back hooves toward the front hooves with the legs then forming a triangle with the abdomen, resulting in an arched back and changed angles of legs. The thick hair coat, providing thermoneutrality down to -50C, reaches 7-9cm in length and affects the perception of body shape. Comparing morphometrics of Huemul and 12 other ungulates revealed that Huemul cannot be associated with rock climbing species, based on proportional metapodial lengths, tibia/metatarsal ratios, and articular stoutness. Proportional leg length is not static within a species and is influenced by ecogeographical, nutritional and physiological factors. Thus, climate, altitudinal hypoxia and locomotor pattern employed according to terrain and predation affect the appendicular skeleton. Nutritional deficiencies are notorious for altering body shape: Andean mountains have been shown to be deficient in iodine and selenium both of which affect bone development and under severe constraint, result in osteopathology. The documented frequent underdevelopment of Huemul antlers and high incidence of osteopathology support the effect from mineral deficiencies. As skeletal proportions are affected by numerous factor and result from adaption to different environmental regimes rather than reflecting phylogeny, large intraspecific variation is expected. Indeed, among the better studied cervids, relative metapodial length varies up to 70%, and populations from different environments can be clearly distinguished. Huemul do not overlap with rock climbing species previously considered analogous, but falls within the range of several other cervids. We caution against the rigid application of modern Huemul habitat occurrence in interpreting past habitat use. Even if rare, the few extra-Andean accounts cannot be considered abnormal outliers. Huemul ecology must be interpreted in terms of first principles rather than applying direct analogues from the present. This allows us to begin to use the past to understand the present instead of repeating the fallacy of imposing the present on the past. Current

efforts to recover remaining Huemul are distinctly based on the assumption that Huemul foremost belong into rugged mountains, because of their supposed special adaptions and resemblance to stereotype ungulates, also erroneously believed to only occur in rugged mountains elsewhere. However, this reflects a lack of awareness of the evolutionary history of Huemul and of their recent ecological realities created from human impacts. We conclude that the present empirical comparisons support many other lines of evidence that Huemul existed in treeless habitat and colonized Andean forests and higher altitudes secondarily. Habitat breath of Huemul is thus more like that found in other closely related Odocoilines, promising tremendous new opportunities for recovery efforts.

Keywords: Huemul, *Hippocamelus bisulcus*, morphometry, skeletal ratios, adaptation, habitat use

The taruka: Past and present conservation actions in Argentina

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The Andean Deer or Taruka (*Hippocamelus antisensis* d'Orbigny, 1834) is a Southamerican threatened cervidae, inhabiting from Peru throughout the Andes in Bolivia, north of Chile and north-west of Argentina. Due to hunting, destruction and occupation of its habitat, its populations have been greatly reduced (IUCN 2008). From a regional assessment made by IUCN specialists, the taruka was re-categorized from Data Deficient to Vulnerable in 2008. The main objective of this work is to present the actual and past conservation issues made in Argentina over the last 10 years. Since 2001, several publications on potential distribution of the taruka were presented helping to improve the knowledge about the geographic distribution of the deer in Argentina. Over the last seven years, five taruka workshops have been made in Argentina gathering different institutions and specialist with the purpose of developing the first National Conservation Plan. Results obtained from these papers showing distribution maps and conclusions from the workshops are presented, highlighting future directions and challenges in the taruka conservation. As a conclusion and in order to achieve a regional scale view, I will present works from Peru and Bolivia on the current conservation actions for this specie.

Keywords: taruka, Argentina, conservation, population, *Hippocamelus antisensis*

Status of conservation of *Hippocamelus antisensis* (taruka) in Bolivia

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The status of conservation of *Hippocamelus antisensis* (taruka) in Bolivia was determined generating information on its present and historical distribution, population situation and the threats it faces. Semi-structured surveys of rural settlers were used and the application of a SIG (Geographic Information System). The survey included 302 settlers in 69 localities in the mountainous range of the Andes, in the counties of La Paz, Oruro, Cochabamba, Potosí, Chuquisaca and Tarija. The potential distribution of taruka in Bolivia covers 135,759 km^2, however, the area with the beststatus of conservation occupies solely 58,510 km^2 and is characterized with high fragmentation. It includes the northern zone of the western and eastern slopes of the mountain range. Fifty eight of the surveyed people (37%) that sometimes saw taruka, indicated that currently they are observed less than five years ago, while 11% (n = 18) mentioned seeing them in greater numbers and 20% (n = 32) equally numerous. The rest no longer see taruka or did not perceive any changes. Taruka were observed most frequently in groups of 3 or more (46%, n = 98), as compared to observations of pairs or solitary individuals, although the average number of individuals per group is low (three individuals), unlike in the past when groups were composed of 10 or more. People frequently observe pairs and solitary individuals which is not common for a species of open habitats. According to local perception, the main cause for reduction of the populations of taruka is hunting (70%, n = 106), reported in 64% (n = 44) of visited localities. In agreement with the data obtained at the moment, we could warn that taruka is "endangered" due to the fragmentation of its habitat, the decrease of its populations and assumed extinctions in 22 of the visited localities (32%), mainly as a consequence of anthropic factors like hunting. Facing this panorama it is a high-priority to recover and conserve the population of taruka by putting into effect a Plan of Action at a national level and to move the species into the category "endangered" to later and in a long term allow a sustainable use. The success of actions for the conservation of taruka will depend on the multidisciplinary and inter-institutional commitment and effort, as well as on its acceptance and the participation of the local communities.

Keywords: taruka, *Hippocamelus antisensis*, conservation, Bolivia, threats

Thirty years in the conservation of Huemul (*Hippocamelus bisulcus*) in Nevados de Chillán

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The Huemul only inhabits the southern regions of Chile and Argentina. In Chile, the Huemul has been protected from hunting since 1929, and has been recognized as endangered since 1996. Conservation efforts on Huemul started in the 1970s through the Corporación Nacional Forestal, and the Comité pro Defensa de la Fauna y Flora together with the Frankfurt Zoological Society began working on Huemul issues in the 1980s. The population of Los Nevados de Chillán–Laguna del Laja discussed here, represents at present the northernmost Huemul population, separated some 300 km from the next populations.

The objetive of this paper is to review the last 30 years of conservation efforts in the Los Nevados de Chillan area. On the one hand, efforts concentrated on improving protection through contracts about land use with private and business land holdings. Other efforts include environmental education programs, purchase of key habitat, creation of new protected areas, and improving infrastructure for wardens. Furthermore, research was undertaken to address dietary behavior, diseases, genetics, and others.

Population size was determined by direct counts and indirect evidence of Huemul presence. Diet was determined by microhistological analysis by identifying plants based on cuticular scale patterns. Gastrointestinal parasites were determined by flotation technique, and genetics was evaluated by studying cytochrome b.

The research projects showed that the population size has diminished over time to currently less than 40 animals separated in two groups, living in a protected area totaling 560.000 ha and consisting of 78.000 ha of National Protected Area, 2.000 ha of private protected area, and the remainder as private areas. The animal's diet is based on grass and leaves of native plants like *Adesmia emarginata*, *Embotrium coccineum*, *Nothofagus antarctica.* The prevalence of gastrointestinal parasites was low, and finally the genetic study showed that there was no difference between Nevados de Chillan and Huemul populations further south of Chile.

Regardless of all institutional efforts, in investigation and land conservation, this population is currently at the brink of extinction possibly due to: its reduced population size; high population fragmentation, with reproductive groups very distant from each other, and geographically isolated (>300 km) from the more numerous and stable population of southern Argentina and Chile.

The recent creation of a center for reproduction by the foundation Huilo-Huilo in the region of Los Ríos has shown itself to be a success with regards to the capture, translocation, conservation, and breeding of the species *ex situ*. This successful experience reinforces the idea proposed in the National Plan for the Conservation of the Huemul of augmenting the population of Huemul in Central Chile, specifically in the Ñuble National Reserve, by means of translocating individuals from donor populations in the south and the creation of a Reproduction Center for scientific purposes that would allow the recovery of the species in this area

Keywords: Huemul, *Hippocamelus bisulcus*, conservation, Central Chile, population trend

Reestablishing a conservation tool for the Patagonian Huemul (*Hippocamelus bisulcus*): Five years of captive breeding

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The Huemul (*Hippocamelus bisulcus),* an endemic deer of Patagonia, is endangered for several reasons, yet efforts to turn the conservation status around for the species have not met with success. Captive breeding projects, though attempted in the past, had been short-termed and with few important contributions. In some cases, methods used for capture and transportation resulted in failures with some or all of the individuals being lost in the process. The Huilo Huilo Foundation started a captive breeding project with the objective to produce sufficient numbers of Huemul for a reintroduction program in the private reserve of the same name at the northern extent of Chilean Patagonia, a place were the species has been extinct since the early 1990s. With government permission, the private reserve built an enclosure of 64 hectars containing primary habitat for the species. After identifying the donating populations, a male and a female were captured in 2005 by teleinjection and chemical immobilization. Two helicopters and a high performance jet were used for the transport of the first couple of animals. Since the initial arrival, animals under ex-situ managements have produced fawns every year, providing strong evidence for the viability of conservation of this species based on captive breeding programs. So far three adults entered the center and one adult was lost from sabotage. The current population consists of four adult males, two adult females and one young of the year. During the autum of 2009, a wild-caught male, who lost a front leg after being seriously wounded from an accident, was transported to a second breeding center in Villarrica, Chile, run by the Fauna Andina Los Canelos Foundation. This foundation has a conservation project for Huemul in the central valley of Chile, a region where this species has possibly been extinct for hundreds of years. This injured male has since been rehabilitated and we are now waiting the arrival of a female for breeding.

Keywords: conservation, neotropical deer, patagonia Huemul, *Hippocamelus bisulcus*, captive breeding

Illegal hunting and cattle, major threats for tarukas

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Tarukas (*Hippocamelus antisensis*) live in the Andes, from northern Peru to northern Argentina, ranging from 2,000-3,500masl at the south to 3,500-5,000masl in Peru and Bolivia. Usually, tarukas live in groups, within rocky outcrops among grasslands. Domestic ungulates increasingly use and invade even the most remote areas used by taruka and might compete with it for food and space, affecting deer populations.
Cattle and sheep ranching are both direct and indirect threats for the taruka. Aside from the competition for space, ranching always include the use of dogs, which can kill adult tarukas, especially when attacking in packs, and kill hidden calves in their first month. A growing threat for tarukas in Peru is illegal hunting, carried out by so called sport hunters, and by personnel working in the mines located in taruka habitat. In the last 15 years it seems that hunters have increased in the high Andes, caused by both the disappearance of terrorist groups in the area and the increase of mining concessions along the high Peruvian Andes. Mining concessions are mostly located on prime typical taruka habitat. Hopefully, legal sport hunters might reverse this trend by working towards a sound management for the species based on trophy hunting on few specimens per year.

Keywords: *Hippocamelus antisensis*, taruka, conservation, Peru, threats, illegal hunting, livestock

Reserve design for an endangered deer: the Huemul (*Hippocamelus bisulcus*) in Chilean Patagonia

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The Huemul is the symbol of Chile. It is an endangered species associated with the Andes of Chile and Argentina and with a world population less than 2,000 individuals. Several reasons have led Huemul to this critical state, such as habitat loss through ranching and the introduction of exotic species, but impacts of these factors are not well known. Since the 80´s the Aysén District in Chile has been recognized as "a Huemul Reserve" due to the existence of some stable Huemul populations like Lago Cochrane National Reserve, which achieved relevancy due to the efforts of CONAF. Recently, new actors have joined in the effort of Huemul conservation, such as NGOs, academic institutions, and the community. In 2004, Conservación Patagónica (CP) acquired the Valle Chacabuco ranch (780 Km^2) to create the Future Patagonia National Park (2,500 Km^2), by linking the neighboring Lago Cochrane and Jeinimeini National Reserves. This new park will respond to the desire of the Chilean State to reestablish territorial continuity and improvement of available Huemul habitat. Huemul is a flag and umbrella species, and thus is of high conservation priority. We have developed five conservation actions. 1) To increase the availability of Huemul habitat. 2) To remove threatening factors such as livestock exploitation, dogs, and poaching via control through establishing park wardens. 3) Generation of basic ecological and biological information through monitoring of marked individuals to estimate distribution and abundance, behavioral patterns, genetic diversity, and sanitary condition. 4) Training courses for park wardens and the local community to exchange experiences. 5) Educational activities such as the Huemul hikes, Huemul scholarships, students and elderly visiting the park, presentations, and radio programs. An important goal is to approach the Huemul conservation from several levels: 1) intraspecific, 2) interspecific, 3) ecosystem, and 4) human dimensions. The results so far show habitat preferences for shrublands and forest in steep mountains, territoriality, high phylopatry, and low genetic variability (2 bottlenecks, a recent one ~ 90 years ago, and an ancient one ~ 10.000 years ago). The current fragmentation of this population in the Cochrane Lake-Chacabuco Valley supports the importance of habitat connectivity for the species in the short term. The creation of protected areas of sufficient size and quality constitutes an effective way to contribute to the species viability, but is also a great challenge. This future protected area also shelters a population of about 2,500 guanacos, which constitute the primary prey of puma (Puma concolor), the top predator in the area. It emphasizes the importance of protecting guanaco and its habitat, and thus to provide alternative prey for puma. In addition, the current role of exotic species such as the European hare (Lepus europaeus), on the abundance of culpeo foxes (Lycalopex culpaeus) and the impact of fox predation on Huemul is our next step because the available information indicates a large impact from mesopredators on young Huemul. In addition, we have found Huemul groups in habitat patches where their presence was unknown, and this constitutes another important advance towards the improvement of connectivity of the population. All the information gathered so far seeks to contribute to the decision making process and the development of coordinated actions to assure the

long-term survival of the Huemul population of this and surrounding areas.

Keywords: Chilean Patagonia, conservation, Huemul, *Hippocamelus bisulcus*, protected areas

Trophic and spatial ecology of Culpeo fox (*Lycalopex culpaeus*), in the Lago Cochrane National Reserve, XI Region, Chile

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There are three wild canids in the genus *Lycalopex* inhabiting Chile. The culpeo fox (*L. culpaeus*) is the largest of them and the one that has the broadest geographical distribution. Within its geographical distribution, culpeo ranges overlap with those of Huemul deer (*Hippocamelus bisulcus*). Here we examine culpeo diet and overlap in space use with Huemul deer to assess it potential role as a fawn predator, as suggested by a previous study. From November 2007 through October 2008, in the Lago Cochrane National Reserve (RNLC) in southern Chile, we studied culpeo diet by analysing contents of 409 fecal samples. Space use of culpeo was studied using radio telemetry and Huemul habitat use was estimated by direct observation of marked individuals.
Results indicate that trophically the culpeo behaves as a generalist and opportunistic predator. Among the 14,092 prey items, that included rodents, lagomorphs, artiodactyls, edentates, birds, insects and fruits, none corresponded to Huemul remains. During the study we could observe an unsuccessful predation event of a Huemul fawn by a culpeo. It seems puzzling that not even the characteristic hairs of Huemul (perhaps coming from feeding on carcasses) were detected in the fox feces. Thus, we found no evidence that culpeo have preyed upon Huemul, neither adults nor fawns.
The special analysis of syntopy showed a marked overlap between 2 culpeo and 8 lactating Huemul female home ranges. However, we could not detect both species at close range simultaneously within days, indicating a level of avoidance, likely determined by the Huemul females. This avoidance behavior may explain the unlikely predation event of a Huemul fawn by a culpeo.
Our results seem at odds with the previous interpretation that the culpeo was an important Huemul fawn mortality agent. We suggest that further monitoring and the use of more sophisticated techniques should be used in order to better interpret the evidence of fawn mortality causes in the area.
Hence, the results suggest that there is no culpeo predation on Huemul deer fawns. Irrespective of the above, there could be non-lethal indirect effects by culpeo on Huemul as yet still unknown and deserving to be studied. It is necessary to continue long-term studies to assess if there is culpeo predation on Huemul deer fawns, and if so, what is the impact of this interaction and/or what non-lethal effects has the culpeo on Huemul deer populations in the LCNR.

Keywords: Huemul, *Hippocamelus bisulcus*, predation, fox, *Lycalopex culpaeus*, fawn mortality, population dynamics

Past and present conservation actions for the Patagonian Huemul in Argentina: The role of protected and non-protected areas

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The Patagonian Huemul (*Hippocamelus bisulcus*), a native deer of Argentina and Chile, has been listed on the IUCN red list as endangered since 1973. In Argentine, despite receiving the highest legal protection plus a National Conservation and Recovery Plan in place since 2005, the recovery of Huemul has not improved in recent times, and instead many subpopulations have continued to disappear, even those within national parks. The situation is particularly grave in that subpopulations are highly fragmented and totaling only 350-600 individuals. Historical events, such as hunting by Indians followed by colonizing Europeans, were likely responsible for Huemul extinctions in more favorable habitats of ecotones and former winter ranges. The failure of populations to rebound today may be due to several factors: a) impact of exotics, b) unavailability of suitable habitat, and c) subpopulations so reduced that they are subjected to constraints of biology of small populations.
Here we provide a brief review of factors a) and b) with emphasis particularly on the suitability of habitat currently used by Huemul. Considering other wild ungulate species, we hypothesize that behavioral patterns, morphophysiological adaptions and population dynamics of Huemul will ultimately be adjusted to forage availability, rather than being fixed traits. However, species plasticity may not suffice if the species is constrained to only a portion of its historic range where acquisition of all its basic nutritional needs are no longer possible. We suggest that lack of recovery of the species in Argentina may hinge on the loss of suitable lower elevational habitat.
The main objective of this work is to review and present conservation strategies that have been practiced over the past 20 years in Argentina and to demonstrate that the emphasis urgently needs to aim at also considering the nutritional ecology if we are to guarantee the species' recovery. Habitat suitability as a pivotal factor influencing the Huemul's demise will be discussed in the context of the role of protected and non-protected areas.

Keywords: Huemul, *Hippocamelus bisulcus*, invasion, exotic herbivores, habitat suitability, nutrition, protected areas

Taruca (*Hippocamelus antisensis*) and domestic livestock: Interactions and demands for resources in Cerro Amarillo, Calilegua National park

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Encargado monumento natural laguna de los pozuelos
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The Cerro Amarillo is the highest mountain range of the Calilegua National park, whose eastern limits are provincial lands with their constant resource utilization from local rural communities. In this sector, the institutional presence of national parks is rather lax which results in the invasion of the park by domestic livestock to areas of high interest for conservation biology and ecology.
In these western high-elevation Andean grasslands with headwaters and abrupt topography, we find some populations of taruca surviving, but anthropic demands are acting on their basic resources needed for survival.
This endemic deer of South America is considered vulnerable by IUCN, whereas in Argentina it is considered endangered (Resolución 1030/04, SAyDS). It is also declared a Natural Monument through national law 24702/96 and provincial Monument in Jujuy by law 5405/04.
In this study we analyzed competition between groups of horses, cattle and sheep in relation to individual taruca, in shared feeding and watering areas, with its potential sanitary risk for wildlife.
We also evaluated the selection of refuge areas, daily movements in search of resources, identifications of threats, loss of habitat, areas used for transit, etc. We looked at habitat use at the scale of the landscape, elevation, slope and vegetation structure.
Cattle and taruca were shown to utilize resoruces in the same space and during the same time; this overlap occurred over a range of hours; and both selected the same forrage species during the critical winter period. We also identified distinct groups or individuals called Fundición, Pueblito and Hermoso; we determined the path of used for diplacement between sectors providing resources and rest areas; we noted that taruca did not make use of forests; and we caution that taruca on this mountain range may be isolated from other populations.

Keywords: taruca, *Hippocamelus bisulcus*, conservation, cattle, protected area, habitat use, competition

Home range of female Pampas deer (*Ozotoceros bezoarticus*) in the Pantanal of Mato Grosso do Sul, Brazil

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The home range is an important information for management and conservation of the species. The Pampas deer (*Ozotoceros bezoarticus*) has been widely studied, especially in Brazil. A study in the region of Brasilia with a male and a female estimated an average home range size of 9.900 and 5.900ha, respectively, by the Minimum Convex Polygon (MCP) method. Two males and two females monitored for one year in Emas National Park had an average home range size of approximately 8235ha. In the Pantanal, the average home range size of this species was 577.7ha by the same method. Those studies used conventional telemetry (VHF) to achieve their results, with no use of GPS collars. Thus, this study estimated the home range size of female Pampas deer in the Pantanal of Mato Grosso do Sul with a greater number of locations with the use of GPS collars. The study area included the farms Nhumirim and Alegria, located in the central region of the Brazilian Pantanal (Nhecolândia). Four free-ranging females of Pampas deer were marked with GPS collars (ATS® G2110), which had their locations recorded every 13 hours for a period of approximately 12 months. The home ranges were analyzed by the extension Animal Movement Analysis version 2.04 of the program ArcView ® GIS version 3.2, using the methods of MCP and 95% fixed kernel probability density independent of the locations. The fixed Kernel method was used to estimate the centers of activity with probabilities of use of 70% and 50%. The home range was analyzed with location points every 13h, 26h and 39h in order to determine the minimum time interval required to achieve the independence of the location points identified by the indices of Schoener and Swihart & Slade. In three of the four females studied, the independence of the points occurred only in the interval of 39h, while in the fourth female no independence occurred in any of the intervals. The average home range size of female Pampas deer was 595.07 ± 215.73ha when estimated by the MPC and 260.16 ± 68.45ha when estimated using Kernel 95%, indicating that the average size of the home range by MPC was 2.3 times greater than by 95% kernel. The individual 002 showed two activity centers set for 50% of the distribution of use, while the individuals 003 and 014 had a single center of activity and the individual 005 showed three activity centers. These centers had sizes between 17.85 and 47.54ha, which represents 5-21% of the total area used by each animal. Furthermore, the results showed that female Pampas deer concentrate 70% of their activities in an area between 16.7 to 40% of its total area. The home ranges found in this study compared with literature data, were much smaller than those obtained in the Cerrado, but similar to those obtained in the Pantanal, corroborating the existence of smaller home ranges for the species in the Pantanal. The reasons for the existence of such huge differences in home range between two ecosystems draws attention and their motives must be determined in future studies.

Keywords: *Ozotoceros bezoarticus*, Pampas deer, home range, ecology, behavior, Brazil

Searching for the last Pampas deer population in São Paulo State, Brazil

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The Pampas deer (*Ozotoceros bezoarticus*) occupies grasslands and cerrado (*stricto censu*) habitats in central and southeastern South America between latitudes 5° and 41°S. Habitat destruction and fragmentation have caused a drastic reduction of the natural geographic area of the species. These factors along with a strong hunting pressure nowadays places *O. bezoarticus* as one of the most endangered neotropical deer. With the exception of big populations in pantanal and part of central Brazil, the species has been restricted to small and isolated populations. In the São Paulo state, where the colonization, agriculture and urban processes were more intense, the impact on the habitat was more drastic. Recent data estimates that grasslands and cerrado areas were reduced to seven percent of the original extension in São Paulo State, which represents just one percent of the total territory. For these reasons, Pampas deer was recently classified as Critically Endangered in the São Paulo`s red list, with the assumption that one last population survives in Santa Bárbara Ecologic Station (SBES), in the outh-central region (22°48'30''S e 49°12'30''W), in a small 2,000 ha fragment of natural habitat. Although in 70 and 80´s and up until 1992, this population was monitored by Dr. Cory Teixeira de Carvalho of the Forestry Institute, no more surveys have been conducted since then to determine the occurrence of the species in the area. The aim of the present study was to find concrete evidence to prove the persistence of this last population in São Paulo. We conducted two field trips in SBES using a scat-detection dog trained to find deer samples. Twenty-four fecal samples were obtained and stored in tubes with silica gel and frozen for DNA preservation. The kit QIAmp ® DNA Stool Mini Kit was used for DNA extraction following the manufacturer protocol. A region of 224 bp of cytochrome b gene was amplified for species identification. This amplification product was subjected to hydrolysis by the restriction enzyme SspI, which generates a cutting pattern able to distinguish between potential species in the area (*Mazama americana*, *M. gouazoubira* and *O. bezoarticus*).
The results were analyzed by electrophoresis in a 3% agarose gel. From the twenty-four extracted samples, eighteen were possible to identify, all of them as *M. gouazoubira*. The results obtained with the samples, suggests either a very low density or local extinction of Pampas deer in SBES. Therefore, it is necessary improve a greater efforts in the field search, including other techniques such as aerial survey and camera trapping to be able to confirm the persistence or local extinction of Pampas deer in the EESB and São Paulo State.

Keywords: *Ozotoceros bezoarticus*, extinction, fecal DNA, RFLP, management, Pampas deer

Identifing non-amazonian Brazilian deer species by PCR/RFLP

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There are six non-amazonian Brazilian deer species and with the increasing use of non-invasive methodologies based on DNA extracted from faeces, skin, hair, hunting products etc, it is necessary to develop protocols to differentiate cryptic samples. There is a methodology based in PCR/RFPL for distinguishing non-amazonian deer species of the genus *Mazama*. This study aimed to establish a PCR/RFLP protocol to discriminate samples from *Blastocerus dichotomus, Ozotoceros bezoarticus,* and non-amazonian *Mazama*. For this, cytochrome B sequences from GeneBank of those species were aligned using the software MEGA 4.0 and then the amplified region by the primers IDMAZH and IDMAZ220 (224bp) were identified and selected. With the software CLC Sequencer Viewer 6.2 were identified the variable and non-variable regions of the cytochrome B fragment among all the species. After that were possible to find that the enzyme SspI separate the non-amazonian *Mazama* (one restriction site) from *B. dichotomus* and *O. bezoarticus* (two restriction sites) and the enzyme TaqαI separate *B. dichotomus* (one restriction site) from *O. bezoarticus* (no restriction site). In order to evaluate these enzymes in biological samples, four DNA samples of *O. bezoarticus* and *B. dichotomus*, extracted from hair and blood were amplified by PCR using the cytochome B primers (IDMAZH and IDMAZ220) and the products were digested by the two enzymes. The results of the PCR and digestions were stained with GelRed™ (Biotium), electrophoresed through an agorose gel (3%) and visualized under UV light, confirming the applicability of this protocol. This PCR/RFLP protocol will contribute for future studies with non-invasive samples where more than one species occur in sympatry as in Pantanal where there are four species: *O. bezoarticus, B. dichotomus*, *M. gouazoubira* and *M. americana*.

Keywords: PCR, RFLP, identification, Mazama, Ozotoceros, Blastocerus, genetic

Genotyping deer faeces at the neotropics

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Mazama genus is composed by five species in Brazil. All of them are extremely shy and have evasive behaviors, what makes the capture almost impossible. Thus, the use of non invasive methodologies is necessary to study the ecology and genetic of these species. One technique that can support good results is the fecal DNA. However, some difficulties rise from this methodology on the neotropic deer studies: (a) Find the scats on the litter (b) samples degradation; (c) PCR Inhibitors; (d) the lack of microsatellite primers specifics for these species. This study aimed to genotype *Mazama americana* field collected faeces with 5 heterologous microsatellite primers. The samples (n=52) were collected in a 600ha forest fragment (21°20'S 47°17'W) during two months in the summer of 2008, with the help of a detection dog and stored at ethanol. Our effort was about 159 hours of effective search in the field. Each sample was classified in "fresh" (with mucus, still bright) and "not fresh" (without mucus, dark, not bright). Of these samples 31% (n=16) were "fresh" and 69% (n=36) were "not fresh". About thirty days after the collection the DNA was extracted using the QIAamp® DNA Stool Mini Kit following the manufacturer's instructions. From the 52 samples collected and extracted, 45 were identified by PCR/RFLP of the cytochrome b as *M. americana*. We tried to amplify by PCR five heterologous microsatellites loci (RT09-120bp; NVHRT16-180bp; BM757-200bp; RT30-210bp and RT01-230bp). The results were stained with GelRed™ (Biotium), electrophoresed through an agorose gel and visualized under UV light. The samples that the reaction did not succeed we repeated it up to 5 times or until it succeeds. Our protocol were the same for all loci and the annealing temperatures started with the same temperature as the established for blood samples and decreased, attempt by attempt, until 3°C lower. We found a negative correlation (-0.74) between amplification success and loci sizes. The "fresh" samples had more success in amplification than the "not fresh" ones in all loci we tested. For "fresh" samples we got an average amplifiability success for all loci of 63.8% and for "not fresh" samples it was 34.5%. These results show the necessity, for this kind of study, of investing time in the samples collect in order to obtain more "fresh" samples. Nevertheless the shorter microsatellite loci primers will probably work better than the longer ones. We suggest also the need to develop specific and short (<180bp) microsatellite loci primes for the *Mazama* genus species.

Keywords: mazama, fecal DNA, microsatellite, neotropic deer, genetic

Protocols for superovulation of Brown brocket deer (*Mazama gouazoubira*)

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Knowledge of assisted reproduction techniques for wild animals is useful for future *in situ* and *ex situ* conservation programs. The present study aimed to establish a superovulation protocol for brown brocket deer (*Mazama gouazoubira*) by evaluating the ovulation rate and the presence of functional corpora lutea (CL) after superovulation using different treatments. For this, two experiments were realized: **(1)** in 2007, six female *Mazama gouazoubira* received an intravaginal device (CIDR®) for 8 days, followed by 0.5mg i.m. injection of estradiol benzoate (OB) at the time of insertion (D-8) and 265µg of cloprostenol ($PGF_{2\alpha}$) at the time of removal (D0). Next, the hinds were divided into 3 groups (n=2): group 1 received an i.m. injection of 600IU eCG **(Treatment A)**, group 2 300IU of eCG **(Treatment B)** and group 3 250IU of FSH+PVP **(Treatment C)**, all on D4 (D-4). The second Experiment **(2)** was developed in 2009 and also used six hinds from the same species divided into 2 groups (n=3): the first received CIDR® for 8 days, followed by 0.25mg i.m. injection of OB on D-8, 700IU of eCG on D-4 and 265µg of $PGF_{2\alpha}$ on D0 **(Treatment D)** and the second received CIDR® for 7.5 days followed by 0.25mg i.m. injection of OB on D-7.5, 130mg of FSH divided into eight equal doses [beginning on D-3 and ending D-0.5] and 265µg of $PGF_{2\alpha}$ on D0 **(Treatment E)**. In each experiment, the treatments were 'crossed over' with 44 day intervals after CIDR® removal. All the hinds received an i.m. injection of $PGF_{2\alpha}$ 14 days after CIDR® removal. Treatment efficacy was evaluated by reproductive behavior, observation of CL and unruptured follicles (over 3mm) via laparoscopy 7 days after the first copulation. In Experiment 1, feces were collected and later analyzed for fecal progestin concentration. The mean ovulation rate (Treatment A = 3.40±0.68 vs. Treatment B = 1.40±0.24 vs. Treatment C = 0.80±0.49); total ovarian stimulation (Treatment A = 4.80±1.02 vs. Treatment B = 1.80±0.37 vs. Treatment C = 1.40±0.60) and mean CL diameter (Treatment A = 7.33±0.76mm vs. Treatment B = 3.94±0.19mm vs. Treatment C = 2.18±0.49mm) in Treatment A were significantly higher than the mean ovulation rates, total ovarian stimulation and mean CL diameter in Treatments B and C. However, the superovulation response was not significantly different between treatment groups (5/6 hinds, 2/5 hinds, and 2/6 hinds, in A, B and C, respectively). The mean fecal progestin concentration at the luteal phase in Treatment A (6277.94±2232.47 ng/g feces) was significantly different from Treatment C (1374.82±401.77 ng/g feces), but not from Treatment B (2970.15±564.38 ng/g feces). The mean ovulation rate in Treatment D (6.0±1.7) was higher than ($P \leq 0.05$) the mean ovulation rates for Treatment E (2.0±0.3), but the superovulation response (6/6 hinds and 4/6 hinds, in D and E, respectively) and the total ovarian stimulation (13.2±2.6 and 10.4±1.1, in D and E, respectively) were not significantly different between treatments. Thus, although fertility was not evaluated directly, Treatment A and D proved capable of induce superovulation in the species *Mazama gouazoubira*, presenting the greatest mean ovulation rates and total ovarian stimulus, with the formation of functional corpora lutea in Treatment A.

Keywords: superovulation, *Mazama gouazoubira*, fecal progestins, Brown brocket deer,

reproduction, conservation

Inventory of deer species in the Jataí Ecological Station, Brazil, using camera traps

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Jataí Ecological Station, located in the city of Luiz Antonio-SP, Brazil, has 4.532 hectares and is situated in the largest preserved areas of Cerrado (which is very similar to savanna ecosystem, and extends to ¼ of the brazilian territory) in the state of São Paulo. A wide variety of other ecosystems is also observed at the reserve, such as aquatics, marshes and non-swamp areas, which make Jataí Ecological Station a reserve where numerous species of the national fauna and flora can be found. Indirect evidence (footprints and feces) suggested the existence of deer in the area, however, little is known about which species currently inhabit the reserve. Based on the absence of data, an inventory of the existing deer species at Jataí Ecological Station was carried out through use of camera traps. Eighteen circular parcels with radius of 200m each, along a 7.200m transect through the reserve were sampled, allowing the analysis of different slopes and environmental gradients in the non-swamp habitat. A total of 18 camera traps were used, one trap for each parcel. Traps were placed where evidence of deer was found, such as: feces, bedding areas, trails and footprints. Traps operated 24h/day for an average period of 43 days. The sample effort of 18212 hours, resulted in a total of 18 deer photographs, a success of obtaining one photo every 1020 hours. From the obtained photographs two species of deer were identified: where 17 photos were of brown brocket (*Mazama gouazoubira*) and one of a red brocket (*Mazama americana*). The obtained results denote the existence of at least these two species of deer at Jataí Ecological Station. One of the species originally found in the region, the Pampas deer (*Ozotoceros bezoarticus*), was not photographed and given the existing data could be locally extinct. The marsh deer (*Blastocerus dichotomus*) was recently reintroduced in the area, however it was not detected by the camera traps given the difficulties in installing this equipment in marsh areas. However, this corroborates information that marsh deer do not inhabit dry areas of the Cerrado. Future studies should be conducted in flooded areas for the identification of this species.

Keywords: Jataí Ecological Station**¡Error! Marcador no definido.**, Mazama, Ozotoceros, Blastocerus, inventory, census, camera traps

Effects of physical and chemical restraint on hematological, blood biochemistry and electrolyte variables of captive Brown brocket deer (*Mazama gouazoubira*)

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Alterations in hematological, blood biochemistry and blood electrolyte variables indicate the consequences of physical or chemical restraints in deer. This study aimed to report the effects of these procedures on the above mentioned variables of captive brown brocket deer (*Mazama gouazoubira*). Six adult deer were physically restrained and randomly anesthetized in a crossover design using three protocols (P1, P2 and P3). P1 - direct induction of anesthesia using a vaporizer setting of 3 Vol.% of isoflurane via face mask. After loss of laryngeal reflexes; the deer were submitted to orotracheal intubation using a cuffed endotracheal tube connected to a semi-closed circle breathing system with an out-of-circle vaporizer calibrated for isoflurane. Anesthesia was maintained with isoflurane diluted in a flow of 60 ml/kg/min of 100% oxygen for a period of one hour and deer were allowed in spontaneous breathing. P2 - oral premedication with midazolam (0.5 mg/kg) and after one hour, induction and anesthesia maintenance with isoflurane, as per P1. P3 - induction of anesthesia with intravenous administration of a combination of ketamine (5 mg/kg), xylazine (1 mg/kg) and atropine sulphate (0.05 mg/kg), followed by isoflurane via face mask permitting intubation and anesthesia maintenance, as per P1. The first blood sample was obtained immediately after physical restraint (M0) and three additional samples were collected during anesthesia at 20 min intervals (M2, M4 and M6). Blood constituents (red blood cells (RBC), white blood cells (WBC), packet cell volume (PCV) and hemoglobin), biochemistry (glucose, urea, creatinine, albumin and total serum protein) and electrolytes (plasma ionized calcium, sodium, potassium and chloride ions, besides osmolality) were determined by manual methods or automated analyzers and cortisol was determined by enzyme immunoassay (intra- and interassay coefficients of variation were 4.8% and 10.7%, respectively; and assay sensitivity was 3.9 pg/well). Data were analyzed by ANOVA using a split plot design; with protocols as the principal source of variation in the whole plot and moments and interactions between protocols and moments as a secondary source of variation in the subplot; followed by the Tukey test using the SAS. $P < 0.05$ was considered significant. Statistical analysis of the results verified significant hemoconcentration; increased WBC counts; and lower cortisol concentrations during M0 compared to M2, M4 and M6. Moreover, observation verified lower lymphocyte counts in P2 compared to P1; decreased urea concentrations during M2 of P2 compared with remaining protocols; and increased glucose concentrations after M0 ($P < 0.01$). The results presented were consistent, since standardization in blood collections was achieved and demonstrated that hemoconcentration and increases in WBC, glucose and cortisol concentrations occurred due to physical restraint. This study also corroborated the early descriptions of α2 agonist induced an increase in blood glucose. Additionally, it was possible suggest the effects

of midazolam premedication in relation to the reduction in stress response based on slight reduction of lymphocyte counts and cortisol concentrations, even though statistically significant differences in cortisol concentrations were not observed between the anesthetic protocols studied. Based on these results, midazolam appears to affect the intensity of the response to stress; however further studies must be conducted on brown brocket deer to evaluate the effect and importance of midazolam on catecholamine release and cortisol concentrations separately.

Keywords: chemical restraint, cortisol, hematology, *Mazama gouazoubira*, physical restraint, plasma electrolytes, serum chemistry, Brocket deer, capture

Karyotype description of the Amazonian Brown brocket deer, *Mazama nemorivaga*

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The Amazonian Brown Brocket deer, *Mazama nemorivaga* is a grayish small-sized deer that inhabits the Amazonian biome. Preliminary descriptions of its karyotype presented chromosome numbers varying from 66 to 73, but did not show chromosome banding. The present work aimed at making a detailed description of the karyotype of this species, through the analysis of G, C, and NOR banding. With this purpose, cultures of peripheral lymphocytes and skin fibroblasts were made from seven animals, three males and four females from Mato Grosso, Pará and Maranhão. At least 20 Giemsa-stained metaphases of each individual were analyzed for chromosome biometry. Chromosomes were classified according to their shape and size into one of the following groups: A (large biarmed chromosomes), C (large acrocentrics), D (small biarmed chromosomes), E (small acrocentrics) and B (supernumerary or B chromosomes). Then, G (GTG), C (CBG) and NOR (Ag-NOR) banding procedures were made and the banded metaphases were photographed and arranged into karyotypes for further comparison. Diploid numbers varied from 67 to 69 plus two to six B chromosomes, NOR bands were present in the telomeres of the two largest E chromosome pairs in almost all animals. Females showed 2n=68, XX + 3 or 4 B's being 66 autosomes from the group E and the X chromosome a medium-sized submetacentric with a small interstitial C-band in the middle of the q arm. The males from Para and Maranhão showed 2n=69, XY1Y2 + 4 to 8 B's, being 66 autosomes from group E, the X chromosome a mediumsized submetacentric similar to the ones seen in the females, a small-sized submetacentric Y chromosome (Y1 or original Y) and one additional small-sized E-like chromosome showing G banding patterns homologous to those in the distal half of the q arm of the X chromosome (Y2). A male from Mato Grosso presented the karyotypic variant 2n=67, XY1Y2 + 6 B's, with two autosomes belonging to the group A, 62 autosomes of the group E, and sex chromosomes equal to those described in the other males. The autosomes of A-group were homologous and metacentrics: the NOR bands were present in the autosomic pairs two and five. These results suggest the existence of karyotypic differentiation between the populations of *M. nemorivaga* from Eastern and Western Amazon suggesting two different evolutionary units/species. Further analyses should be done in order to confirm this hypothesis.

Keywords: Mazama, Brocket deer, banding, conservation, karyotype, genetics

Huemul *(Hippocamelus bisulcus*, Molina 1782) gastrointestinal study in Center-south and South of Chile

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Huemul (*Hippocamelus bisulcus*) has been declared as endangered in Chile and Argentina. Parasites have been described as a cause of reducing populations of Huemul, however there is no information to support it.
With the objective of determine gastrointestinal parasites in Huemul, 200 fecal samples that came from different areas of the regions of Bio Bio, Aysén and Magallanes, where examined using the flotation technique, and to detect *Fasciola hepatica*, the sedimentation method was used. Of all samples, only 44% (n=88) were positive for one or more gastrointestinal parasites. A low prevalence of parasitism was found in Bío Bío (11,8 %), with medium levels in Aysén (27,6 %) and substantially higher levels in Magallanes (87 %). Detected parasites correspond to *Moniezia* in Bio Bio, and *Strongylus, Nematodirus, Moniezia* and coccidian ooquists (*Eimeria*) in Aysén and Magallanes. No evidence of the presence of Fasciola sp. was found in the examined samples. Analyzing seasonal differences, a larger prevalence of positive samples was found during summer (73,3 %) and autumn (63,9 %), decreasing in spring (40,3 %), considerably diminishing during winter (11,8 %).
The overall gastrointestinal parasitism was thus generally low, and no evidence was found for *Fasciola hepatica*. The genera found in this study are also commonly found in domestic livestock, and these should be treated prophylactically if near Huemul, or if using summer ranges shared with Huemul.

Keywords: Huemul, *Hippocamelus bisulcus*, parasite, gastrointestinal, fasciola hepatica, strongylus, nematodirus, moniezia, eimeria, prevalence

Rapid biological assessment through analysis of mitochondrial DNA for identification of deer *Mazama genus* (Rafinesque, 1817) in forest areas and in captivity in the region of the Western Brazilian Amazon

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The Amazonian rainforest has a singular biodiversity and Rapid Biological Assessment can provide valuable and quick information to monitor species diversity. In the western Brazilian Amazon it is not clear if it is inhabited by two or three species of *Mazama* (*M. americana, M. nemorivaga* and *M. gouazoubira*). As brocket deer are sympatric and also are cryptic species, there is a need to apply molecular markers to discriminate species. The objective of this study was to perform a quick survey based on collecting deer feces to isolate DNA and to perform the taxonomic determination using mitochondrial molecular markers. Selected areas of the ecotone region of Cerrado, from the Rondonia and Acre states, were surveyed during January to February 2005. Furthermore, as controls we also collected feces from animals in captivity with known origin from the same areas. Twenty five samples were amplified (PCR) using cytochrome b primers flanking a region of 224 base pairs and subsequently sequenced it. The fragment of 224 bp of cytochrome b has been quite informative in previous studies to identify the species of *Mazama*. For data analysis the sequences were aligned with other South American deer sequences from Genbank using the program CLUSTAL V and checked visually. Haplotypic lineages identified were then analyzed using Kimura 2-parameter model and the number of differences in nucleotide bases to estimate the genetic distance between them, and the UPGMA and "Neighbor-joining" algorithms in the program MEGA. The statistical support for the obtained phylogenetic groups was estimated by a thousand replicates of bootstrap. Through this marker it was possible to classify the different samples as *M. americana* and *M. nemorivaga*, despite the low statistical support for groups in most branches of the obtained phylogenetic tree. The lack of samples classified as *M. gouazoubira* really shows that this species is replaced by *M. nemorivaga* in forest areas of the western Brazilian Amazon. However, the presence of *M. gouazoubira* can not be dismissed due to the phytophysionomies of open areas in the region. The results presented here indicate that this technique could be used for a relatively fast access of the current distribution of the gray *Mazama* species.

Keywords: Amazon, Brazil, Mazama, Brocket deer, mtDNA, genetic, fecal DNA

ADVANCES IN THE CONSERVATION AND ECOLOGY OF PUDU

Domestic dogs as a threat for the conservation of the Southern pudu in Chile: Diagnosis and management alternatives

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The southern pudu (*Pudu puda*) is a vulnerable deer endemic to the South American temperate forests. The pudu is seriously threatened by forest loss and possibly other human-related threats such as the attacks of domestic dogs (*Canis lupus familiaris*), poaching and roadkills. Here we present the results of two studies. The first evaluated whether domestic dogs were likely to be an important threat for pudu conservation. To assess the potential importance of dogs as a threat for pudus we reviewed five years of clinical records of the two main rehabilitation centers of southern Chile and conducted necropsies on animals found in two field sites. Attacks by domestic dogs were the most frequent causes of pudu admissions to rehabilitation centers (50%, n=44) and of deaths of animals found opportunistically in the field (5 of 14). These findings provide support for the hypothesis that in addition to forest loss, dogs are important concerns for pudu conservation. The second study tested the hypothesis that management of domestic dogs is associated to predation rates on wildlife (and pudu). To assess whether the management of domestic dogs influenced predation rates on wildlife, we conducted a comparative-observational study in two localities of southern Chile. In both sites we estimated predation rates on wildlife by analyzing scats and interviewing dog owners. The quality of the nutritional management of dogs was established by clinical examination of dogs and by interviewing the owners. The association between management and predation rates was tested using logistic regression. As expected, we found that food provision by the owner was significantly related to predation pressure exerted by dogs on wildlife, and the association held in the two localities studied. Our data provide support for the hypothesis that poor management of dogs could increase their impact on biodiversity in general and pudus in particular. The inadequate management of dogs is not only a problem for biodiversity and pudus, but also a concern about dog welfare and human health. We suggest that collaborative work between biodiversity conservation, animal welfare and human health professionals will be the most effective way to improve the management of dogs and -in the long run- to decrease their impact on endangered species such as the pudu.

Keywords: *Pudu puda*, domestic dog, predation, Chile, biodiversity conservation

Distribution, deforestation threat and conservation on *Pudu mephistophiles* in Peru

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The Northern Pudu (*Pudu mephistophiles* De Winton 1896) occurs along the northern Andes, from the Central Cordillera of the Colombian Andes to the central eastern Andes of Peru. The overall distribution is split with two populations separated geographically. One population distributes throughout the southern portion of the central Andes of Colombia to the south of the Ecuadorian Andes. The second population is cut off from the first one, and occurs along the central part of the eastern side of the Peruvian Andes, Unpublished records from Peru in the last 25 years show that the northern population distributes farther to the south and the southern population farther to the north. The current known gap between populations is located at the East side of the North Peru Low or Huancabamba Depression, a barrier of low altitude and drier environments along the Huancabamba and Marañón Rivers.

The distribution of the species in Peru overlaps with the area with the largest deforestation of montane and cloud forests in this country. The small area occupied by the northern population in Peru is heavily degraded and deforested, and it is shared with the Ecuadorian dwarf brocket *Mazama rufina*. This northern population endures the largest threat from deforestation, as very few continuous cloud forest and paramo persist in that area.

The southern population of the northern pudu occurs along the northern and central part of the eastern face of the Peruvian Andes. This Peruvian population is protected in some conservation areas, and new ones are being created over its range. However, deforestation has not stopped, and the threat is continuous throughout the Peruvian population distribution. Moreover, the southern or Peruvian population of the species might correspond to a different taxon, at least at subspecies level, increasing the conservation concerns on it.

Keywords: Northern pudu, *Pudu mephistophiles,* conservation, Peru, distribution

Molecular evidence of historical *Pudu puda* Island-Continent isolation: Based on mtDNA control region and cytb

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Island-continent isolation has been an interesting model of study in different current species distribution. In southern Chile during the last glacial maximum, ice sheet advances and retreats led to the formation of the Patagonian archipelago; in which Chiloe Island represents a possible glacial refuge for some *Pudu puda* populations. This native deer is currently distributed along Valdivian temperate rainforests of Chile and Argentina. We used mtDNA control region (CR; 654 bp) and cytochrome-b (Cytb; 734 bp) to understand the effects of this historical isolation over current *Pudu puda* distribution and population structure. Bayesian and Maximun likelihood phylogenetic analysis revealed two significant divergent clades, representing insular and mainland populations. We also found a significant high structure (Φst=0.75) and a sequence divergence percentage of 2.3% between the two clades. Analysis among continental populations showed no clear geographical population structure. Pudu populations were isolated on Chiloé Island from the continent through several past interglacial periods and connected during glacial periods. We calculate the divergence time between the two lineages around 500.000 ya which could suggest isolation during the interglacial periods since the coldest Pleistocene glaciation (~700.000 ya). Therefore, it lead to two reciprocally monophyletic clades and indicates that no recent flow between the two locations occurred. Consequently, we can define two Evolutionary Significant Units (ESUs) which should be considered in the development of future conservation programs for the species.

Keywords: conservation genetics, island-continent isolation, *Pudu puda*, mtDNA, genetics, evolution

Status of the ecology and conservation of the Southern pudu (*Pudu Puda*)

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The objective of this presentation is to examine the current knowledge on the ecology and conservation of the southern pudu deer (*Pudu puda*). I reviewed and examined the technical and grey literature that contribute to the conservation and natural history information on the southern pudu and complemented it with my own field observations. Although it is claimed that the pudu is the world´s smallest deer, this unique species is little known. Only a handful of quantitative studies on the species exist and most come from anecdotal observations. Its distribution is restricted to the southern temperate forests of Chile and neighboring Argentina, from ca. Cauquenes to Aysén, but evidence shows that it may have ranged as far north as Santiago. Unlike other typical deer the pudu has a stout appearance, having short legs and weighting less than 15 kg. It is a solitary forest dweller that conforms to the syndrome of the small solitary forest ruminants. The pudu feeds on a high diversity of native plants –forbs, ferns, saplings and vines- that grows in the understory as well as on some exotic forbs, shrubs, and trees. Pudus have home ranges of about 20 ha. Several external and internal parasites have been found on pudus. The pudu makes up to 49% of the diet of pumas on the mainland cordilleras; foxes appear not to be an important predator of adult pudus. Throughout its range it suffers from intense and recurrent persecution by unleashed domestic dogs. The status of their populations is little known and most records come from animals run over by cars, caught by dogs, or brought to rehabilitation centers, than by actual field observations. Regardless of the above, habitat destruction appear to be another critical threat to its conservation, although it appears to be favored by the abundant forage that growth along forest edges. Poaching for zoos was an important threat in the past. The presence of dogs appears to be the most critical issue for the well being of the species. For the above reasons, pudu populations appear to be declining throughout its distribution. I will provide information from a field study in Chiloé and a pudu released and tracked on the mainland.

Keywords: pudu, *Pudu puda*, conservation, threats, dog predation, habitat modification, diet, parasite

An ex-situ conservation tool for the pudu (*Pudu puda*): Ten years of captive breeding

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The pudu (*Pudu puda*), the smallest deer of the world, has been in several zoos around the world with few results on creating sustainable ex-situ populations. The findings on the management in captivity of these animals will be reported briefly. Fundacion Fauna Andina - Los Canelos at Villarrica, Chile has been working with the species for ten years. The project started with several animals that were turned over from the Chilean government wildlife authority SAG. Most of these individuals had either been attacked by dogs, were illegally owned or escaped attempts to be poached. The center has been able to breed the animals successfully, having a current population of 28 animals, with 75% of those individuals having been captive bred at the center. Even though the center had been able to keep the animals in good health condition and resulted in successful and continuous breeding, the project had to face several difficult situations in terms of the management. During the first year of the project, all of the newborns (2 fawns) died within the first month, following a bout with severe diarrhea. Necropsies showed that the animals died due to necrobacillosis, which occurred when they started receiving vegetable material in their feed. The adults at the center had symptoms of foot rot only a few weeks before. Up until this time, the females at the center had gotten pregnant every year, with cases of necrobacillosis fluctuating every year. Some females became pregnant at six month of age: all of them were individuals in good body condition. Even though the rut season for this species is in autumn (April -May), some females became pregnant in spring (October), thus giving birth in late May. These same observations have been made on wild, free-roaming pudu. During the last three, exceptionally warm springs, several cases of pneumonia caused the death of several newborn and adult animals for the first time. This past summer, the project had a three-month old animal fatality from a fecaloma, a tumor-like accumulation of feces in the rectum. Currently, the 28 animals are kept in a 13-hectare enclosure of native forest, and a release project will soon be started that will include radio-telemetry surveillance and monitoring.

Keywords: pudu, *Pudu puda*, ex situ, captive breeding, management, reproduction, diseases

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