University of Sassari
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Via Muroni 25, I-07100 Sassari, Italy

Dissertation for the Degree of Doctor of Philosophy in Environmental Biology
presented at Sassari University in 2014

XXVII cycle

BEHAVIOURAL ECOLOGY OF ALPINE UNGULATES MATING OPPORTUNITIES, MATE CHOICE AND REPRODUCTIVE SUCCESS IN TWO UNGULATE SPECIES (ALPINE IBEX and ALPINE CHAMOIS)

PH.D. CANDIDATE: ***Federico Tettamanti***

DIRECTOR OF THE SCHOOL: ***Prof. Marco Curini Galletti***

SUPERVISOR: ***Prof. Marco Apollonio***

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‘The best way to have a good idea is to have lots of ideas’
Linus Pauling *(1901 – 1994)*

ABSTRACT

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[ENGHLISH] The crucial decisions in an animal life are how allocate available energy in order to maximize their fitness. In species living in harsh and high seasonal environments, such as in Alpine ecosystems, the maximization of fitness leads the adoption of different life history strategies, permitting to individuals to answer optimally at the trade-offs between life history trait occurring in life. Trade-offs in time and energy allocation shape many aspects of individual's decisions and animal behaviour. Seeing information on life history traits are lacking for females Alpine ibex (*Capra ibex*) and Alpine chamois (*Rupicapra rupicapra*), I provided an investigation on social factors, such as the group member composition, choice of the partner and investments in reproduction in late age. These are traits that impose a trade-off among survival, maintenance and reproduction. First, I demonstrated that the group member composition influence the balance between different activities in males and females ibex. Second, I provided evidences of female ibex sexual behaviour able to distinguish and to prefer males higher in hierarchy. Third I highlighted the importance of the individual quality in dealing the cost of reproduction, in female ibex and chamois, and also in masking reproductive senescence, in chamois. The results indicate as female Alpine species adopt adequate life history strategies over time able to maximize their fitness.

Key words: ageing; group structure; individual quality; mate choice; trade-offs

RIASSUNTO

[ITALIANO] Durante la loro vita gli animali sono tenuti a prendere importanti decisioni su come investire la loro energia nell'obbiettivo di massimizzare la loro fitness. Massimizzare la fitness per gli individui che vivono in ambienti difficili e con un clima molto variabile, come per esempio gli ecosistemi alpini, significa trovare il metodo ottimale per rispondere ai compromessi che si vengono a formare tra le diverse necessità indispensabili per sopravvivere e riprodursi. Il tempo e l'energia a disposizione sono ristretti perciò gli individui devono effettuare delle scelte che influenzano necessariamente il loro comportamento. Visto che le informazioni sulle scelte per massimizzare il fitness effettuate nelle femmine di stambecco alpino (*Capra ibex*) e camoscio alpino (*Rupicapra rupicapra*) non sono molto esaustive e complete si è cercato, con questa tesi, di aumentare le conoscenze in questo ambito. Sono stati analizzati diversi fattori, come i fattori sociali, per esempio la composizione dei gruppi, il comportamento riproduttivo, per esempio la scelta del partner, e l'investimento nella riproduzione in tarda età, che impongono agli individui delle scelte ponderate per cercare di sopravvivere, mantenersi e riprodursi. È stato dimostrato che la composizione dei gruppi influenza fortemente le scelte comportamentali in maschi e femmine di stambecco. È stato evidenziato come le femmine di stambecco cambino il loro comportamento durante il giorno dell'estro e che possono effettuare una scelta del partner. Le femmine scelgono i maschi con una posizione gerarchica migliore, che corteggiano più a lungo e che le proteggono dal disturbo causato dagli altri maschi. Per ultimo è stato mostrato come la qualità individuale ricopre un ruolo fondamentale nel gestire i costi della riproduzione, sia in femmine di stambecco e camoscio. Nelle femmine di camoscio si manifesta una senescenza riproduttiva esclusivamente in tarda età, dove la qualità della femmina influenza la sua capacità a sopportare il declino nella riproduzione. Questa tesi ci indica come sia difficile per le femmine di specie alpine massimizzare la loro fitness e come debbano utilizzare delle strategie differenti in tempi diversi, sottolineando come le femmine di buona qualità possano cambiare più facilmente le loro strategie e di conseguenza aumentare la loro fitness.

Parole chiave: struttura del gruppo; scelta del partner; qualità individuale; invecchiamento; compromessi

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GENERAL INTRODUCTION

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A. The purpose and scope of the research

The aim of this thesis is to increase the knowledge on the factors shaping the survival and reproduction in female ungulates. Female ungulates influence the variability in population growth rate, provide parental care, influence the mating success of males and generally survive more than males. Nevertheless researchers principally concentrated on males life-history traits and those of females have still received little attention. Implications from this research, focalized on females ungulates, extend our knowledge on the life history traits of this sex, investigating their mating opportunities, their mate choice and their reproductive success.

B. Life history theory

Life history theory provides a framework for understanding how organisms should optimally allocate time and energy to life history traits (reproduction, maintenance and survival) in a way to ultimately maximize their fitness (the ability of an individual to spread a set of genes into the population through kin, siblings and offspring, and into the future, through offspring).

Populations of large herbivores are strongly age- and sex-structured. In most species, a polygynous mating system leads to a pronounced sexual size dimorphism, which is correlated with marked sexual differences in life history traits (Clutton-Brock et al. 1982a; Gaillard et al. 2000). Differences can occur in longevity, reproductive efforts and age at maturity (Stearns 1977) not only among sex, but also within sex (Gross 1996; Hamel et al. 2010), highlighting the importance of the individual quality. Based on its quality an individual could invest more in one or another life history traits in order to maximize its fitness. However a beneficial change in one life history trait is linked to a detrimental change in another, inducing a trade-off (the time and energy invested into one biological function is necessarily expended at the detriment of another; Stearns 1989). The most prominent life-history trade-off involves the cost of reproduction, where interact the cost paid in survival and that in reproduction (Stearns 1989). This fundamental compromise leads to an evolution in reproductive strategies (Roff 2002) and generate individual variation, as well as the evolution of social system (Gross 1996). Thereby, individual quality can thus defined as a covariation among life history traits at the individual scale (Hamel et al. 2010).

C. Male life history traits

Studies examining the life history traits of males are common in research and have to be take into account to better understand the life history traits of females. In polygynous species it is common found a large sexual dimorphism, where males are the larger sex and have larger weaponry (such as horn and antlers) compared to those of females (Loison et al. 1999a). This involves large importance of the alternative mating tactics adopted by males during the mating season in order to have access to the other sex (see Isvaran 2005 for a review). In these species it is shown that the maximal reproductive potential occurs when adult body size is reached (Clutton-Brock and Albon 1979; Pelletier and Festa-Bianchet 2006) but, equally, it is not uncommon for young males to pursue a reproductive success (Willisch et al. 2012). Reproductive success in males depends largely in their capacity to monopolize females, which occurs often in old and/or dominant male, whereas for younger/subordinate one depends on the use of alternative mating tactics (Hogg 1987; Isvaran 2005; Saunders et al. 2005; Mainguy et al. 2008).

Senescence (the decline in survival and reproductive success at old age) is a common phenomenon in males ungulates (Loison et al. 1999b) where they first invest in their growth and afterwards start to reproduce (Stearns 1992). Reproduction probability, as survival, declined in older males, showing a reproductive senescence (McElligott et al. 2002). Moreover senescence change their intensity accordingly to the male quality, with good reproducers surviving better than non-reproducers (McElligott et al. 2002), indicating as the quality of individuals could be influence the cost of reproduction (van de Pol and Verhulst 2006; Weladji et al. 2008).

Polygynous ungulates exhibit strict sexual segregation during a large part of the year, gathering only for mating (Bowyer 1984; Ruckstuhl and Neuhaus 2000, 2005). Thus the offspring care fall only on females. Seeing males ungulates do not invest in parental care, they try to maximize the reproductive success in function of the number of females present in their environment (Gross 2005). The lack of parental care in males allows an increase in their secondary sexual characters used as reliable indicators of their condition able to attract female, whereas females should be increase their selective choose of partner to choose a mate in best condition, suggesting the role in shaping sexual selection of parental care (Andersson 1994; Wiley and Poston 1996).

D. Female life history traits

The most important difference between males and females is the reproductive investments in parental care and, accordingly, the change in dealing the cost of reproduction. Females in ungulates, because of their lower potential reproductive output and higher reproductive investments compared to males (Trivers 1972), should not simply attempt to maximize their instantaneous reproductive output, but should allocate resources to reproduction taking into account the possible effects of current reproductive effort on future reproductive potential (Festa-Bianchet and Côté 2008). Moreover females should show an important selection among different mate to increase their reproductive success, seeing their lower potential reproductive output (Clutton-Brock and Vincent 1991). The survival of kid is dependent on maternal care; thus age, reproductive experience, previous reproductive status and therefore individual quality can have strong effects on kid survival (Gaillard et al. 2000). Different studies investigated sexual behaviour, investment into reproduction and survival of females ungulates (Byers et al. 1994; Bérubé et al. 1999; Lovari et al. 2008; Hamel et al. 2010; Martin and Festa-Bianchet 2011; see for a review on sexual behaviour of females Bro-Jørgensen 2011), showing important differences in life history traits between the two sex. Large importance has been given to the differences in phenotypic quality of females in influencing their reproductive success (Gaillard et al. 1992; Cameron et al. 1993; Festa-Bianchet 1998; Hamel et al. 2010; Gaillard et al. 2000; Stopher et al. 2011) but much less is known about how females evidenced a specific sexual behaviour during the mating season (see Bro-Jørgensen 2011 for a review), how females evidenced reproductive senescence (Bérubé et al. 1999; Mysterud et al. 2002; Martin and Festa-Bianchet 2011; Hayward et al. 2013) and how one reproductive attempts influence the probability of reproducing in the following breeding season (Kirkpatrick et al. 1996; Hamel et al. 2010; Weladji et al. 2006, 2008).

i. Female sexual behaviour

Darwin distinguished two selective mechanism in the theory of sexual selection: the intra-sexual competition for mates (male-male competition) and inter-sexual competition (the female choice of mates; Darwin 1871). In polygynous species females should be choosier the most vigor and health male (Trivers 1972; Andersson 1994), seeing the ability to distinguish among male and mate with the higher quality one is determinant of her reproductive success (Krebs and Davies 1978). Nevertheless knowledge on sexual behaviour performed by females is still relatively limited.

Female ungulates have different way to select the best males, such as choosing for the antler and horn size that could be indicate a good genetic quality (Clutton-Brock 1982; Kruuk et al. 2002), for the choice of predetermined mating areas (Carranza and Valencia 1999), for location of their territory (Carranza 1995), on the basis of their ability to acquire resources (Bussiere et al. 2008), for cues (e.g. testosterone; Longpre and Katz 2011), for vocal displays (Charlton et al. 2007; Stachowicz et al. 2014) and for courtship display (Longpre et al. 2011). Some of these options carry genetic benefits and some other non genetic benefits. Because the variance in lifetime reproductive success of females is also due to individual differences in costs and benefits associated with mating, a full understanding of female mating strategies (consequently sexual behaviour) and life history strategies is necessary.

Females show evidence of preferences for both males who provide indirect genetic benefits and males who provide direct benefits. Moreover mate choice can provide direct benefits to females, by reducing male harassment and thus mate cooperatively with preferred males (Bro-Jørgensen 2011). Female mate choice also may influence the paternal investment. Females have indeed been found to prefer males that show care taking abilities, through courtship behaviour (Östlund and Ahnesjö 1998). In fact courtship behaviour may provide information about male's parental ability inducing the females to choice for direct benefits in terms of parental care. Further courtship behaviour may be an honest indicator of male's age, experience and body condition, thus providing evidence of a male's quality (Longpre et al. 2011).

ii. Female reproductive success

Female reproductive success vary in function of the time, from juvenile stage to senescent stage (Gaillard et al. 2000). Investigate the relationship between reproduction and age of females is thus a fundamental step to understand the life history strategies of every individuals (Stearns 1992) and to understand the population dynamics of the species. The senescence hypothesis predicts that older females have fewer resources to allocate to reproduction due to physiological deterioration with ageing (Reznick et al. 2000). Senescence in ungulates females, as already seen in males, is well documented in survival probability (Loison et al. 1999b; Corlatti et al. 2012a), but the change in reproductive effort at the end of life are still lacking (Martin and Festa-Bianchet 2011). In late age females could increase their reproductive effort, according to the terminal investment hypothesis (Clutton-Brock 1984). Moreover individuals could decrease their reproductive success not only by ageing but also by physical deterioration over time, which can vary among individuals (Ricklefs 2008). Senescence in reproductive success could occurs at the older age for all females, predicted by the 'mutation accumulation' (Medawar 1952) and 'antagonistic pleiotropy' theories (Williams 1957) or could occurs for all individuals in their late-life, predicted by the 'damage accumulation' hypothesis (Ricklefs 2000).

Longer-lived individuals are suggested to have greater chances to raise offspring (Weladji et al. 2006), suggesting as survival is the main determinant of female fitness (Hamel et al. 2009). Reproductive success in female could influenced by body mass (Festa-Bianchet 1998), by the age at primiparity (Stearns 1992), by the ability to acquire resources (Weladji et al. 2006), by the environmental conditions early in life (Nussey et al. 2007) and by population density (Gaillard et al. 2000). Investments in present reproduction involves different consequences both in body growth (Hamel et al. 2010) and future reproduction (Weladji et al. 2008). The size reached at the primiparity (generally at 2 or 3 years in large herbivores; Gaillard et al. 2000) influence the energy to allocate to the reproduction in life (Williams 1966). After the first reproduction females attempts to reproduce every year, but some females will not conceive for 1-2 years after weaning an offspring (Gaillard et al. 2000) indicating a cost of reproduction. The consequences of maturing early or late can therefore have long-lasting consequences for individual fitness (Weladji et al. 2008). Positive effect of longevity on female fitness is influenced by the number of breeding attempts, by the experience and by the individual quality (Weladji et al. 2006). Individual quality recover a fundamental role in shaping reproduction during the life. Differences in 'quality' is often overriding the cost of reproduction, with some individuals being able to repeatedly produce numerous offspring over the course of their life (Clutton-Brock et al. 1983), because having more resources to allocate both in reproduction and in maintenance (Reznick et al. 2000).

E. Social interaction and personality

Social structure of a population is an important element influencing life history strategies of individuals (Whitehead 1997). Social interactions are an important evolutionary force in generating individual variation between and within sexes (Gross 1996). Individuals, both males and females, can respond in different way to social interactions showing an individual personality and thus as a results can adopt different life history strategies (Dingemanse et al. 2010). Relationships occurring between individuals influence largely their energetic costly behaviour, influencing directly the fitness (Wey et al. 2008; Tennenhouse et al. 2011). Personality of individuals influence the behavioural decisions in response to specific social interactions or events (Dingemanse et al. 2010) and could also influence the emotions in individuals, often exposed as a specific behaviour (Whitehead 1997) or vocalizations (Briefer 2012).

Females are more subjected to social interaction than males in ungulates. The higher number of social interactions occurs during the breeding and the mating season, when females get in touch with kids of other females (Ruckstuhl and Ingold 1994) and with a high number of males eager to mate (Bro-Jørgensen 2011). Social interaction may degenerate in conflict, both intra and inter sexual. Sexual conflicts in ungulates occur when males rely on sexual harassment a manipulative trait to coerce unwilling females to mate during the rut (Holand et al. 2006; Bro-Jørgensen 2011), whereas during the other periods of the year males could interact aggressively to establish a hierarchy (Bergeron et al. 2010). Also females could incur in intra sexual conflicts to establish a hierarchy (Côté 2000) but the frequency and intensity of those conflicts are lower than in males and secondary sexual characters are thus less developed (Clutton-Brock and Huchard 2013).

F. Alpine ibex and Alpine chamois

Alpine ibex (*Capra ibex)* and Alpine chamois (*Rupicapra rupicapra*) are two bovids living in sympatry in places with short growing season and long hard season (Keller et al. 2005). It is not uncommon see ibex and chamois grazing on the same meadows during some periods of the year.

The Alpine ibex are one of the most sexually dimorphic ungulate species, with adult males being more than twice as heavy and possessing much larger horns than adult females (Loison et al. 1999a; Lüps et al. 2007). The mating period takes place from December to mid January (Apollonio et al. 2013). The birth period, after a gestation of 167 ± 3 days (Stüwe and Grodinsky 1987), occurs from begin of June to early July. Females lactate in summer but it is not rare to still observe suckling behaviours until January. Kids follow their mother immediately after birth until the following spring, and this “following strategy” (Lent 1974) helps reducing the risk of predation (Grignolio et al. 2007a).

The Alpine chamois is a polygynous ungulate native to European mountains that shows moderate sexual dimorphism (Rughetti and Festa-Bianchet 2010). The mating period takes place from November to early December. In female chamois, the first reproduction is approximately at 2 years of age (Loison et al. 1999c). Following a gestation period of ca. 170 days, females usually give birth to a single kid, more rarely to twins, in May or early June (Ruckstuhl and Ingold 1999; Pioz et al. 2008). Kids start following their mothers almost immediately after birth (Ruckstuhl and Ingold 1994). Only females give parental care, thus establishing a strong and close bond with their kid(s) by using the follower tactic (Lent 1974) to reduce predation risk (Ruckstuhl and Ingold 1994). Weaning occurs at *ca*. six months after birth (i.e. November to early December; Ruckstuhl and Ingold 1999), but it is not uncommon to observe suckling behaviours until January.

In male Alpine ibex important determinants of reproductive success is the linear hierarchy established before the mating season, where the heaviest male with longer horns have the higher social rank (Bergeron et al. 2010). Males adopted two alternative mating tactics the coursing and the tending (Willisch and Neuhaus 2009; Apollonio et al. 2013). Generally young subordinate adopted the coursing tactic and have only few opportunities to mate, whereas the dominant one adopted the tending tactic, determinant for reproductive success (Apollonio et al. 2013).

In male Alpine chamois the mating system is still scarcely understood, although is proven the presence of territorial males patrolling a small area in which they try to keep females and chase away intruders, whereas non-territorial males display following behaviour and territory intrusions (von Hardenberg et al. 2000; Corlatti et al. 2012b). This two alternative mating tactics are related to different investments in rutting activity, rather than differences in body size (Corlatti et al. 2012b).

G. Chapters

Much is known on male life history traits of the two species. For females this is not the cases and many topics of the life history traits are yet to investigate for both ibex and chamois females. For example the social interactions with the other sex, the mating strategies and the role of reproductive success and individual quality in shaping the fitness of females and therefore the population dynamics of the two species. Investigate these topics allows us to increase the information on life history strategies adopted by female ibex and chamois to maximise their fitness. I believe that it is very important to co-study mating, reproductive and social traits, especially in females, because they are interrelated and could influence each other.

This thesis investigate different aspects of the life history in female Alpine ungulates. Behavioural and ecological factors are analyzed to contribute to our understanding of how animals answer to the trade-offs occurring in life and how they manage their time in order to maximise the fitness. The aim of the present study is to increase the knowledge on the life history strategies adopted by female to understand the various social, individual and evolutionary factors occurring in the dynamic of Alpine ungulate.

Alpine ungulate provide an excellent model for examining life history strategies, because they are social animals and incur in high energy cost due to the severe winters.

In **chapter I** I investigated, focusing on Alpine ibex, the influence of group social composition on male and female behavioural time budget in mating group, seeing this pattern influence largely the sexual behaviour of both sex (Tennenhouse et al. 2011; L'Italien et al. 2012) and may have drastic consequences on body condition of both, compromising their future fitness (Clutton-Brock 1989). In **chapter II** I explored the sexual behaviour of females during the mating season and the sexual conflicts over the mating system occurring between the sex (Bro-Jørgensen 2011) because females should be extremely selective when choose a partner, since reproduction is much more costly for females than males, as they have a lower potential reproductive output and a higher reproductive investment compared to males. In **chapter III** and **IV** I focused my attention on the importance of individual quality and reproductive success in female Alpine ibex first and Alpine chamois after. I give large importance to understand how change the reproductive performance during the ageing and the variation between individual. Female reproductive success vary in function of the time and thus understand the variation in the last year of life of individuals allow to understand the importance of individual quality and the reproductive senescence.

In **annexe I** I provided a clear example as emotions in ungulates are fundamental to select the appropriate behavioural decisions in response to external or internal events and individuals use both specific vocal and behavioural indicators to expose their emotions. It is not excluded that the same could happen in wild ungulate during the mating season and during the weaning. Whereas in **annexe II** I provided a clear example as the behavioural social interactions between partners influence the fitness of individual.

CHAPTER I

INFLUENCES OF MATING GROUP COMPOSITION ON THE BEHAVIORAL TIME-BUDGET OF MALE AND FEMALE ALPINE IBEX (*CAPRA IBEX*) DURING THE RUT

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Abstract

During the rut, polygynous ungulates gather in mixed groups of individuals of different sex and age. Group social composition, which may vary on a daily basis, is likely to have strong influences on individual's time-budget, with emerging properties at the group-level. To date, few studies have considered the influence of group composition on male and female behavioral time budget in mating groups. Focusing on a wild population of Alpine ibex, we investigated the influence of group composition (adult sex ratio, the proportion of dominant to subordinate males, and group size) on three behavioral axes obtained by Principal Components Analysis, describing male and female group time-budget. For both sexes, the first behavioral axis discerned a trade-off between grazing and standing/vigilance behavior. In females, group vigilance behavior increased with increasingly male-biased sex ratio, whereas in males, the effect of adult sex ratio on standing/vigilance behavior depended on the relative proportion of dominant males in the mating group. The second axis characterized courtship and male-male agonistic behavior in males, and moving and male-directed agonistic behavior in females. Mating group composition did not substantially influence this axis for males. However, moving and male-directed agonistic behavior increased at highly biased sex ratios (quadratic effect) for females. Finally, the third axis highlighted a trade-off between moving and lying behavior in males, and distinguished moving and female-female agonistic behavior from lying behavior in females. For males, those behaviors were influenced by a complex interaction between group size and adult sex ratio, whereas in females, moving and female-female agonistic behaviors increased in a quadratic fashion at highly biased sex ratios, and also increased with increasing group size. Our results reveal complex behavioral trade-offs depending on group composition in the Alpine ibex, and emphasize the importance of social factors in influencing behavioral time-budgets of wild ungulates during the rut.

Keywords: Alpine ibex, behavior, behavioral time budget, group-level, group size, group structure, principal component analyses, social environment, rut, ungulate, trade-offs

Introduction

Many vertebrate species, such as polygynous ungulates, exhibit strict sexual segregation during a large part of the year, gathering only for mating (Bowyer 1984;

Ruckstuhl and Neuhaus 2000, 2005). During the reproductive season however, the formation of mixed groups (hereafter mating groups) enables individuals of different sex, age, experience and behavior to co-occur (Nievergelt 1974; Ruckstuhl and Neuhaus 2000, 2005; Saunders et al. 2005; Turner et al. 2005; Tennenhouse et al. 2011; L'Italien et al. 2012). Because individuals may only allocate a finite amount of time to their daily activities, behavioral trade-offs brought about by social factors, such as mating group composition, may have significant consequences on individual fitness (Turner et al. 2005; Tennenhouse et al. 2011).

In mating groups, important social factors that affect male and female behavioral time budgets and fitness include group size, adult sex ratio, and group member experience or age (Berger 1978; Roed et al. 2002; Turner et al. 2005; Tennenhouse et al. 2011; L'Italien et al. 2012). For instance, biases in the sex ratio of mature adults (the operational sex ratio: Emlen and Oring 1977; De Jong et al. 2012) may affect competition for available mates, with strong consequences on individual and group behavior (Tennenhouse et al. 2011; De Jong et al. 2012; L'Italien et al. 2012). Similarly, individuals of different age are likely to differ in their body size and social rank (Clutton-Brock et al. 1982a; Isvaran 2005; Pelletier and Festa-Bianchet 2006; Willisch and Neuhaus 2009; Apollonio et al. 2013), which in males is often strongly associated with fighting abilities and reproductive success (Roed et al. 2002; Pelletier and Festa-Bianchet 2006; Willisch and Neuhaus 2009; Tennenhouse et al. 2011; L'Italien et al. 2012). Thus, the age structure of males within mating groups, and especially the relative proportion of males of high *vs*. low social rank, is likely to have strong consequences on overall group behavior. Ultimately, by affecting the behavior of males and females, social factors may shape group stability and affect individual fitness (Clutton-Brock 1989; L'Italien et al. 2012). Hence, there is a need to refine our understanding of the proximate effects social factors have on behavior in ungulate mating groups, and how variability in the social composition of mating groups influences individual behavior, with emergent properties at the group level (Marshall et al. 2012).

As most ungulates, Alpine ibex (*Capra ibex*) show strong sexual segregation during a large part of the year, but form mixed sex/age groups during the rut (Nievergelt 1974; Villaret and Bon 1995; Bon et al. 2001). Over the course of the reproductive season ibex exhibit a fission-fusion social system, allowing individuals to freely join or leave any given mating groups (Willisch and Neuhaus 2010). During the rut, male ibex significantly reduce the amount of time spent foraging and lying to the benefit of mating-related activities, whereas females typically decrease the amount of time spent lying to the benefit of moving, standing and social activities (Brivio et al. 2010). Although the rut is generally considered highly energy-demanding for male ungulates (Pelletier et al. 2006; Brivio et al. 2010), male-male agonistic behavior is surprisingly low in the Alpine ibex, owing to a pre-rut establishment of dominance hierarchies and the use of alternative mating tactics in dominant and subordinate individuals (Willisch and Neuhaus 2009; Bergeron et al. 2010; Willisch and Neuhaus 2010; Apollonio et al. 2013). Thus, in addition to adult sex ratio (and mate availability), the proportion of old to young males that differ in dominance rank in mating groups (Willisch and Neuhaus 2009) is likely to strongly influence individual behavior, with emergent properties on male and female group behavior. Further, trade-offs between different activities (*e.g.* maintenance *vs.* agonistic behavior) are also likely to vary depending on group member composition. For instance one might expect subordinate males to invest more time into courtship behavior when group sex ratio is biased towards females, and/or when the ratio of dominant to subordinate individuals in a group is low.

The aim of this study was to investigate how social factors affected the overall time-budgets of male and female group members in mating groups of Alpine ibex. Specifically, we considered the effects of group size, adult sex ratio and the proportion of old and dominant (≥ 9-yrs) to younger (< 9-yrs) males in 45 different mating groups where male and female group time-budgets were recorded, *i.e.* the average time spent by males and females resting, feeding, travelling, standing, courting, and in intra- or inter-sexual agonistic behavior. By potentially modulating mating opportunities (available partners) and/or the amount of harassment experienced by females, we expected group size and the adult sex ratio of mating groups to be important determinants of male and female behavioral trade-offs. In addition, we further considered whether the relative proportion of old (≥ 9-yrs) to young (< 9-yrs) males in mating groups affected both male and female group behavior. Male ibex indeed reach full body size around 8.5-10.5 years of age (Lüps et al. 2007; Parrini et al. 2009), and fully-grown males are the dominant ones (Bergeron et al. 2010) that monopolize most of reproduction by courting and defending receptive females against tentative competitors (Willisch and Neuhaus 2009). For dominant males, competition for access to available mates might increase with increasing male-bias in group sex ratio, to a limit where monopolizing females may be too costly due to a high number of competitors (Tennenhouse et al. 2011; Weir et al. 2011). In addition, previous studies in ungulates have shown that male age-structure in mating groups may have consequent effects on female body condition and receptivity (Valdez et al. 1991; Réale et al. 1996; Komers et al. 1999; Holand et al. 2006).

Methods

Study area and population monitoring

This study was conducted from early December to mid-January 2009-2010, during the mating season of Alpine ibex (Parrini et al. 2003; Parrini et al. 2009; Willisch and Neuhaus 2009). Groups of ibex were monitored (see below) in the South-Eastern part of the Swiss National Park (Val Trupchun; 2060 ha; 1840-2220 m altitude), where an ibex population is followed since 1998. In the Swiss National Park, habitat is dominated by a forest of Swiss stone pine (*Pinus cembra*) and larch (*Larix decidua*) below the tree line (2200 m altitude), and by alpine grasslands and bare rocks above. Annual precipitations amount to *ca.* 700-1200 mm. Both sides of the valley are characterized by avalanche runs and corridors shaped by fallen rocks that offer foraging grounds for ungulates out of high-forest areas (Abderhalden and Campell 2006). In 2009-2010, the Val Trupchun population counted 72 males, 76 females and 4 juveniles born during the year for a total of 152 individuals (see Saether et al. 2002 for more details about census). Although surprisingly low, the juvenile to female ratio observed in this valley is consistent with those observed in previous years, and potentially explained by the fact that juveniles experience high mortality rates due diseases, adverse weather conditions and predation by golden eagles (*Aquila chrysaëtos;* Gauthier et al. 1991; Grignolio et al. 2007a).

Behavioral observations

From the 10th of December 2009 to the 15th of January 2010, we performed behavioral observations on 65 different groups of Alpine ibex. Behavioral observations were performed between 9:00 AM and 16:30 PM. This amounted to a total of 97h50 min of observations that were performed with spotting scopes (20-75X), using the scan sampling method described by (Altmann 1974). The behavior of each individual present in a group was sampled every 3 min, during 1h30 min-long sessions. During each scan, all individuals were distinguishable by their

	Female groups	Male groups
Standing	27.1±3.5^a	39.8±3.5^b
Lying	8.0±2.0^a	5.5±1.7^a
Grazing	52.7±4.4^a	30.2±3.4^b
Moving	10.1±1.1^a	13.1±1.1^b
Courting	NA	10.6±1.5
Intra-sexual agonistic behavior	0.2±0.09^a	0.9±0.4^b
Inter-sexual agonistic behavior	1.8±0.8	NA

Table 1. Mean (± SE) behavioral time budgets (%) of female and male Alpine ibex groups during the mating season. Time budgets are calculated within the 45 different mating groups and averaged (see Methods). Lines not sharing the same superscript are different for $P < 0.05$ (Wilcoxon tests).

location in the group and specific characteristics (either ear tag, horn morphology or coat coloration) and were thus counted only once. Observations were distributed over all daylight hours and performed by two observers that selected 1 to 4 different groups a day. To insure that no group was observed twice in the same day, observations were performed concurrently in different areas (> 1.5 km apart) by the two observers, and each observer selected groups separated by at least 1 km while walking in opposite directions. Groups were observed from a distance of 500-900 m. An ibex group consisted of an assemblage of individuals, each within 50-meters of their nearest neighbor and in visual contact. Individuals' sex was determined by horn morphology (Ratti and Habermehl 1977; Parrini et al. 2009). Approximately 20% of the ibex population in the Swiss National Park is tagged (ear tags and/or colored collars) and monitored, and thus a small fraction of the individuals in our study were of known age. For unmarked males, age was estimated by counting the conspicuous annuli on the outer side of horns as previously described by (Ratti and Habermehl 1977). For unmarked females, age was not estimated because we could not precisely distinguish annuli on their shorter horns from a long distance. Each individual present in the focal group was assigned to one of six age classes including females (unknown age), juveniles (born in June during the year of observation), and males. We assigned males to 3 different age classes: yearlings (1 yr.-old males), young subordinate males from 2 to 8-yrs old, and fully-grown dominant males ≥ 9 yrs-old (Parrini et al. 2009; Willisch and Neuhaus 2009). Behaviors were defined according to (Willisch and Neuhaus 2009). We recorded 6 different behaviors for males and females. Common behaviors included (1) foraging, (2) standing vigilant (scanning the environment) or ruminating, (3) moving (walking and/or running), and (4) lying down flat on the ground either resting or ruminating. Besides, we also recorded (5) male courtship behavior (for a detailed description of those behaviors, see Willisch and Neuhaus 2009) and (6) male and female agonistic behavior. Agonistic behaviors included male and female intra-sexual agonistic behavior (*i.e.* displacing another individual by slowly approaching it, locking or clashing horns), and inter-sexual (male-directed) agonistic behavior. In male-directed agonistic behavior, a female displaced a courting male located at her side away from her by horn contact or by simply initiating a head movement towards him (the male then backed-off before horn contact). Because our interest was to investigate the effects of group composition on the behavioral time budget of mating groups, we discarded from further analyses single-sex groups of ibex that were constituted of only female or male individuals. In total, our analyses were thus conducted on 45 mating groups, for all of which courtship behavior was recorded. Only a small fraction of the animals in this study were tagged and as a result, individuals were counted several times over the season. Thus, to avoid pseudo-replication, we chose to work at the group level, averaging time budgets over mating groups only. Indeed, no group was sampled twice within a given day, nor ibex twice within a given scan. Our data indicate that the different mating groups sampled never had the same composition of individuals and were thus, in effect, different statistical units.

Behavioral time budgets in reproductive groups of Alpine ibex

Within each mating group, we established sex-specific behavioral time budgets by dividing the numbers of scans recorded per sex/behavior by the total number of scans recorded per sex. We used Principal Components Analyses (PCA) to characterize major behavioral axes of Alpine ibex male and female groups, as previously done in large ungulates (Côté et al. 1997; Hamel and Côté 2008). This method produces independent (orthogonal) behavioral axes (principal components, PCs) accounting for the non-independence of behavioral time budgets (Côté et al. 1997; Hamel and Côté 2008). We only retained principal components with eigenvalues >1 which together explained over 70% of cumulative percent variance (Valle, Li et al. 1999). This allowed reducing the dimensionality of our 7 behavioral categories to 3 major axes (PC1, PC2 and PC3; see results) both for males and females. PCAs were applied to correlation matrices of the proportion of time spent in the different behavioral categories, accounting for the fact that some behaviors were largely under-represented in the behavioral time-budget of Alpine ibex (for instance male-male agonistic behavior was extremely low as previously reported by (Willisch and Neuhaus 2010).

Group characteristics and effects on group behavior

Alpine ibex group size was determined by summing all individuals recorded in each specific mating group. Because female age could not be determined precisely, all females were grouped in the same category. Adult Sex Ratio was then calculated as the relative proportion of potentially reproductive males to total female numbers in mating groups using the formula $ASR = males_{\geq 2\text{-yrs}} / (\text{total female number} + males_{\geq 2\text{-yrs}})$ (Tennenhouse et al. 2011; De Jong et al. 2012). An ASR > 0.5 indicates male-bias, an ASR < 0.5 indicates a female-bias, and an ASR = 0.5 indicates an even proportion of males and females in mating groups. In addition, we also calculated the proportion of old dominant (≥ 9-yr) *vs.* young subordinate (< 9-yr) males in each mating groups as $P_{old} = males_{\geq 9\text{-yr}} / (males_{2\text{-}8yrs} + males_{\geq 9\text{-yr}})$. Summary statistics and Spearman rank correlations were used to describe the composition of mating groups during the reproductive season.

We investigated the effect of group social composition on group time budget in male and female ibex separately. Male and female principal components (behavioral axes) were entered as dependent variables in separate Linear Models (LMs), and group size, adult sex ratio and P_{old} as independent explanatory variables. Quadratic influences of adult sex ratio and group size on male and female behavior were tested, by including the quadratic terms adult sex ratio^2 and group size^2 into our models. Our starting models included all two-way interactions between adult sex ratio, group size and P_{old} to account for the fact that the influence of those independent variables on Alpine ibex behavior might have been conditioned by one another. Minimal adequate models were obtained by stepwise model selection using the 'stepAIC' function (library 'MASS') in R. We retained models with the lowest Bayesian Information Criterion (BIC) as best fitting models. To help interpret significant interactions (see Schuett et al. 2011), we report regression lines for each significant effect (*e.g.* group size, Fig. 4) for the minimum, mean and maximum value of its interacting term (*e.g.* adult sex ratio, Fig. 4). All statistical analyses were performed using the R.2.15.1 statistical software (R Development Core Team 2014). Mean estimates are given ± SE, unless otherwise specified.

Ethics Statement

The Research Manager of the Swiss National Park approved the protocol used in this study. No further approval by an Ethics Committee was required, as behavioral observations at a distance were non-invasive in nature, and the Alpine ibex a species classified of least concern (IUCN). Tagging of individual ibex were performed independently of the present study (both prior and during the study) by Rangers of the Swiss National Park, and approved by the Swiss Federal Veterinary Office.

Results

Composition of Alpine ibex groups during the mating season

Mean group size (±SD) across our 45 mating groups was 11.6 ± 5.3 individuals and ranged from 4 to 36 Alpine ibex. The adult sex ratio was 0.54 ± 0.16 and ranged from 0.25 in female-biased mating groups to 0.86 in male-biased mating groups. The proportion of older dominant males (≥ 9-yrs) relative to total male numbers in the group, ranged from 0.0 to 0.75, with a mean of 0.19 ± 0.16. Group size positively correlated with total female number (ρ = 0.71, $P < 0.001$) and total male number (ρ = 0.66, $P < 0.001$) within the group, but was only weakly related to total juvenile number (ρ = 0.29, P = 0.06). As could be expected, total juvenile number positively correlated with total female number ($\rho = 0.30$, $P = 0.04$), but not total male number ($\rho = -0.11$, $P = 0.47$) within the group. Finally, group size did not correlate with adult sex ratio ($\rho = -0.17$, $P = 0.25$), or with the proportion of older dominant males in the group ($\rho = 0.07$, $P = 0.63$). Similarly adult sex ratio did not correlate with the proportion of older dominant males in the group ($\rho = 0.03$, $P = 0.85$).

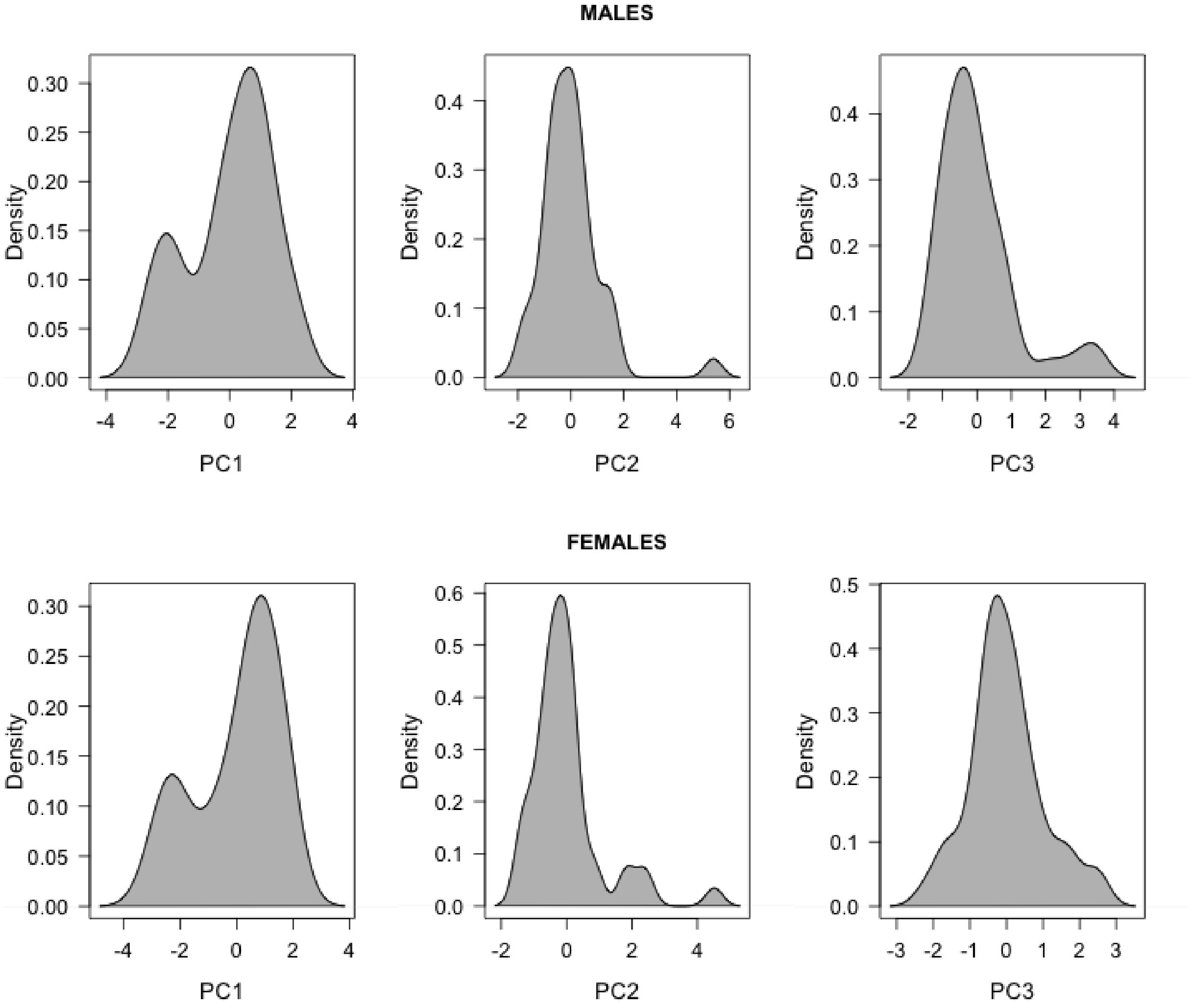

Figure 1. Kernel density distribution of the first three principal components (PC1, PC2, PC3) describing male and female group behavioral time budget in N = 45 different Alpine ibex mating groups. The loadings of the different principal components are given Table 2. Of note, PC1 was bi-normally distributed for both sexes. PC2 and PC3 were normally distributed, apart from a few extreme observations.

Behavioral time budgets in reproductive groups of Alpine ibex

Differences in the behavioral group time budget of males and females within the 45 observed mating groups are reported in Table 1. Both male and female groups spent a substantial amount of time grazing or standing vigilant, substantially less time lying or moving, and only very little time in intra-sexual aggressive behavior. On average, females spent more time grazing than males, whereas males spent more time standing vigilant, moving, and in intra-sexual agonistic behavior (Table 1). There was no significant difference in the amount of time spent by males or females lying.

Principal components analyses of behavioral time budgets

For both males and females, the first 3 axes of the PCA accounted for over 70% of the observed variance in behavioral time budgets (Table 2). In males, the first axis (PC1) loaded strongly and inversely on grazing and standing, distinguishing them from other behaviors. The second axis (PC2) especially distinguished courtship behavior and male-male agonistic behavior, while the final axis (PC3) loaded strongly and inversely on lying and moving behavior. In female groups, PC1 was similar to males, and especially distinguished grazing, standing and lying from other behaviors. In contrast, PC2 loaded similarly on moving and female male-directed agonistic behavior, whereas PC3 inversely distinguished female-female agonistic behavior, moving and lying behavior from other behaviors (see Table 2).

Males				**Females**			
	PC 1	**PC 2**	**PC 3**		**PC 1**	**PC 2**	**PC 3**
Grazing	**0.633**	-0.352	-0.352	**Grazing**	**0.676**	0.028	-0.148
Moving	0.287	0.199	**-0.532**	**Moving**	-0.131	**0.656**	**0.452**
Courtship	0.170	**0.670**	0.157	**Agonistic (F-F)**	0.171	0.052	**0.625**
Agonistic (M-M)	0.010	**0.617**	-0.008	**Agonistic (F→M)**	-0.226	**0.651**	-0.306
Lying	-0.223	0.014	**0.746**	**Lying**	**-0.405**	-0.012	**-0.412**
Standing	**-0.662**	-0.073	-0.358	**Standing**	**-0.530**	-0.378	0.346
SD	1.37	1.16	1.10	**SD**	1.46	1.11	1.00
% variance	31.1	22.5	20.3	**% variance**	35.5	20.4	16.8

Table 2. Independent orthogonal axes (PC1, PC2 and PC3) obtained from a principal component analysis describing the group behavioral time budget of male and female Alpine ibex (Capra ibex) during the mating season. The standard deviation (SD) and the proportion of variance explained by each dominant axis are given.

For both sexes the density distribution of principal components differed between PC1, PC2 and PC3 (see Fig. 1). Mostly, PC2 and PC3 were normally distributed, apart from a right skew in the distribution of PC2 (males and females) and PC3 (males) due to a few extreme observations. For instance, in one group, males spent 31.7% and 15.3% of their behavioral time budget in courtship and male-male agonistic behavior, respectively, explaining the high PC2 value (5.40) observed (Fig. 1). Thus, we ran subsequent model selection for PC2 and PC3 both with and without potential outliers to compare the outputs (see below). In contrast, PC1 was clearly bi-modal for both sexes. Optimal mixture modeling revealed multi-normal distributions for PC1 with means (±SD) of -2.09 ± 0.39 and 0.64 ± 0.80 for males, and means (±SD) of -2.38 ± 0.32 and 0.67 ± 0.80 for females. For both males and females, the first mode of PC1 distribution (low mean PC1) was especially due to groups that spent more time standing vigilant and less time grazing, than groups in the second mode (high mean PC1) (Fig. 1). Interestingly, 8 of the 11 mating groups (72.7%) with a low mean male PC1 (groups in the first mode) also had a low mean female PC1. Conversely, 32 of the 34 mating groups (94.1%) with a high mean male PC1 also had a high mean female PC1. Thus, for subsequent analyses of PC1, data sets were separated according to those different modes. We also modeled the probability for a mating group to end-up in either mode of male and female PC1 distribution, knowing its group size, adult sex ratio and proportion of older dominant males.

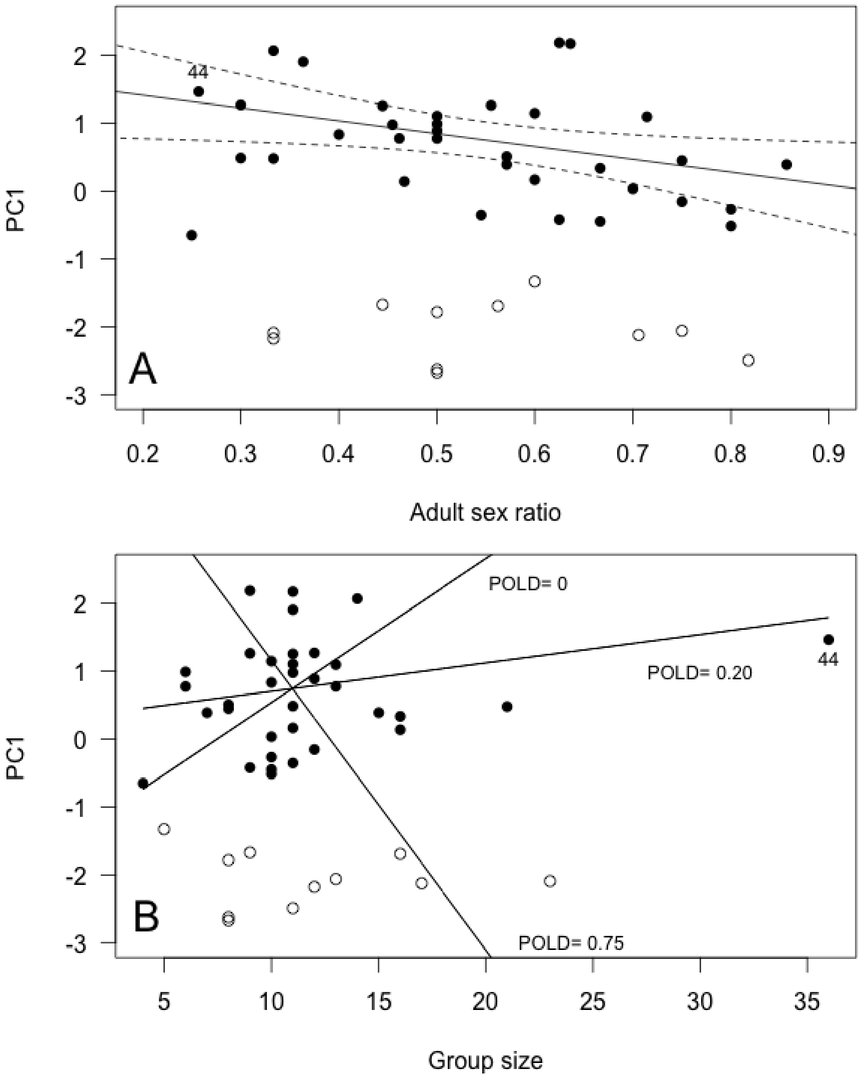

Figure 2. Influence of (A) adult sex ratio and (B) group size on male group behavior (Principal Component 1, PC1) in 45 different Alpine ibex mating groups. High values of PC1 predominantly reflected a high proportion of time spent grazing, and little time spent standing vigilant. The figure presents the best fitting model based on BIC for groups belonging to the second mode of bi-normal PC1 distribution data, excluding mating group n°44 of especially high group size. Groups belonging to the first mode of PC1 distribution are figured as open circles and were not used in the analyses. Panel A: the regression line and 95% confidence intervals are given, holding group size constant at its mean value. Panel B: for illustrative purposes, the interaction between group size and the proportion of older males in mating groups (P_{OLD}) is figured by showing the model regression lines only for the minimum, mean and maximum P_{OLD} observed in mating groups. However, statistics were run on the original continuous variable.

Group characteristics and effects on group behavior

Male group behavior

For males, PC1 was not affected by adult sex ratio, group size or the proportion of older males (≥ 9-yrs) in mating groups when considering the overall data. However, the analyses violated linear model assumptions due to the bimodal distribution of PC1. When restricting our analyses only to mating groups that belonged to the second mode of the bi-normal distribution ($N = 34$ groups with high mean male PC1; see Fig 1), the best model retained adult sex ratio, the proportion of older males (≥ 9-yrs) in the group, group size, group size^2, and a significant interaction between 'group size x proportion of older males in the group' (Table 3). The quadratic effect of group size appeared to be mostly due to one mating group (n°44) of especially high group size (see Fig. 2B). Indeed, when this mating group was removed from the analysis, group size^2 was not retained in the final model (Table 3). Thus, as adult sex ratio increased to favor males, males spent less time grazing and more time standing vigilant. Interestingly, male PC1 was also affected by group size, but the effect differed depending on the proportion of older males in the group. When this proportion was high ($P_{OLD}= 0.75$, Fig. 2), grazing behavior decreased (and standing vigilant increased) rapidly with increasing group size. In contrast, when this proportion was low ($P_{OLD} = 0.20$, Fig. 2), grazing behavior increased (and standing vigilant decreased) slightly as group size increased. When no older males were present ($P_{OLD} = 0$, Fig. 2), grazing behavior increased (and standing vigilant decreased) rapidly as group size increased. Unfortunately, we could not run the model selection on the first mode of male PC1 distribution (groups of low mean male PC1) because of low sample size ($N = 11$ groups). For those mating groups however, simple linear regressions of adult sex ratio, group size, group size^2, or the proportion of older males in the group on PC1 were not significant (all $P > 0.73$). Finally, adult sex ratio, group size and the proportion of older males in a mating group, did not predict the likelihood of a given ibex group to be in one of the 2 modes of male PC1 distribution (GLM with binomial error; all $P > 0.57$).

For male PC2, model selection did not retain any significant effect in the final model when considering the overall data. Removing a potential outlier of especially high PC2 from the analyses (mating group n°5; Fig. 3), we found a significant negative effect of group size on the amount of time spent in courtship and male-male agonistic behavior ($t = -2.05$, $P = 0.046$) (Table 3; Fig. 3). However, the amount of variation explained was weak ($R^2 = 0.07$), and the relationship appeared mostly due to one mating group of especially high group size (mating group n°44; Fig. 3). Indeed, when this mating group was removed from the analyses, the effect of group size was no longer significant ($P = 0.29$).

Response	Independent	Estimate ± SE	*t*	*P*	*N*
PC1 $R^2_{adj} = 0.21$, $F_{5,28} = 2.74$, $P = 0.04$	**Intercept**	-0.92 ± 1.02	-0.89	0.38	35
	ASR	-2.00 ± 0.81	-2.47	0.02*	
	Group size	0.30 ± 0.12	2.51	0.02*	
	Group size^2	-0.004 ± 0.002	-1.88	0.07	
	P_{OLD}	8.55 ± 2.99	2.86	0.008**	
	Group size x P_{OLD}	-0.77 ± 0.27	-2.87	0.008**	
PC1 (outlier removed) $R^2_{adj} = 0.19$, $F_{4,28} = 2.89$, $P = 0.04$	**Intercept**	-0.51 ± 0.94	-0.60	0.55	34
	ASR	-1.89 ± 0.81	-2.34	0.03*	
	Group size	0.21 ± 0.08	2.55	0.02*	
	P_{OLD}	9.35 ± 3.34	2.80	0.009**	
	Group size x P_{OLD}	-0.85 ± 0.31	-2.77	0.009**	
PC 2 $R^2_{adj} = 0.07$, $F_{1,42} = 4.22$, $P = 0.046$	**Intercept**	0.44 ± 0.30	1.47	0.15	45
	Group size	-0.05 ± 0.02	-2.05	0.046*	
PC 3 $R^2_{adj} = 0.22$, $F_{4,40} = 4.15$, $P = 0.007$	**Intercept**	-6.28 ± 2.17	-2.89	0.006**	45
	ASR	22.39 ± 7.21	3.10	0.003**	
	ASR^2	-13.67 ± 5.71	-2.39	0.02*	
	Group size	0.22 ± 0.08	2.82	0.007**	
PC 3 (outliers removed) $R^2_{adj} = 0.11$, $F_{3,36} = 2.69$, $P = 0.06$	**Intercept**	-1.84 ± 0.92	-2.00	0.05	41
	ASR	4.27 ± 1.83	2.33	0.02*	
	Group size	0.14 ± 0.07	1.91	0.06	
	ASR x Group size	-0.40 ± 0.16	-2.51	0.02*	

Table 3. Final model estimates explaining the variation observed along male behavioral principal components (PC1, PC2, PC3) in mating groups of Alpine ibex according to: group size, group size^2, adult sex ratio (ASR and ASR^2), and the proportion of old (≥ 9-yrs) males relative to all males of reproductive age (POLD). Starting models included all second order interactions. The presented models are those retained by stepwise model selection based on Bayesian Information Criterion (BIC). For PC1, only groups belonging to the second mode of the bi-normal distribution (of sufficiently large sample size) were included in the analyses. In addition, for PC1 and PC3, models excluding potential outliers are also presented (see text) *P < 0.05, **P < 0.01, ***P < 0.01.

In contrast, we found complex interacting linear and quadratic effects of adult sex ratio and group size on male PC3 (Table 3). The quadratic relationship however, was mainly due to mating groups of especially high PC3 (mating group n°2, 3, 25 and 34; Fig. 3). Indeed, removing those groups from the analyses, the squared effect of adult sex ratio was no longer significant, and the best fitting model only retained the linear interacting effects of adult sex ratio and group size on PC3 (Table 3). Thus it appeared that group size affected male PC3 differently depending on adult sex ratio (Fig 4). When adult sex ratio was biased to favor males (ASR= 0.86, Fig 4), lying behavior increased and moving behavior decreased with increasing group size. In contrast, at equilibrated sex ratio, the inverse relationship was observed (ASR = 0.55; Fig 4), and the effect was even more pronounced as sex ratio bias increased to favor females (ASR = 0.25; Fig 4).

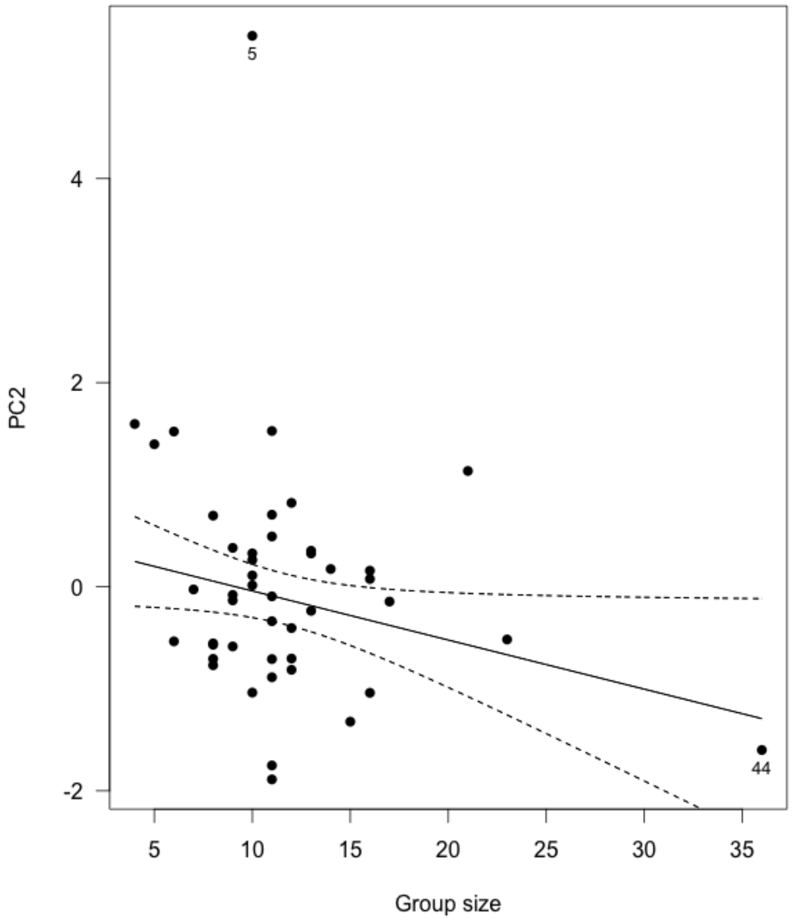

Figure 3. Influence of group size on male group behavior (Principal Component 2, PC2) in 45 different Alpine ibex mating groups. High values of PC2 predominantly reflected high male group time budget spent in courtship and intra-sexual (male-male) agonistic behavior. The figure presents the best fitting model (significant regression line and 95% confidence intervals) based on BIC for the data excluding mating group n°5, of especially high PC2. Note that regression line is no longer significant when mating group n°44 is also excluded from the analyses.

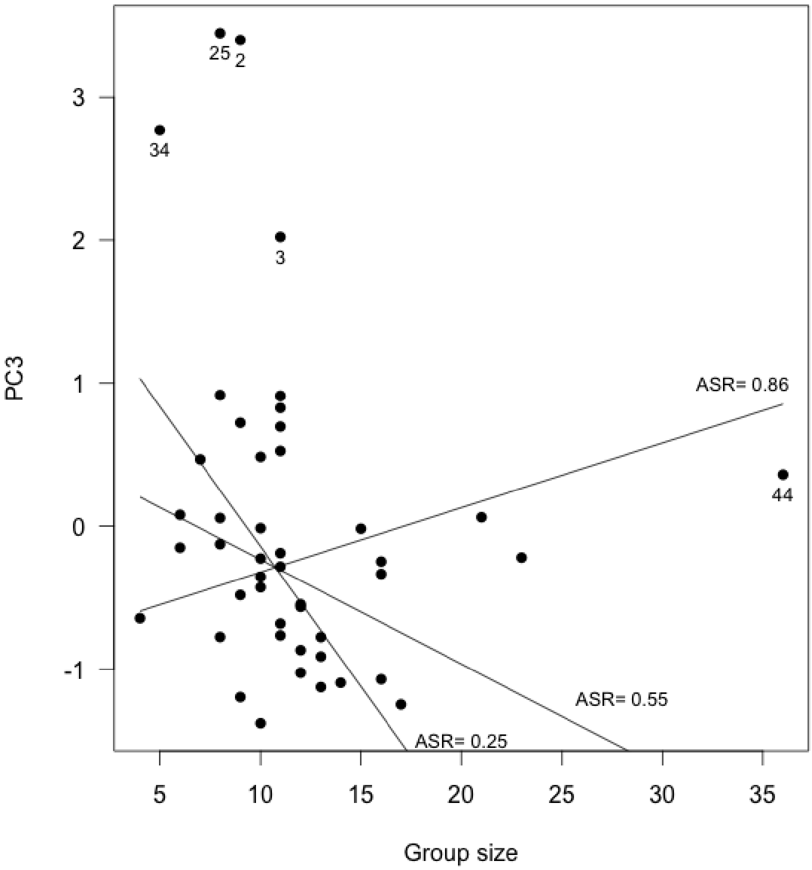

Figure 4. Influence of group size on male group behavior (Principal Component 3, PC3) in 45 different Alpine ibex mating groups. High values of PC3 predominantly reflected a high amount of time spent lying and little time spent moving. The figure presents the best fitting model retained by BIC for the data excluding mating groups n°2, 3, 25, 34 and 44, of especially high PC3 (see text). For illustrative purposes, the interaction between group size and adult sex ratio (ASR) is figured by showing the model regression lines only for the minimum, mean and maximum (ASR) observed in mating groups. However, statistics were run on the original continuous variable.

Female group behavior

For females, the best model fit on PC1 retained a negative quadratic effect of adult sex ratio when considering the overall data (-2.40 ASR^2, P =0.05). Again however, this model was not appropriate given the bimodal distribution of female PC1. However, restricting our analyses only to mating groups that belonged to the second larger mode of the distribution (N = 35 groups with high mean PC1; Fig. 1) yielded similar results (Table 4).

Response	Independent	Estimate ± SE	*t*	*P*	*N*
PC1	**Intercept**	1.35 ± 0.28	4.83	< 0.001***	35
$R^2_{adj} = 0.16, F_{1,33} = 7.28, P = 0.011$	**ASR^2**	-2.19 ± 0.81	-2.70	0.011**	
	Intercept	6.17 ± 1.75	3.53	0.001**	45
PC 2	**ASR**	-21.90 ± 6.04	-3.62	< 0.001***	
$R^2_{adj} = 0.26, F_{3,41} = 6.25, P = 0.001$	**ASR^2**	20.55 ± 5.41	3.80	< 0.001***	
	Group size	-0.07 ± 0.03	-2.50	0.02*	
	Intercept	2.74 ± 1.46	1.88	0.07	45
PC 3	**ASR**	-19.31 ± 5.27	-3.66	< 0.001***	
$R^2_{adj} = 0.38, F_{4,40} = 7.68, P < 0.001$	**ASR^2**	16.19 ± 4.69	3.45	0.001**	
	Group size	0.34 ± 0.08	4.35	< 0.001***	
	Group $size^2$	-0.009 ± 0.002	-4.08	< 0.001***	

Table 4. Final model estimates explaining the variation observed along female behavioral principal components (PC1, PC2, PC3) in 45 mating groups of Alpine ibex according to: group size, group $size^2$ and adult sex ratio (ASR and ASR^2). Starting models also included the proportion of old (≥ 9-yrs) males (relative to all males of reproductive age) in mating groups, and all second order interactions. The presented models are those retained by stepwise model selection based on Bayesian Information Criterion (BIC). For PC1, only groups belonging to the second mode of the bi-normal distribution (of sufficiently large sample size) were included in the analyses. Statistics excluding potential outliers are reported in the text. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.01$.

Interestingly, for both models, the effect of adult sex ratio on PC1 was similar in direction and magnitude. Thus, as adult sex ratio in mating groups increased to favor males, females spent less time grazing and more time standing vigilant (Fig. 5). Again, we could not run the analyses on the first mode of female PC1 distribution (groups of low mean female PC1) because of low sample size (N = 10). However, simple linear regressions of adult sex ratio or its quadratic term on PC1 were not significant (all P > 0.19). Finally, adult sex ratio, group size and the proportion of older males in a mating group, did not predict the likelihood of a given ibex group to be in one of the 2 modes of female PC1 distribution (GLM with binomial error; all P > 0.57).

For PC2, the best model fit retained significant linear and quadratic effects of adult sex ratio, and a significant negative effect of group size (see Table 4; Fig. 6A and 6B). Removing potential outliers (mating groups n° 16 and 44; see Fig. 6A and 6B) from the analysis did not change the results for adult sex ratio (-13.61 ASR, + 12.21 ASR^2, all P < 0.05). Not surprisingly however, the effect of group size on PC2 was no longer significant (P = 0.12).

Thus, when adult sex ratio was highly biased towards males or females, females increased the amount of time spent moving and the amount of time spent in male-directed agonistic behavior. In contrast, those behaviors appeared to decrease at equilibrated sex ratios (Fig. 6A). The amount of time spent moving and in male-directed agonistic behavior also appeared to decrease with increasing group size, but only when one especially large group (group n°44; Fig. 6B) was kept in the analyses.

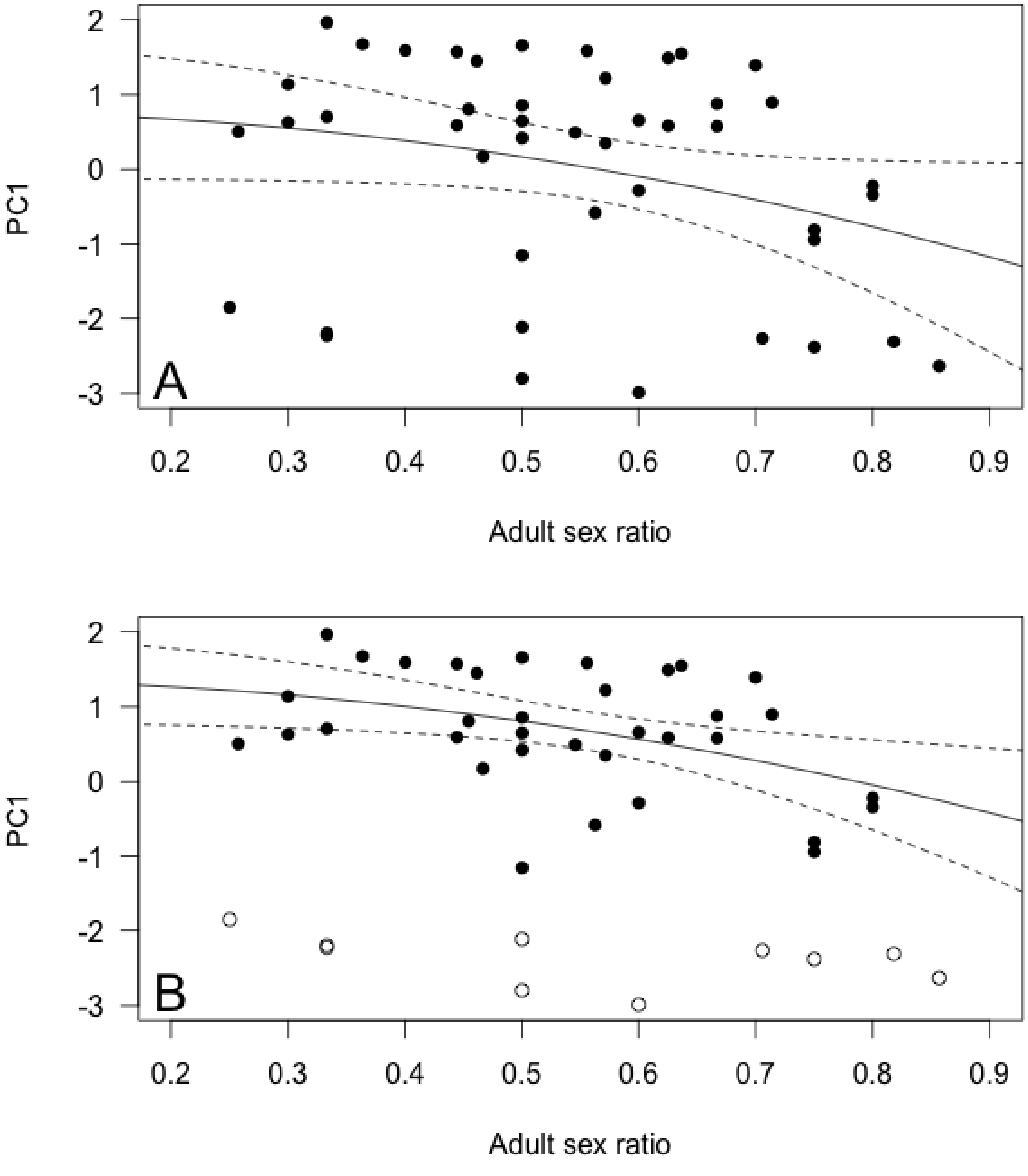

Figure 5. Influence of adult sex ratio on female group behavior (Principal Component 1, PC1) in 45 different Alpine ibex mating groups. High values of PC1 predominantly reflected high female group time budget spent grazing, and little time spent standing vigilant or lying. The figure presents the best fitting model (significant regression line and 95% confidence intervals) based on BIC for **(A)** the overall data (N= 45 mating groups); **(B)** the groups belonging to the second mode of bi-normal PC1 distribution (N = 35 mating groups, filled circles). Groups belonging to the first mode of PC1 distribution are figured as open circles and were not used in the analyses presented panel B.

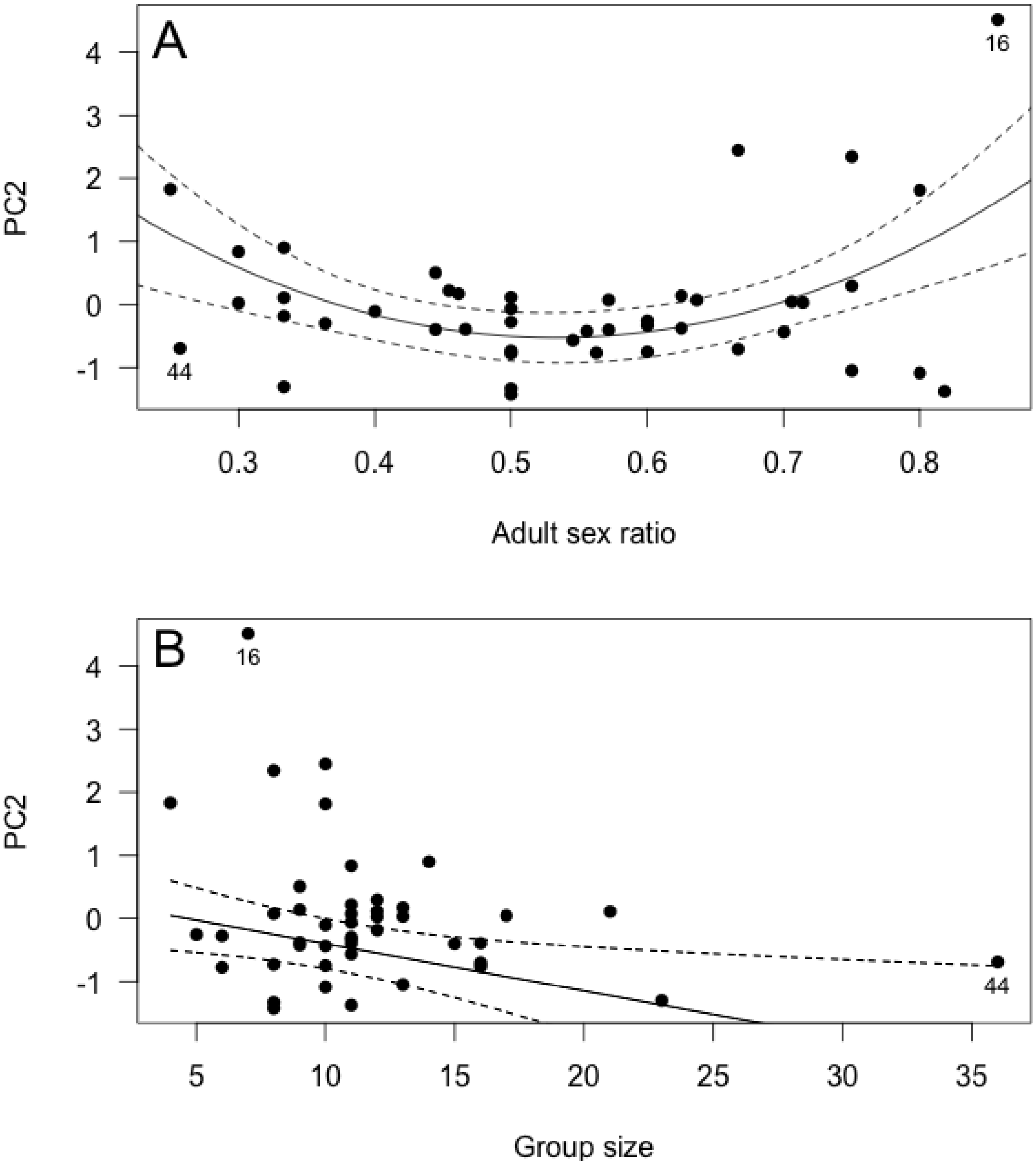

Figure 6. Influence of (A) adult sex ratio and (B) group size on female group behavior (Principal Component 2, PC2) in 45 different Alpine ibex mating groups. High values of PC2 predominantly reflected high female group time budget spent in moving behavior and in inter-sexual (male-directed) agonistic behavior. The figure presents the best fitting model (significant regression lines and 95% confidence intervals) based on BIC for the overall data (N = 45 mating groups). Potential outliers (mating groups n° 16 and 44) are shown as filled circles (see text for statistics without those mating groups).

Finally, both adult sex ratio and group size had significant linear and quadratic effects on PC3 (Table 4, Fig. 7A and 7B). Again, excluding group n°44 from the analyses (high group size) did not change the effects for adult sex ratio (-17.36 ASR, +14.74 ASR^2, all *P* < 0.01), but removed the quadratic effect of group size on PC3 retaining only a significant positive component (+0.12, *P* < 0.001). Thus, it appeared that as group size increased, females spent more time moving and in intra-sexual (female-female) agonistic behavior, and less time lying (Fig. 7B). Similarly, females spent more time in intra-sexual agonistic and moving behavior (and less time lying) at highly biased sex ratios, but showed the opposite pattern at more equilibrated sex ratios.

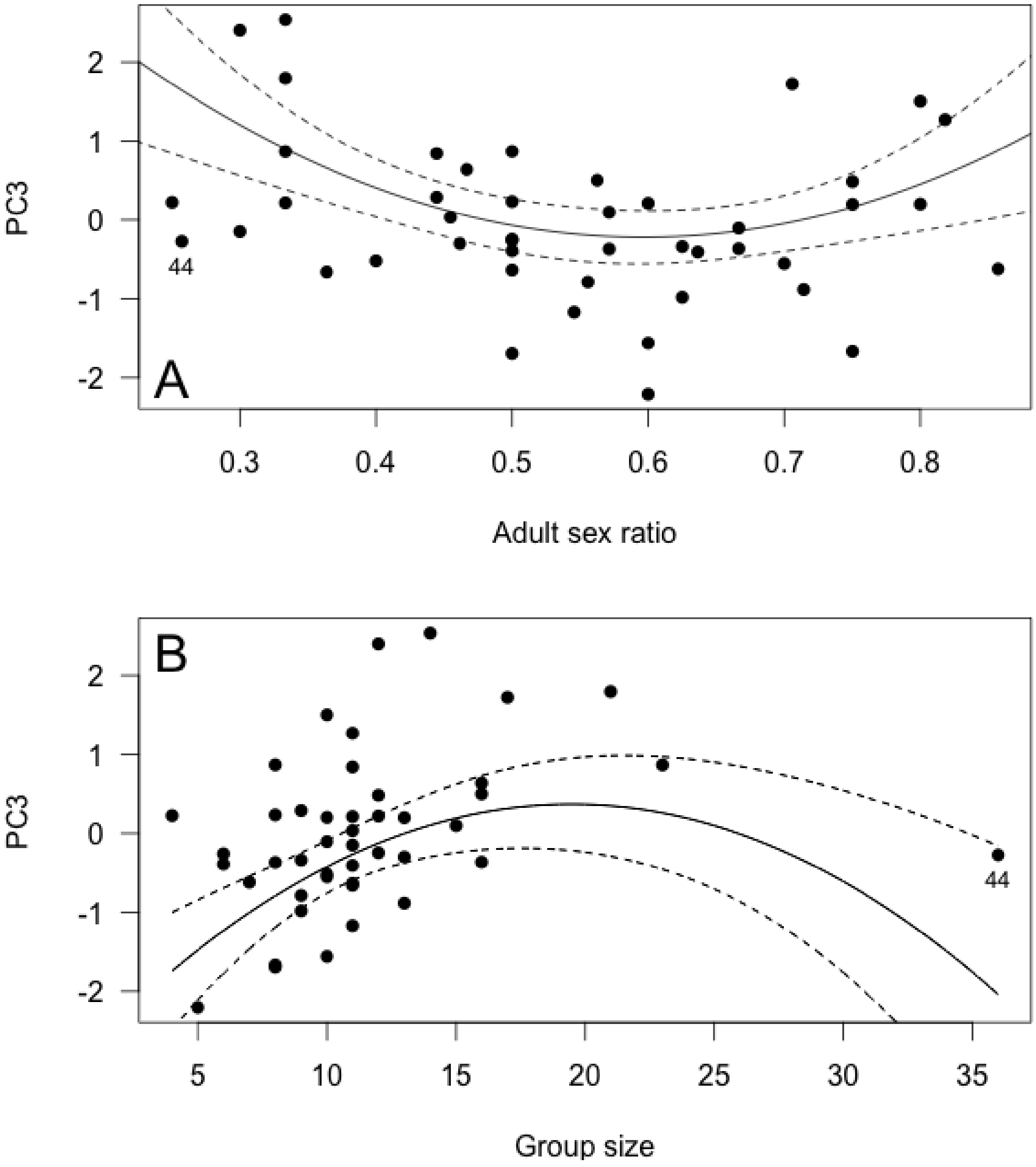

Figure 7. Influence of (A) adult sex ratio and (B) group size on female group behavior (Principal Component 3, PC3) in 45 different Alpine ibex mating groups. High values of PC3 predominantly reflected high female group time budget spent in intra-sexual (female-female) agonistic behavior and moving behavior, and little time spent in lying behavior. The figure presents the best fitting model (significant regression lines and 95% confidence intervals) based on BIC for the overall data (N = 45 mating groups). A potential outlier (mating group n° 44) is shown as a filled circle (see text for statistics without that mating group).

Discussion

Our study underlines important effects of group social composition on the behavioral time budget of Alpine ibex during the rut. Both male and female ibex group behavior indeed varied according to the underlying properties of mating groups. Importantly, because only a small fraction of individuals in our study were tagged, and could be reliably re-identified over the season, we conducted our analyses at a group- (rather than individual-) level to avoid pseudoreplication. Nonetheless, it should be noted that the observed effects of social group composition on ibex behavior likely occurred first of all at an individual level. Further data on individually marked animals are thus required to determine how variable individual behavior in mating groups is, and what the precise links between individual-level behavior and emergent group properties are (Marshall et al. 2012).

For both males and females, PC1 presented a bimodal distribution with some groups spending more time in vigilance than in grazing (low PC1), or the opposite (high PC1). Social factors (group size, adult sex ratio, and the relative proportion of older males within the group) did not predict the probability for a given group to be in either mode of PC1 distributions. However, it is interesting to note that the majority of the groups separated in male PC1 distribution were also those separated in female PC1 distribution. This suggests that male and female ibex in mating groups had synchronized activities in terms of grazing *vs.* vigilance behavior, consistent with the idea that behavioral synchrony may be important in maintaining group cohesion (Ruckstuhl and Neuhaus 2001), even during the mating season. When focusing only on the second mode of PC1 distributions of larger sample size, we found that adult sex ratio, group size, and the proportion of older males (> 9-yrs old) within a mating group, had varying effects on the amount of group time-budget spent in vigilance/grazing by males and females. For both sexes, vigilance behavior increased at the detriment of grazing when adult sex ratio in mating groups was biased towards males. Previous studies in ungulates have found that vigilance/foraging trade-offs could be influenced by herd sex ratio, due to sex-specific differences in energy requirements (Liley and Creel 2008) or variation in predation risk (Childress and Lung 2003). Predation of adult ibex however is negligible in the Alps (Grignolio et al. 2007a), and it is unlikely that the effect of sex ratio on male and female vigilance/grazing behavior was a consequence of changes in individual predation risk. Rather, our results suggest that the time devoted to vigilance/grazing was highly responsive to the social environment.

Monitoring group social composition and/or the behavior of social conspecifics is an important component of ungulate social behavior (Cameron and du Toit 2005; Lung and

Childress 2007). For females, vigilance group-behavior might have increased with adult sex ratio because of higher sexual harassment in male-biased groups (Réale et al. 1996; Bro-Jørgensen 2011). This suggestion is consistent with the fact that female group time-budget devoted to moving and agonistic behavior towards males (female PC2) also increased in male-biased mating groups. Although we recorded courtship behavior in all of the mating groups, we unfortunately have no data on female receptivity in this study. However, it is likely that in highly male-biased groups, female aggressiveness towards males reflected low female receptiveness. Alternately, sexually receptive females might have allocated less time to feeding and more time observing potential suitors when more males are present in the group (Rasmussen 1985; Bro-Jørgensen 2011). Indeed, recent studies have suggested that female mate choice may be more common in ungulates than previously thought (see Bro-Jørgensen 2011 for a review). In this regard, it is interesting to consider whether female male-directed agonistic behavior in male-biased groups might have been an expression of female mate choice (Bro-Jørgensen 2011), used to displace non-favored suitors.

Although we observed very little female-female aggressiveness overall, we found that the group time-budget spent by females in intra-sexual agonistic behavior and moving behavior increased at the detriment of time spent lying (female PC3), with increasing group size and increasing sex ratio bias towards males or females (quadratic effect). Higher female-female agonistic behavior in larger groups may reflect feeding competition for limited high quality food patches (Thouless 1990) or better positions within the group. For instance, it would be interesting to know whether female aggressiveness allows to acquire more central positions within mating groups which might offer social thermoregulatory benefits (Gilbert et al. 2010; Robert et al. 2013) by sheltering individuals from detrimental weather conditions. The reason why female-female aggressiveness would increase at highly biased male or female sex ratios is unclear. Female aggressiveness and reproductive success are typically related to dominance status and age in mountain goats (Greenberg-Cohen et al. 1994; Côté 2000; Shargal et al. 2008) and it is possible that in ibex, variation in the female composition of mating groups in terms of age/dominance affected female-female aggressiveness at highly biased sex ratios. However, we could not determine female age from a distance in the present study, and further investigations should thus consider whether similar patterns occur in mating groups where females of known age/dominance status are monitored.

For males, the increase in vigilance group time-budget (male PC1) that occurred in male-biased groups may have reflected a greater proportion of time allocated to monitoring conspecific behavior when numerous males were present in mating groups. Whereas the number of competitors within a group appeared to influence vigilance behavior, it is interesting to note that neither sex ratio nor group size (see below) had an effect on the amount of male group time-budget devoted to courtship or male-male agonistic behavior (male PC2). This result is consistent with the knowledge that strong social hierarchies are established prior to the mating season in Alpine ibex (Bergeron et al. 2010), dominant males monopolizing most of reproductive females. Male-male agonistic behavior is thus surprisingly low during the rut in the species (Willisch and Neuhaus 2010), in agreement with our own observations. In addition, we found that male group time budget devoted to vigilance (but not to courtship or male-male agonistic behavior) also increased with group size at the detriment of grazing, but only when groups were constituted primarily of older (> 9-yrs) males. In contrast, when mating groups were biased towards younger males, male group time-budget was actually more devoted to grazing than to vigilance behavior. Because dominance is related to body size in the Alpine ibex (Bergeron et al. 2010) and male ibex reach full body size at 8.5-10.5 years of age (Lüps et al. 2007; Parrini et al. 2009), it is likely that age-related dominance patterns in mating groups affected the amount of time devoted to monitoring the behavior of social competitors *vs.* time spent foraging (*i.e.* more time spent in vigilance when numerous dominant males were present in the group). Although we actually did find a negative effect of group size on the amount of time males devoted to courtship and male-male agonistic behavior (male PC2), this effect was essentially driven by a mating group of especially high group size (group n°44) that spent little time in those behaviors, and should thus be treated with caution. Indeed, mating groups of high group size are rarely observed during the rut (F.T. *personal observations*), and mating group n°44 was observed at low altitude on Jan. 13th, following a particularly heavy snowfall event. Snow cover is known to strongly limit the movement capacity of Alpine ibex (Parrini et al. 2003; Grignolio et al. 2004), and may have constrained individuals to move towards less snow-covered areas at lower altitudes, gathering into a larger group. Under those conditions, it is possible that males may have been less motivated to engage into courtship or male-male aggressive behavior, as Alpine ibex males are known to decrease their investment in mating behavior in high snow cover conditions (Apollonio et al. 2013). A similar explanation may also hold true for females, explaining why females in this mating group invested relatively little time moving or in male-directed agonistic behavior (low female PC2). Finally, we found that male group time-budget spent lying increased at the detriment of time spent moving with increasing group size, but only when groups were especially biased towards males (male PC3). A high male-bias may have limited the opportunities for male-female social interactions

(Tennenhouse et al. 2011), explaining why male movements generally decreased in such groups. Interestingly, this suggestion is somewhat supported by the observation that the opposite trend occurred in highly female-biased groups (males group time budget increased in time spent moving at the detriment of time spent lying).

Because the mating season of Alpine ibex occurs during winter at high altitude in the Alps, it is characterized by harsh environmental condition. Indeed, both males and females spent a substantial portion of their time budget grazing, although females dedicated substantially more time to this activity than males, consistently with the idea that food and nutrient availability are essential determinants to female ungulate reproduction (Cameron et al. 1993; White et al. 1997; Testa and Adams 1998; Neuhaus and Ruckstuhl 2002; Grignolio et al. 2004; Hamel and Côté 2009; Parker et al. 2009). What is perhaps surprising is that relatively little time was spent by males in active courtship behavior, whereas proportionally more time was actually spent in vigilance or grazing behavior. Whereas this is likely due (at least partly) to dominance hierarchies being established before the rut, it should be kept in that physical activity linked to social interactions may have consequent effects on the energy budget of those animals. Thus, behavioral adjustments in male and female Alpine ibex may follow a trade-off minimizing costly activities while maximizing reproductive success depending on group social composition. Linking the energetics of social behaviors (Viera et al. 2011) to group composition and individual fitness during the mating season opens exciting perspectives for future research.

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CHAPTER II

SEXUAL BEHAVIOUR IN A POLYGYNOUS MATING SYSTEM: FEMALES HAVE THEIR SAY

Federico TETTAMANTI, Stefano GRIGNOLIO, Nicoletta SICA and Marco APOLLONIO

Manuscript

Abstract

In mammals researches are dominated by male mating behaviours on courting patterns, leaving aside the female sexual behaviour. Anyway, in polygynous species females should be extremely selective when choose a partner, since reproduction is much more costly for females than males, as they have a lower potential reproductive output and a higher reproductive investment compared to males. Here we investigated the mating strategies of females in a species with marked sexual dimorphism and marked polygynous mating system, the Alpine ibex (*Capra ibex*). Females' sexual behaviour and their responses to males courting them were recorded during three different mating seasons. We observed the changes in behaviour of females during the oestrus day and their attitudes to answer to three different kind of male courting behaviours. Females increased their time in standing, moving, resting, aggressive behaviour and decreased the time spent in feeding behaviour when in oestrus, whereas when courted by the chosen male they increased the time spent in feeding behaviour at the detriment of the other four behaviour. Female seems choose the male higher in hierarchies and that allows her direct benefit, such as decreasing harassment of other male and increasing the time spent to feeding. Our results reveal complex inter sexual conflicts and a mutual beneficial cooperation between female and the chosen male. We emphasize the presence of direct female mate choice, consonant with the mating tactics adopted by males, which can increase the strength of the sexual selection on males.

Key words: *Capra ibex*; Alpine ibex; mate choice; oestrus day; time-budget; aggressive behaviour; sexual conflicts

Introduction

Sexual selection is described as the male competition for mates (intrasexual competition) and the female choice of mates (intersexual competition; Darwin 1871). Sexual selection is largely explained by sexual behaviour (Clutton-Brock 2007) and it includes thus both male and female mating strategies. The two topics have been investigates in different depth as regards of the analyzed species. The intrasexual competition is well studied and there is evidence for a specific behavioural variation in mating strategies for males (Isvaran 2005; Neff and Svensson 2013). Intersexual competition was investigated in a broad range of taxa (birds: Hill 2006; fish: Janetos 1980; frogs: Akre et al. 2011), but information in mammals is still lacking (see Clutton-Brock 1989; Bro-Jørgensen 2011) and less is known about the specific behaviour performed by females during the mating season (Wiegmann et al. 1999; Byers et al. 2006). Although the females behaviour and their responses to the mating strategies are relatively unknown, these topics are necessary to understand females preferences and, consequently, individual fitness (Byers et al. 1994; Roberts and Dunbar 2000; Longpre et al. 2011). The challenge for the researchers is to investigate the behavioural patterns during mating season focusing on female sex. This approach allows substantial progress in understanding sexual selection.

In polygynous species sexual selection are most extreme, with males having larger body size and able to monopolize the access to females (Kirkpatrick 1987). In these species it is often assumed as adaptive for females to carefully choose the most vigorous and healthy male (Andersson 1994), in relation to their greater investment in the offspring (Trivers 1972; Clutton-Brock and Vincent 1991). Mate choice in female is a major determinant of their future reproductive success (Emlen and Oring 1977). The existence of female mate choice in most ungulates is further supported by the theory of sexual selection that predict that the sex with the lower reproductive rate, like female in polygynous species (Clutton-Brock and Vincent 1991), should choose among different mate to increase its reproductive success (Andersson 1994). Instead, until some years ago, female mate choice was considered unimportant, seeing there was the crude assumptions about the monopolizazion of the mating by males.

Many ungulates species, being strongly sexually dimorphic, have been used to illustrate polygynous mating systems (Clutton-Brock 1989). However quite rarely behavioural patterns related to a female preferences for mate in ungulate have been investigated (see for a detailed reviewer about this Bro-Jørgensen 2011). Females are proved to choose the mate by particular physical characteristics (Clutton-Brock 1982), by quality of the defended territory (Carranza 1995) or by male behaviour as patterns (Longpre et al. 2011). To find a definition for ‘female mate choice’ in polygynous ungulates is very difficult, also because males mating strategies could obscure those of females and because females were considered submissive to mating tactics performed by males (Clutton-Brock et al. 1982b; Hogg and Forbes 1997; Isvaran 2005; Saunders et al. 2005; Pelletier et al. 2006). An important step to understand the female role during the mating system in ungulates is to obtain information on their different behaviours in the fertile days in combination to the male mating strategies adopted (e.g. the courtship behaviour performed could be an indicator among high and low quality males: Longpre et al. 2011). A variety of behavioural changes in females were observed during the oestrus day but never connected with an expression of female mate choice (Clutton-Brock et al. 1982a). Accurate observations on these context could give useful insights to understand the exact role of the sexual selection and sexual conflicts in polygynous ungulates.

Alpine ibex are among the most suitable species to investigate the mating strategies of females, due to the marked dimorphism between the sexes and the polygynous mating system. Male ibex have larger and heavier horns than females, and are about double the weight of adult females (Ruckstuhl and Neuhaus 2001). Ibex show strong sexual segregation outside the mating period (Bon et al. 2001; Grignolio et al. 2007b). During the mating season, occurring in December - January, mixed sex/age groups are formed and the social group composition has a strong impact on group behaviour (Chapter I). Male ibex adopt alternative mating strategies related to their age (Willisch and Neuhaus 2010; Apollonio et al. 2013).

The only insight on female ibex behaviour during the mating season is that female group time-budget devoted to moving and aggressive behaviour towards males increase in male-biased mating group, indicating a likely expression of female mate choice (Chapter I), hence may arise change in sexual conflicts over mating system. In other ungulate species, when conflicts with non-preferred males are exacerbated, females become more likely to mate cooperatively with high quality males (Bro-Jørgensen 2011). Polygynous species, as Alpine ibex, are expected to experience stronger sexual selection on males, and in turn sexual conflict, than monogamous species (Emlen and Oring 1977). Interestingly, sexual conflicts are expected to be greatest when sex difference in the opportunity for sexual selection is large. In polyginous ungulates the greater opportunity for sexual selection, or in other words the larger variance in mating success, is found in males (Clutton-Brock and Vincent 1991), leaving open the possibility of intersexual conflicts. Females mate choice could be cooperative with respect to high quality males, but conflicting with the other males (Bro-Jørgensen 2011).

In this study, we investigated female Alpine ibex behaviour during the mating season and we aimed to present evidence of behaviours consistent with female mate choice. Recent studies suggested that female sexually selected behaviours might be common in ungulates, especially those living in harsh environments (see Bro-Jørgensen 2011 for a review; Buzzard et al. 2014). In this regard, it is interesting to consider different predictions investigating the female mating strategies in Alpine ibex. First, we expect females to show an important change in their behaviour during the oestrus day; second, we consider females able to discriminate between males and to exert an active preference for mating with particular categories of males; third, we predict that females modify their behaviour in relation of the nearby male and the one chosen; fourth, given the greater variance in mating success for male ibex we expect the presence of strong intersexual conflicts during the day of oestrus, where aggressive behaviours performed by females towards unwanted males could be common.

Methods

Study Area

The study was carried out in the Gran Paradiso National Park (hereafter GPNP; 45°38′ N, 7°12′ E), a protected areas where hunting is strictly prohibited and where only one predators is sedentary, the Golden eagle (*Aquila chrysäetos*), whereas for the wolf (*Canis lupus*) no stable presence was evident in the years of data collection. Predation by Golden eagle is limited and focused only on young ibex of few months of age. Behavioural data were collected in the Levionaz valley, located at the centre of the GPNP, where the highest density of Alpine ibex is observed. Habitat is characterized by cliff, slopes and alpine meadows (mainly *Carex curvula* and *Festuca* spp.). During the rutting periods the temperature in the study area ranged from -15°C to +9.4°C and the snow cover averaged was 46.7 cm.

Each year the personnel of the GPNP capture some ibex in the study area of Levionaz. Males were tagged with ear tags of different colors and females with colored collars, permitting to determine precisely the identity of each individual. The age of each captured individual was exactly determined by counting the number of annual incremental growth rings on the horns (Ratti and Habermehl 1977). Individuals were captured by chemical immobilization following the methodology described by Sica (2014). During the study period the Levionaz valley population estimated by total counts (Jacobson et al. 2004) on average 64 males, 62 females, 14 yearlings and 24 juveniles born during the year for a total of 163 individuals. In the study area approximately the 85% of males and the 20% of females were marked.

Data collection

This study was conducted during the mating season in 2011-2012, 2012-2013 and 2013-2014 occurring from early December to mid-January (see Apollonio et al. 2013). From the first day of December to the 12th of January we performed focal animal sampling (Altmann 1974) on 26 different individual tagged female ibex. Behavioural observations were performed between 8:30 AM and 17:00 PM using a spotting scope (20 – 60x). Focal females were observed from a distance of 500-700 m and, thus, no disturbance is caused to. In the first two mating seasons (2011-2012 and 2012-2013) we recorded the behaviour of tagged females when males court them. Explorative analyses on this first period showed a change in the female behaviours when they were in oestrus days. Thus, during the last year of the study (2013-2014) observations were implemented only in those females that exhibit the behavioural patterns typical of the oestrus period.

During the first parts of the study (2011-2012 and 2012-2013) only tagged females were observed, to avoid pseudoreplication. During the second part (2013-2014) we started to observe also untagged females to extend the observed sample size. For unmarked females, age was not estimated because we could not precisely distinguish annuli on their horns from a long distance. Female ibex in captivity had an average oestrus cycle length of 20 days ± 1 day (Stüwe and Grodinsky 1987). The time between different observations of untagged females it is always larger or smaller than 20 days. This means that no untagged females were sampled twice. Oestrus day refers to the day of observation where a focal female was observed engaged in mount or copulation. The oestrus length of a female ibex is unknown but this reproductive cycle can last about 28.7 ± 11.5 h in domestic goats (Fonseca et al. 2005).

During the observation we recorded also the identity of tagged males. In the case of unmarked males, the age was estimated by the body size and the size of the horns and later compared with tagged males nearby. We recorded the following behavioural states for females: foraging, standing either vigilant (scanning the environment or check the other individuals) or ruminating, moving (walking and/or running), lying down flat on the ground either resting or ruminating. Besides we also recorded females behavioural events performed towards males, called females aggressive behaviours (Chapter I): displace males (movements with the horn performed by female to displace the male in courtship behaviour), horn contact (when female crash with their horn towards them of the male), escape (when female move quickly and fast away the male) and jump (when females before the horn contact towards them of the male stands up on his hind legs). Whereas for males we recorded different courtship behaviour performed towards the focal female and were regrouped into three groups (for a detailed description of those behaviours, see Willisch and Neuhaus 2009): (1) control (merge standing near females, when male tend female; following, when male follow in the movements the female; sniff, males sniff the place where females peed); (2) non-intensive courtship (merge low-stretch and masturbation); and (3) intensive courtship (merge touch, tongue flick and front kick).

In female aggressive behaviour, focal female displaced a courting male located at her side away from her by horn contact or simply by initiating a head movement towards him (the male then backed-off before horn contact). Moreover female could be show an escape from the courting male (quickly movement in the opposite direction of the males) or a jump (when females before the horn contact stands up on his hind legs) towards a courting male. For each female we established five behavioural activity budgets: foraging, standing (standing and standing ruminating), resting (resting and resting ruminating), moving (moving and running) and aggressive (see above) behaviours. Each behavioural variable were calculated as a matrix where the first column is the number of 'successes' (the total second a female was seen in one of the five specific behaviour) and the second column is the number of 'failures' (the total second spent by female in remaining behaviours). The obtained matrix are used as dependent variable in the statistical models. The same method is used also for the three behaviour variables of males (control, non-intensive and intensive).

Statistical analysis

Female mate choice was investigated using generalized linear mixed effects models (GLMMs) with the restricted maximum-likelihood method in the R package *lme4* (Bates et al. 2014). To account for the binomial distribution of our response behaviours (feeding, standing, moving, resting, aggressive behaviours for females and control, non-intensive, intensive behaviours for males) the error distribution of our models was set to 'binomial', whereas for other response variables to 'poisson' (total second courtship behaviours for males, age and hierarchies of males). All models included female identity, male identity and year of observation as random effects to account for the non-independence of repeated measures on the same individuals, while the latter allowed accounting for annual variation. We ran nine different GLMMs with the data from the first part of the study (2011-2012 and 2012-2013) using as explicative variables the presence or absence of a mount or a copulation during the observation to investigate the change in the five behavioural variables of females (feeding, standing, moving, resting, aggressive behaviour, all as binomial denominator) and in the three behavioural variables (control, non-intensive and intensive behaviour, all as binomial denominator) plus the total second observed in courting behaviour of males. The same for the entire period of observation (2011-2012, 2012-2013 and 2013-2014) where the presence or absence of a mount or a copulation was used to investigated variation in age of male observed in courtship behaviour (in seconds) and in female aggressive behaviour (in seconds) during three different periods of the mating season (I: from 1st of December to 15th December; II: from 16th to 31th December; III: from 1st of January to 15th January). Lastly, the observations where copulation was observed during the entire study period were used to investigate the changes occurring in the five female behavioural variables when courted by the male performing copulation respect when courted by other male during the same day and to understand the quality (age and hierarchy, where the higher male in hierarchy has the higher number on the scale of hierarchy) and the behaviour (non-intensive and control) of the chosen male by female for copulation .

To represent significant effect of each model we used the package *effects* (Fox 2003) in R. All statistical analyses were performed using the R 3.0.3 statistical software (R Development Core Team 2014).

Results

In three years we observed 26 females courted by males for a total of 271 h of focal observations for a total of 81observations (3.19 ± 2.51 observations per females; minimum-maximum: 1-10 observations). The mean (± SE) number of males courting per females was 4.01 ± 2.65 (minimum-maximum: 1 - 11). Mean duration of observation on focal females was 4.31 h ± 1.86 h (minimum-maximum: 0.43 h - 8.20 h). A total of 39 mounts and 7 copulations were observed during this study. On average each females received 2.0 ± 3.7 mounts and engaged in 0.27 ± 0.67 copulations. In three years only one female was observed copulate with two different males during the same day, otherwise none of the other females copulated with >1 male. Three females were mounted by 4, 3 and 2 different males; otherwise only a single male mounted all other females.

Variation of male courtship behaviour during the mating season (2011-2012-2013)

We observed 84 males courting females during the entire study period for a total of 97.57 h of courting behaviour. The engagement in courtship behaviours for males changed during the three periods (GLMMs *poisson* family; *e*: -0.07, *se*: 0.003, *Z* = -23.43, *p* = <0.001) and during the first period the mean age of males engaged in courtship is significantly lower than during the other two (unpaired t-test: I-II, 199 *df*, *p* = 0.029; I-III, 68 *df*, *p* = 0.034; Fig. 1).

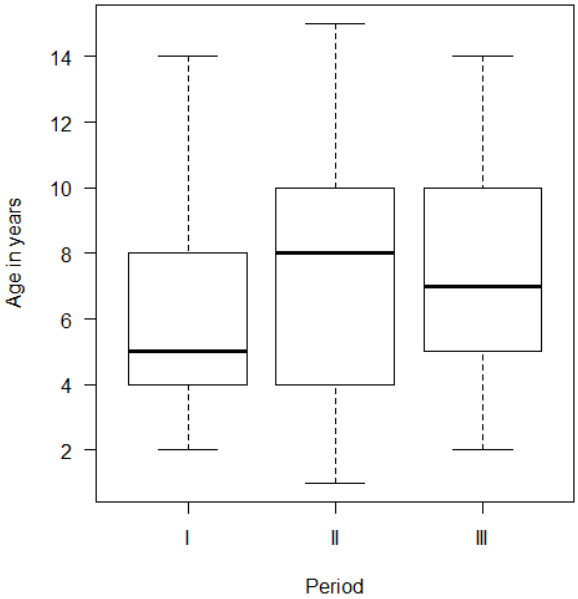

Figure 1. Median age of male Alpine ibex engaged in courting behaviour in three different periods of the mating season. Periods: I, 1st – 15th of December; II, 16th – 31th of December; III, 1st – 12th of January.

Changes in female activity budget while a mount or a copulation was observed (2011 and 2012)

Female behaviour was analyzed during the two first years of study (2011 and 2012) for a total of 173 observations on 19 females to investigate if there are changes in the behaviour females when observed during the oestrus day. In total we observed 54.72 h of courtship behaviour. 12 mounts and/or copulations were observed on 12 different females. The models showed that female allocated an overall greater percentage of their time to standing, resting, moving and in aggressive behaviours during the observation were mount or copulation were observed, whereas decrease the percentage in the time spent feeding (see Tab. 1).

Female behaviours	*Fixed effects*	*Estimate*	*SE*	*Z value*	*p-value*
Feeding	MCobs	-1.41	0.04	-35.18	*< 0.001*
Standing		0.50	0.04	12.49	*< 0.001*
Moving		0.36	0.08	4.27	*< 0.001*
Resting		5.40	0.15	36.08	*< 0.001*
Aggressive		0.48	0.11	4.41	*< 0.001*

Table 1. Effects of mount or copulation on the proportion of time spent by female Alpine ibex in feeding, standing, moving, resting and aggressive behaviours. *MCobs* / Mount or Copulation observed. Data used are from mating season 2011-2012 and 2012-2013. Intercept and random effects Identity of females, Identity of males and Year are not shown. All behaviours were corrected for the binomial family.

Female aggressive behaviour during the different periods of the mating season while a mount or a copulation was observed (2011-2012-2013)

Seeing the change in behaviour of females during the day where mount or copulation was observed (the oestrus day), the variations in the aggressive behaviour were investigated in the three periods. The models were applied only on aggressive behaviour to observe if females changed their behaviour accordingly the change in the time spent by young males in courtship behaviour. Aggressive behaviour increased in the first period and it is correlated with the interaction between period and day of oestrus (GLMMs *binomial* family, e: 0.34, se: 0.06, Z = 5.84, p = <0.001; Fig. 2).

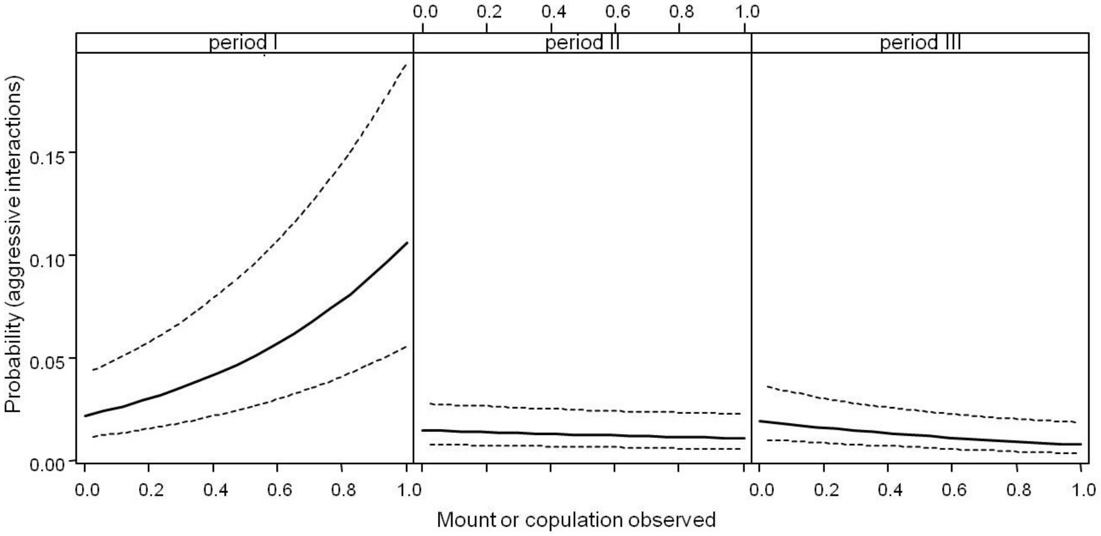

Figure 2. Probability to observe female aggressive behaviour during the three different periods in relation with oestrus day. The vertical axis is labelled on the probability scale for aggressive behaviour. Periods: I, 1st – 15th of December; II, 16th – 31th of December; III, 1st – 12th of January.

Changes in male activity budget while a mount or a copulation was observed (2011 and 2012)

Change in activity budget of males was analyzed on the first two year of this study, observing the change of their behaviour in function of the observation of the oestrus day of females. Analyzes were performed using observations on 19 different females for a total of 173 males observed in courting behaviour. In total we observed 86 different males. Males increased non-intensive and intensive behavioural patterns whereas decreasing the control ones when a female was in oestrus. Consistently the total time spent by males in courting behaviour increase when a female was in oestrus (Tab. 2; Fig. 3).

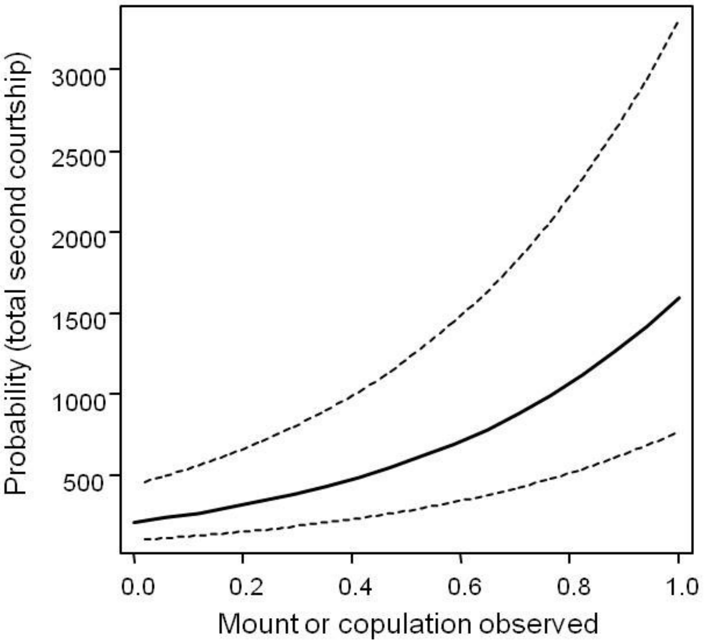

Figure 3. Probability to observe the change in the activity budget of male Alpine ibex during the days without and with mount or copulation. The vertical axis is labelled on the probability scale to total second courtship behaviour.

Male behaviours	*Fixed effects*	*Estimate*	*SE*	*Z value*	*p-value*
Control	MCobs	-1.03	0.04	-27.62	*< 0.001*
Non-intensive		0.72	0.04	19.13	*< 0.001*
Intensive		0.67	0.07	8.99	*< 0.001*
Total		2.01	0.01	140.10	*< 0.001*

Table 2. Effects of mount or copulation on the proportion of time spent by male Alpine ibex in control, non-intensive, intensive and total second courtship (Total) behaviours. *MCobs* / Mount or Copulation observed. Data used are from mating season 2011-2012 and 2012-2013. Intercept and random effects Identity of females, Identity of males and Year are not shown. Control, non-intensive and intensive behaviours were corrected for the binomial family; total second courtship (*Total*) behaviour was corrected for the poisson family.

Changes in female activity budget depending on the quality of males (2011-2012-2013)

Using the entire data set we analyzed 26 different females engaged in total with 243 males courting them (one same male could be counted twice). Male age and males dominance rank were positively correlated (*Pearson* correlation test: r = + 0.63, *p* < 0.001). GLMMs, using a *poisson* family, with behaviour females as responses variable were fitted with only age of males as explicative variable. When females are courted by older males spent more time in feeding behaviour, decreasing consequently the time in standing, resting and aggressive behaviours. The inverse happens when females were courted by younger males. Moving behaviour was not changed by females in function of males age (Tab. 3).

Female behaviour	*Fixed effects*	*Estimate*	*SE*	*Z value*	*p-value*
Feeding	Age	0.57	0.01	75.68	*< 0.001*
Standing		-0.26	0.01	-32.12	*< 0.001*
Moving		0.03	0.02	1.56	0.118
Resting		-0.94	0.08	-12.21	*< 0.001*
Aggressive		-0.28	0.02	-12.64	*< 0.001*

Table 3. Effects of age of males on the proportion of time spent by female Alpine ibex in feeding, standing, moving, resting and aggressive behaviours. Intercept and random effects Identity of females, Identity of males and Year are not shown. Data used are from all study period.

Female choice (2011-2012-2013)

To investigate proper female mate choice we selected only the observations were a copulation was observed (males observed during the days when a copulation was observed=24, copulations observed=7). General linear mixed models showed that the chosen male by female to copulate was preferably the older one and the highest in hierarchy (Fig. 4 A and B, respectively). Moreover the chosen male allocated less time in non-intensive courtship behaviour but spent more time in control behaviour (Fig. 4 C and D, respectively). Results of models are showed in Table 4.

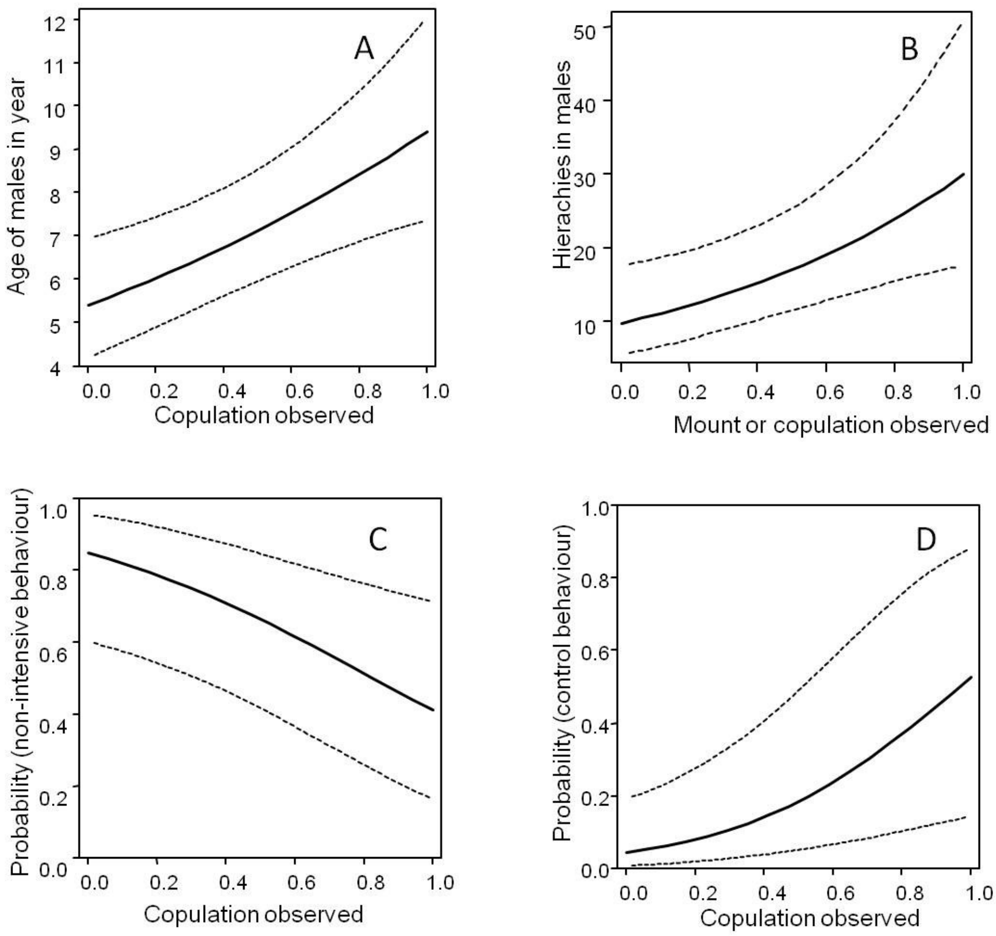

Figure 4. Probability in the change of the age (A) and hierarchies of males (B) on the moment of copulation and the probability to observe non-intensive (C) and control behaviour (D) in the male that copulate. Intensive behaviour was not showed.

Responses	*Fixed effects*	*Estimate*	*SE*	*Z value*	*p-value*
Age males	Acceptance	0.56	0.18	3.18	*0.002*
Hierarchies		1.12	0.40	2.82	*0.005*
Control behaviour		3.19	1.05	3.03	*0.003*
Non-intensive behaviour		-2.08	0.80	-2.60	*0.009*
Intensive behaviour		0.41	1.21	0.34	0.734

Table 4. Selection criteria for female to accept a given males. To investigate female mate choice we selected only the observations were copulation was observed (males observed during the day when a copulation was observed = 24, copulations observed = 7). Intercept and random effects Identity of females, Identity of males and Year are not shown. Data used are from all study period.

Females showed an increase in moving behaviour when courted by the chosen males, whereas other behaviours seemed not influenced by the males identity, although a trend is observable (Fig. 5 and Tab. 5).

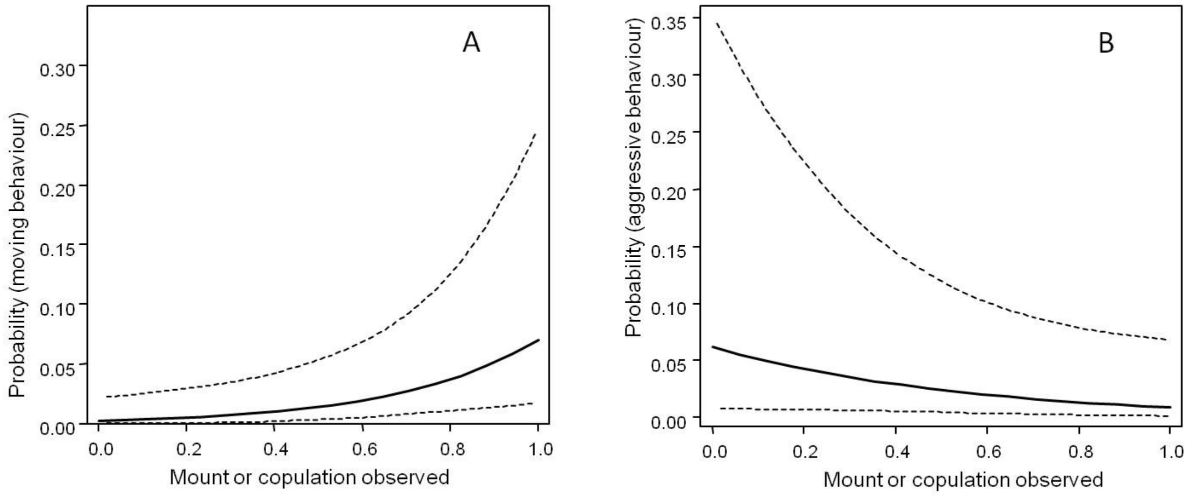

Figure 5. Probability to observe the change in the activity budget of female Alpine ibex in function of the chosen male (male that performed the copulation). The vertical axis is labelled on the probability scale to each behaviours. (A) Moving and (B) aggressive.

Female behaviour	*Fixed effects*	*Estimate*	*SE*	*Z value*	*p-value*
Feeding	*Chosen male*	2.39	1.63	1.46	0.144
Standing		0.83	1.86	0.45	0.654
Moving		3.34	1.23	2.73	*0.006*
Resting		3.44	3.13	1.10	0.273
Aggressive		-1.96	1.22	-1.57	0.112

Table 5. Effects of the chosen males on the feeding, standing, moving, resting and aggressive female behaviours. Intercept and random effects Identity of females, Identity of males and Year are not shown. Data used are from all study period (males observed during the day when a copulation was observed = 24, copulations observed = 7).

Discussion

In this study we provided the first investigation on the mating behaviour of females Alpine ibex, giving insights to understand sexual conflicts and mate choice by female in ibex. Our study showed change in female time-budget during the day of the oestrus, as well as aspects of female mating strategies in Alpine ibex related specifically to copulation.

Our study supports the view that female ungulates change their behaviour when in oestrus (Clutton-Brock et al. 1982a; Morrison 1960; Ozoga and Verme 1975). Female ibex increased the time spent in standing, moving, resting, aggressive behaviour and decreased the time spent in feeding behaviour when observed in oestrus (Tab. 1). Results in accordance with Chapter I, that demonstrated as group of females with a high presence of males allocated less time to feeding and more time to other behaviour, especially to observe potential suitors. We investigated the female responses to different male courting them, showing an individual correspondence to previous observation done at group level. Moreover as we observed considerable changes during the oestrus day, we concluded that the increase in aggressive behaviour is a expression of direct female mate choice and not of low female receptiveness (as suggested in Chapter I). Direct mate choice means that females actively discriminates among possible mates, affecting their chances of mating (Wiley and Poston 1996).

Direct mate choice is clearer when the behaviour towards the chosen male was investigated. Even if not significant (due to the low number of copulations observed), we observed a pattern of aggressive behaviour decreasing towards the chosen male (Fig. 5, B). Besides, we found that chosen male was engaging in control behaviours of female at the detriment of time spent courting them with non-intensive behaviours (Tab. 4, Fig. 4). This result provide evidence of female preferences for males who protect them against harassment of other males (Clutton-Brock et al. 1993; Carranza and Valencia 1999). Females seems gain direct benefits when courted by the chose male with the reducing of male harassment (see Clutton-Brock 1992; Carranza and Valencia 1999) and with the increasing of time spent in feeding behaviour in his proximity (Tab. 3). Moreover, after the selection of a male, females increased their movement to avoid harassing males and stay close to selected one (Tab. 5, Fig. 5 A). Accordingly, we observed that the male engaging in longer courtship behaviour (more often control behaviour) have the higher change to be choose by females (Tab. 2, Fig. 4).

Females seems choose the male according to the guarding ability (as suggested in *Oreotragus oreotragus*: Roberts and Dunbar 2000) preferring males protecting them rather than harassing them (in agreement with the study on male ibex mating behaviour: Willisch and Neuhaus 2000; Willisch et al. 2012; Apollonio et al. 2013) and according to the courtship display, choosing the male performing the longer courtship behaviour, considered as a honest indicator of a male fitness in other species (Seymour and Sozou 2009; Longpre et al. 2011). In domestic goats courtship rate is correlated with testosterone concentrations and oestrus goats prefer males with higher testosterone concentrations (Longpre et al. 2011), leaving open this possibility also in Alpine ibex. Interestingly, female ibex do not prefer male courting them at higher rate (which are the young male) but prefer male courting them at lower rate, the opposite of the preferences in female domestic goats (Longpre et al. 2011). This change of preference in ibex could be strongly influenced by the adverse condition with severe winter (when the mating season occurs; Apollonio et al. 2013) and thus both male and female have to choice the less expensive tactics in term of energy. Female ibex exacerbated the conflicts with the non-preferred males and mate cooperatively with preferred males. The two sexes seems to establish a mutually beneficial cooperation.

Mating behaviour of males changed substantially when observed courting females during the oestrus day. All males increased their time spent in non-intensive and intensive behaviours at the detriment of control as well as increased the total time in courtship behaviours. This means that during the oestrus day females were more subjected to harassment of males, and their prefer the male less harassing (see above). As expected male age and male hierarchies are positively correlated (Bergeron et al. 2010), with females preferring for copulation the older one and, consequently, the higher in hierarchies (as suggested by the paternity analyses performed by Willisch and colleagues (2012) on a population of reintroduced ibex in the Swiss Alps).

The sexual conflicts exacerbated with the non-preferred males were also visible when the mating season was divided into three periods (I: from 1st of December to 15th December; II: from 16th to 31th December; III: from 1st of January to 15th January). During the first period the mean age of males observed in courting behaviour is significantly different from the other two periods (Fig. 1). Moreover female aggressiveness towards males was different among the three period (Fig. 2): they showed an higher aggressive rate during the first period when in oestrus than during the other ones. This suggest that during the first periods females were more frequently approached by young males, were subjected to a higher harassment rate and therefore they rejected these disturbing, unwanted males. Interestingly, in the second two periods (II and III) when the mean age of males courting females increased females decreased their aggressive behaviour during the day of oestrus.

Our results, even if based on small sample size due to big difficult to observe copulation and not in a deficiency of observations, highlighted as the sexual conflicts in Alpine ibex are more accentuated as expected by previous research. The investments in mating courtship for males represent a substantial fraction of their total energy budget (Willisch and Neuhaus 2010), ultimate they have to court a female for long time to be considered a possible choice. Female mating strategy in Alpine ibex seems consistent with the picture given of the mating systems in males (Willisch and Neuhaus 2009; Apollonio et al. 2013). Even so, it should be noted that further genetics and spatial study are still necessary to understand thoroughly the female mate choice in Alpine ibex.

Seeing the difficult weather condition of the mating season in ibex and thus the attention to do not spent lot of energy, males establish hierarchy during the spring because more easy to take energy after the fight (Bergeron et al. 2010). We assume that this hierarchy is confirmed by sexual selection performed by females because, as we have reported, females should choose the male higher in hierarchy (see Willisch et al. 2012). In this case male competition for mates inevitable depends on condition set by females and, consequently, that male competition for mate is inseparable from what is called indirect female choice (Wiley and Poston 1996).

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Ethical note

Our experimental protocol complied with the current Italian laws. The capture protocol was authorised by ISPRA (Italian Institute for Environmental Protection and Research). No further approval by an Ethics Committee was required, as behavioural observations at a distance were non-invasive.

CHAPTER III

FEMALE QUALITY AND THE COST OF REPRODCUTION EXPLAIN AGE-RELATED VARIATION IN REPROCUTIVE SUCCESS IN THE ALPINE IBEX

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Manuscript

Abstract

Variations inter-individual in traits associated with the cost of reproduction imply to investigate on the presence of individual 'quality'. We used 13 years of data on weaning success of Alpine ibex (*Capra ibex) t*o investigate the importance of the reproduction cost and of the individual in quality variations in an emblematic mammal species of the Alpine ecosystem. Weaning success of female Alpine ibex incurs in a decline from 7 years of age, but notably it is not found the common plateau in reproductive performance showed by other ungulate species. We showed an important effect of individual quality on weaning success, with females that successfully wean a kid in a given year more likely to wean another kid two year later. This result highlight the cost of reproduction. Interestingly females able to wean a kid every two year, produce kids with higher survival probably than kids of females unable to maintain this performance. No senescence was detected in this population. These results pointed out the importance of the individual quality in reproduction and in manage life history trade-off.

Key words: Alpine ibex; individual quality; ungulate; costs of reproduction; successful; unsuccessful

Introduction

Life-history theory assumes that reproduction is costly (Stearns 1992), and the frequent declines in reproductive success at older age observed at the population level are often due to the inability of very old individuals to cope with the costs of a reproductive event (Reznick 1985). There is however accumulating evidence that differences in 'quality' between individuals is often overriding the cost of reproduction, with some individuals being able to repeatedly produce numerous offspring over the course of their life (Clutton-Brock et al. 1983; Festa-Bianchet 1989). These individual differences in quality can also largely account for individual variation in the rates of senescence within a species (Nussey et al. 2013). It is necessary, therefore, to investigate the importance of individual quality when modeling heterogeneity in the rates of ageing (Weladji et al. 2008) and in analyses of life-history strategies (Nussey et al. 2008; Hamel et al. 2009).

Because individual 'quality' is an ambiguous concept, it must be defined to avoid mis-interpretation (Wilson and Nussey 2010). Following the seminal works conducted by Reznick and colleagues (2000) and Wilson and Nussey (2010), individual quality can be defined by the positive covariation between reproduction and survival. This positive covariation is probably best explained by high-quality individuals having more resources to allocate to both reproduction and maintenance (Reznick et al. 2000). Should the use of resources is the proximate mechanism driving quality differences among individuals, the study of individual quality likely to be particularly relevant in species living in harsh and high seasonal environments, such as in Alpine ecosystems, where resources are difficult to monopolize, acquire and store (Marell et al. 2006). Indeed, Alpine ecosystems impose large trade-off between survival and reproduction due to the short growing season and long harsh winter season (Keller et al. 2005). Two non-exclusive, contrasting, mechanisms are therefore likely to explain age-related variation in reproductive success: on one hand, the ‘cost of reproduction hypothesis’ predicts that females that successfully produced an offspring should have a lower reproductive success in their following reproductive attempt if compared to unsuccessful females (Mundinger 1981), and on the other hand the ‘individual quality hypothesis’ predicts that females being able to produce offspring should have a higher chance to reproduce again in the following breeding event than unsuccessful females (Hamel et al. 2009). Accordingly, previous studies on female ungulates living in Alpine ecosystems have reported large trade-offs between mass gain and reproduction – weaning costs in mountain goats (*Oreamnos americanus*: Hamel and Côté 2009), but also a positive relationship between individual reproduction success and survival in reindeer (*Rangifer tarandus*: Weladji et al. 2006) and in Alpine chamois (*Rupicapra rupicapra*: Chapter IV), with successful females being more likely to survive until the next year and reproduce again successfully.

In this study we investigated the importance of the cost of reproduction and of the inter-individual variation in quality in an emblematic mammal species of the Alpine ecosystem, the Alpine ibex (*Capra ibex*). Using data on the weaning success of individually tagged females living in the Gran Paradiso National Park, we studied first age-related variation in weaning success at the population level. We applied then a individual age reverse approach allowing us to separate processes occurring at the within-individual level (i.e. cost of reproduction) from processes explained by individual variation in reproductive success (i.e. individual quality; van de Pol and Verhulst 2006; Reed et al. 2008; Martin and Festa-Bianchet 2011; Froy et al. 2013; Bize et al. 2014; Chapter IV).

Material and Methods

Study species and study area

The Alpine ibex are a sexually dimorphic species, with adult males being more than twice as heavy and possessing much larger horns than adult females (Loison et al. 1999a; Lüps et al. 2007). The mating period takes place from December to mid January (Apollonio et al. 2013; Chapter I). The birth period, after a gestation of 167 ± 3 days (Stüwe and Grodinsky 1987), occurs from begin of June to early July. Females lactate in summer but it is not rare to still observe suckling behaviours until January, likely for social bonds between mother and kid. Kids follow their mother immediately after birth (following strategy; Lent 1974) until the following spring to reduce the risk of predation (Grignolio et al. 2007a).

This study was conducted in an area of integral protection, where human harassment and hunting are forbidden, in the Italian Alps: the Gran Paradiso National Park (GPNP). Data were collected in the Northwest part of the GPNP (Valsavarenche; 1700 ha; 1700-3300 m altitude). Valsavarenche habitat is predominated by larch below the tree line and by very steep rocks and high meadows above (Grignolio et al. 2004). Each year from 1956 the GPNP ibex population was counted by means of a total census by park rangers (see Jacobson et al. 2004 for more details) recording the number of females, males and juveniles (see Tab. 1 for a summary of the data recorded during this study).

Year	Females	Males	Juveniles	Proportion of tagged females in the population (%)
2000	118	107	30	10.2
2001	119	94	23	13.4
2002	123	107	54	14.6
2003	112	106	28	24.1
2004	107	105	54	20.6
2005	112	95	38	19.6
2006	68	81	23	26.5
2007	60	77	13	23.3
2008	68	76	25	14.7
2009	46	64	13	13
2010	49	51	29	10.2
2011	58	60	20	17.2
2012	61	65	6	21.3
2013	68	68	9	19.1

Table 1. Alpine ibex (*Capra ibex*) present in the Levionaz Valley (Gran Paradiso National Park, Italy) during the study period.

Data analysis

From 2000 to 2013 the GPNP personnel captured by chemical immobilization 50 adult females and fitted them with radio-collars (for more details see Sica 2014). The exact age of each individual was determined by the GPNP vet by counting the number of annual incremental growth rings on the horns (Ratti and Habermehl 1977). Tagged females have been the subject of an individual-based study on their reproductive success. Each month during the year and especially from May-April to January, researchers and rangers of the GPNP made a great effort to identify tagged females and to monitor their presence and reproductive status. As a consequence, the annual rate of re-sightings of each tagged female was 100% from their year of tagging to the year of their last observation within the population. Regular observations and individual identification allowed mother-offspring relationships to be determined with certainty. Note that particular attention was made in winter to observe tagged females for a long time period to be sure of the presence or absence of a kid, due to the high probability for the kid to death and due to the greater distance between female and kid than in summer. Tagged female annual reproductive success was scored using a binomial score, where for each year females observed at least once with a kid from June to January received a score of 1, or a score of 0 is never observed with a kid. Annual weaning success was scored using the same approach based on the presence or absence of a kid in January. Weaning success was recorded in January because is the limit before which mortality is mostly dependent on maternal care (influenced by the environmental resources available), whereas after that mortality is care independent (because depend principally by the body condition with which the kid begin the winter and by the harshness of winter; see Gaillard and Yoccoz 2003).

Age-related variations in female reproductive performances were monitored from the year of tagging to the year of their last appearance in our population survey.

Variation in breeding and weaning success

We investigated age-related variation in female annual breeding and weaning success at the population level using the full dataset (i.e. 210 observations from the 50 ear-tagged females). The relationship between dependent variables and various life-history traits was analyzed using generalized linear mixed effects models using restricted maximum-likelihood method (GLMMs). To account for the binomial distribution of our response variables, the error distribution of our models was set to ‘binomial’. All the models included as random effect the individual identity (ID) that allowed accounting for the non-independence of repeated measures on the same individuals. All models were fitted using the *lme4* package (Bates et al. 2014) in R. We built six different GLMMs to test whether the shape of the relationship with annual breeding and weaning success was best explained by linear, quadratic and/or logarithmic age functions. Two of this six models are built using breakpoints, entered as fixed effects, identified using the R cran package "*segmented*" (Muggeo 2008; Tab. 2; following Berman et al. 2009 and Froy et al. 2013). Breakpoints are useful to quantify an abrupt change of the response variables. The different models were compared based on Akaike Information Criterion (AIC). The model with the lowest AIC was retained as the best model (Tab. 2).

(A) Model ***AGE***		AIC	Δ AIC
	No effect of age		
BS ~ 1		277.50	+ 11.90
WS ~ 1		230.15	+ 3.88
	Effect of age		
BS ~ linear(age)		277.30	+ 11.70
WS ~ linear(age)		230.76	+ 4.49
	Quadratic effect of age		
BS ~ quadratic(age)		**265.60**	**0**
WS ~ quadratic(age)		**226.27**	**0**
	Logarithmic effect of age		
BS ~ log (age + 1)		279.30	+ 13.70
WS ~ log (age + 1)		231.95	+ 5.68
	Existence of one segment		
BS ~ I^{st}		278.20	+ 12.60
WS ~ I^{st}		231.93	+ 5.66
	Existence of two segments		
BS ~ I^{st} + II^{st}		280.00	+ 14.40
WS ~ I^{st} + II^{st}		226.99	+ 0.72

(B) ***Slope*** of each segments between the breakpoint

	BS		WS	
	Estimate	SE	Estimate	SE
slope before the break point (0-7)	0.1166	0.0711	0.1404	0.0494
slope after the break point (8-19)	- 0.0352	0.0114	-0.0488	0.0144

Table 2. Candidate models used to explain the effect of age on breeding success (BS) and weaning success (WS) of ibex females in Gran Paradiso National Park (A). The best model, selected using AIC values, is reported in bold. Age is divided into one breakpoint both for breeding and weaning success, identified using the "*segmented*" package in R 3.0.3. Slopes of each the two segments (before and after the breakpoint) are reported in the second part of the table (B) for breeding and weaning success.

Weaning success had clearly a more direct effect on individual fitness and population dynamic. Hence, in the follow steps of our analyses we used this data in a data centering approach to distinguish between and within individual patterns observed at the population level (van de Pol and Verhulst 2006).

In the second part of our analyses we focused on the second share of the bell shape relationship between annual weaning success and age (age related decline or ageing; in Fig. 1 from 7 years of age, which is the peak of reproduction for ibex females). To restrict our analyses to senescing females, only individuals fulfilling those three conditions were kept in the analyses: (i) an age at death greater than or equal to 9 years, (ii) with minimum 2 - maximum 5 years before death and (iii) accordingly, with at last 2 years of observation before death. We kept an age at death ≥ 9 years because allowed us to keep in our analyses females from 7 years of age, age where began the decline of weaning success and where was observed the maximum weaning success in the population. 2 years of observation of females were necessary to investigate the effect of weaning success at year *t-1* on the weaning success at year t.

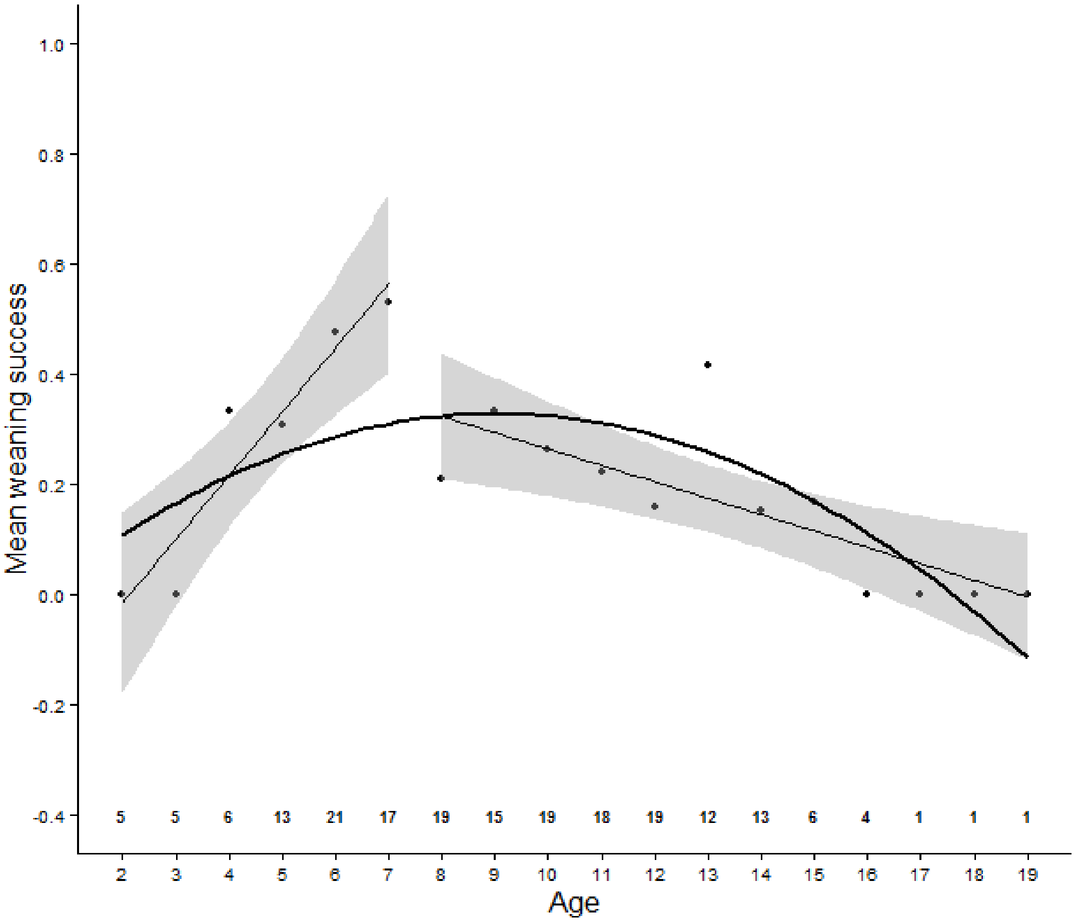

Figure 1. Mean breeding success of ibex female in relation to age in Levionaz valley in the Gran Paradiso National Park, Italy. The curve in bold, obtained from result of the best models (BS ~ quadratic(age)), shows the quadratic fit of breeding success in relation to age. Regression lines are fitted as solid and black lines for each break points obtained using the package *segmented*. Regression lines are shown with the 95% confidence intervals. Data are on 210 observations from 50 females. The number of females observed for each age is reported above the X-axis.

In the analyses we considered the followed variables:

- *WS* (weaning success at year *t*): female with or without kid in January of the next year; it is the response variables;
- *AD* (age at death): age at last appearance, age when female was considered dead; AD was used to investigate selective appearance or disappearance and AD^2 allow testing for quadratic effects of AD;
- *YBD* (years before death): last years of life of the individual, with 0 denoting the final year of life; YBD was used to investigate senescence *per se* and YBD^2 allow testing for quadratic effects of YBD;
- WS_{t-1} (weaning success at year *t-1*): weaning success in the past attempt; WS_{t-1} were entered to test whether WS in year *t* was influenced by carry over costs associated with a successful reproduction in year *t-1*;
- WS_{t-2} (weaning success at year *t-2*): weaning success in the two past attempt; WS_{t-2} were entered to test whether WS in year *t* was influenced by carry over costs associated with a successful reproduction in year *t-2*;

Age at death is estimated in function of the last year of appearance of each female. In 14 years of study with marked female, individual emigration was never observed. Thus if a female was not observed in the subsequent year, she was considered dead. This restriction of our data set allows us to investigate our hypothesis using an age-reverse approach. Age-reverse approach is a method centering our females on their age at death, thus expressing the age term as years before death, with age 0 denoting the final year of life. The age-reverse approach permits to focus on age-related variation during the last years of life of each individuals (for similar approach see van de Pol and Verhulst 2006; Reed et al. 2008; Martin and Festa-Bianchet 2011; Froy et al. 2013; Bize et al. 2014; Chapter IV). At the end, our trimmed dataset counted 61 observations from 18 individuals. To test the effects of the different variables presented above on weaning success at year *t* (without weaning success at year *t-2*, see below) we set six different GLMMs (as reported in Tab. 3). A model selection was performed and the model with the lowest AIC was retained as the best. When the ΔAIC between the best competing models was < 2, the most parsimonious model (the one with the least terms) was retained as the best fit. As weaning success at year *t-1* influence weaning success at year *t* (see Results section), we ran a separate GLMMs model based on a smaller data set (removing the unknown data of weaning *t-2* for different individuals and obtaining 48 observations on 17 females) to test the effects of the weaning success at year *t-2* on year *t*.

Survival analyses of kid

All kids observed every year from 2000 to 2012 were used to estimate the survival probability from July to May of the following year. Survival of kid were investigated in function of: (i) the age of females, (ii) the weaning success at year *t-1* and (iii) at the year *t-2*. To examine if survival of kid changed in relation to these variables, the survival analyses were performed using the *survival* package (Therneau and Lumley 2014) in R and the *survreg* function based on Weibull distribution.

All statistical analyses were performed using the R 3.0.3 statistical software (R Development Core Team 2014).

Response variable	Model	Fixed effect(s)	AIC	Δ AIC
	1	$AD + AD^2 + YBD + YBD^2 + WS_{t-1} + AD^* WS_{t-1} + YBD^* WS_{t-1}$	69.1	+ 1.9
	2	$AD + AD^2 + YBD + YBD^2 + WS_{t-1} + AD^* WS_{t-1}$	67.9	+ 0.7
WS	3	$AD + AD^2 + YBD + YBD^2 + WS_{t-1}$	70.6	+ 3.4
	4	$AD + AD^2 + YBD + WS_{t-1}$	68.8	+ 1.6
	5	$AD + AD^2 + WS_{t-1}$	67.2	0
	6	**$AD + WS_{t-1}$**	**68.1**	**+ 0.9**

Table 3. The six generalized linear mixed effects models fitted to investigate the various factors that could affect weaning success (WS). Intercept and random effect individual identity are not shown. *WS* / weaning success at year *t*: female with or without kid in January of the next year; *AD* / age at death: age at last appearance, age when female was considered dead; *YBD* / years before death: last years of life of the individual, with 0 denoting the final year of life; WS_{t-1} / weaning success at year *t-1*: weaning success in the past attempt. The retained model with the ΔAIC < 2 from the lowest AIC values and with the least terms is reported in bold.

Results

51 female ibex were captured (N = 50) at a mean (±SE) age of 7.42 ± 3.21 years of age (min – max: 2 – 14 years). In total 210 years/female (n = 210) were examined in relation to weaning success. Mean age at disappearance from the dataset (i.e. death) was of 12.53 ± 3.23 years of age (minimum – maximum: 6 – 19 years). In the trimmed data set ($n = 61$; $N = 18$) used to analyze the reproductive senescence the mean age at tagging was 7.63 ± 3.00 years of age (minimum – maximum: 2 – 13 years) and mean age at death was 13.71 ± 1.72 years (minimum – maximum: 10 – 16 years). To investigate the effects of weaning succes at year *t-2* on year *t* a smaller data set was used, including 17 females and 48 years/female.

Population level variation in breeding and weaning success

The model fitting a quadratic effect of age is the best one to explain variation in female weaning (Fig. 1; GLMM, age: $e = 0.61 \pm 0.29$ SE, $z = 2.12$, $P = 0.034$; age^2: $e = -0.04 \pm 0.06$, $z = - 2.36$, $P = 0.018$) and breeding (Fig. 2; GLMM, age: $e = 0.86 \pm 0.28$ SE, $z = 3.03$,

$P = 0.002$; age^2: $e = -0.05 \pm 0.02$, $z = -3.32$, $P < 0.001$) success at the population level (Tab. 2 (A)). Females showed a significant increase in weaning success up to 7 years of age (breakpoint) immediately followed by a significant decline in both weaning and breeding success to old age (Tab. 2 (B), Fig. 1 and 2).

Factors influencing variation in weaning success during the ageing

The best model retained age at death and weaning success at year *t-1* (Tab. 2). Whereas to investigate weaning success at year *t-2* a separated model was set to allow the use of more data in the first model. The decreasing of weaning success at older age was explained by the presence of *selection per se*, investigated using age at death (GLMMs, *estimate*: -0.74; *se*: 0.34; *Z value*: -2.17; *p-value*: 0.029). Besides, Alpine ibex females showed a variation in weaning success at year *t* following their weaning success at year *t-1* (GLMMs, *estimate*: -2.08; *se*: 1.08; *Z value*: -1.91; *p-value*: 0.055) and *t-2* ($N = 17$, $n = 48$; GLMMs, *estimate*: 3.3; *se*: 1.19; *Z value*: 2.789; *p-value*: 0.005). Females who reproduced successfully two year before (year *t-2*) were more likely to be successful again in their reproductive attempts at year *t*, whereas the opposite is observed for one year before (year *t-1*; Fig. 3). Instead females were not likely to show senescence in old age (years before death not retained into the best model).

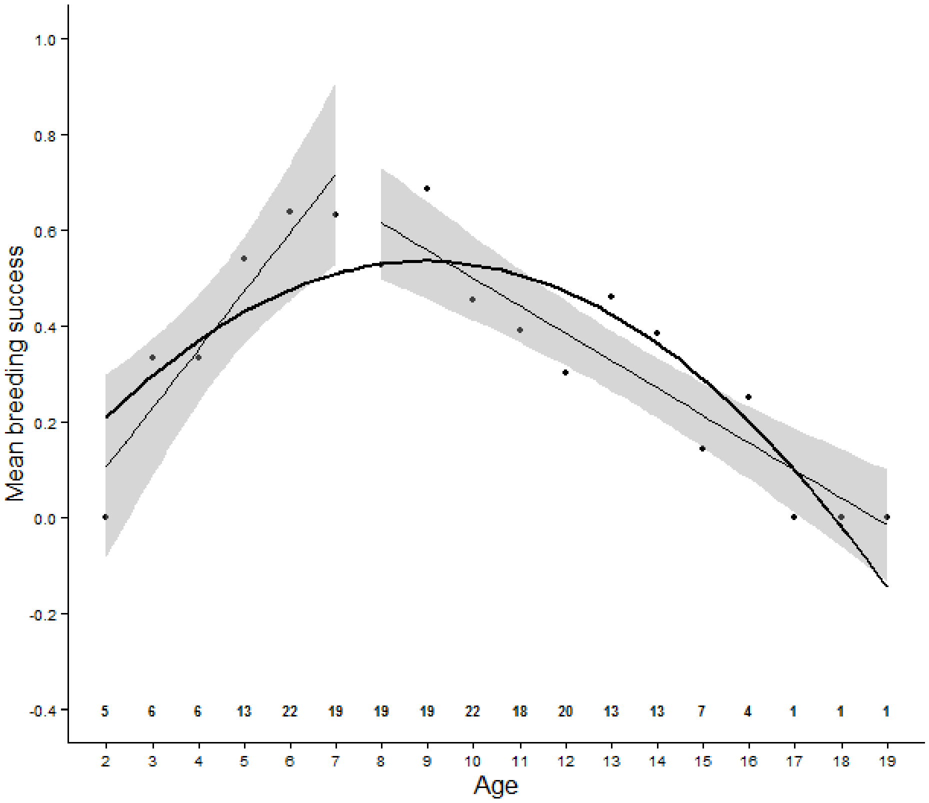

Figure 2. Mean breeding success of ibex female in relation to age in Levionaz valley in the Gran Paradiso National Park, Italy. The curve in bold, obtained from result of the best models (WS ~ quadratic(age)), shows the quadratic fit of weaning success in relation to age. Regression lines are fitted as solid and black lines for each break points obtained using the package *segmented.* Regression lines are shown with the 95% confidence intervals. Data are on 195 observations from 50 females. The number of females observed for each age is reported above the X-axis.

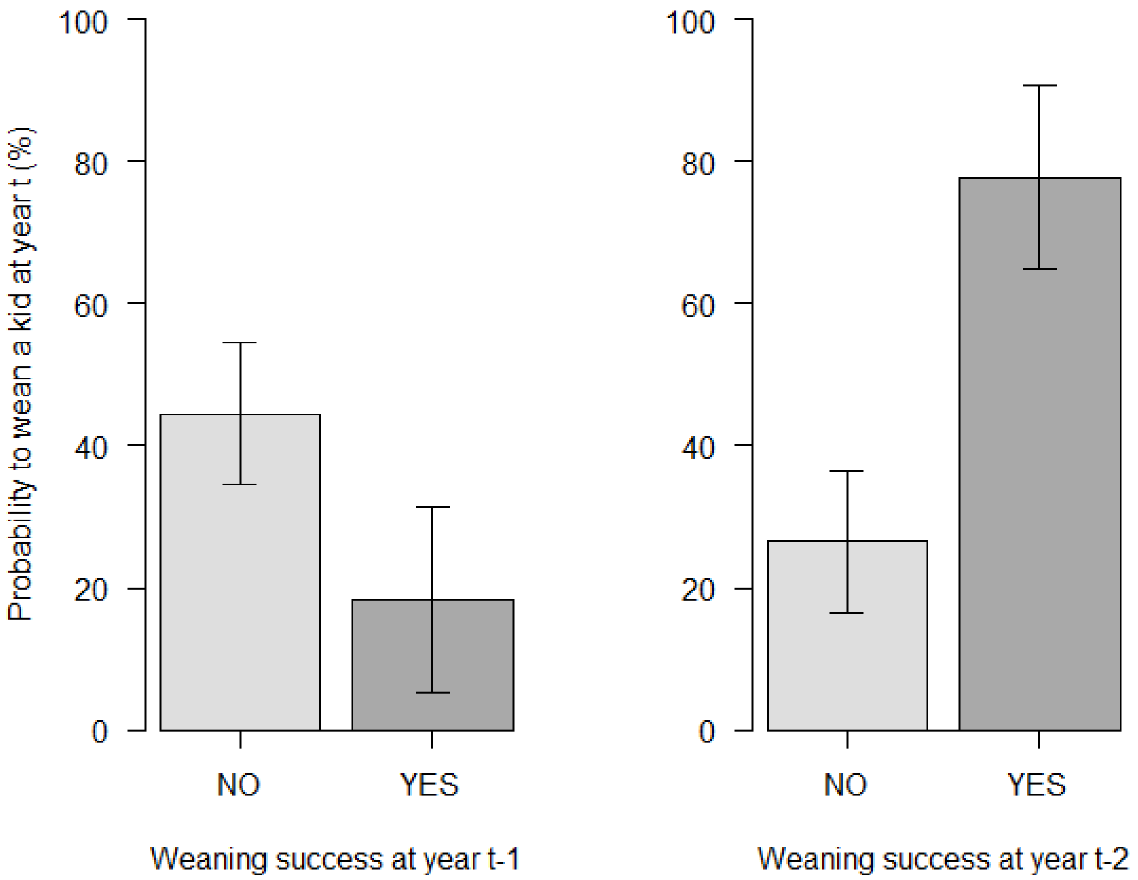

Figure 3. Probability for Alpine ibex females to wean a kid during the year *t* in relation to the weaning success during the year *t-1* and *t-2* in Levionaz valley in the Gran Paradiso National Park, Italy.

Survival analyses of kid

The risk of death decrease with each passing month for kids (*scale* = 0.67; Fig. 4 A, B). Different pattern were observed when survival was investigated using (i) age of females separated into two groups, one from 2 to 7 years of age and the second from 8 to 19 years of age (p = 0.24, mean month at death for kid of females from first group is April whereas for the second group is January); (ii) females that weaned a kid at year *t-1* showed a reduced survival at year *t* of their kid (mean month at death: January) whereas the estimate of survival for the kid are higher if females did not weaned a kid at year *t-1* (mean month at death: March; p = 0.22; Fig. 4, A); (iii) the opposite happens when the weaning success at year *t-2* was observed, indeed kid of females weaning a kid at the year *t-2* had higher probability to survive than those of females don't weaning a kid (p = 0.13; mean month death for kid with female weaning a kid at year *t-2* was nor detected because high survival probability during each months, whereas for the other the mean month of death is February; see Fig. 4, B).

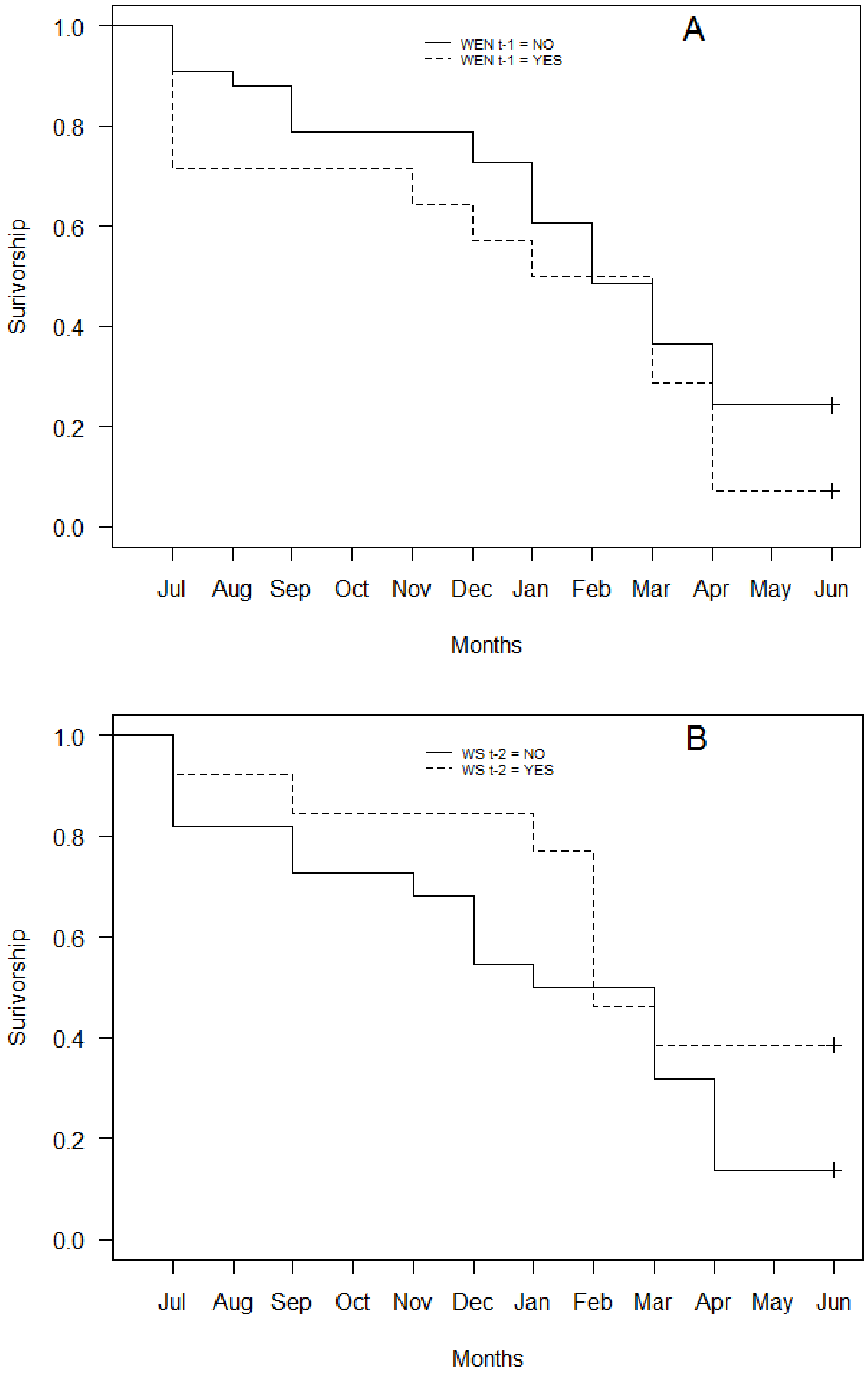

Figure 4.Survivorship probability for kid during one year in functions of (A) the weaning success at year *t-1* and (B) the weaning success at year *t-2* in Levionaz valley in the Gran Paradiso National Park, Italy.

Discussion

Our results emphasize that female Alpine ibex showed the classical pattern of increase of weaning and breeding success through early adulthood followed by a decline in late age (Fig. 1 and 2) as reported in wandering albatross (*Diomedea exulans*: Froy et al. 2013) and in

Alpine chamois (Chapter IV). We pointed out the presence of the cost of reproduction in female ibex depending on previous reproduction and we highlighted the presence of successful females in the population, which also seems to influence the survival of kids. We disentangle age-related variation in reproductive performances driven by a demographic effects (i.e., population effects) from those occurring at an individual level (i.e., senescence *per se*).

The age-related variation in female ibex breeding success (Fig. 2) was previously found in other ungulates. Generally ungulates showed a plateau where breeding success remain stable for some years before their decline (see for example Alpine chamois: Chapter IV; fallow deer, *Dama dama*: San José et al. 1999; bighorn sheep, *Ovis canadensis*: Festa-Bianchet and King 2007), whereas ibex differs from this pattern with an immediate decreasing in breeding success just after the reach of the peak (around 7 years of age). This immediate decrease is probably due to the harsh environmental condition of the mating season (i.e. during the winter; Apollonio et al. 2013) when some females older than 7 years of age could incur in a deficiency of body condition precluding their future reproduction, as previously reported for *Alces alces* (Testa and Adams 1998). Moreover, the kids produced by females of 7 years of age or younger seems to have a higher probability to survive till the next year than the one produced by older females.

Surprisingly, the decrease in female ibex weaning and breeding success observed at the population level was not explained by within-individual decline in reproductive success, differently from other ungulates (e.g. in bighorn sheep: Martin and Festa-Bianchet 2011). This result may however have been the outcome of small sample size and the absence in the dataset of long-lived females. Otherwise our results could be an indication of the absence of senescence in female ibex. Accordingly, females ibex showed very low senescence rate in survival (Toigo et al. 2007), suggesting the possibility of a similar patterns in weaning success. Interestingly, another interpretation of this result is that we do not account for reproductive senescence because it starts some year after the survival senescence (as happens in bighorn ewes; Festa-Bianchet and King 2007). Absence of senescence could be due to intrinsic individual factors. Females could vary their weaning experience (Curio 1983) and thus respond differently to daily energetic requirements (Hamel and Côté 2008). Thereby the individual level recovers a fundamental role in explaining the variation in weaning success. In our dataset there were five barren females: their investments in reproduction is zero, also when observing the first years from their appearance in the study population. It could be that our analyses is affected by these females and that, in order to adequately disentangle the effectively presence of selection per se, as well as of senescence, more long-term data on the reproduction of a larger individual sample are necessary to increase the power of the results.

Alpine ibex females showed a variation in weaning success with the successful weaning at year *t-1* that have a negative effect on weaning success at year *t* but, contrary, weaning at year *t-2* have a positive effect on weaning at year *t*. Females who reproduced successfully two year before (year *t-2*) were more likely to be successful again in their year *t*, whereas the contrary happens in respect to year *t-1* (Fig. 3). These results suggest a cost of reproduction in this species, because weaning reduces maternal body condition (Pomeroy et al. 1999) and accordingly influences largely the future reproductive success (Hamel and Côté 2008). Parental care in Alpine ibex lasts at last three months (Grignolio et al. 2007b), but it is not uncommon see suckling behaviour until January. Weaning females showed important changes in the home range size used in summer compared to the non-lactating ones. Moreover, the mothers select first rocky slopes and secondly Alpine meadows, whereas the other females make an inverse selection of these areas (Grignolio et al. 2007b). Furthermore Grignolio and colleagues (2007a) indicated predator avoidance as another source of energetic costs for lactating females. Thereby, the use of high-quality vegetation by weaning females is reduced and concentrated in a few weeks per year; in this time they have to lactate the kids and to accumulate body reserves to survive the next winter. These constrains could be the cause for which maternal care seems prevent the immediate reproduction during the subsequent year (as happens in *Bison bison*: Kirkpatrick et al. 1996). Our results highlighted the presence of successful and unsuccessful females (Fig. 3). Successful females increase their probability to wean a kid at year *t* if during the year *t-2* they weaned a kid (Fig. 4, B). This suggests a better body-condition of successful females and a better capacity to manage the trade-off between their own survival and reproduction (Stearns 1992). The same distinction in female were observed in Alpine chamois (Chapter IV) and in reindeer (*Rangifer tarandus*: Weladji et al. 2008). Successful females had kids with higher survival. Kids with mothers that weaned a kid during the year *t-2* and not during the *t-1* showed a tendency to higher survival than the kid born from females not weaning a kid during the year *t-2* and weaning a kid during the *t-1,* even if these differences were not significant (Fig. 4 A, B). These results support the cost of reproduction and suggest that the successful females could have a better fitness than the unsuccessful one.

Using the reverse-age approach and the mixed effects models we are able to disentangle the inter-individual effects (quality) from the intra-individual effects (cost of reproduction) on the variation in female Alpine ibex weaning success. In Alpine ecosystems the individual quality explains large variation in weaning success. We highlighted the presence of successful and unsuccessful female Alpine ibex and we founded an important cost of reproduction which could affect the survival of kids. More long-term data on reproduction of the same female and more extensive behavioural studies are necessary in order to better understand the differences between these two kinds of females and to investigate the presence or absence of reproductive senescence in the population of Alpine ibex.

Acknowledgments

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CHAPTER IV

SENESCENCE IN BREEDING SUCCESS OF FEMALE ALPINE CHAMOIS (*RUPICAPRA RUPICAPRA*): THE ROLE OF FEMALE QUALITY AND AGE

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Abstract

Although numerous studies have reported reproductive senescence in wild animal populations, we still know very little on inter-individual differences in rates of ageing and on the factors accounting for these differences. To investigate age-related variation in breeding success in a natural population of Alpine chamois (*Rupicapra rupicapra*), we used 15 years of data obtained by monitoring individual ear-tagged females. Analyses at the population level confirmed the occurrence of a decline in female breeding success, which is most noticeable from 9 years of age onward. Using an age-reverse approach, we showed that senescence in females' breeding success occurs at same age, since only very old individuals (older than 16 years) showed a decline in breeding success in the years preceding death. Interestingly, we also found evidence that "success comes from success", as females that gave birth in a given year were more likely to procreate again in the following year. Moreover, results showed that discrepancies between successful and unsuccessful individuals tend to become more relevant in the oldest age classes. There was no evidence of a terminal allocation. These results emphasize the importance of age-dependent effects and individual quality in shaping reproductive senescence in a wild long-lived ungulate.

Key words: ageing; age-dependent; longevity; individual quality; mammal

Introduction

Senescence, denoted by a decline in survival and reproductive success at old age, is now well documented to occur not only in humans and domesticated animals, but also in wild populations of birds and mammals (e.g., Loison et al. 1999a; Mysterud et al. 2002; van de Pol and Verhulst 2006; Jones et al. 2008; Reed et al. 2008; Berman et al. 2009; Froy et al. 2013; Nussey et al. 2013). Interestingly, whatever the species (humans, domesticated or wild species), there is often a large inter-individual variation in the rates of senescence within the same species (Nussey et al. 2013). Since the study of senescence is always performed on a group of individuals (i.e., a population; see Loison et al. 1999a), as a first essential step, it is necessary to disentangle age-related variation in reproductive performance driven by demographic effects (i.e., population effects) from those occurring at an individual level (i.e., senescence *per se*). For example, if individuals with a lower than average reproductive success also tend to live longer, they will be overrepresented in older age classes (Vaupel et al. 1979). In this case, a decline in reproduction of older females might be wrongly interpreted as senescence, rather than due to the death of individuals with a higher reproductive success (van de Pol and Verhulst 2006). Thereafter, as a second and complementary step, it is necessary to investigate the importance of individual quality in modeling heterogeneity in the rates of ageing (Weladji et al. 2008).

Central tenets of life-history theories are that allocation to reproduction is traded off against allocation to somatic maintenance and, thus, that reproduction comes at a cost of longevity (Stearns 1989). Despite experimental evidence demonstrating the occurrence of a longevity cost of reproduction at the within-individual level (Clutton-Brock et al. 1989; Daan et al. 1996; Robinson et al. 2012; Santos and Nakagawa 2012), among-individual studies often report a positive association between reproduction and survival, suggesting that longer-lived individuals also have greater chances to raise offspring (Weladji et al. 2006; Hamel and Côté 2009). This positive association is thought to be rooted, for instance, in inter-individual variation in the capacity to monopolize, store or acquire resources, with high-quality individuals having more resources to allocate to both reproduction and maintenance (Reznick et al. 2000). As 'reproductive costs' and 'individual quality' are the primary factors driving the co-variance between reproduction and longevity, Weladji and colleagues (2008) have proposed two 'extreme' scenarios: the 'individual cost-only' and the 'individual quality-only'. According to the predictions of the 'cost-only' scenario, females that successfully produced an offspring should have a lower reproductive success in their following reproductive attempt if compared to unsuccessful females, whereas the 'individual quality-only' scenario predicts that females being able to produce offspring should have a higher chance to reproduce again in the following breeding event than unsuccessful females. Life-history theory also postulates that individuals should increase their allocation to reproduction as their future reproductive prospects decline (Stearns 1992), thus leading to a maximal allocation of resources in the last reproductive attempt (also referred to as the 'terminal allocation'; Clutton-Brock 1984 and Froy et al. 2013). Interestingly, all these processes can further interact, for example, when only the high-quality individuals have enough remaining resources to allocate to reproduction at the end of their life. To adequately address and disentangle these different hypotheses, long-term data on the reproduction of the same individuals should be investigated, by means of state-of-the-art statistical models that allow to distinguish the between-individual effects (i.e., selection, quality) from the within-individual effects (i.e. senescence *per se*, cost; van de Pol and Verhulst 2006).

In this study we analysed data on breeding success of female Alpine chamois (*Rupicapra rupicapra*) living in a protected area where human disturbance and hunting were forbidden. The Alpine chamois is a polygynous ungulate native to European mountains that shows moderate sexual dimorphism (Rughetti and Festa-Bianchet 2010). The mating period takes place from November to early December. In female chamois, the first reproduction often occurs at 2 years of age (Loison et al. 1999b). Following a gestation period of ca. 170 days, females usually give birth to a single kid, more rarely to twins, in May or early June (Ruckstuhl and Ingold 1999). Kids start following their mothers almost immediately after birth (Ruckstuhl and Ingold 1994). Only females give parental care, thus establishing a strong and close bond with their kid(s) by using the follower tactic (Lent 1974) to reduce predation risk (Ruckstuhl and Ingold 1994). Weaning occurs at *ca*. six months after birth (i.e. November to early December; Ruckstuhl and Ingold 1999), but it is not uncommon to observe suckling behaviours until January. Our aim was to investigate the importance of individual quality, reproduction success and terminal allocation in shaping the rates of senescence in breeding success of female Alpine chamois.

Materials and methods

Study site and data collection

Data were collected in the Swiss National Park, an area of integral protection covering 172.4 km^2 in South-Eastern Switzerland. The habitats of the Park are dominated by forests of Arolla pine (*Pinus cembra*) and larch (*Larix decidua*) below the tree line (2200m altitude) and by alpine grasslands and bare rocks above it. Annual precipitations amount to 700-1200 mm of rain, and the growing period above the tree line is ca. 80 days per year. The Swiss National Park was created in 1914 and chamois hunting ceased in 1920. Their only predator is the golden eagle (*Aquila chrysäetos*) that can prey upon small kids only. During the study period the number of chamois within the protected area was naturally fluctuating over the years from a maximum of 1677 individuals (9.7 individuals per Km^2; 791 females, 442 males and 444 individuals younger than two years) to a minimum of 1163 individuals (6.7 individuals per Km^2; 544 females, 332 males and 287 individuals younger than two years; official census data of the Swiss National Park).

Each year since 1995, one to nineteen female chamois have been live-trapped and individually identified with numbered ear tags. In total, 102 females were ear-tagged between 1995 and 2010, with 72% of them tagged before 2001. At capture, the exact year of birth was determined by counting the number of annual incremental growth rings on the horns (Schröder and von Elsner-Schack 1985). Ear-tagged females have been the subject of an individual-based study on reproductive success since 1995. Each year, the Rangers of the

Swiss National Park made a great effort to monitor ear-tagged females, and, in particular, to observe whether these females were followed by kids or not. Most of the sightings of chamois were made during the birth period (May-June). Reproductive performance of each ear-tagged female was monitored from the year of tagging to the year of their last observation within the population, with an annual rate of re-sightings of 1 for each female between these two events. Annual breeding success of ear-tagged females was scored using a binomial score, i.e., each individual received either a score of 1 when observed to be followed by a kid during May-June, or a score of 0 if observed without a kid. Thus, this measure of annual breeding success takes into account both the likelihood for a female to give birth and the early survival of its offspring. The history of breeding success of each ear-tagged female is fully known from its year of tagging to its last appearance in the population.

Ethical note

Female Alpine chamois were tagged by rangers of the Swiss National Park under the legal authorization of the Swiss Veterinary Office.

Data analyses

Age-related variations in female annual breeding success were analyzed using generalized linear mixed effects models (GLMMs) with the restricted maximum-likelihood method in the R package *lme4* (Bates et al. 2014). To account for the binomial distribution of our response variable breeding success the error distribution of our models was set to 'binomial' and a 'logit-link' function adopted. All the models included individual identity and year of observation as random effects. The former allowed us to account for the non-independence of repeated measures on the same individuals, while the latter allowed to account for annual variation in breeding success. We ran two different sets of analyses, the first aimed at exploring age-related variations in female breeding success at the population level, and the second focused on discriminating between-individual and within-individual effects in female breeding success.

Variation in female breeding success at the population level

We investigated age-related variation in female breeding success at the population level using the full dataset (i.e. 654 observations from the 102 ear-tagged females). We used different GLMMs to test whether the shape of the relationship with breeding success was best explained by linear, quadratic and/or logarithmic age functions; the null model included year and individual identity as random terms (see Table 1). We also ran threshold models to identify breakpoints in age-related variation in female breeding success using the R cran package "*segmented*" (Muggeo 2008; Berman et al. 2009; Froy et al. 2013; Table 1). Breakpoints are useful to quantify an abrupt change in the response variable. The different models were compared according to the Akaike Information Criterion (AIC). The model with the lowest AIC was retained as the best model (Table 1).

(A) Model ***AGE***

			AIC	Δ AIC
BS ~ 1	No effect of age on breeding		670.85	46.82
BS ~ linear(age)	Linear effect of age		672.19	48.16
BS ~ quadratic(age)	**Quadratic effect of age**		**624.03**	**0**
BS ~ log (age + 1)	Logarithmic effect of age		668.92	44.89
BS ~ I^{st}	Existence of one segment	I^{st}:0-21	657.12	33.09
BS ~ $I^{st} + II^{st}$	Existence of two segments	I^{st}:0-5; II^{st}:6-21	656.38	32.35
BS ~ $I^{st} + II^{st} + III^{st}$	Existence of three segments	I^{st}:0-5; II^{st}:5-13; III^{st}:13-21	644.91	20.88

(B) ***Slope*** of each segment before and after the breakpoints

	Estimate	SE
slope before the first breakpoint (5 years)	0.1721	0.0401
slope between the breakpoints (5 and 13 years)	- 0.0111	0.0116
slope after the second breakpoint (13 years)	-0.0621	0.0181

Table 1. Null, linear, quadratic, logarithmic and threshold models used to explain the effect of age on breeding success (BS) of female chamois in the Swiss National Park (A). The best model, selected using AIC values from the model presented in (A), is reported in bold (age: $e = 0.68 \pm 0.12$ SE, $z = 6.29$, $P < 0.001$; age^2: $e = -0.04 \pm 0.01$, $z = - 6.74$, $P < 0.001$). (B) shows results of the best threshold model predicting two breakpoints and three segments, permitting to understand the inflections points in age-related variation in female breeding success. Slopes of each of the three segments are reported.

Between-individual and within-individual variation in breeding success

The population-level approach indicated a bell-shape relationship of female breeding success in relation to age (see the Results section), and therefore we used a data-centering approach to distinguish the between- from the within-individual contribution to the declining breeding success at older age (van de Pol and Verhulst 2006). We did not investigate the increase in female breeding success over the first years of life because most individuals were most likely tagged after they had already reproduced once or more (mean tagging age was 6.13 years), thus hindering a careful examination of age-related reproductive improvement over the first reproductive attempts. To make sure that only the females possibly showing ageing effects were taken into account, analyses were restricted to the observations fulfilling the three following conditions:

(i) We only kept records of individuals that appeared for the last time in our dataset no later than 2010. It follows that those individuals that have not been re-sighted for at

least two years were considered dead. In this regard, we had no cases of females disappearing for one or more years and then being re-sighted inside the study area the following year. Moreover, regular findings of marked chamois carcasses by park rangers supported this approach. Thus, hereafter we refer to the age at last appearance as age at death;

(ii) At the population level, female breeding success was reaching a plateau between 5 and 13 years of age, followed by a marked decline after 13 years of age (Fig. 1). To include information on pre-senescent females in the analyses, we kept the observations of individuals aged 9 years or older and of an age at death of 11 years or more. We used 9 years of age as a cut-of-point since it is halfway between 5 and 13 years of age, when the highest estimated productivity was observed. We considered age at death of 11 years or more because individuals should have been observed for at least three consecutive years before age at death to be included in the analyses (see also point (*iii*));

(iii) To investigate within-individual changes in reproductive performances, we only considered those individuals which have been consecutively observed at least during their three last years of life. Furthermore, to strictly focus on the effects of senescence *per se*, we restricted our dataset to observations collected in the last five (maximum) or three (minimum) years of life.

In total, we limited our dataset to 206 observations carried out on 44 females (see the Results section), and to study senescence *per se*, we used an age-reverse approach by centering our data on the age at death (for a similar approach see Reed et al. 2008; Martin and Festa-Bianchet 2011; Froy et al. 2013), i.e., by expressing the age term as years before death, with age 0 denoting the final year of life. This approach represents a powerful tool to compare senescence in breeding success in individuals with varying life expectancies. In the first statistical model of factors which are expected to account for variation in breeding success (BS), we entered age at death (AD) as a continuous variable to test for selective disappearance (i.e., between-individual effect); years before death (YBD) as a continuous variable to investigate senescence *per se* (i.e., within-individual effect); a one-level factor for female breeding success in the past attempt (BS_{t-1}), to test whether breeding success in year t was influenced by carry-over costs associated with a successful reproduction in year $t-1$ (Clutton-Brock et al. 1983; but see Festa-Bianchet 1989); and a two-level factor for final breeding attempt (FBA), to test for terminal allocation, comparing the final breeding attempt (value 1) with the previous one (value 0; Bouwhuis et al. 2009 and Froy et al. 2013). Our first statistical model thus included the following fixed terms:

$$BS = AD + YBD + BS_{t-1} + FBA + AD^2 + YBD^{\wedge 2} + AD*YBD + AD*BS_{t-1} + AD*FBA.$$

The terms age at death squared (AD^2) and years before death squared (YBD^2) allowed testing for quadratic effects of age at death or years before death on breeding success, while the interactions between age at death and years before death (AD*YBD), age at death and breeding success in the past attempt (AD*BS$_{t-1}$) and age at death by final breeding attempt (AD*FBA) allowed testing for differences in the rate of senescence, costs of reproduction, and terminal allocation of individuals that disappeared early or late from the population. The minimal adequate model was obtained by backwards model selection based on the Akaike Information Criterion (AIC) and least significant *p*-values (starting with interactions), to retain the best fitting model with the lowest AIC. All statistical analyses were performed using the R.2.15.1 statistical software (R Development Core Team 2014).

(A) Model ***breeding success***	AIC	ΔAIC
BS ~ 1	209.65	25
BS ~ AD + YBD + BS$_{t-1}$ + FBA + AD^2 + YBD^2 + AD*YBD + AD* BS$_{t-1}$ + AD*FBA	188.22	3.57
BS ~ AD + YBD + BS$_{t-1}$ + FBA + AD^2 + YBD^2 + AD*YBD + AD*BS$_{t-1}$	186.78	2.13
BS ~ AD + YBD +BS$_{t-1}$ + FBA + AD^2 + AD*YBD + AD*BS$_{t-1}$	185.89	1.24
BS ~ AD + YBD + BS$_{t-1}$ + FBA + AD*YBD + AD*BS$_{t-1}$	185.12	0.47
BS ~ AD + YBD + BS$_{t-1}$ + AD*YBD + AD*BS$_{t-1}$	**184.65**	**0**

(B) Results best model ***breeding success***

Factor	Effect size	*SE*	*z*-value	*P*-value
AD	-0.891	0.214	-4.166	<0.001
YBD	-2.992	1.157	-2.587	0.009
BS$_{t-1}$	-6.367	2.956	-2.154	0.031
AD*YBD	0.189	0.189	2.535	0.011
AD*BS$_{t-1}$	0.491	0.491	2.508	0.012

Random effects	Variance	*SD*
Female identity	<0.001	0.0002
Year of observation	1.49	1.222

Table 2. GLMM models on breeding success (BS) during the last 5 years before death in function of age at death (AD), years before death (YBD), breeding success at year t-1 (BS t-1) and final breeding attempt (FBA) (A). Female identity and year of observation were fitted as random effects. * stands for an interaction. The best model, selected using AIC values, is reported in bold. (B) provides the estimates of the fixed and random terms of the best model.

Results

Considering the entire dataset (*n* = 654, *N* = 102), mean (± SE) age of females at tagging was 6.13 ± 0.15 years (minimum – maximum: 6 months to 17 years) and mean (± SE) age at death was 13.63 ± 0.17 years (minimum – maximum: 1 to 21 years). In the trimmed dataset used to analyze the reproductive senescence (*n* = 206, *N* = 44), the mean (± SE) age at death of females was 15.44 ± 0.18 years (minimum – maximum: 11 to 20 years).

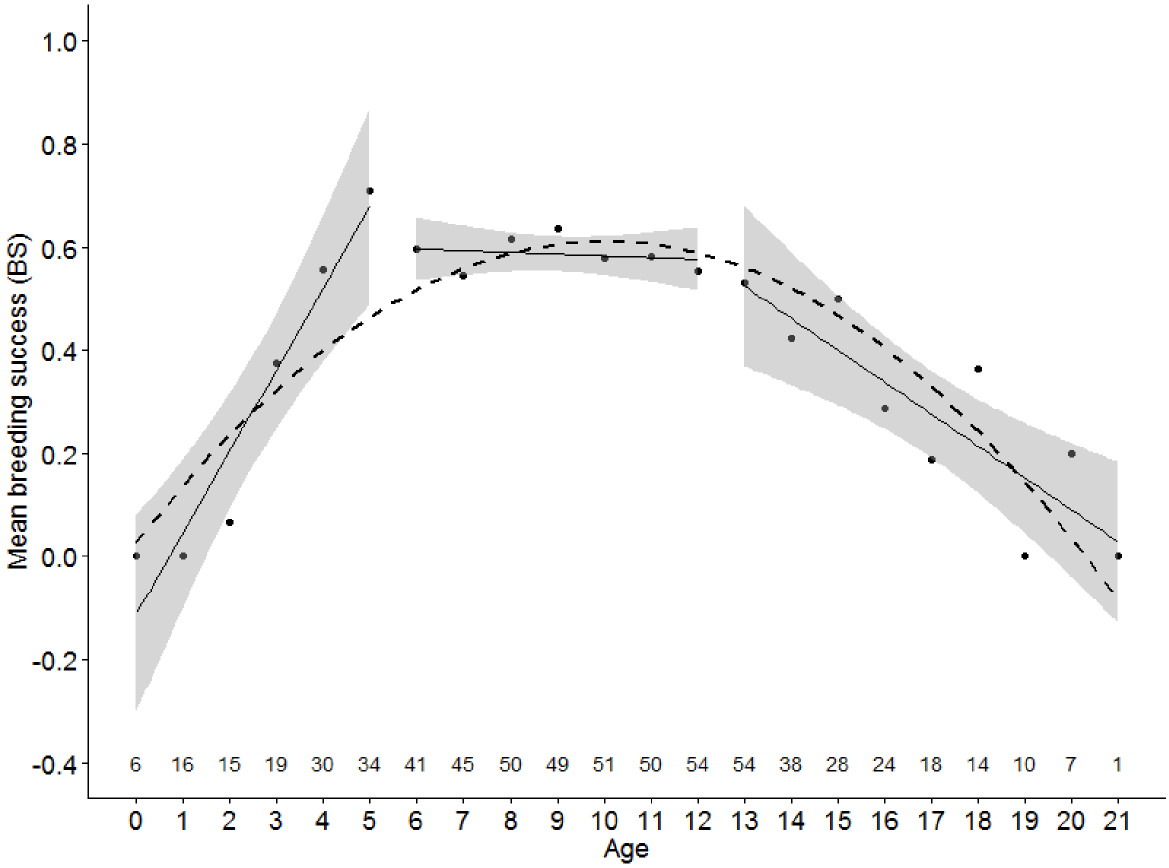

Figure 1. Mean breeding success (BS) of female chamois in relation to age in Swiss National Park. The dashed curve, obtained from result of the best models (BS ~ quadratic(age)), shows the quadratic fit of breeding success in relation to age, and the solid regression lines are fitted for each break points, obtained using the R package *segmented*. Regression lines are shown with the 95% confidence intervals and the slope of each regression line is reported in Table 2. Data are based on 654 observations from 102 female Alpine chamois. The number of females observed for each age is reported above the X-axis.

Variation in female breeding success at the population level

The model fitting a quadratic effect of age best explained the variation in female breeding success at the population level (Table 1A, Fig. 1). Additional analyses using breakpoints showed that this bell-shaped variation in female breeding success was best described by identifying two breakpoints, i.e., by subdividing the females' reproductive life history into three segments (Table 1B). Female breeding success increased until 5 years of age (i.e., the first break point), reached a plateau between 5 and 13 years of age (i.e., the second break point), declined after 13 years of age (Fig. 1).

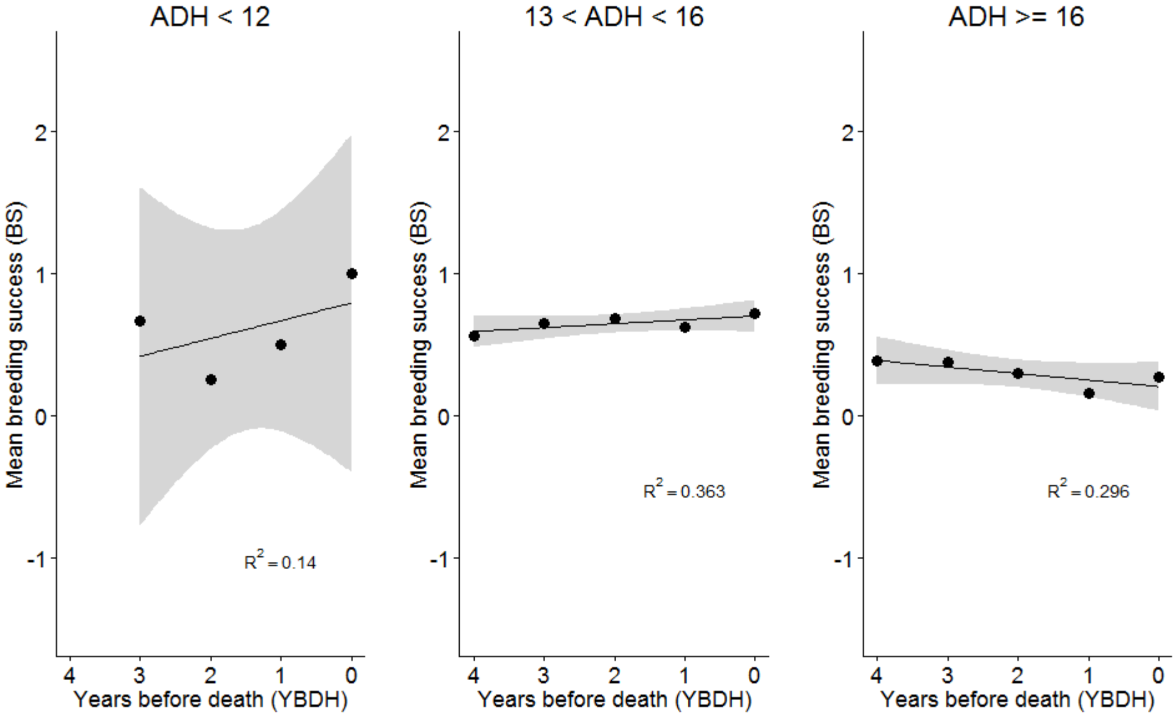

Figure 2. Mean breeding success in relation to years before death (YBD) of female chamois in Swiss National Park. For illustrative purpose, individuals were divided into three age at death (AD) classes (*n*: number of observations; N: number of individuals): AD < 12 (*n* = 15; N = 4); 13 < AD < 16 (*n* = 100, N = 22); AD > 16 (*n* = 74, N = 18). Each dot represents a mean breeding success value per YBD per AD class. Fitted lines are shown with 95% confidence intervals. R^2 and the slope of each of the three segments are reported in the panels.

Between-individual and within-individual variation in breeding success

The decrease in breeding success at older ages was best explained by a model retaining as explanatory variables age at death, years before death, breeding reproductive success of the previous year, and the interactions between age at death and years before death, and between age at death and breeding success in the past attempt (Tab. 2). The age at death by years before death interaction revealed that intra-individual decline in breeding success (i.e., measured by years before death) was becoming foremost apparent in females that died at an old age (i.e., measured by age at death) (see Fig. 2). The age at death by breeding success in the past attempt interaction was explained by the fact that females who reproduced successfully the year before (year *t-1*) were more likely to be successful again in their following reproductive attempt (year *t*; i.e., measured by breeding success in the past attempt), and the difference between successful and unsuccessful females was more marked in long-lived than in short-lived individuals (i.e., measured by age at death; Fig. 3). The inspection of the random terms showed that the variation in annual breeding success was strongly influenced by the effect of the year (*var* = 1.49), but not by individual identity (*var* = 0). Effects of the final breeding attempt, either alone or in interaction with other terms, were not included in the best model.

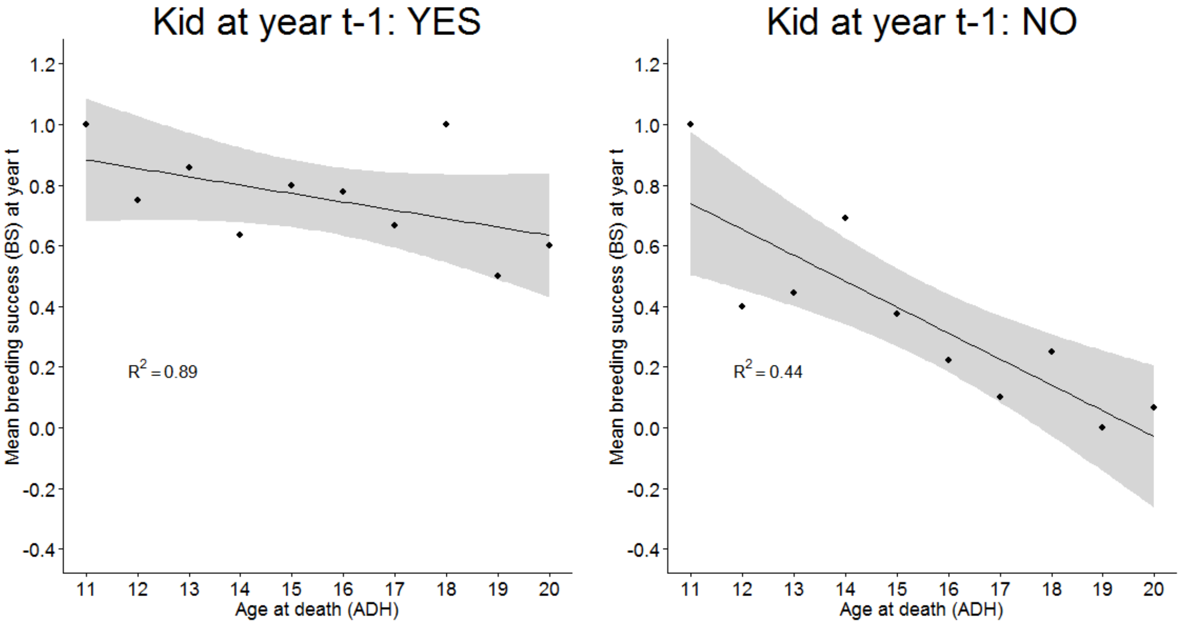

Figure 3. Mean breeding success at year t in relation to age at death (AD) and breeding success at year t-1 of female chamois in Swiss National Park. Left panel: 81 observations from 29 female that successfully produced a kid at year *t*; right panel: 84 observations from 34 females that failed to produce a kid at year *t-1*. Regression lines are shown with 95% confidence intervals. R^2 of each regression line are reported in the panels.

Discussion

Our analyses of a natural population of Alpine chamois showed that the likelihood for females to give birth follows a classical bell-shaped curve in relation to age (Fig. 1). Females were less likely to reproduce in either young- and old-age classes. However, the underlying mechanisms explaining the lower reproductive success in young- and old-age classes with respect to middle-age classes are most likely different. Since in the present study female chamois were tagged on average at 6.13 years and most of the increase in breeding performance was observed before 6 years of age, our data did not allow to address the mechanisms accounting for an increase in reproductive performance from young- to middle-age classes. However, our data were suitable to disentangle the between-individual effects (selection, quality) from the within-individual effects (senescence *per se*) of the decreasing reproductive success in old-age classes. Analyses at the individual level, in fact, showed that senescence in females' breeding success occurs at the same age, as the reduction in breeding success with increasing age was observed only in very old individuals (Martin and Festa-Bianchet 2011). Our results also indicated the presence of *successful* and *unsuccessful* females in the population: females which had been observed with a kid were more likely to be observed again with another kid the following year, whereas unsuccessful females were more likely to remain unsuccessful also in the following year. This strong dichotomy between *successful* and *unsuccessful* females became foremost evident at older ages, thus suggesting that females were not all ageing at the same rate. Indeed, *unsuccessful* females suffered a stronger decrease in breeding success at older ages than *successful* females. Also, no evidence was found to support the occurrence of a terminal allocation (i.e., an increase in reproductive success in the ultimate reproductive attempt).

To highlight that senescence in breeding success in female Alpine chamois occurs at the same age, we used a reverse-age approach, where “years before death” was used as an alternative measure of age (e.g., Reed et al. 2008; Martin and Festa-Bianchet 2011; Bize et al. 2014; Froy et al. 2013). In so doing, we quantified senescence *per se*. Results showed that the decline in breeding success became evident only in long-living female chamois (age at death ≥16; Fig. 2), whereas no decline in breeding success was observed in female chamois dying between 9 and 16 years of age (Fig. 2). Thus, the present results indicate that senescence occurred at same age in all individuals at very old age. Clear evidence of senescence in breeding success occurring at the same age among individuals has been previously reported for common guillemots (*Uria aalge;* Reed et al. 2008), Asian elephants (*Elephas maximus*; Robinson et al. 2012) and bighorn sheep (*Ovis canadensis*; Martin and Festa-Bianchet 2011), while evidence of senescence in reproductive effort occurig at different ages among individuals has been reported in Black-legged kittiwakes (*Rissa tridactyla*; Coulson and Fairweather 2001) and bighorn sheep (*Ovis canadensis*; Martin and Festa-Bianchet 2011). A previous study on the same population of Alpine chamois focusing on adult survival showed that this species has a very high survival rate (i.e., >0.90), which does not differ between sexes and remains constant from young (2-7 years) to middle-age (8+) classes (Corlatti et al. 2012a). Since our results show that senescence in breeding success occurs in very late (16+ years), additional data will be certainly helpful to address the occurrence of a similar decline in survival rate in very old individuals.

The inclusion of breeding success in the preceding year in the analyses of breeding success in the year *t* turned out to be an important effect of individual quality in the Alpine chamois, indicating the presence of two extremely different kinds of females (*sensu* Weladji et al. 2008): *successful* females that can successfully breed offspring over several consecutive years without any reduction in reproductive performance, and *unsuccessful* females that remain unsuccessful breeders over the years. Positive links between past reproductive success and current ones have been previously reported in male fallow deer (*Dama dama*; McElligott et al. 2002), in female reindeer (*Rangifer tarandus*; Weladji et al. 2006 and Weladji et al. 2008) and in female subantarctic fur seals (*Arctocephalus tropicalis*; Beauplet et al. 2006). The amount of energy that a female can allocate to reproduction depends, on the one hand, on the amount of resources available in the environment (shaped by environment productivity and variability; Hirshfield and Tinkle 1975) and on the other hand on its ability to gather, process and store resources from the environment (Festa-Bianchet 1989). The strong effect of past reproductive success on the future one suggests that the *successful* females were consistently better than the *unsuccessful* ones at extracting resources from the environment and at allocating them to reproduction.

When analyzing individual reproductive tactics, we also tested the occurrence of a terminal allocation but we found no evidence that female Alpine chamois increased their breeding success in their last reproductive attempt. For male chamois, instead, the patterns of reproductive allocation have been demonstrated to be a continuum from downturns in reproduction efforts at old age to terminal allocation (Mason et al. 2011). In general, this study highlighted that long-term individual monitoring is essential to study lifelong variations in animal reproductive performance, and, in so doing, (*i*) to distinguish the processes occurring at the population level from those occurring at the individual level; (*ii*) to test the importance of ‘reproductive costs’ and ‘individual quality’ as the primary factors driving the co-variance between reproduction and longevity; and (*iii*) to determine the occurrence of a terminal allocation. Moreover, our findings supported the existence of a strong heterogeneity in individual quality and the need to take it into account when addressing evolutionary processes (e.g., ageing) as well as in population dynamics studies.

Acknowledgments

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CONCLUSIONS AND PERSPECTIVES

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This research provided an integrative view of the various factors which influence life history strategies in female ungulates and, accordingly, the different ways available to maximize their fitness. Social interactions, reproduction and survival have been analyzed to increase the knowledge in Alpine ibex and Alpine chamois and to obtain information on biology of ungulate species in general. This work is focused on (*i*) investigate group social interactions and changes in behaviour in the two sex during the mating season; (*ii*) understand the changes in female sexual behaviour during the oestrus, able to indicate a mate choice; (*iii*) investigate the factors accounting for an age-related decline in reproductive success in female Alpine ungulate.

In the chapter I the factors shaping social interactions in mating group of Alpine ibex were analyzed. The objective of the study was understand the variation in group time-budget of females and males when changed the social structure of mating group. Change in aggressive behaviour (displacing another individual by slowly approaching it, locking or clashing horns) was observed in female group at a highly male-biased sex. This could indicate a female mate choice or simply low female receptiveness. Chapter I provided clear evidence of complex behavioural trade-offs depending on group composition, suggesting the importance of social factor in dealing interactions between and within sex and in influencing the strategies to maximize fitness.

On the basis of these results Chapter II focused on female sexual behaviour, testing the hypothesis of the presence in Alpine ibex of female mate choice. The study showed a change in female time-budget during the day of the oestrus, as well as some features of female mating strategies related specifically to copulation. Males with higher hierarchical position are preferred by female, because courting them for long time and protect them from the harassment of other males. Chapter II provided a new insight of the sexual behaviour in ibex, showing as the sexual conflicts are more accentuated as expected and that fitness of female may be influenced by the choice of the best partner.

Chapter III and IV investigated the reproduction and survival in female ibex and chamois, focusing on age-related variation in breeding and weaning success. Age has very strong effects on both reproduction and survival (Gaillard et al. 2000). The objective of these two chapters was disentangle age-related variation in reproductive performances driven by a demographic effects (population effects) from those occurring at an individual level (senescence *per se*). Seeing the two species examined are long-living species, it is expected an age at maturity delayed in the life and a reproductive success until late age. To understand which factors (such as individual quality, senescence, selective appearance or disappearance of individuals and the cost of reproduction, investigated using the effects of previous reproduction on current reproduction) influenced the reproduction in late life in individuals and populations it is important to increase the knowledge on the pattern of our population. These two studies demonstrate as breeding successes decline with age in Alpine chamois and Alpine ibex. It is highlighted the importance of the females quality in dealing the reproductive cost at late age. Successful female ibex wean a kid every two years, showing an important cost of reproduction, whereas the unsuccessful one seems can not overcome this cost. Instead in Alpine chamois successful females are able to give birth every year, even in older age the difference between successful and unsuccessful become more relevant. Successful females increased their fitness until late age because more able to respond to the reproductive senescence, whereas unsuccessful females evidenced a rapid decline of their reproductive capacity. Chapter III and chapter IV evidenced an important role of the individual quality in reproductive capacity at late age and a declining in reproductive only in very old age. Reproduction is a well know critical component of population dynamics and understand the variation in late age between and within individuals allowed us to increase the knowledge on life history strategies and accordingly discern how individuals maximize their fitness.

Finally, the ways to maximize fitness for a ibex and chamois females are multiple and complex. Females adopt adequate life history strategies according to their quality, choosing the social group with carefulness and selecting the best partner to increase their reproductive success in future. Some females have better quality, genetics and/or physiologics, in overcoming the cost, in acquiring and/or directing energy into reproduction and thus they are able to maximize their fitness more than the others one.

Recent researches pointed out the decrease in the number of Alpine chamois in the Alps (Mason et al. 2014) and also on decrease of the only natural population of Alpine ibex (von Hardenberg et al. 2014). The importance to investigate the female life history traits is not only necessary to understand which factors could influence the pattern of the populations, but also the causes of these decreases. Large importance is given to the climate change (Büntgen et al. 2014) but we must not forget that also hunting and purely the individual quality play an important role in shaping the population dynamics. My research want give a boost to the researches on life history traits on females. Genetics, dynamic, spatial, survival and behavioural studies are essential to increase our knowledge on female Alpine ungulates and be able to explain the life history strategies of females and consequently to understand the decline in our Alpine species.

ANNEXE I

EMOTIONS IN GOATS: MAPPING PHYSIOLOGICAL, BEHAVIOURAL AND VOCAL PROFILES

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Abstract

Emotions are important because they enable the selection of appropriate behavioural decisions in response to external or internal events. Techniques for understanding and assessing animal emotions, and particularly positive ones, are lacking. Emotions can be characterized by two dimensions; their arousal (bodily excitation), and their valence (negative or positive). Both dimensions can affect emotions in different ways. It is thus crucial to assess their effects on biological parameters simultaneously, so that accurate indicators of arousal and valence can be identified. In order to find convenient and non-invasive tools to assess emotions in goats (*Capra hircus*), we measured goat physiological, behavioural and vocal responses in four situations: 1) control situation (no external stimulus, neutral); 2) anticipation of a food reward (positive); 3) food-related frustration (negative); 4) isolation away from conspecifics (negative). These situations were characterised by different levels of arousal, assessed *a posteriori* by heart rates measured during the tests. We found several clear, reliable indicators of arousal and valence. During situations of higher arousal, goats had lower heart-rate variability (RMSSD) and higher respiration rates. They displayed more head movements, moved more, had their ears pointed more often forward and less often on the side (horizontal), and produced more calls. They also produced calls with higher fundamental frequencies and higher energy distribution. In positive situations, goats had their ears less often orientated backward and spent more time with their tails up than in negative situations. Furthermore, they produced calls in which the fundamental frequencies were less variable. Our methods for assessing the effects of emotional arousal and valence on biological parameters could lead to more effective monitoring and understanding of animal emotions, as well as to a better understanding of the evolution of emotions through cross-species comparisons.

Keywords. Acoustic communication, arousal, *Capra hircus*, positive emotions, ungulates, valence, vocal analysis

Introduction

Although the existence of animal emotions has been suggested since Darwin (1872), techniques for understanding and assessing these affective states, and particularly positive ones, are still lacking. The discovery of clear emotional indicators is crucial for many disciplines, including animal behaviour, neuroscience, psychopharmacology and animal welfare (Mendl, Burman, & Paul, 2010). Emotions are composed of four components: neurophysiological, behavioural, cognitive and subjective (Keltner & Lerner, 2010). While there is evidence for a subjective, conscious component of emotions only in humans, the other components can potentially be used as indicators in non-human animals (Mendl et al., 2010).

Unlike the "discrete emotion approach", which suggests the existence of a small number of fundamental emotions, the "dimensional approach" proposes to characterize emotions according to their two main dimensions: arousal (bodily activation or excitation; e.g. calm versus excited) and valence (negative or positive; e.g. sad versus happy; Russell 1980). This approach is very promising for the study of animal emotions (Mendl et al., 2010). Its recent use has allowed substantial progress to be made in identifying animal behavioural (e.g. pigs, *Sus scrofa*, Imfeld-Mueller, Van Wezemael, Stauffacher, Gygax, & Hillmann, 2011; review, Murphy, Nordquist, & van der Staay, 2014; sheep, *Ovis aries*, Reefmann, Bütikofer Kaszàs, Wechsler, & Gygax, 2009a), physiological (e.g. hens, *Gallus domesticus*, Davies, Radford, & Nicol, 2014; sheep, Reefmann, Bütikofer Kaszàs, Wechsler, & Gygax, 2009b) and cognitive indicators of emotional arousal and valence (goats, Briefer & McElligott, 2013; rats, *Rattus norvegicus*, Burman, Parker, Paul, & Mendl, 2008; review, Mendl, Burman, Parker, & Paul, 2009). In addition, the relationship between an individual's inner state and the vocalisations it produces suggests that vocalisations are promising indicators of emotions (Briefer, 2012; Manteuffel, Puppe, & Schön, 2004).

Indicators of animal emotional arousal have been extensively studied in negative situations (e.g. stress, fear in farm animals, Forkman, Boissy, Meunier-Salaün, Canali, & Jones, 2007). Conversely, studies of arousal indicators during situations of positive valence are rare. Indicators that could allow us to differentiate between negative and positive situations (i.e. valence indicators) have also been poorly studied. Finding indicators of valence requires comparing animals that are exposed to negative versus positive situations. Yet, changes in parameter values between neutral and negative situations are often easier to detect than between neutral and positive situations, because negative emotions often trigger higher arousal levels than positive ones (Boissy et al., 2007). Another concern regarding research on indicators of emotions is that very few studies have investigated both arousal and valence in a given species (but see Gogoleva et al., 2010; Soltis, Blowers, & Savage, 2011). Additionally, the emotional situations that are used often differ in both dimensions simultaneously, or may differ in more than simply the emotions they trigger (e.g. comparing the effect of pain as a negative situation versus food reward as a positive one). This results in confusion about which dimension affects the measured parameters. More precise arousal indicators could assist in identifying and thus minimizing stress during negative situations, while more accurate valence indicators could allow us to distinguish between negative and positive situations. This would then lead to enhanced animal wellbeing by promoting situations that trigger positive emotions (Boissy et al., 2007).

In this study, we investigated indicators of both emotional arousal and valence in domestic goats. Goats are highly social and vocal animals that in the wild (feral goats) live in complex fission-fusion societies (Stanley & Dunbar, 2013). This species should benefit from behavioural or vocal expression of emotions, as a mean to regulate social interactions within groups (Panksepp, 2009). Goats have good cognitive abilities, such as perspective taking (Kaminski, Call, & Tomasello, 2006) and conspecific gaze following (Kaminski, Riedel, Call, & Tomasello, 2005). They have the ability to use indirect information (i.e., the absence of food; Nawroth, von Borell, & Langbein, 2014b) and human pointing and touching cues (Nawroth, von Borell, & Langbein, 2014a) to find a reward. They also have good visual discrimination learning abilities (Langbein, Nürnberg, & Manteuffel, 2004) and long-term memory (Briefer, Haque, Baciadonna, & McElligott, 2014; Briefer, Padilla de la Torre, & McElligott, 2012). The most common goat vocalisation is the contact call, which is used to maintain contact at relatively close distance (Briefer & McElligott, 2011a). Goats produce two kinds of contact calls: closed-mouth contact calls and open-mouth contact calls (Ruiz-Miranda, Szymanski, & Ingals, 1993). Contact calls contain information about individuality (Briefer & McElligott, 2011a), age, sex and body size (Briefer & McElligott, 2011b), kinship and even group membership of the producer (Briefer & McElligott, 2012). Playback experiments have shown that these vocalisations allow mothers and kids to recognize each other from at least one week postpartum (Briefer & McElligott, 2011a), and that mother goats remember the calls of their kids for up to one year after separation (Briefer et al., 2012). Goat behaviour and vocalisations have been shown to be affected by the degree of social isolation (complete or partial), suggesting the existence of indicators of negative arousal (Siebert, Langbein, Schön, Tuchscherer, & Puppe, 2011). In terms of potential indicators of valence, goat patterns of behaviour, sympathetic reactions and brain activity have been shown to differ between positive and negative situations (i.e. different valence; Gygax, Reefmann, Wolf, &

Langbein, 2013). In this study, we assessed physiological, behavioural and vocal profiles linked to both arousal and valence, by testing which dimension was most responsible for changes in the measured parameters.

We combined new frameworks recently adapted from humans to animals to analyse vocalisations (source-filter theory; Taylor and Reby 2010), and emotions (Mendl et al., 2010), in order to find non-invasive indicators of emotions in goats. We placed goats in four situations likely to induce different emotional arousal and valence: control (neutral), anticipation of food reward (positive), food frustration (negative) and social isolation (negative). Physiological stress (nonspecific response of the body to any demand made upon it), and thus heart rate, increases with arousal, whether the situation is positive or negative (Seyle, 1976). For this reason, we assessed the arousal triggered by our experimental emotional situations by comparing the heart rates of goats in response to the tests. In the absence of well-established valence indicators in the literature, we inferred the valence of our situations based on knowledge of the function of emotions and on goat behaviour. We tested the hypothesis that emotional arousal and valence in goats are indicated by particular physiological, behavioural and vocal profiles. For instance, we expected physiological parameters linked to the autonomic nervous system (e.g. heart rate and respiration rate) to be affected by arousal, while behavioural and vocal parameters could indicate both dimensions (Briefer, 2012; Imfeld-Mueller et al., 2011; Reefmann, Wechsler, & Gygax, 2009). We defined the parameters that changed according to increased arousal levels as reliable indicators of arousal. Similarly, we defined the parameters that changed consistently from negative to positive valence as reliable indicators of valence.

Methods

Subject and Management Conditions

The study was carried out at a goat sanctuary (Buttercups Sanctuary for Goats, http://www.buttercups.org.uk), Kent (UK). We tested 22 adult goats (11 females and 11 castrated males), which were fully habituated to human presence and could be led around using a lead rope (Baciadonna, McElligott, & Briefer, 2013; Briefer & McElligott, 2013), between May and June 2011. They were aged 3-17 years and of various breeds (Table 1). They had been at the sanctuary for at least 2 years (range = 2-11 years). Five of these goats (three females and two males) had been rescued and brought to the sanctuary because of poor welfare conditions (three goats) or because they had been found abandoned (two goats). These five goats had been at the sanctuary for at least 4 years in 2011. The other goats had been housed in good conditions and were brought to the sanctuary because their owners could not keep them anymore.

Goat	Sex	Age (years)	Breed	Number of calls
1	Female	11	Anglo-Nubian	8
2	Female	16	British Toggenburg	0
3	Female	8	Pygmy Goat	6
4	Female	11	Golden Guernsey	12
5	Female	7	British Alpine	2
6	Female	5	British Alpine	8
7	Female	14	British Toggenburg	10
8	Female	7	British Saanen	19
9	Female	7	British Toggenburg	3
10	Female	13	British Toggenburg	20
11	Female	17	British Saanen	0
12	Male	11	Pygmy Goat	5
13	Male	10	Golden Guernsey	17
14	Male	8	Pygmy Goat	0
15	Male	10	British Toggenburg	2
16	Male	7	British Toggenburg	11
17	Male	9	British Saanen	8
18	Male	4	Boer	12
19	Male	7	British Toggenburg	10
20	Male	3	Boer	9
21	Male	9	Mixed Breed	6
22	Male	6	Golden Guernsey	2

Table 1. Characteristics of the goats used: sex, age and breed; as well as the number of calls analyzed for each goat.

All goats at our study site are released into a large field during the day. At night, they are kept indoors in individual or shared pens (2 or 3 goats, average size = 3.5 m^2) with straw bedding, within a larger stable complex. Routine care of the animals is provided by sanctuary employees and volunteers. Goats have ad libitum access to hay, grass (during the day) and water, and are also fed with a commercial concentrate in quantities according to their state and age. Every stable is cleaned on a daily basis. All goats are inspected each day by the sanctuary employees and volunteers, and are checked regularly by a vet and given medication when appropriate.

Situations inducing Emotional States

We designed three brief situations (≤ 5 min) of various arousal and valence, which were likely to elicit vocalisations by the goats (anticipation of food reward (positive), food frustration (negative) and social isolation (negative)). In addition, these situations were compared to a neutral, control situation. To avoid stress linked to isolation, goats were tested in familiar pairs (identical during the whole experiment) during the control, anticipation of food reward and food frustration situations, but not during the isolation situation. When tested in a pair, the two goats were located in adjacent pens. They could thus hear and see each other, but not physically interact.

We introduce the experimental situations here and give more details about the procedure in the next section (*Experimental Procedure*). i) During the control situation, goats were left unmanipulated in a pen with hay ("Control"). This situation did not elicit any calls, but allowed us to obtain baseline values for physiological and behavioral data. ii) The positive situation was the anticipation of an attractive food reward that the goats had been trained to receive during three days of habituation ("Feeding"). iii) After goats had been tested with the Feeding situation, they were tested with a food frustration situation. This consisted of giving food to only one of the goats in the pair and not to the subject ("Frustration"). iv) The second negative situation was brief isolation, out of sight from conspecifics behind a hedge. For this situation, goats were tested alone and not in a pair ("Isolation").

Determination of Arousal Levels and Valence of the Situations

The relative level of activity of the autonomic nervous system is determined to a great extent by current emotions (von Borell et al., 2007). Heart rate is a well-recognized indicator of physiological stress (e.g. Forkman et al., 2007), which is linked to emotional arousal during situations of both positive and negative valence (Seyle, 1976). Therefore, to determine arousal levels, we assessed between-situation differences in heart rate. By contrast, no clearly established indicator of emotional valence exists for goats or related mammals in the literature, because studies that have investigated both emotional arousal and valence simultaneously (in order to highlight clear indicators of valence that are not influenced by arousal) are rare. The valence of our situation was thus inferred from knowledge of the function of emotions and of goat behaviour.

Positive emotions result from encounters with rewarding stimuli that enhance fitness. They trigger approach behaviour towards the reward (Mendl et al., 2010). Negative emotions result from encounters with punishing stimuli that threaten fitness, and they trigger avoidance behaviour (Mendl et al., 2010). Accordingly, situations that involve a stimulus that can enhance fitness (e.g. food reward) and that an animal would want to approach can be assumed to be positive. By contrast, situations that involve a stimulus that somehow threaten fitness and that an animal would try to avoid can be assumed to be negative.

The Control situation was assumed to be neutral. There is some evidence suggesting that appetitive-anticipation of a reward induces positive emotional states (e.g. satisfaction/contentment; Boissy et al., 2007; Moe et al., 2009; Spruijt, van den Bos, & Pijlman, 2001). We thus assumed the Feeding (anticipation of food reward) situation as positive for goats, because this situation would enhance fitness through the acquisition of a food reward. However, failure to obtain expected food is likely to lead to a high-arousal negative state (Mendl et al., 2010; Rolls, 2005). The Frustration situation was therefore considered negative for the goats, because this situation would (in the wild at least) threaten fitness through the lack of food intake. Finally, isolation (even during a short period) for social herd-living animals such as goats, can be stressful (Price & Thos, 1980; Siebert et al., 2011). Consequently, we considered the Isolation situation to be also negative for goats, because it could potentially threaten fitness through greater exposure to predators.

Technique used for Data Collection

We collected physiological data using a small wireless non-invasive monitor, fixed to a belt placed around the goat's chest (MLE120X BioHarness Telemetry System, Zephyr). For detailed behavioural analyses, all tests were video recorded using a Sony DCR-SX50E camcorder. Finally, vocalisations were continuously recorded during the tests at distances of 3-5 m from the vocalising animal using a Sennheiser MKH-70 directional microphone (frequency response 50 – 20.000 Hz; max SPL 124 dB at 1 kHz), connected to a Marantz PMD-660 numeric recorder (sampling rate: 44.1 kHz).

Experimental Procedure

During the Control, Feeding and Frustration situations, goat pairs were tested in two indoor adjacent pens, 3 m^2 each, within a familiar larger stable complex. During the Isolation situation, they were tested individually in a 3.4 m^2 and 2.10 m high outdoor pen made of gaited hurdles, in a familiar field (usual daytime range) out of sight from other goats. To minimize stress linked to novelty, the emotional tests were preceded by three days of habituation. During these three days, goats were gradually habituated to the set-up, to the measurement equipment (i.e. wireless non-invasive monitor) and to the Feeding and Isolation situations (both repeated once per day, during the three habituation days). They were not habituated to the Control situation (everyday situation), nor to the Frustration situation, in order to ensure that food was always expected during the Feeding situation. Goats were then tested over three days with one test per day (i.e. Feeding, Frustration and Isolation situations).

i) The Control situation (no habituation) was carried out on two consecutive days. On each of these two days, we placed the pair of goats in adjacent pens for 5 min, with hay in the feeders, and left them undisturbed. ii) For the Feeding situation, each pair of goats was placed in the indoor pens for 5 min pre-test with hay in the feeders. At the end of the 5 min pre-test, during the first habituation day, an experimenter (same person throughout the experiment) presented two buckets with food (commercial concentrate for goats with fresh chestnut tree leaves) simultaneously to the two goats for 1 min, before giving them the food. During the second and third habituation days, and during the test day, the hay was removed for 2 min before the food presentation, in order to increase food motivation. Then, the experimenter presented the food simultaneously to the two goats for 20 s, walked outside the stable complex for 1 min, and walked back towards the goats while shaking the food bucket to make it obvious, and presented the food again for 20 s. Then, he walked away a second time for 1 min, walked back and gave them the food. This allowed us to obtain three events (before the first and second 20 s presentation and before giving them food), when the experimenter approached the goats with food, resulting in the production of vocalisations. iii) The Frustration situation (no habituation) started in the same way as the Feeding situation, with 5 min pre-test (with hay), followed by 2 min during which the hay had been removed. Then, the experimenter presented the food buckets (similar buckets and food as during the Feeding situation) during 20 s simultaneously to the two goats and gave food to only one of the goats in the pair, while the subject did not receive food. This lasted for 4 min, after which the experimenter also gave food to the subject. iv) To habituate the goats to the Isolation situation, goats were placed in pairs during the first two habituation days, in two identical and adjacent pens, out of sight of the other goats, for 5 min. On the last habituation day, the adjacent pen was removed and each goat was placed in the test pen alone. During the test day, the Isolation situation started with 5 min pre-test, during which the subject was placed in the indoor pen used for the other situations, with hay and with the paired goat in the adjacent pen. This allowed the subject to settle down after it was equipped with the heart-rate monitor. Then, the subject was placed alone in the outdoor isolation pen and left there for 5 min. At the end of the 5 min, it was returned to the other goats.

The 22 goats were tested in every situation (repeated measure design). During habituation days, goats were placed in both the Feeding and Isolation situations each day in a random order. During the three test days (one test per day), the order of the emotional situations was pseudo-random. The only constraint was that, for each individual, the Feeding situation always preceded Frustration situation (1-6 days before), in order to ensure a positive state during the Feeding situation. Within a pair, both goats were tested with the Control and Feeding situations at the same time. This prevented food frustration if one of the goats was not simultaneously tested. They were tested with the two other tests (Frustration and Isolation) on different days for each goat in the pair. The Control situation was carried out on different days as the emotional situations.

Response Measures

Physiological measures

We measured heart rate and respiration rate, which are likely to be affected by emotions (Reefmann et al., 2009b; von Borell et al., 2007), and are part of the of the stress response of the Sympatho-Adreno-Medullary axis (SAM; i.e. neuronal or "fight-or-flight" response), as opposed to the stress response of the second main stress axis, the Hypothalamo–Pituitary-Adrenocortical axis (HPA; i.e. humoral response). Because vocalisations are affected by the autonomic nervous system (ANS; Scherer, 1986, 2003), vocal parameters are more likely to be correlated with the SAM response indicators than the HPA response indicators (e.g. cortisol), at least over short time scales (Schrader & Todt, 1998). Before the beginning of the experiment, we quickly clipped a small patch of hair below the heart-rate monitor, in order to improve the contact between the electrodes and the body. Goats were equipped with the monitor before each situation, on both the habituation and test days. This technology was also removed immediately after each test. ECG gel was applied on the parts of the belt containing the electrodes before each use. The data (continuous ECG trace and breathing wave, i.e. inhalation/exhalation cycle) were then transmitted and stored in real time to a laptop using LabChart software v.7.2 (ADInstrument) for later analyses. During the tests, one experimenter, who was concealed in a pen close to the subjects, quietly recorded comments into the software indicating important events (e.g. when the other experimenter was presenting food to the goats, leaving or coming back during Feeding situation, or when the paired goat was given food and finished eating during the Frustration experiment). This allowed us to measure physiological parameters at the exact times when these events occurred.

When possible (i.e. good quality signal; clearly visible heart beats on the ECG trace and respiration on the breathing wave), we analysed data for each situation over three 10 s sections, in which the software could track the heart beats (ECG trace) and the inspiration–exhalation cycles (breathing wave) accurately (Reefmann, Wechsler, et al., 2009: mean ± SD for each section: Control, 10.03 ± 0.03 s; Feeding, 9.26 ± 0.19 s; Frustration, 9.83 ± 0.10 s;

Isolation, 9.69 ± 0.14 s). For the Control situation, the three sections were collected at the beginning, middle and end of the time starting 1 min after the tested pair of goats was placed in the adjacent pens with hay in the feeder. This allowed the goats to settle down before we started data collection. For the Feeding situation, the first section corresponded to the time just before the first food presentation, the second section to the time when the experimenter came back with the food for the first time (i.e. after opening the door to enter the stable complex), and the third section to the time when the experimenter came back with the food for the second time (i.e. after opening the door to enter the stable complex and just before giving the food). The data were thus collected only when the subject could hear/see the experimenter coming towards them with food, and was likely to be experiencing a positive state. This also ensured that our data would reflect an anticipatory state, as opposed to a consummatory state while feeding or a post-consummatory state after feeding, which are likely to be of different arousal (Spruijt et al., 2001). For the Frustration situation, the three sections were collected at the beginning, middle and end of the time starting when the other goat in the pair received food, until this goat finished eating. This ensured that the data were collected while the subject could see and hear the paired goat eating. The subject was thus likely to be experiencing a negative state of frustration throughout data collection. By contrast, the situation in which both goats were not eating (after the pair goat finished) or the situation in which the subject was itself eating are likely to trigger different arousal and valence and were thus not considered for analyses. Finally for the Isolation situation, the three sections were collected at the beginning, middle and end of the time starting 1 min after the subject was placed in the isolation pen, until the end of the Isolation situation 4 min later (isolation duration = 5 min). This allowed the goat to settle in the isolation pen and to be likely to be experiencing a negative state of isolation, before we started data collection.

From the ECG trace and breathing wave, we analysed the following parameters: heart rate, heart-rate variability (root mean square of successive R-R interval difference, “RMSSD”) and respiration rate. The heart-rate (beats/min) and respiration-rate (breaths/s) averages were obtained automatically from the software. Individual inter-heart-beat (RR) intervals (ms) were also extracted, in order to calculate RMSSD (ms).

Behavioural measures

For a similar reason than for the physiological parameters (i.e. to measure parameters during well-defined positive/negative emotional state), for the Feeding situation, we analysed the behaviour of the goats during the two events when the experimenter came back from outside the stable complex towards the goats with food (i.e. when the goats could see the experimenter bringing them food; mean total time scored per goat ± SD = 29.55 ± 5.54 s). For the Frustration situation, we analysed the behaviour during the time starting when the other goat in the pair received food, until this goat finished eating (i.e. when the goats could see or hear the other goat eating; mean total time scored per goat ± SD = 176.50 ± 35.91 s). For the Isolation and Control situation, we analysed the behaviour during the last 4 min of the test (i.e. 1 min after the subject was placed in the test pen; time scored per goat per test = 240 s).

We scored behavioural parameters that could potentially be affected by emotions (Boissy et al., 2011; Reefmann, Bütikofer Kaszàs, et al., 2009a; Reefmann, Wechsler, et al., 2009). The following parameters were scored using CowLog 1.1 (open source software for coding behaviours from digital videos; Hänninen & Pastell, 2009); the time spent with the tail raised (i.e. tail raised above the perpendicular to the backbone), the duration of locomotion (defined as at least two legs moving), the number of rapid head movements (i.e. < 1 s in duration), and the number of calls produced. We also scored the time spent with the ears orientated forward (tip of the ear pointing forward), backwards (tip of the ear pointing backward), horizontal (perpendicular to the headrump axis), or asymmetrical (right and left ears in different positions, such as one pointing forward and the other one horizontal or backwards; Boissy et al., 2011; Reefmann, Bütikofer Kaszàs, et al., 2009a).

	Abbreviation	**Correlated with**	**Parameter**
Physiology	RMSSD (ms)	HR	Root mean square successive difference
	RespRate (breaths/s)	HR	Respiration rate
Behaviour	HeadMov (min^{-1})	EarsHoriz	Number of rapid head movement per minute
	Locomotion	EarsForw, EarsAsym, TailUp	Proportion of time spent moving
	EarsForw	Locomotion, EarsHoriz EarsBack, EarsAsym, TailUp	Proportion of time spent with the ears orientated forward
	EarsHoriz	HeadMov, EarsForw, TailUp	Proportion of time spent with the ears orientated horizontally
	EarsBack	EarsForw, EarsAsym	Proportion of time spent with the ears orientated backward
	EarsAsym	Locomotion, EarsForw, EarsBack	Proportion of time spent with the ears asymmetrical (different orientation for the right and left ears)
	TailUp	Locomotion, EarsForw, EarsHoriz	Proportion of time spent with the tail up
	Calls (min^{-1})	None	Number of calls per minute
Vocalisations	Dur (s)	None	Duration of the call
	F0mean (Hz)	F0end, F0range, FMextent, AMextent, Q25%, Q50%	Mean F0 frequency value across the call
	F0end (Hz)	F0mean, F0range, Q50%, Q75%	Frequency value of F0 at the end of the call
	F0range (Hz)	F0mean, F0end, FMextent	Difference between F0Max and F0Min
	FMextent (Hz)	F0mean, F0range	Mean peak-to-peak variation of each F0 modulation
	AMextent (dB)	F0mean	Mean peak-to-peak variation of each amplitude modulation
	Q25% (Hz)	F0mean, Q50%, Q75%	Frequency value at the upper limit of the first quartiles of energy
	Q50% (Hz)	F0mean, F0end, Q25%, Q75%	Frequency value at the upper limit of the second quartiles of energy
	Q75% (Hz)	F0end, Q25%, Q50%	Frequency value at the upper limit of the third quartiles of energy
	F1mean (Hz)	F2mean	Mean frequency value of the first formant
	F2mean (Hz)	F1mean, F3mean, F4mean	Mean frequency value of the second formant
	F3mean (Hz)	F2mean, F4mean	Mean frequency value of the third formant
	F4mean (Hz)	F2mean, F3mean	Mean frequency value of the fourth formant

Table 2. Abbreviations, definition and correlations for the physiological, behavioural and vocal parameters. Correlations across individuals between a given parameter and others within its category (physiological, behavioural or vocal) are indicated when significant (Spearman's rank correlation; $P < 0.05$).

We calculated the rate of occurrence (per minute) for the number of rapid head movements and for the number of calls, and the proportion of the total time spent performing the behaviour, for the other behaviours (Table 2). Therefore, the difference in duration between the various situations was taken into consideration. When the head, ears or tail were not easily observable because of the position of the camera or goat in the pen, the proportion of behaviour was calculated over the time during which we were able to score the behaviour accurately, instead of the total time. On two occasions, it was not possible to score the position of the ears and the head movements (one goat during the Feeding situation) or the position of the tail (one goat during the Feeding situation). Therefore, sample sizes differ slightly between parameters (see sample sizes in Table 3).

	AROUSAL							**VALENCE**								
	0		**1**					**Negative**		**Neutral**		**Positive**				
Parameter	**Mean**	**SD**	**Mean**	**SD**	χ^2_1 *(N)*	*p*		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	χ^2_1 *(N)*	***P***	
RMSSD	**0.05**	**0.42**	**-0.07**	**0.40**	**7.05 (311)**	**0.008**	>	0.01	0.42	0.03	0.41	-0.10	0.41	1.58 (311)	0.21	
RespRate	**-0.10**	**0.28**	**0.17**	**0.26**	**76.67 (311)**	**<0.0001**	<	0.05	0.32	-0.12	0.25	0.18	0.23	0.75 (311)	0.39	
HeadMov	**-0.56**	**0.76**	**0.54**	**0.80**	**35.59 (85)**	**<0.0001**	<	**-0.23**	**0.70**	**-0.41**	**0.99**	**0.86**	**0.86**	**16.58 (85)**	**<0.0001**	NC
Locomotion	**-0.18**	**0.44**	**0.18**	**0.58**	**9.39 (88)**	**0.002**	<	0.05	0.55	-0.17	0.42	0.08	0.61	0.00 (88)	0.96	
EarsForw	**-0.03**	**0.09**	**0.03**	**0.08**	**11.36 (83)**	**0.0008**	<	0.01	0.07	-0.10	0.07	0.06	0.06	2.14 (83)	0.14	
EarsHoriz	**0.29**	**1.21**	**-0.57**	**0.63**	**15.43 (83)**	**<0.0001**	>	-0.52	0.71	1.42	0.77	-0.74	0.04	0.21 (83)	0.65	
EarsBack	**0.37**	**0.99**	**-0.15**	**1.20**	**4.56 (83)**	**0.033**	>	**0.63**	**0.77**	**-0.01**	**1.16**	**-0.91**	**1.03**	**31.50 (83)**	**<0.0001**	>
EarsAsym	**0.30**	**1.11**	**-0.24**	**1.17**	**4.56 (83)**	**0.033**	>	**0.32**	**1.11**	**0.34**	**1.10**	**-0.89**	**0.84**	**14.75 (83)**	**0.0001**	NC
TailUp	**-0.20**	**0.97**	**0.27**	**0.78**	**5.64 (85)**	**0.018**	<	**-0.25**	**0.92**	**0.16**	**0.90**	**0.50**	**0.68**	**10.78 (85)**	**0.001**	<
Calls	**-0.46**	**1.03**	**0.36**	**1.11**	**11.61 (85)**	**0.0007**	<	0.23	1.13	-1.09	0.15	0.52	1.09	0.03 (85)	0.87	

Table 3. Effect of arousal and valence on physiological and behavioural parameters. Residuals of the models controlled for locomotion (physiology only), sex, age, individual identity and breed (mean ± SD; raw values are listed in Appendix Table A2), along with statistical results (χ^2 values, sample size (*n*) and *p* values). The direction of the effect is indicated ("<" indicates an increase with arousal level or from negative to positive valence, whereas ">" indicates a decrease; NC indicates that the effect was not consistent, i.e. increase followed by decrease or vice-versa). Significant results are shown in bold.

Vocal measures

Vocalisations were imported into a computer at a sampling rate of 44.1 kHz and saved in WAV format at 16-bit amplitude resolution. We used Praat v.5.3.41 DSP Package (Boersma & Weenink, 2009) and Seewave (Sueur, Aubin, & Simonis, 2008) for subsequent analyses. Calls were visualised on spectrograms in Praat (FFT method, window length = 0.03 s, time steps = 1000, frequency steps = 250, Gaussian window shape, dynamic range = 60 dB). We selected all good quality calls recorded during each situation (total = 180 calls; 40 for Feeding, 80 for Frustration and 60 for Isolation; 8.18 ± 7.76 calls per goat; range = 0 (3 goats) – 30; Table 1). Because calls were produced intermittently by the goats (unlike physiological and behavioural data that could be acquired continuously), we used a more opportunistic approach in order to obtain adequate sample sizes of vocalisations. During the Feeding situation, we analysed calls produced between the time when the experimenter approached the goats for the first time with the food, until they were rewarded. During the Frustration situation, we analysed calls produced between the time when the other goat received food and the time preceding the return of the experimenter toward the subject to give it food. During the Isolation situation, we analysed all the calls produced while the subject was in the isolation pen. Calls were never produced in bouts. Because consecutive calls produced in bouts are more likely to be homogeneous, we therefore avoided pseudoreplication (Reby, Cargnelutti, & Hewison, 1999).

According to the source-filter theory of voice production (Fant, 1960), mammal vocalisations are generated by vibrations of the vocal folds (source, determining the fundamental frequency, “F0”), and are subsequently filtered by the supralaryngeal vocal tract (filter, producing amplified frequencies called “formants”, Titze, 1994; Taylor and Reby, 2010). We extracted source- and filter-related vocal parameters as well as intensity and duration measures using a custom built program in Praat. This program batch processed the analyses and exporting of output data (Charlton, Zhihe, & Snyder, 2009a; Reby & McComb, 2003). The vocal parameters measured are listed in Table 2 and the analyses are detailed in the Appendix.

The settings for the analyses were adapted from Briefer & McElligott (2011a). Goat contact calls vary between individuals, particularly at the level of F0-related parameters, formant-related parameters and energy quartiles (see Table 2 for definitions; Briefer & McElligott, 2011a). Therefore, the most appropriate settings to accurately detect F0 (i.e. pitch floor and pitch ceiling) and formants (i.e. maximum number of formants and maximum formant value; see Appendix for details) with Praat differed between individuals. In order to prevent biases linked to the settings used for the analyses, the same settings were used for all calls (i.e. produced during all situations) of a given individual. We included 13 vocal parameters in our analyses. Some parameters (formants) could not be measured in every call, resulting in a small proportion of missing values. Therefore, the sample size (number of calls) differs between the vocal parameters (see sample size in Table 5).

Statistical Analysis

First, in order to investigate which physiological, behavioural or vocal parameters would be more useful as indicators of emotions (as opposed to which ones were correlated and therefore redundant), we tested for potential correlations between the mean parameter values for each individual using Spearman's rank correlation. Then, to test for differences in heart rate between situations and determine arousal levels, we carried out a linear mixed effects model (LMM; lmer function, lme4 library; Pinheiro & Bates, 2000) in R 3.0.2 (R Development Core Team, 2013). This model included heart rate as a response variable, and the sex and age of the goats as fixed factors to control for their effects. The situation (Control, Feeding, Frustration and Isolation) was included as a fixed factor. Finally, the breed, and identity of the goats nested within the test pair were included as crossed random factors, in order to control for repeated measurements of the same subjects, for the impact of the goats within a pair on each other, and for breed differences (Table 1). Then, two-by-two comparisons between the four emotional situations were carried out using LMMs including the same control, fixed and random factors. We applied a Bonferroni correction at $\alpha = 0.017$ (0.05/3 comparisons) for these *post-hoc* tests. Based on these results, we ranked the situations according to the heart rate values they triggered; we attributed the highest arousal level to the situation triggering the highest heart rate and the lowest arousal level to the one triggering the lowest heart rate. Situations that did not differ in heart rate were considered to be of the same arousal level.

We ran further models to test the effects of arousal and valence on the other physiological, behavioural and vocal parameters measured (Table 2). Separate LMMs were set up for each parameter. All of these models included the parameter as a response variable and the same control and random factors as listed above for heart rate (sex and age of the goats as fixed factors; breed, and identity of the goats nested within the test pair, as crossed random factors). The proportion of time spent moving (Locomotion) was also included as a fixed factor for the physiological parameters, to control for its effect. The extent of mouth opening influences the resonant properties of the vocal tract (Titze, 1994). Several vocal parameters thus differ between closed- and open-mouth calls (Sèbe, Duboscq, Aubin, Ligout, & Poindron, 2010). For this reason, we added the type of call (open-mouth, closed-mouth or mixed, referring to calls containing both open-mouth and closed-mouth parts) as a fixed factor in the models carried out on the vocal parameters to control for the potential differences between call types (see Appendix Table A1 for the results of the control factors).

Because arousal levels and valence were correlated (Spearman's rank correlation: $r = 0.15$, $p = 0.006$), they were not included as factors in the same models. Instead, we first ran one set of models with arousal level (1-2; see Results, *Determination of Arousal Levels using Heart Rate*) as a fixed effect and another set with valence (Control situation = 0; Feeding = +1; Frustration and Isolation situations = -1) as a fixed effect. Then, for each parameter that was significantly affected by both arousal and valence, we used a model selection procedure based on the Akaike's information criterion adjusted for small sample size (AIC_C) to identify which of arousal or valence best explained the parameter (Burnham & Anderson, 2002). We used AIC_C instead of AIC, because AIC_C converges to AIC as sample size increases and should be used by default (Symonds & Moussalli, 2011). When the difference between the AIC_C values of two models (ΔAIC_C) is less than 2 units, both models have support and can be considered competitive. Models with ΔAIC_C ranging from 3 to 7 have considerably less support by the data, and models with $\Delta AIC_C > 10$ are poorly supported. Akaike weights (ωi) indicate the probability that a particular model has more or less support from the data among those included in the set of candidate models (Burnham & Anderson, 2002; Burnham, Anderson, & Huyvaert, 2011).

To test for further differences between the Frustration and Isolation situations, which were both assumed to be of negative valence, we carried out further LMMs. We included in these models (one for each parameter) the same control and random factors as mentioned above (sex and age of the goats, Locomotion (physiology only) and call type (vocalisations only) as fixed factors; breed, and identity of the goats nested within the test pair as crossed random factors, and the situation (Frustration and Isolation) as a fixed factor (see Appendix Table A1 for these results)).

The residuals were checked graphically for normal distributions and homoscedasticity. To satisfy assumptions, we used log transformations for RMSSD, ResRate, F0mean, F0end, F0range, FMextent, AMextend, Q50%, Q75% (see Table 2 for abbreviations). Some of the behavioural parameters measured in proportions were logit-transformed (EarsForward, HeadMov, and Locomotion). These log- and logit-transformed physiological, behavioural and vocal parameters were then entered into models fitted with Gaussian family distribution and identity link function. None of the other behavioural parameters (see Table 2) met statistical assumptions despite logit transformation. They were thus transformed to binomial data (behaviour occurs = 1; does not occur = 0), and entered into generalised linear mixed models, fitted with binomial family distribution and logit link function (glmer function, lme4 library). For each model, we assessed the statistical significance of the factors by comparing the model with and without the factor included using likelihood-ratio tests (LRT). To this aim, and in order to compare models with AIC_C, all models were fitted with maximum likelihood estimation. The significance level was set at $\alpha = 0.05$. All means are given with standard deviations.

Ethics

Animal care and all experimental procedures were in accordance with the ASAB/ABS Guidelines for the Use of Animals in Research (Guidelines for the Use of Animals, 2014).

The research plan was reviewed by the UK Government Home Office inspector for Queen Mary, University of London. All measurements were non-invasive, and the negative situations (Frustration and Isolation) lasted not more than 5 min each. The stress levels of the goats were monitored throughout the tests (ECG trace and breathing wave were transmitted and displayed, in real time on the laptop) to ensure that subjects did not become overly stressed, in which case the test would be halted and the subject would not be tested anymore. However, this did not happen and none of the goats had to be removed from the experiments.

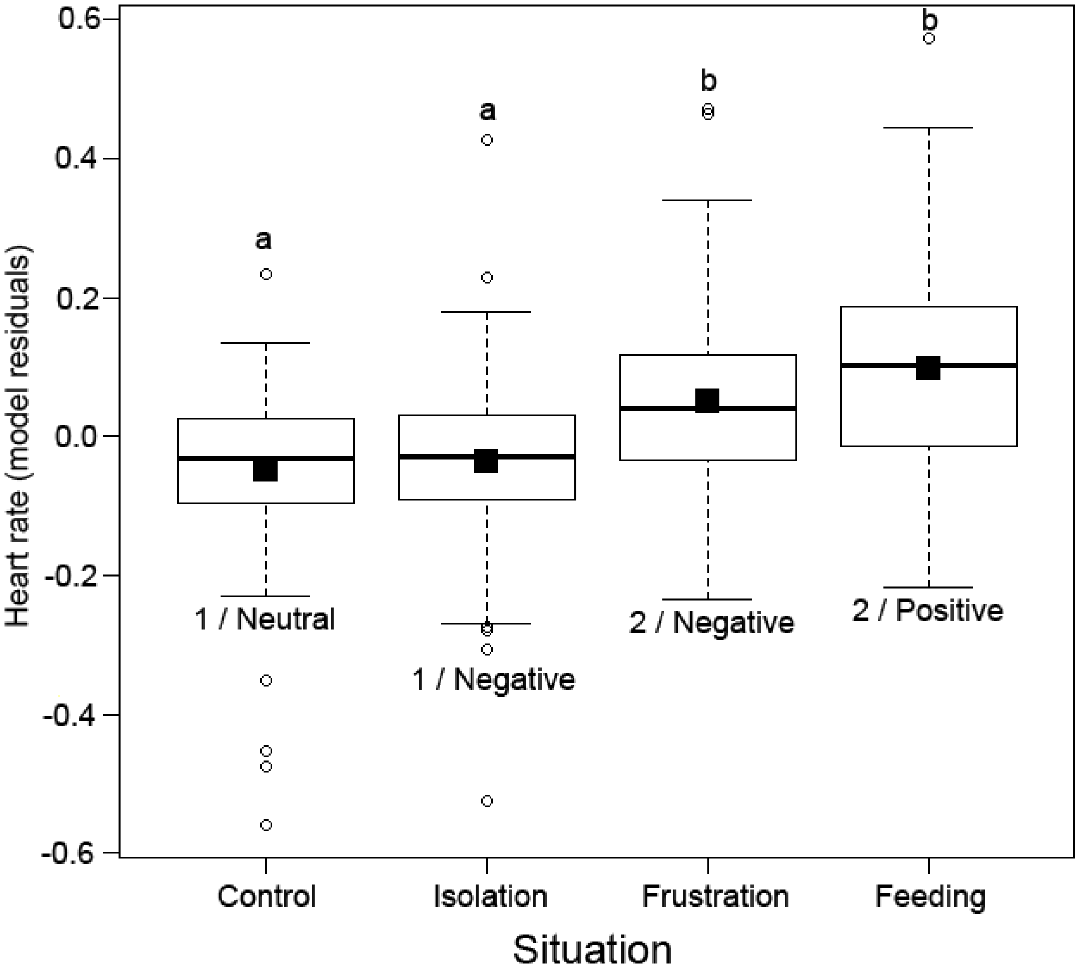

Figure 1. Heart rate as a function of emotional situations. Model residuals of heart rate controlled for sex and age of the goats, individual identity, test pair and breed for each of the experimental situations (Control, Feeding, Frustration and Isolation situations; box-and-whiskers plot, the horizontal line shows the median, the box extends from the lower to the upper quartile, and the whiskers to the most extreme data point). The black squares indicate the mean. Same letters (a, b) indicate that situations did not significantly differ. Based on these results, situations marked with an (a) received an arousal level of 1, situations marked with a (b) received an arousal level of 2. Arousal levels (1 or 2) and valence (Neutral, Positive and Negative) corresponding to the situations are also indicated under the box-and-whiskers plot (arousal / valence).

Results

Determination of Arousal Levels using Heart Rate

The analysis of heart rate as a function of the emotional situations revealed two arousal levels (1 and 2; Fig. 1). Heart rate differed according to the situation (LMM: X^2 = 53.03, df = 3, $p < 0.0001$), with the lowest value occurring during the Control situation. These heart rates were not different from those obtained for the Isolation situation (Control vs Isolation; LMM: X^2 = 0.68, df = 1, p = 0.41). Therefore, we attributed an arousal level of 1 (lowest) to the Control and Isolation situations. The heart rates measured during the Isolation situation were significantly lower than those measured during the Frustration situation (Isolation vs Frustration; LMM: X^2 = 12.24, df = 1, p = 0.0005). Finally, the rates for the Frustration situation were not significantly different from those obtained for the Feeding situation, after Bonferroni correction (Frustration vs Feeding; LMM: X^2 = 4.36, df = 1, p = 0.037; Bonferroni, α = 0.017). Frustration and Feeding situations thus received an arousal level of 2. To summarize, the arousal levels based on heart rate were 1 for the Control and Isolation situations (mean ± SD = 108.75 ± 15.38 beats/min) and 2 for the Frustration and Feeding situations (mean ± SD = 123.00 ± 23.52 beats/min).

Physiological Responses

Correlation analyses between the mean physiological parameters of each goat showed no associations between RMSSD and RespRate (Spearman's rank correlation: r = -0.09, n = 22 goats, p = 0.69). The models investigating the link between physiological parameters and emotional arousal and valence of the situations revealed that the two measured parameters (RMSSD and RespRate) were influenced by arousal and not by valence (Table 3; Appendix Table A2). RMSSD decreased and RespRate increased with arousal (Table 3). To summarise, RMSSD and RespRate were good indicators of arousal, as they were clearly more affected by arousal levels than valence. During higher arousal situations, goats had lower heart-rate variability (RMSSD) and higher respiration rates. There were no clear physiological indicators of valence.

Behavioural Responses

Correlation analyses between the mean behavioural parameters of each goat showed several associations between most of the ear positions (EarsForw, EarsHoriz and EarsAsym) and some of the other behavioural parameters (HeadMov, Locomotion and TailUp; Table 2). EarsBack was only correlated with other ear position parameters (EarsForw and EarsAsym).

Finally, the number of calls per minute (Calls) was not correlated with any of the other behavioural parameters.

The analyses of behavioural parameters in relation to the emotional arousal and valence of the situations revealed that all measured parameters were affected by arousal (Table 3; Appendix Table A2). HeadMov, Locomotion, EarsForw, Calls and TailUp increased with arousal, while EarsHoriz, EarsBack and EarsAsym decreased. Four of the parameters were also affected by valence (HeadMov, EarsBack, EarsAsym and TailUp; Table 3; Appendix Table A2). EarsBack decreased and TailUp increased from negative to positive valence. HeadMov and EarsAsym were not affected consistently by valence (level: HeadMov, - > 0 < +; EarsAsym, - < 0 > +). AIC_C comparison revealed that the variation in HeadMov was better explained by arousal (100% chance to be the best model) than valence, while variation in EarsBack and EarsAsym was better explained by valence (100% and 99% chance respectively to be the best model) than arousal. For TailUp, the ΔAIC_C was 5.13, indicating that the model including valence had considerably more support by the data than the model including arousal. The model including valence had 93% chance to be the best model (chance level = 50%; Table 4).

Parameter	**Arousal/ Valence**	**AIC_C**	**ΔAIC_C**	***wi***
HeadMov	**A**	**215.47**	**0.00**	**1.00**
	V	234.49	19.01	0.00
EarsBack	A	115.60	26.94	0.00
	V	**88.66**	**0.00**	**1.00**
EarsAsym	A	125.39	10.19	0.01
	V	**115.20**	**0.00**	**0.99**
TailUp	A	109.17	5.14	0.07
	V	**104.03**	**0.00**	**0.93**

Table 4. Results of AIC comparisons for behavioural parameters significantly affected both by arousal and valence. The best fit (arousal or valence based on lowest AIC_C) for a given response variable (set of models) is indicated in bold. The fit of the models is assessed by Akaike's information criterion corrected for small sample sizes (AIC_C). ΔAIC_C gives the difference in AIC_C between each model and the best model. The Akaike's weights (*ωi*) assess the relative support that a given model has from the data, compared to other candidate models in the set.

To summarise, HeadMov, Locomotion, EarsForw, EarsHoriz and Calls were good indicators of arousal, as they were clearly more affected by arousal levels than valence. During high arousal situations, goats displayed more head movements, moved more, had their ears pointed more often forward and less often to the side (horizontal) and produced more calls. By contrast, EarsBack and TailUp were good indicators of valence, as they changed consistently from negative to positive valence (unlike EarsAsym), and were more affected by valence levels than arousal ($\Delta AIC_C > 5$). In positive situations, as opposed to negative ones, goats had their ears orientated backward less often and spent more time with the tail up.

Vocal Responses

Correlation analyses between the mean vocal parameters of each goat showed several associations between most of the F0-related parameters (F0mean, F0end, F0range and FMextent; Table 2). Some of these F0-related parameters (F0mean and F0end) were also correlated with energy quartiles (Q25%, Q50%, and Q75%), which were all correlated to each other. AMextent was only correlated with F0mean. F1mean was correlated with F2mean. Finally, F2mean, F3mean and F4mean were all related to each other.

Our analyses of vocal parameters as a function of the arousal levels and valence of the situations revealed six parameters that were influence by arousal (Table 5; Appendix Table A2). F0mean and F0end (which characterise F0 contour over time), as well as Q25%, Q50%, and Q75% increased with arousal, while F1mean decreased. F4Mean also tended to increase, but the effect of arousal on this parameter was only marginally significant (p = 0.055). Two parameters were influence by valence (Table 5; Appendix Table A2). F0range and FMextent (which characterise F0 variation within the call), all decreased from negative to positive valence. The other parameters were neither affected by arousal, nor by valence (Table 5).

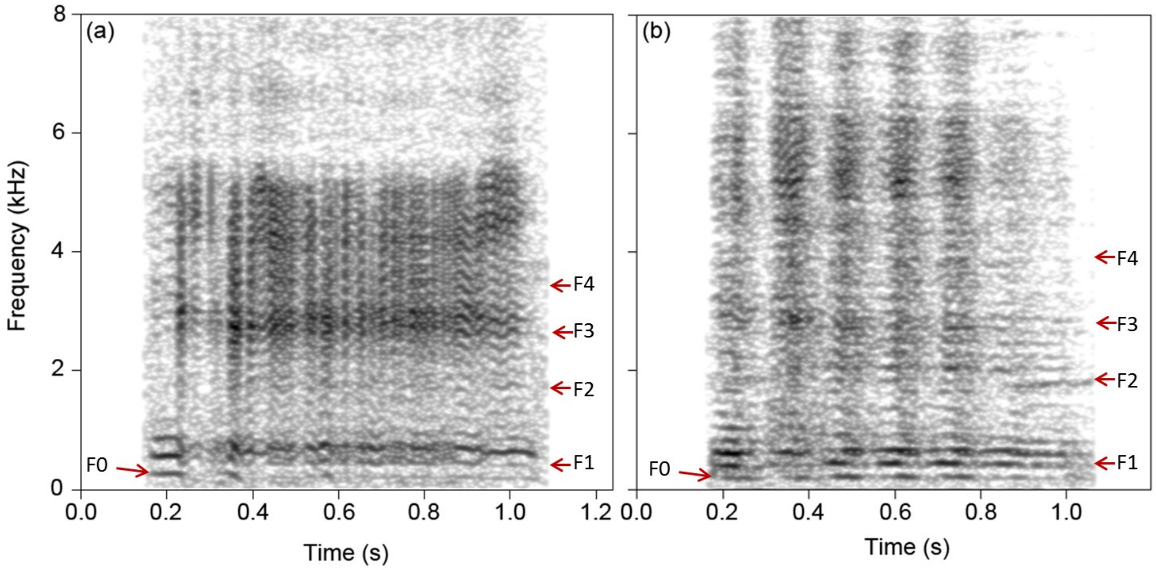

Figure 2. Spectrograms of negative and positive calls. (a) call produced during the negative situation; (b) call produced during the positive situations by the same goat. Positive calls have a lower fundamental frequency (F0) range and smaller frequency modulations than negative calls. The first four formants are also indicated on the right of the spectrogram. These calls are available as audio files (Audio A1).

	AROUSAL							**VALENCE**						
	0		**1**					**Negative**		**Positive**				
Parameter	**Mean**	**SD**	**Mean**	**SD**	χ^2_1 (*N*)	*p*		**Mean**	**SD**	**Mean**	**SD**	χ^2_1 (*N*)	***P***	
Duration	-0.01	0.13	0.00	0.11	0.35 (158)	0.55		0.00	0.12	-0.01	0.11	0.63 (158)	0.43	
F0mean	**-0.02**	**0.10**	**0.01**	**0.10**	**4.67 (158)**	*	<	0.00	0.11	0.01	0.10	0.23 (158)	0.63	
F0start	**-0.03**	**0.19**	**0.02**	**0.16**	**5.24 (158)**	*	<	-0.01	0.17	0.02	0.16	0.70 (158)	0.40	
F0end	**-0.05**	**0.19**	**0.03**	**0.15**	**13.36 (158)**	***	<	-0.01	0.17	0.04	0.15	3.43 (158)	0.06	
F0range	0.04	0.38	-0.02	-0.47	1.20 (158)	0.27		**0.05**	**0.43**	**-0.16**	**0.43**	**6.88 (158)**	**	>
F0AbsSlope	0.06	0.39	-0.03	0.34	3.17 (158)	0.07		**0.04**	**0.37**	**-0.14**	**0.29**	**8.19 (158)**	**	>
F0var	**0.10**	**0.40**	**-0.05**	**0.43**	**6.91 (158)**	**	>	**0.05**	**0.41**	**-0.18**	**0.43**	**9.66 (158)**	**	>
FMextend	0.09	0.44	-0.04	0.59	3.20 (158)	0.07		**0.05**	**0.52**	**-0.17**	**0.58**	**5.26 (158)**	*	>
AMextent	0.01	0.43	0.00	0.31	0.03 (158)	0.85		-0.02	0.36	0.07	0.31	2.06 (158)	0.15	
Q25%	**-20.02**	**88.36**	**10.01**	**73.00**	**7.10 (158)**	**	<	-2.39	77.25	8.36	87.38	0.57 (158)	0.45	
Q50%	**-0.10**	**0.37**	**0.05**	**0.28**	**12.50 (158)**	***	<	-0.01	0.33	0.05	0.29	1.25 (158)	0.26	
Q75%	**-0.08**	**0.50**	**0.04**	**0.32**	**5.84 (158)**	*	<	-0.01	0.40	0.04	0.35	0.68 (158)	0.41	
F1mean	**40.15**	**106.75**	**-17.27**	**81.65**	**16.56 (112)**	***	>	-4.37	94.77	15.69	87.77	1.07 (112)	0.30	
F2mean	11.58	152.13	-3.05	101.45	0.46 (95)	0.50		0.72	122.46	-2.35	77.90	0.02 (95)	0.90	
F3mean	-6.08	102.16	1.80	118.64	0.14 (98)	0.71		3.46	120.46	-11.11	94.95	0.36 (98)	0.55	
F4mean	**-36.93**	**90.44**	**11.04**	**146.92**	**3.68 (92)**	**0.055**	<	5.44	136.34	-19.16	141.12	0.68 (92)	0.41	

Table 5. Effect of arousal and valence on vocal parameters. Residuals of the models controlled for sex, age, breed and call type (mean ± SD; raw values are listed in Appendix Table A2), along with statistical results (χ^2 values, sample size (*N*) and *p* values). The direction is indicated for the significant and marginally significant (0.06 ≥ p ≥ 0.05) effects ("<" indicates an increase with arousal level or from negative to positive valence, whereas ">" indicates a decrease). Significant and marginally significant results are shown in bold.

To summarize, F0mean, F0End, Q25%, Q50%, Q75% and F1Mean were good indicators of arousal, as they were clearly more affected by arousal than valence. With an increase in arousal, goats produced calls with higher F0 and higher energy distribution (i.e. energy quartiles). Furthermore, the first formant frequency decreased, while the fourth formant tended to increase. By contrast, F0range and FMextent were good indicators of valence, as they were clearly more affected by valence than arousal. In positive situations, as opposed to negative ones, goats produced calls with a lower fundamental frequency range and smaller frequency modulations (i.e. calls with less frequency variations; Fig. 2 and Audio A1).

Discussion

We investigated changes in physiological, behavioural and vocal parameters between situations potentially triggering different arousal and valence, in order to identify non-invasive indicators of emotions in goats. We found physiological, behavioural and vocal indicators of arousal. All these parameters changed consistently with increasing arousal and were clearly more affected by arousal than valence. We also found behavioural and vocal indicators of valence that changed consistently from negative to positive valence, and were clearly more affected by valence than arousal. Arousal indicators could help to identify and therefore minimize stress during negative situations. By contrast, valence indicators could help to differentiate between negative and positive situations, in order to reduce negative emotions and increase positive ones. In particular, the behavioural indicators that we found are relatively easy to observe. The potential to more effectively monitor animal emotions (and therefore also moods) is critical to our overall understanding of animal behaviour and wellbeing in general (Boissy et al., 2007; Mendl et al., 2010; Nettle & Bateson, 2012), as well as our understanding of the evolution of emotions.

Physiological Indicators

We use heart rates in this study to determine arousal levels triggered by the various situations. We found that this parameter was higher during the food frustration and anticipation of food reward situations than during the isolation and control situations. Isolation in social species normally induces high stress levels and an increase in heart rates (e.g. in ungulates; cattle, *Bos taurus*, Boissy & Le Neindre, 1997; sheep, Reefmann, Wechsler, et al., 2009; goats, Aschwanden, Gygax, Wechsler, & Keil, 2008). Therefore, it is surprising that our social isolation situation induced heart-rate values that were similar to the control situation. Our tests were preceded by three days of habituation, in order to minimize stress linked to novelty. Goats seemed to decrease stress-related behaviours over the days of habituation (EFB, personal observation), suggesting that they could have fully habituated to the isolation situation after three days. This differs from Siebert et al. (2011), which did not find clear evidence for habituation to repeated isolation sessions in the behavioural and vocal responses of goats. This could be due to the much longer isolation sessions used by Siebert et al. (2011; 30 min) compared to ours (5 min).

The physiological measures that we collected (heart rate, heart-rate variability and respiration rate) are involved in the sympathomedullary pathway axis (SAM) stress response, which prepares the animal to react to a stressor (Cannon, 1929; Seyle 1976). Stress has been defined as a nonspecific response of the body to any demand made upon it, whether positive or negative (Seyle, 1976). An increase in both negative and positive arousal is thus normally accompanied by (among other parameter changes), an increase in heart rate (used in our study to determine the levels of arousal triggered by the various situations), and an increase in respiration rate (von Holst, 1998). Accordingly, we found that respiration rate increased with increasing arousal (heart rate), independently of the valence of the situation.

Heart rate represents the interaction between sympathetic (increases heart rate) and vagal (reduces heart rate) regulation. By contrast, heart rate variability mainly depends on vagal influences and thus indicates when the vagal branch of the autonomic nervous system is activated (von Borell et al., 2007). This parameter increases (i.e. more variable heart rate due to an increase in successive R-R interval difference) when the parasympathetic system is activated. In our study, heart rate variability (RMSSD; root mean square of successive R-R interval difference) increased with decreasing arousal levels (heart rate), indicating that the parasympathetic system was activated during our low arousal situations. According to our criterion, RMSSD was thus also an accurate indicator of arousal. Our two physiological parameters (RespRate and RMSSD) were not correlated and therefore are both good, independent indicators of arousal.

We did not find any good physiological indicator of valence. High vagal tone and vagal activation have been suggested to indicate efficient autonomic regulatory activity and be associated with positive emotions, thus implying that RMSSD could be a good indicator of valence, notably in pigs (Zebunke, Langbein, Manteuffel, & Puppe, 2011) and sheep (Reefmann, Wechsler, et al., 2009). However, these studies did not control for the effect of arousal, as they compared situations of opposite valence, but also of different arousal (e.g. grooming as positive versus isolation as negative). In our study, RMSSD was not influenced by valence, despite controlling for the degree of movement (Locomotion). Similarly, Gygax et al. (2013) did not find any difference in RMSSD between two situations of different valence and likely similar arousal in goats. We suggest that heart rate variability, in a similar manner to most physiological parameters, is affected by arousal more than valence and thus constitutes an indicator of arousal.

Behavioural Indicators

The stress response prepares an animal to be more alert and vigilant, and to behave appropriately when faced with a stressor (e.g. Cannon, 1929). When the stimulus triggering the change in arousal enhances fitness (e.g. food reward), the animal should approach it, whereas when the stimulus threatens fitness (e.g. predator), the animal should avoid it (e.g. flight; Mendl et al., 2010). Accordingly, in our study, goats displayed more head movements and moved more during high arousal than low arousal situations, independently of the valence. Similarly, in Siebert et al. (2011), goat locomotion increased with arousal between complete isolation and partial isolation (supposedly higher arousal than complete isolation, because of the permanent sensory feedback from adjacent pen mates). In our study, goats also had their ears pointed forward more often and less often to the side (horizontal), which could indicate vigilance. Call rate generally increases with arousal in most species (Briefer, 2012). Accordingly, our results showed an increase in the number of calls per minute (Calls) with arousal, independently of valence (i.e. in both negative and positive situations).

We identified two promising behavioural indicators of valence. Goats had their ears orientated backward less often and spent more time with the tail up in positive situations compared to negative ones. Ear and tail positions were also suggested as indicators of emotions notably in sheep (Boissy et al., 2011; Reefmann, Bütikofer Kaszàs, et al., 2009a; Reefmann, Wechsler, et al., 2009), and pigs (Reimert, Bolhuis, Kemp, & Rodenburg, 2013). Reefmann, Bütikofer Kaszàs, et al. (2009) and Reefmann, Wechsler, et al. (2009a) showed that horizontal ears are associated with positive situations in sheep. However, in these studies, some of the positive situations (e.g. feeding on fresh hay or grooming) and negative situations (e.g. separation from group members) might have differed also in arousal levels. In a similar manner to our study, pigs in negative situations (anticipation of aversive situation) had their tails more often in a low position and ears more often backward than positive situations (anticipation of a rewarding situation; Reimert et al., 2013). By contrast, sheep tails were held up during separation from group members (negative), but not during rumination (intermediate) or when feeding on fresh hay (positive; Reefmann, Bütikofer Kaszàs, et al., 2009a). Alternative techniques for measuring ear and tail movements (e.g. non-invasive electromyography measuring ear and tail muscle activity) could help to obtain more precise results and allow accurate cross-species comparisons. Because ear position in our study was correlated with other behavioural parameters and clearly indicated both arousal (EarsForward and EarsHorizontal) and valence (EarsBackward), we suggest that the observation of ear positions is a promising tool to assess emotions.

Vocal Indicators

We found that parameters describing F0-contour (source-related parameters; F0mean, and F0End) increased with arousal levels. This could have resulted from an increase in the tension of the vocal folds after contraction of the cricothyroid muscles, or from stronger sub-glottal air pressure (Fant, 1960; Titze, 1994). We also found that higher arousal levels were characterized by higher energy quartiles (Q25%, Q50%, Q75%). Filter-related parameters (i.e. formants and the energy distribution) mostly depend on the shape and length of the vocal tract, and can be modified by laryngeal retraction (e.g. goats, dogs *Canis familiaris*, pigs and cotton-top tamarins *Sagunius oedipus;* Fitch, 2000; fallow deer *Dama dama*; McElligott et al., 2006). Mammals could also potentially constrict their pharynx (i.e. increase the tension of vocal tract walls), which results in a shift in energy distribution towards higher frequencies, but this phenomenon has, until now, only been studied in humans (Scherer, 1986) and birds (Riede, Beckers, Blevins, & Suthers, 2004). The shift in the energy distribution towards higher frequencies that we observed could thus be caused by a less pronounced retraction of the larynx or/and an increase in pharyngeal constriction with an increase in arousal. Although the energy distribution mainly depends on the filter process, our correlations between vocal parameters showed that this parameter was correlated with F0 (and therefore with its harmonics). As a result, the increase in energy quartiles with arousal could also have been a side effect of the increase in F0. Similar increases in F0 and energy quartiles with arousal are commonly found in humans (Scherer, 2003), other mammals (Altenmüller, Schmidt, & Zimmermann, 2013; Briefer, 2012; Lingle, Wyman, Kotrba, Teichroeb, & Romanow, 2012), and even birds (zebra finch, *Taeniopygia guttata*, Perez et al., 2012), suggesting highly conserved vocal indicators of arousal throughout evolution.

Surprisingly, the increase in energy quartiles with arousal was only accompanied by a marginally significant increase in the fourth formant (F4; $p = 0.055$). By contrast, the first formant (F1) decreased. These discrepancies were confirmed by a lack of correlation between energy quartiles and formants. Higher formants (e.g. F3, F3) depend mainly on the length of the vocal tract (Fant, 1960; Fitch & Hauser, 1995), while lower formants (e.g. F1, F2) depend more on the shape of the vocal tract. Our results could be explained by a less pronounced retraction of the larynx, which shortens the vocal tract and induces a higher F4, with an increase in arousal (Fant, 1960; Titze, 1994). The decrease in F1 might require more subtle changes in the configuration of the pharyngeal regions and oral and nasal cavities, and opening of the mouth. Indeed, mouth opening/closing and lip protrusion/retraction or lip rounding/spreading can also be used to modify formant frequencies, at least in primates (Hauser, Evans, & Marler, 1993; Hauser & Ybarra, 1994; Riede, Bronson, Hatzikirou, & Zuberbühler, 2005). However, this suggestion requires further analysis of goat behaviour while vocalizing (Riede et al., 2005). Several of our vocal parameters indicating arousal (F0mean, F0End, Q25-Q75% and F1Mean) were correlated with each other. Energy quartiles (Q25-Q75%) are easier to measure than F0 or formants and could constitute ideal indicators of arousal in goats and maybe other ungulates as well.

We found that during positive situations, goats produced calls with lower fundamental frequency range (F0range), as well as smaller frequency modulations (FMextent) than during negative situations. The fundamental frequency thus presented less variation during positive than negative emotions. These measures characterising F0 variation (F0Range and FMextent) were correlated. F0Range was more clearly affected by valence than FMextent. This parameter, which is also easier to measure than FMextent, could thus be selected as a clear valence indicator in goats. A decrease in F0range from negative to positive situations has also been observed in humans (Hammerschmidt & Jürgens, 2007), and elephants (*Loxodonta africana,* Soltis et al., 2011). Similarly, lower variation in F0 (cumulative variation of F0) in positive than in negative situation has been found in dogs (Taylor, Reby, & McComb, 2009). During positive emotions, it thus seems that vocal folds vibrate at a more stable rate than in negative emotions, resulting in more stable F0 over time. However, very few studies have been carried out on vocal indicators of valence and it is thus difficult to make general conclusions on the evolution of vocal correlates of valence.

Conclusion

By merging recent frameworks developed to measure animal vocalisations (source-filter theory; Taylor and Reby 2010) and emotions (Mendl et al., 2010), we have identified several non-invasive, promising indicators of arousal and valence. These physiological, behavioural and vocal indicators could be very useful to differentiate situations eliciting negative emotions from those eliciting positive ones, in order to promote the implementation of positive animal states (Boissy et al., 2007). Further experiments validating these indicators using different emotional situations (e.g. partial versus total isolation; Siebert et al., 2011), could allow us to determine the extent to which these indicators can be used across contexts. We believe that our approach, which simultaneously measures the effects of emotional arousal and valence, could lead to more accurate monitoring of animal emotions and a more comprehensive understanding of the evolution of emotions.

Appendix

Acoustic Analysis

Here, we provide a detailed description of the acoustic analysis. The source-related acoustic features (fundamental frequency, F0), filter-related acoustic features (formants and energy quartiles), and intensity features that we measured (13 parameters) are detailed below (Praat commands are indicated in brackets).

Source-related acoustic features were measured by extracting the F0 contour of each call using a cross-correlation method ([Sound: To Pitch (cc) command], time step = 0.01 s, pitch floor =100-150 Hz, pitch ceiling = 300-600 Hz). For each extracted F0 contour, we measured the following vocal parameters: the frequency value of F0 at the end (F0end) of the call, the mean F0 frequency values across the call (F0mean), and the F0 frequency range (F0range). To characterize F0 variation along the call, we measured the mean peak-to-peak variation of each F0 modulation (FMExtent, Charlton et al., 2009a; Charlton, Zhihe, & Snyder, 2009b).

Filter-related (formants) acoustic features were measured by extracting the contour of the first four formants of each call using Linear Predictive Coding analysis (LPC; [Sound: To Formant (burg) command]: time step = 0.01 s, maximum number of formants = 4–5, maximum formant = 3000–5500 Hz, window length = 0.05 s). To check if the Praat software accurately tracked the formants, the outputs of the LPC analysis were visually inspected together with the spectrograms. Spurious values were deleted and we corrected for octave jumps when necessary. For each call we collected the mean (F1-4mean) values of the formants. Finally, we measured the frequency values at the upper limit of the first (Q25%), second (Q50%) and third (Q75%) quartiles of energy, using a linear amplitude spectrum applied to the whole call.

We measured intensity characteristics by extracting the intensity contour of each call [Sound: To Intensity command]. We then included the mean peak-to-peak variation of each amplitude modulation in our analyses (AMextent; see Charlton et al. 2009b for details of these parameters). We also included the total duration of each call (Dur).

		Sex		**Age**		**Call type**		**Frustration vs Isolation**	
	Parameter	χ^2_1	***p***	χ^2_1	***p***	χ^2_1	***p***	χ^2_1	***P***
Physiology	**RMSSD**	0.17	0.68	0.12	0.73	-	-	3.60	**0.058**
	RespRate	8.15	**0.004**	3.79	**0.052**	-	-	27.44	**<0.0001**
Behaviour	**HeadMov**	0.01	0.94	3.22	0.07	-	-	26.53	**<0.0001**
	Locomotion	4.41	**0.036**	0.94	0.33	-	-	8.07	**0.005**
	EarsForward	3.37	0.07	0.00	1.00	-	-	0.70	0.40
	EarsHorizontal	0.20	0.65	0.02	0.89	-	-	1.15	0.28
	EarsBackward	0.17	0.68	0.77	0.38	-	-	4.64	**0.031**
	EarsAsymetric	0.00	0.98	0.26	0.61	-	-	0.12	0.73
	TailUp	0.03	0.87	2.06	0.15	-	-	4.61	**0.032**
	Calls	0.38	0.54	2.45	0.12	-	-	0.03	0.86
Vocalisations	Duration	3.60	0.06	1.96	0.16	1.11	0.57	0.41	0.52
	F0mean	1.79	0.18	0.03	0.85	8.17	**0.017**	5.74	**0.017**
	F0end	3.10	0.08	0.39	0.53	2.09	0.35	8.44	**0.004**
	F0range	0.06	0.80	0.78	0.38	9.74	**0.008**	0.22	0.64
	FMextent	0.10	0.76	0.61	0.44	7.84	**0.020**	0.53	0.47
	AMextent	0.13	0.72	0.34	0.56	0.40	0.82	0.33	0.56
	Q25%	9.33	**0.002**	3.53	0.06	37.87	**<0.0001**	6.37	**0.012**
	Q50%	3.47	0.06	0.02	0.89	18.98	**<0.0001**	8.64	**0.003**
	Q75%	1.43	0.23	0.03	0.87	3.64	0.16	4.55	**0.033**
	F1mean	0.16	0.69	0.27	0.60	18.55	**<0.0001**	21.71	**<0.0001**
	F2mean	0.08	0.77	0.08	0.78	2.13	0.35	0.52	0.47
	F3mean	0.37	0.54	0.20	0.65	4.21	0.12	0.08	0.78
	F4mean	0.34	0.56	0.12	0.73	2.80	0.25	4.02	**0.045**

Table A1. Control factors and differences between Frustration and Isolation situations. Effects of the control factors (age, sex and call type), as well as difference between the Frustration and Isolation situations (both assumed of negative valence), for physiological, behavioural and vocal parameters (linear mixed-effects models and generalized linear mixed models, compared with likelihood-ratio tests). Bold font indicates significant ($p < 0.05$) and marginally significant ($0.06 < p \leq 0.05$) effects. The direction of the significant changes was assessed from residuals of the models. Sex (female, “F”; or male, “M”) affected RespRate (F > M), Locomotion (F < M) and Q25 (F > M). Age tended to affect RespRate (marginally significant decrease with age). The type of call (closed mouth “CM”, open mouth “OP” or mixed call “Mi”) affected F0-related parameters (F0mean: Mi > OP > CM), the variation in F0 (F0range and FMextent: Mi > OP > CM), the energy quartiles (Q25% and Q50%: Mi > OP > CM) and F1mean (OM > Mi > CM). The difference between the Frustration (“Fr”) and the Isolation situations (“I”) was marginally significant for RMSSD (Fr < I), and significant for RespRate (Fr > I), HeadMov (Fr > I), Locomotion (Fr > I), EarsBackward (Fr < I) and TailUp (Fr > I). Concerning vocal parameter, this difference was significant for F0-related parameters (F0mean: Fr > I), the energy quartiles (Q25-Q75%: Fr > I), F1mean (Fr < I) and F4mean (Fr > I). In addition, the time spent moving (Locomotion) was included as a control factor for the physiological indicators. It had a significant effect on RespRate (LMM: $X^2 = 6.03$, $df = 1$, $p = 0.014$), but not on RMSSD (LMM: $X^2 = 0.09$, $df = 1$, $p = 0.77$).

		AROUSAL				VALENCE					
		0		1		Negative		Neutral		Positive	
	Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Physiology	HR (beats/min)	108.75	15.38	123.00	23.52	115.74	21.94	107.79	12.25	126.37	24.71
	RMSSD (ms)	31.66	19.65	26.83	15.21	29.90	18.53	31.21	19.49	26.15	13.34
	RespRate (breaths/s)	0.32	0.10	0.42	0.11	0.38	0.13	0.31	0.08	0.42	0.09
Behaviour	HeadMov (min^{-1})	7.16	10.61	22.02	26.08	9.73	13.48	9.68	14.95	29.80	31.17
	Locomotion (%)	3.78	3.57	7.69	8.60	5.87	5.49	3.49	2.38	7.71	10.81
	EarsForward (%)	61.57	29.80	82.02	27.04	74.80	25.97	41.93	26.79	92.54	19.14
	EarsHorizontal (%)	16.25	25.08	0.21	1.11	0.25	1.14	35.99	26.32	0.00	0.00
	EarsBackward (%)	12.63	14.18	15.37	21.04	19.72	17.74	9.13	14.88	6.39	17.62
	EarsAsymetric (%)	8.61	15.10	2.58	5.57	2.84	3.10	16.16	20.07	1.86	7.26
	TailUp (%)	33.56	42.01	57.07	46.23	35.15	45.67	46.34	42.25	64.54	43.58
	Calls (min^{-1})	0.45	1.59	3.36	4.40	1.55	2.74	0.00	0.00	4.67	5.16
Vocalisations	Dur (s)	0.84	0.21	0.78	0.16	0.81	0.19	-	-	0.78	0.15
	F0Mean (Hz)	253.40	105.98	228.30	56.56	240.42	81.58	-	-	223.51	58.76
	F0End (Hz)	189.30	62.83	190.49	51.26	189.88	56.23	-	-	190.83	52.14
	F0Range (Hz)	115.75	84.85	91.29	52.63	106.75	69.21	-	-	73.85	44.95
	FMExtent (Hz)	71.16	42.52	75.37	105.98	79.99	99.20	-	-	52.87	36.92
	AMExtent (dB)	11.76	7.46	11.96	5.52	11.68	6.49	-	-	12.63	5.14
	Q25% (Hz)	241.83	135.94	255.42	109.55	251.98	120.39	-	-	247.09	114.39
	Q50% (Hz)	437.61	263.19	453.05	209.33	446.91	226.02	-	-	451.38	238.04
	Q75% (Hz)	1020.65	814.46	997.37	659.22	998.30	689.43	-	-	1029.04	797.02
	F1Mean (Hz)	694.97	163.87	655.17	113.25	659.00	132.24	-	-	696.33	125.15
	F2Mean (Hz)	1651.64	369.82	1596.72	289.56	1621.11	307.98	-	-	1566.03	305.97
	F3Mean (Hz)	2544.34	419.27	2549.80	368.42	2573.22	370.67	-	-	2469.27	400.37
	F4Mean (Hz)	3283.72	471.22	3423.91	521.30	3420.27	497.53	-	-	3290.92	557.30

Table A2. Raw values of the physiological, behavioural and vocal parameters measured for each arousal level and valence of the situations (Mean ± SD).

Audio A1. Negative and positive calls. Audio files corresponding to the calls presented in Fig. 2; one call produced during the negative situation followed by a call produced during the positive situation by the same goat.

ANNEXE II

FITNESS SURFACES FOR NEST-DEFENCE BEHAVIOR IN THE ALPINE SWIFT: EFFECTS OF PARTNERSHIP, SOCIAL AND ECOLOGICAL CONDITIONS

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Abstract

Understanding behavioral diversity in nature requires studying the adaptiveness of mean behavioral trait value and trait variability concurrently, and the selective forces maintaining them across ecological and social contexts. Here, we investigate how selection conditionned by social and ecological heterogeneity co-acts on mean behavioral trait and trait variability. In colonial Alpine swifts, we analyze selection gradients acting on heritable adult nest-defense behavior. We consider how interactions between parents behavioral diversity (means and CVs derived from repeated yearly behavioral scores), and environmental stochasticity in yearly ressources, parental partnership and social group behaviors affect the growth of cross-fostered offspring. Our results suggest that selection on nest-defense behavior is conditioned by behavioral interactions between sexual partners and with the social environment. Nestlings grow significantly faster when parents are dissimilar in terms of mean behavior and behavioral variability, and when fathers display similar mean behavior to their colonial conspefics. In contrast, we found no evidence that selection on adult behavioral traits is influenced by yearly ecological conditions as reflected by colonial nestling mass growth. Our study suggests a more important influence of parental role division and social interaction over ecological factors as drivers of the evolution and maintenance of behavioral diversity in the wild.

***Keywords**:* Alpine swift, behavioral mean and variability, cross-fostering experiment, environmental heterogeneity, fitness surface, nest-defense behavior, reaction norm, social behavior

Introduction

Behavior is a frontline response to environmental change and is often regarded as a highly variable trait (Piersma and Drent 2003). Therefore, widespread reports of behavioral consistencies across time/contexts in nature (Réale et al. 2000, Dingemanse et al. 2002, Bize et al. 2012), call into question the best way to study the adaptiveness and diversity of behavioral traits (Dall et al. 2004, Sih et al. 2004, Reale et al. 2007, Dingemanse et al. 2010). Although individuals within populations often display consistent differences in mean levels of specific behaviors (Wilson 1998) (*e.g.* differences in aggressiveness) they usually retain a certain amount of intra-individual variation around their mean behavior (Frost et al. 2007, Dingemanse et al. 2010, Westneat et al. 2011, Betini and Norris 2012). Accordingly, the first challenge we face to unravel the selective advantage of behavior is to embrace both behavioral mean and behavioral variability in one integrative framework (Westneat et al. 2011, Betini and Norris 2012). In addition, an individual's behavior and its fitness consequences (*e.g.* reproductive performance) often varies with biotic and abiotic factors (*e.g.* predation, climate) or a combination of the two (*e.g.* effect of climate on food abundance) (Réale and Festa-Bianchet 2003, Dingemanse et al. 2004, Boon et al. 2007), and in many cases is influenced by social partner and/or peer behavior (*e.g.* cooperation, competition) (Both et al. 2005, van Oers et al. 2005, Schuett et al. 2011). Thus, a second challenge is accounting for such sources of environmental variability *simultaneously* when considering the effects of behavioral mean and variability on fitness. Previous studies have separately investigated selection acting on behavioral mean (often refered to as personality) and/or variability (often refered to as plasticity) with respect to fluctuations in ecological/social conditions (Réale and Festa-Bianchet 2003, Dingemanse et al. 2004, Boon et al. 2007, Quinn et al. 2009, Adriaenssens and Johnsson 2013), or documented the consequences of individual behavioral mean, and behavioral mean interactions with pair or social partners, on fitness (Both et al. 2005, Sih and Watters 2005, van Oers et al. 2005, Schuett et al. 2011). However, to the best of our knowledge no empirical study has yet considered in the same analysis how both behavioral mean and variability around the mean may be co-evolving depending on both ecological and social factors. Yet, understanding how an individual's fitness may simultaneously be shaped by the interplay between its mean behavior, the amount of behavioral variation it exhibits, and the social and ecological environment it experiences, is necessary to move towards an integrative understanding of the evolutionary function of behavior.

Here, we use experimental data collected over 7 years on wild Alpine swifts (*Apus melba*) to investigate the strength and shape of selection (Lande and Arnold 1983) acting on mean parent nest-defense behavior and variability in parent nest defense behavior, computed as the mean and CV of nest-defense behavioral scores for adults scored at least three different times over at least three different years. As nest-predation is an important source of mortality for birds (Martin 1993), including in the Alpine and other swift species (Arn 1960), nest-defense behavior is expected to be under strong selection. Alpine swifts build open nests that lay flat on the substrate (rock-fissures in natural cliffs or roof-floors in urban environments) and are indeed known to suffer from nest-predation by rats (*Rattus rattus*), domestic cats (*Felix catus*), stone martens (*Fouina fouina*), carrion crows (*Corvus corone*), and European jackdraws (*Corvus monedula*; Arn 1960 & P.B., *unpublished data*). Their mean nest-defense behavior is heritable (Bize et al. 2012); a prequiste for evolutionary responses. Adult swifts also retain intra-individual variation around their mean nest-defense behavior (Bize et al. 2012), enabling us to consider selection on both behavioral mean and variability concurrently. Further, in Alpine swifts, bi-parental care is essential to fledge offspring (P.B., *unpublished data*). An individual's breeding success thus depends on the behavior of its partner, providing strong scope for interactions between parents' mean behavior and the range of behavioral variation they exhibit. As colonial breeders, those birds also provide a good opportunity to integrate how the yearly behavior of colonial conspecifics, a reflection of social group composition, might influence selection on behavioral mean/variability in nest-defense behavior. Finally, Alpine swifts are aerial predators that forage exclusively on insects caught during flight. Thus, acute or sustained detrimental weather conditions have immediate effects on food availability and on nestling growth rates and survival (Bize et al. 2006b), so that good and poor environmental years are reflected in the annual mass growth of colonial offspring. This enables us to integrate fluctuations in yearly environmental conditions when considering the effects of parental nest-defense behavior (both mean and variability) on fitness.

Over 2003-2009, we exchanged offspring between 178 nests at the egg or hatchling stage, which were as a consequence food-provisoned by foster parents, and monitored their body mass growth. As is the case for many birds, nestling mass growth is a strong predictor of recruitment as a breeder, and thereby a good proxy to fitness, in the Alpine swift (see *General methods* section). This experimental cross-fostering design insured that variation in offspring growth trajectories was foremost related to variation in parental nest-defense behavior while excluding possible confounding genetic and early environmental effects (Bize et al. 2012). We tested for selection gradients (quadratic and correlational selection) acting on parents' behavioral mean/variability in nest-defense behavior by: (1) investigating the effects of parents' behavioral mean ($\mathrm{MEAN}_{\mathrm{father}}$, $\mathrm{MEAN}_{\mathrm{mother}}$ and quadratic terms) and variability ($\mathrm{CV}_{\mathrm{father}}$, $\mathrm{CV}_{\mathrm{mother}}$ and quadratic terms), and their interaction, on nestling growth; and (2) integrating into our analyses interacting effects of parental behavior with social (mean annual colony nest-defense behavior, $\mathrm{MEAN}_{\mathrm{col}}$) and ecological (mean annual nestling mass growth of the colony, $\mathrm{GROWTH}_{\mathrm{an}}$) environmental factors (see below) (Lande and Arnold 1983).

Methods

General methods

Since 1999, swifts have been monitored in two Swiss colonies located some 21km apart, in the clock towers of Bienne (*ca.* 100 breeding pairs; 47°10'N, 7°12'E) and Solothurn (*ca.* 50 breeding pairs; 47°12'N, 7°32'E). In each year, regular visits to the colonies make it possible to precisely determine the fate of each breeding attempt. Either feathers or a drop of blood was sampled from adults in order to sex individuals from DNA (Bize et al. 2012). Hatchlings were individually recognized with a nontoxic colour mark before being ringed at 10 days of age, and nests visited at least every 10 days to monitor individual nestling-growth throughout the season. Fledging occurs 50 to 69 days after hatching (hatching day being defined as day 0) (Bize et al. 2006a). Body mass of each nestling was measured to the nearest 1-mg using a digital scale at least at three different ages between day 5 and 35 after hatching. Body mass-gain was first derived for each nestling from the slope of a linear regression of body mass against age, and subsequently averaged within nest. This index of nestling mass growth is a good proxy to fitness in the Alpine swift, as illustrated by its strong relationship with the odds of nestlings to survive and recruit into the adult population, after controlling for year and colony-dependent effects. Over 1181 nestlings, the odds of recruiting into the adult population increased by 61% for a body mass gain increase in 1g/day (odds ratio of recruiting *vs.* not recruiting = 1.613, CI_{95} = 1.21-2.16) during nestling's growth period (logistic regression likelihood ratio test; $\chi^2 = 10.86$, $P = 0.001$, $N = 1181$ nestlings). In addition, gain in nestling body mass is strongly affected by weather conditions (Bize et al. 2006b) and parental food provisoning behavior (Bize et al. 2006c).

Parental nest-defense behavior

Between 2003 and 2011 parental nest-defense behavior was scored along a shy-bold continuum based on how breeding adults behaved towards an experimenter during their approach and capture while sitting on eggs or young chicks (Bize et al. 2012). Adult swifts (including Alpine swifts) have been subjected to nest-predation over their evolutionary history [28-29], and from an evolutionary perspective predation-risk and human-caused disturbance stimuli are likely analogous selective pressures (Frid and Dill 2002). Both divert time and energy from fitness-enhancing activities, and thus an animal's response to human disturbance most probably follows the same economic rules as prey facing predators (Frid and Dill 2002). Nest-defense behavior was always scored by the same observer (P.B.) over the entire study period using an arbitrary discrete variable divided in five scores and ranging from 0 to 2 (Bize et al. 2012). The score of 0 was assigned to birds which flushed from the nest upon first audio and/or visual detection of the experimenter, 0.5 to birds which remained on the nest during approach but flushed before hand capture, 1 to birds that remained motionless during approach and thus were hand captured on nest, 1.5 to birds that remained motionless during experimenter approach but subsequently moved towards the experimenter's hand, flapped their wings and clawed on the approaching hand, and 2 to birds that behaved aggressively towards the experimenter both during approach and capture. Hence, our scoring system reflects a gradual increase in the chance for an incubating adult Alpine swift of being spotted and caught on its nest by an intruder. It can be interpreted along a shy-bold axis of risk taking, with birds assigned a score of 0 being the shyest (flying away from their nest before being spoted) and those assigned a score of 2 being the boldest (staying on their nest and actively defending it). Nest-defense behavior is both repeatable (r = 0.273) across the years, and heritable (h^2 = 0.146) (Bize et al. 2012). As adult behavior was scored repeatedly over the years, a measure of individual's mean nest-defence behavior was obtained by averaging the individual's behavior over the years (behavioral mean). In addition, we calculated individual's behavioral variability as coefficients of variation (CVs; standard deviation over the mean) around individuals mean nest-defense behavior, as previously suggested by (Schuett et al. 2010, 2011). This second axis of behavioral variation thus ranges from high consistency (CV = 0) to high variability (CV = 1) in nest-defence strategies. Parents' behavior mean and variability in nest-defense were only calculated for adults which had been scored at least three different times over at least three different years. Although bird behaviors were described using 5 discret scores, inter-individual variation in nest-defense behaviour is most likely continuous (see Figure 1 in Bize et al. 2012). Indeed, the computation of mean individual behavioural scores based on discrete scores collected over at least three different years allowed transforming our discrete variable in a continuous variable better reflecting the natural variation observed for this trait (Bize et al. 2012). In the present study our objective was to analyse selection on among-individual variation in behavioral mean and variability in nest defense, which is a critical step to understand the evolution and maintenance of behavioral diversity in natural populations. However, everything remains to be done to identify the proximate factors accounting for among-individual variation in behavioral mean and variability. Here, behavioral variabiliy (CVs) can thus encompas both adaptive responses as well as random noise, and we have no a priori on the environmental factors shaping this variability.

Cross-fostering design

Experimental cross-fostering designs are typically used in behavioral research to dissociate genetic from environmental (*e.g.* post-natal environment) factors (Schuett et al. 2011, Bize et al. 2012). Here, in order to insure that parental effects on offspring development occurred via post-hatching behavioral effects and were not confounded with genetic or maternal effects, we investigated the influence of parental behavior (mean nest-defense behavior and its variability) in 178 families where eggs or hatchlings had been swapped between pairs of nests. To insure that parental effort was not manipulated, nests were pair-matched on the criteria that eggs/nestlings were laid/hatched on the same dates, and that nests contained the same number of eggs or hatchlings, so that our cross-fostering design did not alter laying date or clutch/brood size.

Fitness selection analysis

Selection gradients acting on nest-defense behavior in the Alpine swift, were considered by investigating the interacting effects of foster mother and foster father's mean nest-defence behavior ($MEAN_{mother}$ and $MEAN_{father}$) and variability around the mean (CV_{mother} and CV_{father}) on nestling mass growth (MG; g/day) in a formal selection analyses (see Table 1; Lande and Arnold 1983). Besides parents' nest-defense behavior mean and CV, we included in the same statistical model the annual mean body mass growth of all colony nestlings ($GROWTH_{an}$) and the annual mean nest-defense behavior of all colony adults ($MEAN_{col}$) as two additional explanatory variables (Table 1). This allowed us to account for the influence of social and ecological factors on nestling development. For instance, annual mean growth in body mass reflected whether environmental conditions were good or bad, with high annual growth supported by favourable environmental conditions and high food abundance. Annual colony behavior on the other hand related to the grand mean of individual mean nest defense behavior (see above). Because individual nest defense behavior is heritable (Bize et al. 2012), years of high and low colonial nest defense behavior are expected to reflect yearly variation in social group composition. Both $GROWTH_{an}$ and $MEAN_{col}$ were annually and colony centered variables, and thus controlled for year and colony-dependent effects. In addition, we specified clutch size as a covariate in our analyses to account for the known negative trade-off between growth and number of offspring in the Alpine swift (Bize and Roulin 2006). Parents' identities (ring number) were included as random effects in our models to account for repeated measurements on the same individuals as parents bred several times over the years, and thus appeared more than once in the data set. All terms were standardized before analyses, so that model estimates were comparable. Non-significant terms were deleted sequentially ($P > 0.05$) starting with 3-way interactions. AICc and BIC selection criterion led to consistent results, and the final model had the lowest AICc scores ($\Delta AIC = 71.35$ with the full model and 6.42 with the next best fit; AIC weight = 0.96).

All analyses were done using the JMP® v.9.0.0 (SAS Institute Inc.) software. Standard least square mixed models were fitted by Restricted Maximum Likelihood (REML). Significant results are reported for $P \leq 0.05$. Number of observations is reported as *n*, number of individuals as N_{males} and $N_{females}$, respectively.

Rational for inclusion	**Model term**
Covariate: controls for the trade-off between number and growth of offspring	Brood size
Directional and stabilizing selection gradients acting on parents behavior	Father's mean nest defense behavior ($MEAN_{father}$) Father's variability in nest defense behavior (CV_{father}) Mother's mean nest defense behavior ($MEAN_{mother}$) Mother's variability in nest defense behavior (CV_{mother}) Father's mean nest defense behavior^2 ($MEAN^2_{father}$) Father's variability in nest defense behavior^2 (CV^2_{father}) Mother's mean nest defense behavior^2 ($MEAN^2_{mother}$) Mother's variability in nest defense behavior^2 (CV^2_{mother})
Effects of ecological and social conditions of the year on nestling mass growth	Annual colony nestling mass growth ($GROWTH_{an}$) Annual colony nest-defense behavior ($MEAN_{col}$)
Correlational selection gradients acting on parents behavior: interactions with annual ecological conditions	$MEAN_{mother}$ x $GROWTH_{an}$ CV_{mother} x $GROWTH_{an}$ $MEAN_{father}$ x $GROWTH_{an}$ CV_{father} x $GROWTH_{an}$
Correlational selection gradients acting on parents behavior: interactions with annual social conditions	$MEAN_{mother}$ x $MEAN_{col}$ CV_{mother} x $MEAN_{col}$ $MEAN_{father}$ x $MEAN_{col}$ CV_{father} x $MEAN_{col}$
Correlational selection gradients acting on parents behavior: interactions with partner's behavior	$MEAN_{mother}$ x $MEAN_{father}$ CV_{mother} x CV_{father}
Effects of pair behavioral type on nestling mass growth conditional on annual social and ecological conditions	$MEAN_{mother}$ x $MEAN_{father}$ x $MEAN_{col}$ CV_{mother} x CV_{father} x $MEAN_{col}$ $MEAN_{mother}$ x $MEAN_{father}$ x $GROWTH_{an}$ CV_{mother} x CV_{father} x $GROWTH_{an}$

Table 1. Model construction and rational for terms explaining nestling mass growth in colonial Alpine swift (*Apus melba*).

Results

Our experimental cross-fostering design revealed that nestling body mass growth, and in turn parental fitness, was strongly influenced by the behavior of foster parents while controlling for possible confounding genetic and early parental effects on offspring growth trajectories (see Table 2). Not surprisingly, nestling body mass growth was negatively associated with brood size (estimate = -0.10 ± 0.03) and positively associated with the mean annual mass growth of chicks in a given colony (0.17 ± 0.04). Taking those effects into account, our analysis reveals selection acting on parental behavior in relation to the behavior of their social partner, and in relation to the social environment at large (see significant interactions, Table 2).

Term	Estimate (β)	CI 95%	DFDen	t Ratio	Prob>|t|
Intercept	2.83 ± 0.05	[2.73; 2.93]	61.06	56.85	<.0001***
Brood size	**-0.10 ± 0.03**	**[-0.16; -0.03]**	**162.54**	**-3.00**	**0.003****
MEAN_{mother}	-0.07 ± 0.04	[-0.14; 0.01]	18.36	-1.84	0.081
CV_{mother}	-0.06 ± 0.03	[-0.12; 0.01]	38.39	-1.66	0.106
MEAN_{father}	0.04 ± 0.05	[-0.05; 0.13]	63.75	0.89	0.376
CV_{father}	-0.03 ± 0.04	[-0.12; 0.06]	58.52	-0.66	0.510
MEAN^2_{mother}	0.00 ± 0.02	[-0.04; 0.04]	32.57	-0.09	0.929
CV^2_{mother}	0.04 ± 0.02	[0.00; 0.08]	67.27	1.98	0.052
MEAN^2_{father}	-0.04 ± 0.03	[-0.1; 0.02]	100.55	-1.43	0.157
CV^2_{father}	-0.02 ± 0.02	[-0.07; 0.03]	59.13	-0.79	0.431
MEAN_{col}	0.02 ± 0.04	[-0.06; 0.10]	124.35	0.56	0.576
GROWTH_{an}	**0.17 ± 0.04**	**[0.09; 0.25]**	**151.72**	**4.28**	**<.0001*****
MEAN_{father}* MEAN_{col}	**0.10 ± 0.04**	**[0.01; 0.18]**	**101.91**	**2.30**	**0.024***
MEAN_{mother}*MEAN_{father}	**-0.10 ± 0.04**	**[-0.18; -0.02]**	**87.76**	**-2.41**	**0.018***
CV_{mother}*CV_{father}	**-0.08 ± 0.04**	**[-0.15; -0.01]**	**90.77**	**-2.18**	**0.032***

Table 2. Mixed model estimates (±SE) for selection acting on Alpine swift (Apus melba) nest-defence behavior. The overall model explained 35.5% of variation in nestling mass growth. Data is for *n* = 178 cross-fostered nests; $N_{male\ parents}$ = 95 and $N_{female\ parents}$ = 93. Variation in nestling growth was analyzed in relation to foster mother's mean behavioral trait value (MEAN_{mother} and MEAN^2_{mother}) and variability around the mean (CV_{mother} and CV^2_{mother}), and foster father's mean behavioral trait value (MEAN_{father} and MEAN^2_{father}) and variability around the mean (CV_{father} and CV^2_{father}), and accounted for potential influences of social (yearly mean colony nest-defence behaviour, MEAN_{col}) and non-social (yearly mean colony chick mass growth, GROWTH_{an}) environmental factors. 95% confidence intervals are given. Significant values are indicated for $^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}P\leq0.001$

First, foster father and mothers' mean (MEAN_{father} and MEAN_{mother}) nest-defence behavior interacted to affect nestling growth (Table 2). Nestling mass growth was highest when both parents displayed opposite behavior, for instance high nest defence behavior for fathers and low nest defence behavior for mothers (Fig. 1A). In contrast, nestlings raised by foster parents that displayed similar mean nest-defence behavior displayed the lowest growth rates. Interestingly, we found similar patterns for foster parents' behavioral variabiliy (CV_{father} and CV_{mother}). Parents of similar behavioral variability were the worst combination for the offspring (low body mass growth), whereas more dissimilar parents in terms of consistency raised nestlings that grew better (Fig. 1B).

Second, foster father's mean nest-defence behavior also interacted with the annual mean nest-defense behavior of all colony adults (MEAN_{col}) to affect nestling growth (Table 2). Fathers that displayed high (low) nest-defence behavior, i.e. high (low) MEAN_{father}, raised nestlings that grew heavier on average, but only in years when the overall nest-defence behavior of the colony was high (low) (Fig. 1C). In contrast, there appeared to be a substantial fitness cost for fathers to display high (low) nest-defence behavior when the overall nest-defence behavior of the colony was low (high) (Fig. 1C).

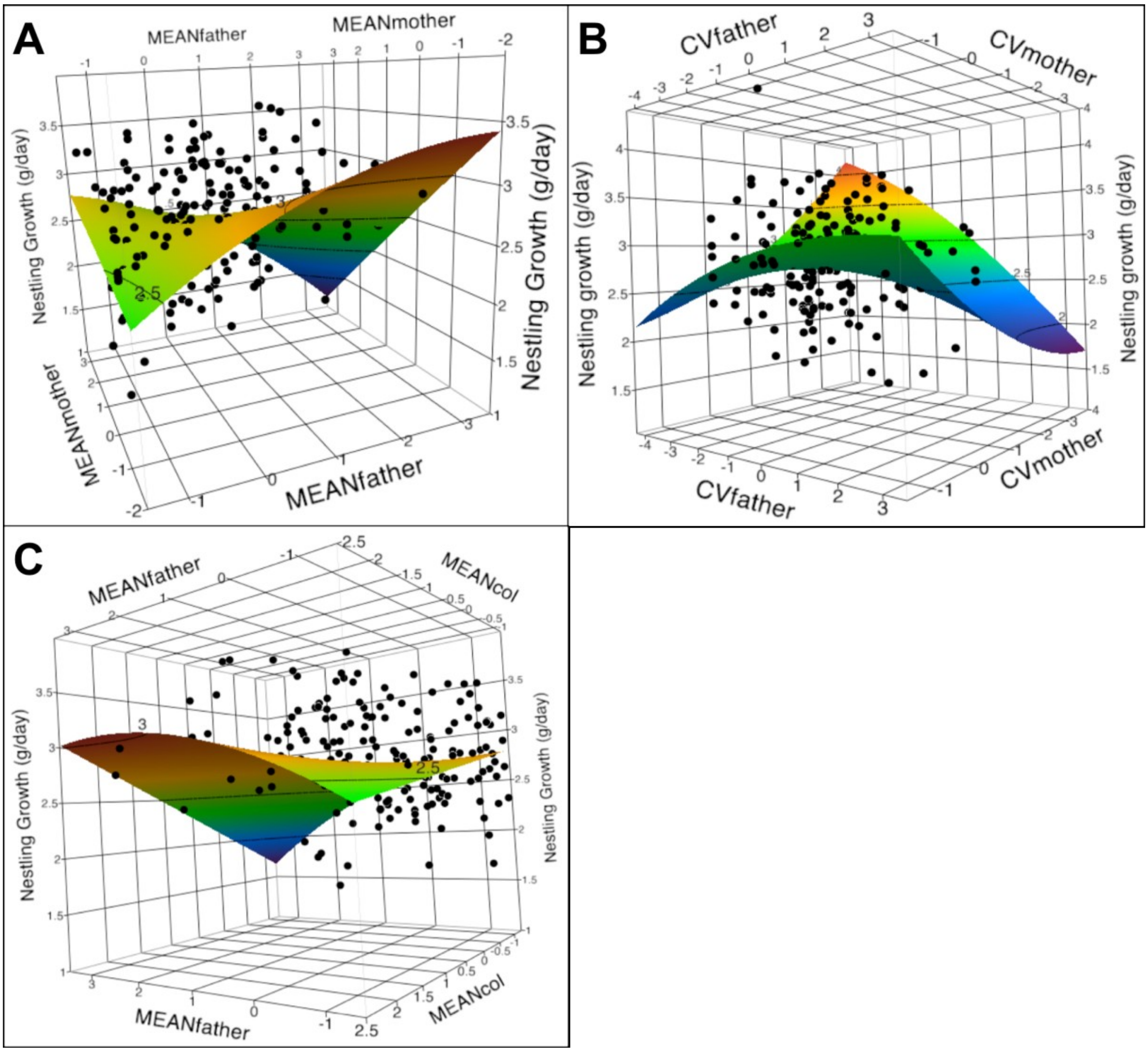

Figure 1. Fitness surfaces for the effects of parental mean nest-defence behavior and variability in nest-defence behavior on nestling growth in the Alpine swift (Apus melba). The modeled surfaces and raw data points present **A.** The interactive effects between foster father's mean nest-defense behavior ($\mathrm{MEAN}_{\mathrm{father}}$) and foster mother's mean nest-defense behavior ($\mathrm{MEAN}_{\mathrm{mother}}$) on nestling mass growth (in g/day). **B.** The interactive effects between foster father's variability in nest-defense behavior ($\mathrm{CV}_{\mathrm{father}}$) and foster mother's variability in nest-defense behavior ($\mathrm{CV}_{\mathrm{mother}}$) on nestling mass growth. **C.** The interactive effects between foster father's mean nest-defense behavior ($\mathrm{MEAN}_{\mathrm{father}}$) and the mean nest-defence behavior of the colony ($\mathrm{MEAN}_{\mathrm{col}}$) on nestling mass growth. Data are standardized. Increasing negative values in nest-defense behavior reflect increasing shyness, whereas increasing positive value reflect increasing boldness. Increasing negative values in CVs reflect decreasing variability around the mean (higher consistency in nest-defence behavior), whereas increasing positive values reflect increasing variability around the mean (higher variability in nest-defence behavior).

Finally, we did not find any significant interaction between foster parents' behavior and the environmental conditions of the year, as reflected by average annual nestling mass growth in the colony (no significant interaction with parental behavior was retained in the final model).

Discussion

In the colonial Alpine swift, nestlings rely entirely on their two parents to be fed, and our experimental cross-fostering design demonstrates that nestling body mass growth is related to the nest-defense behavior of foster parents. Because nestling body mass is strongly associated with recruitment in this species (see *General Methods* section), it suggests that nest-defense behavior is under selection in adult Alpine swifts. Selection on male and female parents' mean behavioral value and its variability proves however to be complex. At the breeding pair level, nestlings raised by parents with dissimilar behavioral traits, either in terms of mean or variability (CV), grew at faster rates (Fig 1A & B). At the colony level, nestlings raised by fathers whose mean behavior deviates from the yearly mean of the colony grew at slower rates (Fig. 1C). Finally, although Alpine swifts forage exclusively on aerial insects, and as a result the growth of their nestlings is strongly influenced by yearly variation in abiotic environmental conditions (Bize et al. 2006b), we found no evidence that selection on adult behavioral traits was influenced by variation in environmental conditions. Altogether, the present findings shed light on the importance of behavioral interactions between mating partners and group members (rather than abiotic environmental factors) in promoting and maintaining the diversity of animal behaviors in natural populations.

For species providing extended parental care such as the Alpine swift, parents face the dilemma of having to trade-off limited time and energy resources to competing fitness-enhancing activities (*e.g.* nest defense *vs.* offspring provisioning). Such trade-offs relying on the same limited capital predict negative relationships between nest-defense behavior and foraging/offspring provisioning, and thereby nestling growth (*e.g.* Markman et al. 1995, Komdeur and Kats 1999). In species with bi-parental care, an optimal resolution of this trade-off can be achieved if each parent specializes itself in a different task. This is well-known in raptors for instance where males usually specialize in provisioning food to their mate and their brood while females incubate the eggs, brood the chicks and defend the nest (Sunde et al. 2003). Alpine swift parents do not show sex-specific parental role division, both parents being involved in incubating, brooding, food provisioning and nest defense. Nonetheless, the fact that mean nest defense behavior is repeatable in this species (Bize et al. 2012) suggests among-individual variation in this task. The present findings of significant interactions between nest defense behavioral traits (mean and CV) of male and female parents, dissimilar parents being fitter, points towards an important influence of parental role division in maintaining the diversity of behaviors observed among individuals in a species with no previously known sex-specific division of parental duties. Those intriguing results raise the question of whether other behavioral combinations (e.g foraging behavior) may be under similar selection. The few other studies that investigated pairing among individuals showing consistent differences in their behaviors, revealed greater fitness returns in pairs of individuals with similar rather than dissimilar mean behaviors (Budaev et al. 1999, Both et al. 2005, Schuett et al. 2011, Gabriel and Black 2012). Those contrasting findings call for more comprehensive research on the role of sexual conflicts and selection in driving behavioral diversity (Schuett et al. 2010). Here, it is also important to note that although parental provisioning has been recently suggested to play a key role in linking behavioral consitency with fitness (Mutzel et al. 2013), direct evidence for a chain of causation between parent nest-defense behavior, food provisioning, nestling growth and fitness is still needed in the Alpine swift.

Alpine swifts are colonial birds that breed and forage in groups, and conversely to our findings at the pair level, at the colony level we observed greater yearly fitness returns for parents (though males only) that showed similar mean behavior to the yearly grand mean of the colony. As for our finding at the pair level, the proximate mechanisms remain to be unveiled, but again point towards a significant role of social interactions in shaping behavioral diversity. Selection for pairing of individuals with similar behaviors has been suggested to favor coordination in parental duties (Both et al. 2005, Schuett et al. 2011, Gabriel and Black 2012), and here the same arguments can be extended to the group level if compatibility among group members promotes cooperative behaviors. Alternatively, a positive association across the years between an individual's mean behavior and the grand mean behavior of its conspecifics could merely arise from a third latent environmental variable similarly affecting all colonial birds that display the same mean behavior. Because Alpine swifts are aerial insectivores, yearly variations in environmental conditions could be independently influencing nestling growth and favoring particular parental behavioral traits. However, we found no evidence supporting this later hypothesis. Finally, it is interesting to note that our selection analysis indicated weakly balancing selection on mother's behavior variability, suggesting that selection might favor the maintenance of 'moderate' variability in nest defense behavior over strong consistency or strong variability in this behavior.

To sum-up, whereas this study does not address the mechanistic links relating chick-growth to the nest defense behavior of their foster parents *per se*, our experimental (cross-fostering) results highlight a central role of behavioral interactions between pair partners and the social environment at large in shaping the evolution and maintenance of nest defense behavior. The present findings should stimulate future studies to consider both ecological and social sources of selection in shaping behavioral diversity in the wild.

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