

Hormones, parasites and male mating tactics in Alpine chamois: identifying the mechanisms of life history trade-offs

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Alternative mating tactics (AMTs) may show different trade-offs between current reproduction and survival; however, the proximate mechanisms underlying this pattern remain unclear. Among them, the relationship between reproductive effort and parasite resistance mediated by hormonal secretion has received increasing attention. We monitored 19 marked adult male chamois, *Rupicapra rupicapra*, within the Gran Paradiso National Park (Italy) between the pre- and post-rut 2011, to investigate the trade-off between mating effort and parasites associated with AMTs, and the underlying physiological mechanism. Territorial males sharply increased mating effort, faecal androgen and cortisol metabolites, and parasite levels during the rut, whereas nonterritorial ones displayed a similar pattern only for androgen metabolite levels. During the rut, territorial males invested more in rutting activities, while having higher levels of hormone metabolites and greater faecal counts of parasites than nonterritorial males. Before and after the rut, differences between male types (territorial and nonterritorial males) were smaller. Our analysis suggests that a trade-off between mating effort and parasitism exists, and that the proximate mechanism underlying this pattern may be found in the secretion of androgen metabolites. The greater investment in rutting activities, which territorial males make, suggests potentially high mating benefits. However, mating benefits could be counterbalanced by greater risk of injuries, consumption of fat reserves and higher hormone levels, which might favour the suppression of immunological defence and the subsequent decrease in parasite resistance.

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In species with intense competition for mating, the pursuit of reproductive success may foster the development of alternative mating tactics (AMTs, Taborsky et al. 2008). Our understanding of the evolution and maintenance of AMTs may benefit from the comparison of the physiological and life history trade-offs underlying their expression (Garant et al. 2003; Oliveira et al. 2008).

From a life history perspective, individuals that invest a lot in current reproduction may have lower levels of survival or future reproduction (Stearns 1992). However, the proximate mechanisms underlying this trade-off are still poorly known (Cox et al. 2010). It is widely assumed that reproductive effort, defined as the allocation of energy resources to current reproduction, is traded off

against the individual's ability to defend itself against pathogens (reviewed in Lochmiller & Deerenberg 2000; Zuk & Stoehr 2002; but see the study of Hörak et al. 2006 on rats, *Rattus norvegicus*). Indeed, several studies have found a positive correlation between reproductive effort and parasitism in various taxa (e.g. zebra finches, *Taeniopygia guttata*: Deerenberg et al. 1997; collared flycatchers, *Ficedula albicollis*: Nordling et al. 1998; tree swallows, *Tachycineta bicolor*: Ardia et al. 2003; bighorn sheep, *Ovis canadensis*: Pelletier et al. 2005).

The physiological basis of this trade-off includes the role of the immune function. The steroid hormone testosterone may promote male reproductive effort (Knapp 2003; Hirschenhauser & Oliveira 2006) and enhance mating potential (Folstad & Karter 1992; Ezenwa et al. 2012; but see the recent study of Nie et al. 2012 on the giant panda, *Ailuropoda melanoleuca*) while simultaneously suppressing the immune function (e.g. in Alpine ibex, *Capra ibex*: Decristophoris et al. 2007). Immunosuppression may also be related to changes in secretion of glucocorticoids (Ezenwa et al.

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2012), which share several enzymatic pathways in common with androgens (Knapp 2003). The increase in parasite infection (Folstad & Karter 1992; Decristophoris et al. 2007; Hau 2007), in turn, draws resources that may be used for other activities such as reproduction and survival, reducing the host's fitness (Lehmann 1993).

The expression of AMTs normally involves hormonal regulation: for example, dominant males usually have higher levels of testosterone than subordinates (Taborsky et al. 2008), as shown in bison bulls, *Bison bison*, by Mooring et al. (2004). This may lead to different mating potential and parasite susceptibility between AMTs. Therefore, the interplay among hormone levels, mating effort and parasite susceptibility may help us to understand the maintenance of alternative tactics, as it may ultimately contribute to shaping the differential trade-off between individual reproductive success and individual opportunities of survival (see, e.g. in fur seal, *Arctocephalus forsteri*: Negro et al. 2010; Grant's gazelle, *Nanger granti*: Ezenwa et al. 2012).

The Alpine chamois, *Rupicapra rupicapra*, is a nearly monomorphic ungulate, with males weighing 30–40% more than females only at the start of the rut (Garel et al. 2009; Rughetti & Festa-Bianchet 2011), but sharing all other morphological features, with only slight differences. Its mating system is still scarcely understood, although recent studies suggest the adoption of a low-risk strategy by males (Bocci et al. 2010; Rughetti & Festa-Bianchet 2011; Corlatti et al. 2012). Behavioural observations suggest that, during the rut, territorial males patrol a relatively small area in which they try to keep females and chase away intruders, whereas nonterritorial males display following behaviour and territory intrusions (Krämer 1969; von Hardenberg et al. 2000). Given the limited sexual size dimorphism and the rich behavioural repertoire of chamois (Lovari 1985), we suggest that mating effort of territorial and nonterritorial males may be related to different investment in rutting activity, rather than to the differential body and weapon size found in other polygynous ungulates (e.g. Alpine ibex: Willisich & Neuhaus 2010).

In this study we aimed to (1) investigate the temporal variation in mating effort and related physiological traits between AMTs in rutting male chamois, (2) investigate differences in levels of mating effort, hormone secretion and parasite counts between AMTs, and (3) explore the physiological mechanism underlying the expression of mating effort and parasite susceptibility between AMTs. Specifically, we had three hypotheses. (1) The levels of inter- and intra-sexual interactions (i.e. mating effort), hormone metabolites and parasites would peak during the rut in both AMTs. (2) Territorial males would show a greater number of interactions (with both males and females) and higher hormone and parasite levels than nonterritorial males during the rut. Before and after the mating period, AMTs should show smaller differences for each of these variables. (3) The increase in parasite susceptibility, following greater mating effort, would be mediated by testosterone, in accordance with the immunocompetence handicap hypothesis (Folstad & Karter 1992).

METHODS

Study Site and Population

The study site was located in the upper Orco Valley, within the Gran Paradiso National Park (hereafter GPNP, Western Italian Alps, 45°26'30"N, 7°08'30"E) and covered an area of ca. 10 km² between 1800 and 3000 m above sea level. The valley, east to west oriented, is characterized by low precipitation rates (daily mean: 5.4 mm in spring, 7.1 mm in autumn) and average temperatures between -4.2 °C in winter and +9.5 °C in summer (Brambilla et al. 2006). Meadows of coloured fescue, *Festuca varia*, dominate the south-

facing slope, whereas woods of larch, *Larix decidua*, and patches of alder shrubs, *Alnus viridis*, are prevalent on the north-facing slope. The GPNP has been protected since 1922, and the chamois population in the study site shows densities of ca. 20 individuals/km² (L. Corlatti, unpublished data; GPNP census data). Chamois survival in the Park is limited mainly by winter starvation (Peracino & Bassano 1987; Rughetti et al. 2011).

For this study, 19 adult male chamois were darted by the personnel of the GPNP, with a CO₂ injection rifle (DAN-INJECT, model JM Standard) from a mean distance \pm SD of 41.5 \pm 6.1 m. Chamois were darted by surprise (i.e. park wardens used hiding places, mainly rocks, prior to darting). No chase occurred, as this method is not suitable for mountainous, rocky habitats. Whenever the chamois climbed on rocks, the approach immediately stopped, to prevent any potentially harmful situation. The syringes were filled with a combination of xylazine (40 mg/animal) and ketamine (20 mg/animal), with the aim to reduce any negative effects of alpha-agonist on the rumen, heart and breathing activity (Bassano et al. 2004). The mean induction time was 16.9 \pm 12.4 min. The chemical immobilization of chamois allows handling with minimum levels of stress, as opposed to mechanical capture (i.e. using traps and nets), which may have a strong impact on animals.

After sedation had occurred, it took on average 10.0 \pm 6.4 min to reach the animal. We estimated the age of each individual (relative to May 2011) by counting horn notches (Schröder & von Elsner-Schack 1984), collected biometric measurements (e.g. weight, horn length) and monitored physiological parameters (i.e. temperature, heart and breathing activity). Finally, we equipped all chamois with individually recognizable GSM-GPS Pro-Light collars (Vectronic Aerospace GmbH, Berlin, Germany). Ten animals were equipped with 2D collars (630 g, 1.9% of the mean weight, 2.4% of the minimum weight) and nine animals were equipped with 1D collars (450 g, 1.5% of the mean weight, 1.8% of the minimum weight). The mean handling time was 32.2 \pm 16.2 min.

The sedative effect was reversed by an injection of atipamezole (5 mg/animal; Dematteis et al. 2009). The mean recovery time, recorded from the moment of the administration of the antagonist, was 5.4 \pm 2.7 min. During this time, a veterinarian kept the animals under direct observation. The entire procedure, from darting to release, took on average 63.1 \pm 20.0 min, and was always performed with the assistance of a veterinarian.

The behavioural changes following the use of xylazine and ketamine are related to the dose administered to the animal: with the use of 40 mg of xylazine, rapidly antagonized by antagonists (atipamezole), we did not record any changes in social behaviour of Alpine chamois. Similarly, following captures we did not observe alterations in the behaviour of chamois equipped with radiocollars, and to date we have no evidence for long-term effects, as already shown by Nussberger & Ingold (2006).

These methods are in line with the Italian law, as the capture of any wild animal within the GPNP can be made only after receiving authorization from the Italian Ministry of Environment, with the permission of ISPRA (The Institute for Environmental Protection and Research).

Territorial Males Versus Nonterritorial Males

The existing literature lacks unambiguous definitions of territoriality. Maher & Lott (1995, page 1589) reviewed 48 definitions of territoriality in vertebrate species, and suggested that territory could be defined as a 'fixed space from which an individual, or group of mutually tolerant individuals, actively excludes competitors for a specific resource or resources'. This definition includes both spatial and behavioural elements, and was previously used in the same population by von Hardenberg et al. (2000). They showed

that territorial male chamois have small and exclusive home ranges, and a high degree of intrasexual aggressiveness; in addition, territorial males tend to win almost all male–male interactions within their territory. To distinguish between territorial and non-territorial males, we assumed that during the rutting season: (1) territorial males would show a higher degree of site fidelity and hence they should have smaller home ranges than nonterritorial males; and (2) territorial males would win more intrasexual interactions (i.e. they exhibit land ownership) compared to non-territorial males.

In the rutting season 2011 (early November–early December), for 19 males, we therefore calculated the following.

(1) Individual home ranges were calculated using high-quality GPS locations (i.e. with at least four satellites and dilution of precision values lower than 10; Lewis et al. 2007), collected every hour (mean number of fixes \pm SD = 437 ± 127). Violation of data independence is unlikely to affect home range estimation when fixed kernel density (KD) is used (De Solla et al. 1999). With large sample sizes the 90% density threshold is recommended (Börger et al. 2006), provided an appropriate bandwidth (i.e. smoothing parameter) is chosen (Wand & Jones 1995; Kie et al. 2010). To account for this, we calculated the 90% KD in R 2.14.1 (R Development Core Team 2011) as follows: we first represented our study area as a grid and set the resolution of grid cells to 25 m \times 25 m. Using the 'ks' library (Duong 2007) we kernel-smoothed the data for each individual track with the plug-in bandwidth selector ('hpi') developed by Wand & Jones (1995). As a result, each cell was assigned a density of usage (between 0 and 1). Finally, we calculated the number of grid cells within the 0.90 (i.e. 90%) density threshold using the 'GenKern' library to fuzzy match the nearest value to the given threshold, and calculated the home range by multiplying the number of grid cells obtained by the resolution specified (625 m²). The main advantage of this procedure lies in the better performance of plug-in bandwidth selectors over cross-validation selectors (Duong 2007; Chacon & Duong 2010), which might over- or undersmooth data (Kie et al. 2010).

(2) We also calculated the ratio of intrasexual interactions won (IW) as the number of interactions won divided by the total number of interactions. Behavioural data were recorded throughout the mating season, during independent (i.e. not aimed at recording activity budget, see below) hourly sessions of ad libitum observation (Altmann 1974). Each individual was observed for at least 7 h during the rut, with the exception of four animals, which were only observed for 2 h. We considered the focal animal as involved in intrasexual interactions if he displayed direct or indirect forms of aggressiveness (Lovari 1985) towards one or more males. We counted the aggressive interactions displayed by the focal male and if the animal interacted with more males at the same time (e.g. three males) we counted more interactions (i.e. three interactions). We considered the focal male as winner if the antagonist male(s) was chased away or displayed submissive behaviour patterns (Lovari 1985); vice versa, when the focal male was chased away after an interaction, or showed submissive behaviours, he was considered a loser. If none of the rival males displayed submissive behaviours or moved away after interactions had occurred, we considered the interaction had ended in a tie.

To distinguish between territorial and nonterritorial males, in R 2.14.1 we combined individual KD home ranges and IW values (Table 1) in a data matrix and used the Mahalanobis distance (a measure based on correlations between variables, Mahalanobis 1936) as a distance metric to perform multivariate hierarchical clustering (Everitt et al. 2011), using the function `hclust` from the 'stats' library. The main advantage of Mahalanobis distance over Euclidean distance is the possibility to account for correlations in the data set and for objects at different scales (i.e. with unequal

Table 1

90% Fixed kernel density home range, proportion of intrasexual interactions won, total number of interactions and mating behaviour of adult male chamois ($N = 19$) in the GPNP during the 2011 rut

Animal ID	90% Fixed kernel density Home range (ha)	No. of interactions won/total no. of interactions	Total number of interactions	Mating behaviour
M1	4.56	0.93	16	Territorial
M4	3.13	1.00	6	Territorial
M5	12.00	0.88	8	Territorial
M7	237.38	0.00	1	Nonterritorial
M8	16.63	0.00	12	Nonterritorial
M9	23.75	0.08	14	Nonterritorial
M11	7.81	1.00	5	Territorial
M12	13.06	0.38	11	Nonterritorial
M13	2.25	1.00	6	Territorial
M14	3.00	0.90	10	Territorial
M15	16.00	0.00	9	Nonterritorial
M16	5.75	1.00	5	Territorial
M17	4.63	1.00	6	Territorial
M18	16.94	0.50	12	Nonterritorial
M19	13.00	1.00	3	Territorial
M21	290.31	0.00	2	Nonterritorial
M22	75.50	0.46	11	Nonterritorial
M23	598.50	0.00	2	Nonterritorial
M24	8.75	0.25	5	Nonterritorial

variances) returning a standardized, uncorrelated data matrix (Everitt et al. 2011). According to the definition of territoriality given above, territorial males should be clustered as the group with smaller KD home ranges and higher IW values.

We investigated differences in age during the rut, body weight and maximum horn length at capture (Table 2) between territorial and nonterritorial males. Age was compared using the Wilcoxon rank sum test for independent samples. Body weight was analysed by fitting a linear model (LM), using body weight as a response variable, mating behaviour as a predictor, age (in months) and date

Table 2

Date of capture, age (in months, assuming 1 May as date of birth), body weight (in kg) and maximum horn length (in cm) at capture of territorial and nonterritorial male chamois ($N = 19$) in the GPNP

Mating behaviour	Animal ID	Date of capture	Age at capture (months)	Body weight at capture (kg)	Maximum horn length at capture (cm)
Territorial	M1	12 Feb 2010	57	28.5	22.2
	M4	2 Apr 2010	83	30.2	22.5
	M5	12 May 2010	72	29.8	23.0
	M11	10 Nov 2010	90	34.4	22.0
	M13	11 Nov 2010	54	40.8	22.0
	M14	17 Nov 2010	102	31.6	21.5
	M16	23 Nov 2010	78	24.6	19.8
	M17	2 Dec 2010	79	28.4	22.6
	M19	14 Dec 2010	91	30.4	20.5
				Mean = 30.9	Median = 22
			SD = 4.53	QR = 21.5–22.5	
Nonterritorial	M7	31 May 2010	73	25.4	22.0
	M8	01 Jun 2010	61	26.4	22.8
	M9	26 Oct 2010	138	38.6	22.0
	M12	10 Nov 2010	42	32.6	19.5
	M15	17 Nov 2010	78	33.4	24.5
	M18	03 Dec 2010	91	27.8	24.5
	M21	22 Dec 2010	91	26.0	23.3
	M22	22 Dec 2010	55	29.8	21.5
	M23	28 Jan 2011	152	26.0	24.4
	M24	10 Mar 2011	70	28.6	21.1
			Mean = 29.5	Median = 22.4	
			SD = 4.25	QR = 21.6–23.9	

The results of the comparisons of body weights and horn lengths between territorial males and nonterritorial males (estimated through linear models and generalized linear models, respectively) are given in the text.

of capture (transformed in Julian date) as covariates. Horn length was analysed by fitting a generalized linear model (GLM), using horn length as a response variable, mating behaviour as a predictor and age (in months) as a covariate.

Activity Budget, Hormone Metabolites and Faecal Parasite Counts

We carried out data collection over three periods in 2011: prerut (1 October–3 November), rut (4 November–3 December) and postrut (3 December–31 December). In chamois, the first signs of rutting behaviour usually occur in early November (see Lovari & Locati 1991; Pérez-Barbería et al. 1998; Willisch & Ingold 2007). This timing appears rather stable, also in captive chamois (Forthman Quick & Pappas 1986). Moreover, there are physiological indications that the rut occurs in November, that is, when spermatogenesis and the weights of the testis and epididymis reach their maximum levels (Martinez-Pastor et al. 2005).

Activity budget data were collected using scan sampling at 5 min intervals (Altmann 1974) for 2 h (1 h + 1 h) within each period, for each individual. Behavioural categories included foraging, lying down (which typically includes rumination), moving, standing, interactions with males or females (cf. Lovari 1985). In this study, we used only the sum of inter- and intra-sexual interactions (hereafter called 'number of interactions'), as a proxy of mating effort. Between the pre- and postrut 2011 we observed 17–19 marked animals (overall observations of 100 h) with 15 × 60 binoculars and 20–60 × 65 spotting scopes from at least 50 m distance to avoid disturbance. Observations were evenly distributed throughout daylight hours.

We collected faecal samples to measure the concentrations of faecal cortisol (FCM) and androgen (FAM) metabolites, as well as faecal counts of larvae of bronchopulmonary parasites. For each observed individual, we collected one fresh faecal sample per period, for an overall 51 scats. Faecal sampling was evenly distributed throughout each period for both territorial and non-territorial males. Within 10 h from sampling, each scat was divided into two fractions and put in separate plastic bags: the one for FCM and FAM analyses was frozen at –20 °C, and the one for the analysis of faecal counts of parasite larvae was kept refrigerated at +4 °C.

A total of 0.5 g of each well-homogenized faecal sample was extracted with 5 ml aqueous methanol (80%; Palme & Möstl 1997). All samples were analysed in duplicate. FCM and FAM levels were measured with an 11-oxo-aetiocholanolone enzyme immunoassay (EIA) and a testosterone EIA, respectively. Details of the two EIAs, including cross-reactions of the antibodies, are given by Möstl et al. (2002) and Palme & Möstl (1994), respectively. The sensitivity of these methods was 3 and 0.3 pg/well. The intra- and interassay coefficients of variation were 9.7 and 12.5% and 7.9 and 12.9%, for the two assays, respectively. These methods proved suitable for a number of ruminant species (e.g. Möstl et al. 2002; Huber et al. 2003; Hoby et al. 2006; Kleinsasser et al. 2010).

To identify and quantify bronchopulmonary larvae, we used the zinc sulphate flotation method and the McMaster counting procedure (Thienpont et al. 1979). Zinc sulphate solution was diluted at 33% to obtain a specific density of 1200 kg/m³. Two grams of faeces were mixed with the solution and subsequently filtered with a strainer. The two chambers of the McMaster slide were then filled using a Pasteur pipette. Counting, performed using a light-optical microscope at 100× magnification, started 5 min after loading the slide. We summed all the counted larvae, and the sum was multiplied by 50 to obtain the number of larvae per gram of faeces. The multiplication factor depends on the dilution of the faecal sample and on the area of the McMaster slide.

Mating Effort, FAM, FCM Levels and Faecal Parasite Counts (Hypothesis 1)

To investigate the variation in the number of interactions and in the faecal counts of parasite larvae over the three periods, we fitted generalized linear mixed models using the function `glmmPQL` ('MASS' library) separately for territorial and nonterritorial males, assuming a Poisson distribution corrected for overdispersion (as the residual deviance of each model was greater than its residual degrees of freedom). The number of interactions and the faecal counts of parasite larvae were separately set as response variables, the time period was used as a categorical predictor and the age as a covariate (i.e. the models fitted for territorial and nonterritorial males were: number of interactions ~ time period + age; faecal counts of parasite larvae ~ time period + age). We accounted for autocorrelation over different periods by setting animal identity as a random factor.

To investigate the variation in FAM and FCM levels over the three periods, for territorial and nonterritorial males we fitted linear mixed-effect models (LME) using the function `lmer` ('lme4' library) using FAM and FCM levels, separately, as response variables. The time period was set as a categorical predictor and we used age as a covariate (i.e. the models fitted for territorial and nonterritorial males were: FAM ~ time period + age; FCM ~ time period + age). We accounted for autocorrelation over different periods by setting animal identity as a random factor. Prior to analysis, FAM and FCM were log transformed and the assumption of normality was tested using the Shapiro–Wilk test (FAM levels: territorial males: $W = 0.962$, $P = 0.404$; nonterritorial males: $W = 0.946$, $P = 0.168$. FCM levels: territorial males: $W = 0.979$, $P = 0.842$; nonterritorial males: $W = 0.949$, $P = 0.199$).

Finally, for each of the eight fitted models, we ran a post hoc test, using the `glht` function (with user-defined contrasts) from the 'multcomp' library, to compare each period with every other period in a pairwise manner. The `glht` function provides a convenient framework to test multiple hypotheses in several parametric models, including linear and nonlinear mixed-effect models (Bretz et al. 2011).

Territorial Versus Nonterritorial Males Within Periods (Hypothesis 2)

To test for differences between territorial and nonterritorial males, within each period we used the Wilcoxon rank sum test for independent samples for the number of interactions and the faecal counts of parasite larvae, and the Student's *t* test for FAM and FCM levels. Following the design of hypothesis 2, for the pre- and postrut we used two-tailed tests, whereas for the rut we used one-tailed tests.

Effect of FAM on Mating Effort and Faecal Parasite Counts (Hypothesis 3)

We used confirmatory path analysis (Shipley 2000a) to test prespecified hypotheses for the causal relationships among our variables. Specifically we wished to test the hypothesis that FAM directly influences the number of interactions and faecal counts of lungworm larvae, thus mediating the effect of AMTs. Standard path analysis methods, such as those implemented in structural equation models (SEM), compare the observed covariance matrix with the covariance matrix predicted by the tested causal model. SEM, however, cannot deal with data having a hierarchical structure, such as the data in the present study, owing to the repeated measurements conducted on the same individuals. We therefore used the *d*-sep test, developed by Shipley (2000b), which tests the

conditional probabilistic independences implied in the directed acyclic graph of the hypothesized causal model and which can be easily generalized to deal with data having an underlying hierarchical or multilevel structure (Shipley 2009). To perform the d-sep test using R 2.14.1 (R Development Core Team 2011) we followed the instructions provided by Shipley (2009) to which we refer readers for more details. To test in R the single conditional independencies implied by the models, we used linear mixed models using the function `lme` ('nlme' library) and generalized linear mixed models using the function `glmmPQL` ('MASS' library) according to the nature of the dependent variable in the test. An overall test of the fit of the causal model is provided by the Fisher *C* statistic, which combines the probabilities of all independence claims implied by the model and which follows a chi-square distribution with $2k$ degrees of freedom (where k is the number of independence tests in the model). The path model is thus considered to fit the data when the *C* value is not significant ($P > 0.05$; Shipley 2000a, 2004).

All statistical analyses were performed using R 2.14.1. The significance level P was set at 0.05. All graphs refer to untransformed data, except for the number of interactions, which was transformed to percentage to facilitate interpretation.

RESULTS

Territorial Males Versus Nonterritorial Males

The cluster analysis confirmed the grouping of nine ($N = 9$) males with very small home ranges and very high values of IW (Fig. 1, Table 1). The territoriality hypothesis for these individuals is therefore strongly supported from both the spatial and behavioural viewpoints. Territorial males occupied exclusive (i.e. nonoverlapping) home ranges in the lower part of the study site; their median home range was 4.63 ha (quartiles range [QR]: 3.13–7.81), much smaller than nonterritorial males' ($N = 10$) median home range,

20.34 ha (QR: 16.16–196.91). The IW value of territorial males (median = 1; QR: 0.93–1) was higher than that of nonterritorial males (median = 0.04; QR: 0–0.34). Territorial and nonterritorial males did not show substantial differences in median age: 7 years for both groups (QR territorial males (T): 7–8; QR nonterritorial males (NT): 6–8; Wilcoxon rank sum test: $W = 48.5$, $N_{NT} = 10$, $N_T = 9$, $P = 0.803$). Body weight did not differ between territorial males and nonterritorial males (LM: $t_{15} = 0.707$, $P = 0.491$; Table 2). The length of the longest horn measured at capture also did not differ between territorial and nonterritorial males (GLM: $z_{16} = -0.281$, $P = 0.779$; Table 2).

Mating Effort, FAM, FCM Levels and Faecal Parasite Counts (Hypothesis 1)

In territorial males, the number of interactions drastically increased from the prerut to the rut, and subsequently dropped in the postrut (Fig. 2a, Table 3). FAM and FCM levels, as well as faecal counts of parasite larvae, followed the same pattern (Fig. 2b, c, d), albeit FAM levels and faecal counts of parasite larvae remained significantly higher in the postrut than in the prerut (Table 3).

Nonterritorial males constantly increased their investment in inter- and intrasexual interactions over the time periods, although the difference was nearly significant only between the pre- and the postrut (Fig. 2a, Table 3). FAM levels were significantly higher during the rut, but FCM levels did not differ between periods (Fig. 2b, c, Table 3). Faecal counts of parasite larvae were significantly lower in the prerut (Fig. 2d, Table 3).

Territorial Versus Nonterritorial Males Within Periods (Hypothesis 2)

Territorial and nonterritorial males did not show differences in the number of interactions before the rut (two-tailed Wilcoxon rank sum test: $W = 29$, $N_{NT} = 8$, $N_T = 9$, $P = 0.464$). During the

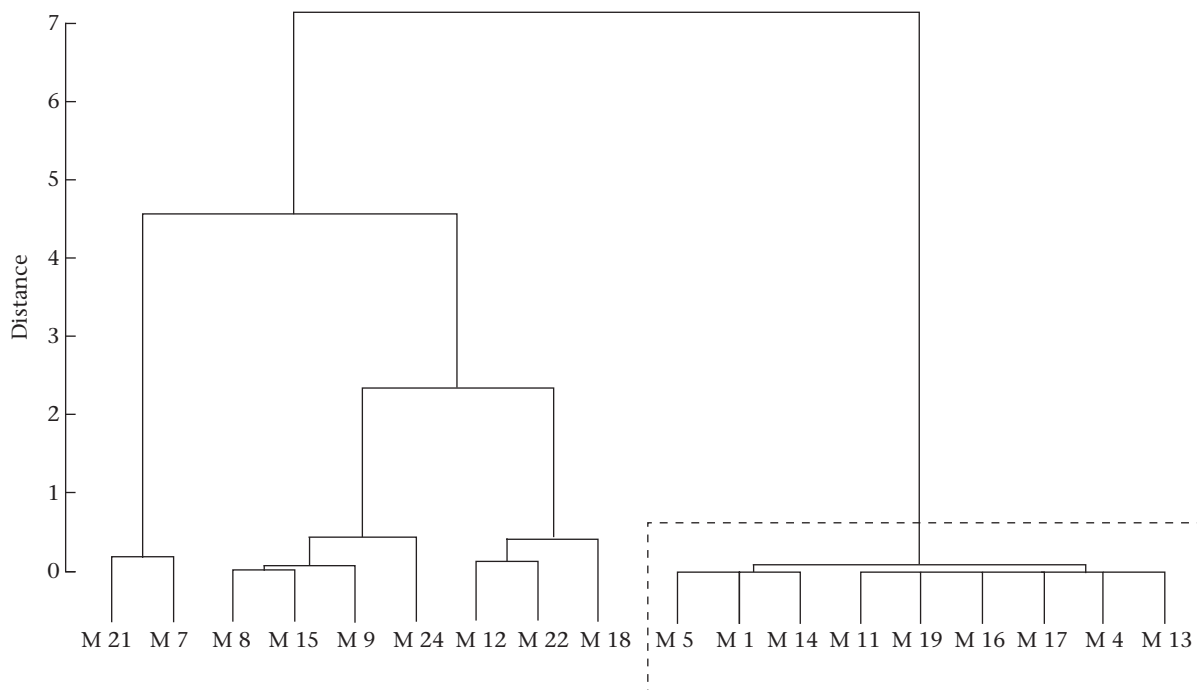


Figure 1. Dendrogram of the hierarchical clustering of male chamois ($N = 19$) marked in the GPNP, based on Mahalanobis distance using home range size and proportion of intrasexual interactions won during the 2011 rut. The dashed rectangle includes individuals labelled as territorial males. One male (M23) is not represented in the dendrogram because its distance was well above 7: given its large, undefended home range (Table 1), it was labelled as nonterritorial.

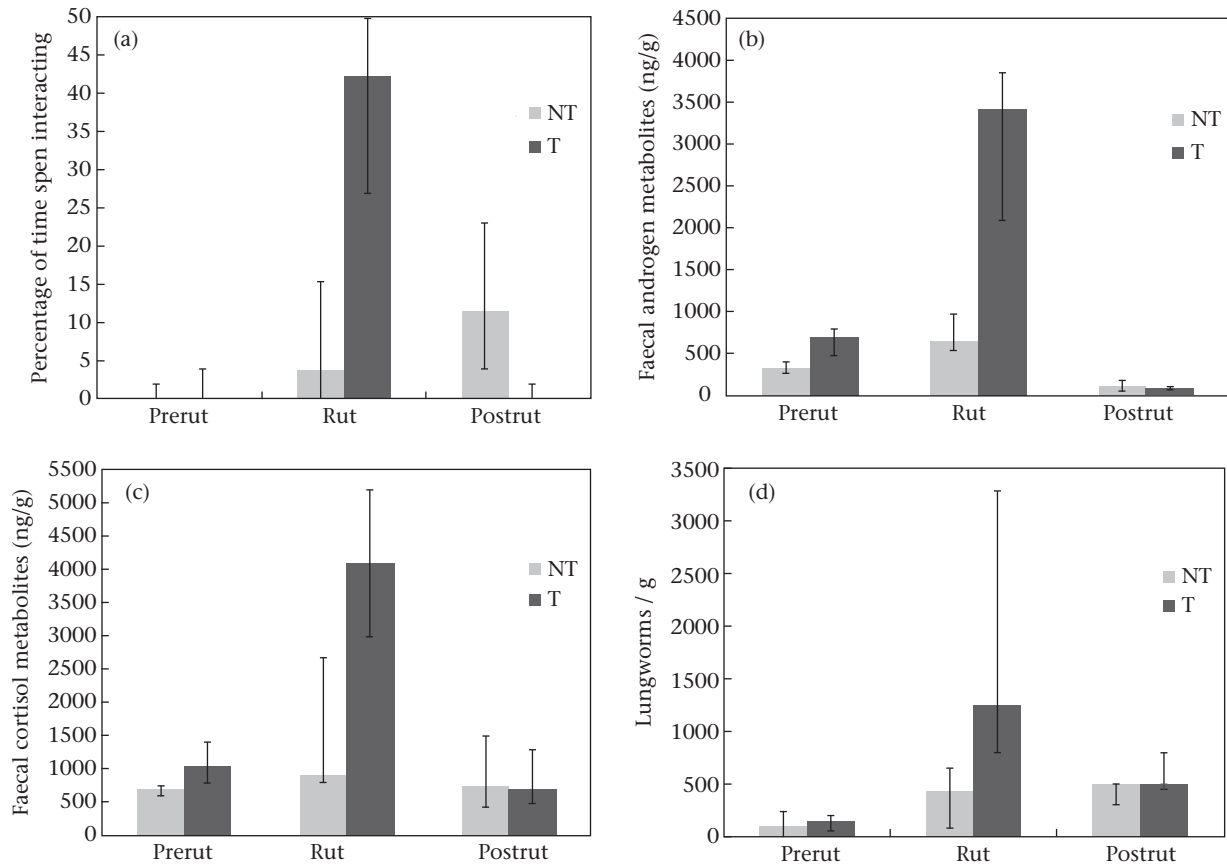


Figure 2. (a) Percentage of time spent interacting, (b) levels of faecal androgen metabolites (ng/g), (c) levels of faecal cortisol metabolites (ng/g), and (d) faecal counts of parasite larvae (number of lungworms/g) of territorial (T) and nonterritorial (NT) male chamois from the pre- to the postrut 2011 in the GPNP. Values are medians \pm interquartile ranges.

rutting period, territorial males interacted significantly more often (one-tailed; $W = 2$, $N_{NT} = 9$, $N_T = 9$, $P < 0.001$) than nonterritorial males, whereas nonterritorial males interacted more often than territorial males in the postrut (two-tailed; $W = 61$, $N_{NT} = 9$, $N_T = 8$, $P = 0.014$; Fig. 2a). FAM levels were significantly higher in territorial males in the prerut (two-tailed Student's t test: $t_{14,334} = -3.647$,

$P = 0.003$). The comparison between FAM levels did not show significant differences between AMTs during the rut (one-tailed; $t_{14,355} = -1.320$, $P = 0.104$), yet we found evidence for a medium–large effect size (Cohen's $d = 0.7$, 95% CI = 0.4–0.9; Nakagawa & Cuthill 2007). FAM levels were similar in both male types in the postrut (two-tailed; $t_{14,371} = 0.272$, $P = 0.789$). The level of FCM

Table 3

Post hoc multiple comparisons for models analysing temporal variations in number of interactions, faecal counts of lungworm larvae (estimated through generalized linear mixed models), faecal androgen (FAM) and cortisol (FCM) metabolites (estimated through linear mixed effect models) in territorial ($N = 9$) and nonterritorial ($N = 10$) male chamois, in the GPNP, in 2011

Mating tactic	Between-periods comparison		Interactions	FAM	FCM	Lungworms
Territorial males	Prerut/Rut	Estimate	-1.365	-0.469	-0.530	-2.570
		Z	-2.862	-2.458	-3.052	-5.429
		P	0.011	0.037	0.007	<0.001
	Prerut/Postrut	Estimate	1.456	0.869	0.152	-1.412
		Z	1.420	4.560	0.874	-2.775
		P	0.315	<0.001	0.657	0.014
	Rut/Postrut	Estimate	2.822	1.338	0.681	1.158
		Z	2.947	7.018	3.926	4.484
		P	0.008	<0.001	<0.001	<0.001
Nonterritorial males	Prerut/Rut	Estimate	-1.001	-0.422	-0.254	-0.896
		Z	-1.314	-2.580	-1.724	-2.334
		P	0.379	0.027	0.196	0.050
	Prerut/Postrut	Estimate	-1.613	0.533	-0.019	-1.153
		Z	-2.252	3.358	-0.130	-3.172
		P	0.060	0.002	0.991	0.004
	Rut/Postrut	Estimate	0.612	0.955	0.236	-0.256
		Z	1.296	5.694	1.560	-0.883
		P	0.389	<0.001	0.263	0.648

Significant results are shown in bold.

(Fig. 2c) was significantly higher in territorial males during the rut (one-tailed; $t_{14.981} = -2.135$, $P = 0.025$), whereas FCM levels did not differ before (two-tailed; $t_{15.904} = -1.121$, $P = 0.279$) and after (two-tailed; $t_{14.849} = 0.237$, $P = 0.816$) the rut. Similarly, lungworm burdens did not differ between male types in the prerut (two-tailed; $W = 40.5$, $N_{NT} = 10$, $N_T = 9$, $P = 0.739$) and postrut (two-tailed; $W = 31.5$, $N_{NT} = 9$, $N_T = 9$, $P = 0.448$), but were significantly greater for territorial males during the rut (one-tailed; $W = 11.5$, $N_{NT} = 8$, $N_T = 9$, $P = 0.010$; Fig. 2d).

Effect of FAM on Mating Effort and Faecal Parasite Counts (Hypothesis 3)

The best fitting path analysis model is represented as a directed acyclic graph in Fig. 3. In this model, time period is a causal parent of FAM, FCM, mating effort and faecal counts of parasite larvae. FAM is a causal parent of FEC, ME and FCM, while it depends directly on the alternative mating tactic (AMT). This model is not rejected by the data ($C = 16.653$, $P = 0.275$) and thus it can be accepted as a plausible explanation of the cause–effect relationships among the variables. This model suggests that the effect of alternative mating tactics on mating effort and level of parasite infection is mediated through the effect of FAM. While territorial behaviour, through the mediation of FAM, increases the number of social interactions (a proxy of increased mating opportunities), FAM directly increases the level of parasite infection (measured as FEC) and the level of stress measured as FCM.

DISCUSSION

Territorial males sharply increased mating effort, faecal androgen and cortisol metabolites and parasite levels during the rut, whereas nonterritorial males displayed a similar pattern only for faecal hormone metabolites. During the rut, territorial males had higher reproductive effort, higher levels of faecal hormone metabolites and greater faecal counts of parasite larvae than nonterritorial males. Before and after the rut, differences between males were smaller. A path analysis model suggests that the increase in mating effort and faecal counts of larvae of bronchopulmonary parasites were mediated by faecal androgen metabolite secretion.

Territorial Males Versus Nonterritorial Males

To date, it has been unclear whether the nonterritorial mating tactic would be limited exclusively to the extreme age classes.

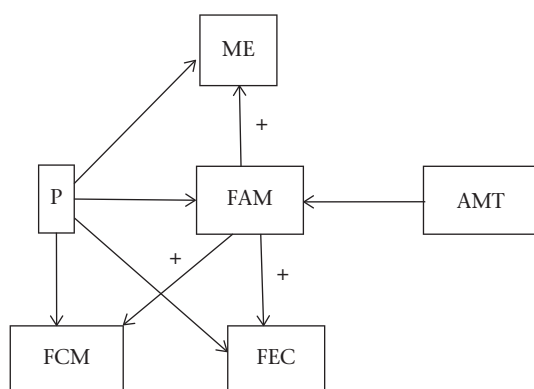


Figure 3. Directed acyclic graph of the path analysis model depicting the proposed causal links among alternative mating tactics (AMT, i.e. territorial or nonterritorial), faecal androgen metabolites (FAM), faecal cortisol metabolites (FCM), faecal counts of parasite larvae (FEC) and period of the year (P). The sign next to the arrow linking two variables indicates the direction of the relationship.

While it is likely that very young and very old males would be excluded from the competition for land ownership, for both social and physiological reasons (Bubenik & Schwab 1975), we showed that the nonterritorial tactic is also widespread in adult individuals. Territorial males can be as young as 5.5 years (M13) and they may hold a territory at least until the age of 9.5 (M14). This is not surprising, as male Alpine chamois are fully grown when they are 4.5 years old, and their decline in body weight begins after a further 5–6 years (Bassano et al. 2003; Garel et al. 2009). The early onset of territoriality may be indirectly supported by our preliminary results on individual reproductive success (M16 and M17 successfully sired kids when they were 5.5 years old; L. Corlatti, unpublished data). Territorial males' median home range was in line with von Hardenberg et al. (2000; mean 95% MCP: 3.3 ha; SE \pm 0.9) and the median IW value of territorial males, too, was similar to that found by von Hardenberg et al. (2000; 95.3% of interactions won). The lack of morphological differences (i.e. body weight and horn length) between AMTs may suggest a weak pressure of sexual selection, compared to other species (e.g. Alpine ibex), which in turn would support a weak level of polygyny in Alpine chamois, as already hypothesized by Bocci et al. (2010), Rughetti & Festa-Bianchet (2011) and Corlatti et al. (2012).

Mating Effort, FAM, FCM Levels and Faecal Parasite Counts

When females are aggregated, the temporal distribution of the oestrus is likely to influence the intensity of rutting activity (Emlen & Oring 1977; Ims 1989). Territorial males invested in mating activities almost exclusively during the rut, whereas nonterritorial males interacted more often in the postrut. Most female chamois synchronize their oestrus over a few days in the second half of November (Krämer 1969). During this time, territorial male chamois therefore increased the intensity of rutting behaviours to ensure access to females. This may have precluded access to females by nonterritorial males, which, in turn, may have concentrated their reproductive effort afterwards, when territorial males stopped rutting and a small number of females might still be receptive.

FAM levels significantly increased during the rut for both AMTs. This pattern is in line with the sexual cycle of chamois and other artiodactyls (Mooring et al. 2004; Hoby et al. 2006), and it is unlikely to be the result of a dilution effect caused by seasonal variations in nutritional strategies (Klasing 2005), such as rut-induced hypophagia (Willisch & Ingold 2007). During the rut, territorial males' feeding rate was similar to that registered in spring, whereas nonterritorial males' feeding rate was similar to that registered in the prerut (L. Corlatti, personal observation); despite the similarities in feeding rates between the mating season and previous periods, FAM levels in the rut were clearly higher for both male types.

The relationship between FCM levels and the biological cycle of ungulates is somewhat controversial. Although some studies suggest that the onset of the rutting season may trigger the peak of glucocorticoid secretion (Creel 2001; Mooring et al. 2006), several ungulate species do not show this pattern (e.g. white-tailed deer, *Odocoileus virginianus*; Bubenik et al. 1983; chital deer, *Axis axis*; Chapple et al. 1991; red deer, *Cervus elaphus*; Huber et al. 2003; Pyrenean chamois, *Rupicapra pyrenaica*; Dalmau et al. 2007). These latter studies, however, did not take into account potential differences between alternative male tactics. In our population, the level of FCM increased during the rut only in territorial males: if all males were considered, the effect of the rutting season would be diluted, possibly leading to undetectable variations. The peak of FCM levels for territorial males is unlikely to be caused by reduced nutritional intake (for the same reasons seen above for FAM levels).

Parasite burden showed a peak during the rut only in territorial males. This trend is in line with the pattern of FAM and FCM, as already shown by Hoby et al. (2006), and suggests that, if variation in parasite burdens was due to immunosuppression (Folstad & Karter 1992), this effect may be marked in territorial males. Faecal counts of parasite larvae in nonterritorial males, on the other hand, may be largely influenced by the lungworm seasonal cycle, which shows a peak during the autumn months (Štefančíková 1994; Štefančíková et al. 2011).

Territorial Versus Nonterritorial Males Within Periods

The mating effort of territorial males was greater than that of nonterritorial males during the mating period. The territorial strategy entails the maintenance of a mating ground long before the rut (i.e. from May to June, von Hardenberg et al. 2000); such an investment of energy suggests potentially higher mating benefits (i.e. greater access to females) during the mating season. This hypothesis is supported by our results on mating success, as all observed matings occurred between 16 and 25 November, and were monopolized by territorial males (L. Corlatti, unpublished data).

Territorial males had higher levels of FAM in the prerut, possibly related to the early occupancy of territories (von Hardenberg et al. 2000). Dominant males usually have a higher level of androgen metabolites than subordinate males during the rut (Mooring et al. 2004; Oliveira et al. 2008). In our population, territorial and nonterritorial chamois did not show significant differences, but a clear trend for higher levels of FAM in territorial males during the rut.

FCM levels during the rut were significantly higher in territorial males. Although it has been often argued that social subordinates would show elevated levels of cortisol metabolites, there is increasing evidence that dominant individuals have high glucocorticoid levels more often than do subordinates (Creel 2001; Mooring et al. 2006; Oliveira et al. 2008). Our results are consistent with the 'stress of domination' hypothesis: individuals engaged in energetically expensive behaviours are more stressed than subordinate individuals (Mooring et al. 2006). Our path analysis model, which highlights a direct causal link between FAM and FCM, supports this hypothesis. In the long term, several weeks of high FCM levels may lead to overall reduced survival (Sapolsky 1992; Möstl & Palme 2002).

The infections caused by protostrongylid species are among the most common diseases in wild chamois populations. Pulmonary nematodes may activate bacterial pneumonia, a common cause of mortality in Alpine chamois (Richomme et al. 2006). As such, the greater parasite infections observed during the rut in territorial males, compared to nonterritorial males, may be an important cost in terms of overwinter survival, also considering the high mass loss that occurs during the rut (Rughetti & Festa-Bianchet 2011).

Effect of FAM on Mating Effort and Faecal Parasite Counts

Androgens are widely known to be mediators of aggressive and courtship behaviours (Knapp 2003; Hirschenhauser & Oliveira 2006), while simultaneously suppressing the immune function (Folstad & Karter 1992). Several studies found a positive correlation between reproductive effort and parasitism in various taxa (Deerenberg et al. 1997; Nordling et al. 1998; Pelletier et al. 2005), but the causal relationships among behavioural, endocrine and health variables remain unclear.

Our analysis confirmed the existence of a life history trade-off among reproductive opportunities on one side and metabolic stress and increased level of parasite infection on the other; moreover, we showed how the mechanism underlying such

a trade-off is mediated by androgens. As far as the expression of AMTs normally involves hormonal regulation, AMTs may entail different trade-offs between opportunities to invest in reproduction and in survival.

Conclusions

Our results suggest that, for the given mating season, variations in mating effort are positively associated with variations in physiological costs and that such a trade-off is mediated by androgens. They also suggest that AMTs, in rutting male chamois, may have different costs and benefits. Territorial males invest more in rutting activities, potentially gaining mating benefits, but may pay several costs, owing to greater energy consumption, higher levels of hormone metabolites and greater parasite susceptibility. On the other hand, nonterritorial males, which invest less in rutting, may benefit from saving energy, having lower levels of hormone metabolites and fewer lungworms.

Different strategies of energy allocation during the rut may eventually lead to different survival probabilities (Garel et al. 2011). The estimation of mortality rates, however, requires long-term studies and large sample sizes (see Corlatti et al. 2012), which we were unable to record. Furthermore, our results do not allow us to investigate whether a tactic is a short-term strategy or a fixed characteristic of a given individual. AMTs that diverge only in behavioural traits should be more flexible than AMTs that imply morphological variations (Taborsky et al. 2008). In that context, it may be possible that (owing to the high cost of territoriality) some males might breed in alternate years. Such year skipping is recognized in other species (e.g. water pythons, *Liasis fuscus*: Madsen & Shine 1999; Alpine ibex: Toïgo et al. 2002). Moreover, Lovari et al. (2006) showed the occurrence of alternative strategies of space use in male chamois (i.e. resident and migrant), possibly associated with AMTs. In the study area, snowfalls tend to move females to lower altitudes at the peak of the rut, where resident, mostly territorial males would monopolize mating. When snowfalls are late, nonterritorial males would have an advantage. Yearly variations in individual status and meteorological conditions may therefore lead to different strategies of energy allocation in AMTs.

To understand whether such clues to life history trade-offs may result in different lifetime fitness trade-offs, long-term investigations of marked individuals are needed. Studies on survival, reproductive success, flexibility and frequency dependence may eventually help disclose the mechanisms underlying the maintenance of AMTs in Alpine chamois.

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collected data in the field, carried out the parasitological and (part of) the hormone analyses and participated in writing up this paper. A.v.H. did the confirmatory path analysis and participated in writing up this paper. B.B. participated in the capture of all animals and in writing up this paper. R.P. supervised the hormonal analyses and participated in writing up this paper. S.L. supervised all stages of this work, took part in the data collection, and participated in the preparation of all drafts of the manuscript.

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