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FEMALE COMPETITION AND THE ROLE OF TESTOSTERONE IN A

POLYGYNOUS SYSTEM

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ABSTRACT

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FEMALE COMPETITION AND THE ROLE OF TESTOSTERONE IN A POLYGYNOUS SYSTEM

Chairperson: Dr. Vanessa Ezenwa

Testosterone plays an important role in male competitive ability, and it may play a similar function in females. Female-female competition for resources is often associated with high testosterone, both within females and in comparison to males. Positive associations between testosterone and competition are most likely to be seen in systems with high female-female competition. Resource defense polygyny may be one such system since females congregate on high-quality nutrient sources, leading to substantial opportunities for interference competition. I studied female Grant's gazelle (*Nanger granti*), a classic resource defense polygynous species, to investigate female competition and its relationship to testosterone. I found substantial evidence for competition between females. Females frequently engaged in agonistic behavior, on average initiating over two agonistic bouts per hour, and higher-ranking females initiated more agonism than lower-ranking females. Testosterone appeared to play an important role in competitive ability. Immunoreactive fecal testosterone metabolites (fT) were strongly positively correlated to both dominance rank and number of agonistic bouts initiated per hour, but only agonism was associated with fT when dominance and agonism were accounted for simultaneously. Females had similar fT concentrations as males. fT was positively associated with immunoreactive fecal glucocorticoid metabolites (fGCM), and this relationship was not due to associations between dominance and fGCM. Month also influenced testosterone physiology. Females had lower fT during a drought than after rains had begun. In addition, fT increased more from August to November in high-ranking females than in low-ranking females. My results suggest that female-female competition may be substantial in resource defense polygynous systems. Testosterone might play an important role in mediating competitive ability in females, but it was also associated with higher glucocorticoid secretion. Future studies on females would be well served to investigate the consequences of testosterone secretion and how they relate to female competition.

ACKNOWLEDGEMENTS

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Introduction

Androgens play an important role in male competition (Hirschenhauser and Oliveira 2006), and they may serve a similar function in females. In males, androgens are associated with a suite of physiological and behavioral traits that increase competitive ability, including muscle hypertrophy, aggression, reproductive behavior, and territory defense (Wingfield *et al.* 2001). In females physiological effects of androgens appear broadly similar, as they are associated with muscle hypertrophy, female-female aggression, sexual behavior, and reproductive status (reviewed in Staub and De Beer 1997). Androgens play a particularly important role in shaping aggressive behavior. Androgens generally tend to make future aggressive bouts more intense, but usually do not cause increases in the frequency of aggression. Observed relationships between androgens and aggression are thus generally a result of aggression increasing androgens rather than vice versa (Sapolksy 1998). Nonetheless, if behavioral effects of androgens are similar in males and females, there may be predictable associations between testosterone and female behavior.

Androgens positively associate with female aggression in many settings (Gill *et al.* 2007). Androgens can both increase in response to aggression and have activational effects on aggression in females, leading to positive associations between testosterone and aggression (Albert *et al.* 1992; Staub and De Beer 1997). Two lines of evidence suggest that androgens play an important role in female-female agonism over competition for resources: variation in androgens among females, and male-female androgen ratios. Among females, maximum testosterone levels are higher in avian social systems where female-female competition is more intense (Ketterson *et al.* 2005). In addition, dominance rank is positively associated with androgens among females in several mammals (Clarke and Faulkes 1997; Beehner *et al.* 2005; Dloniak *et al.* 2006; Shargal *et al.* 2008). Male:female androgen ratios can also be a marker of female competition, which was first noted in studies of phylogenetically closely related species (Sannen *et al.* 2003). Female bonobos (*Pan paniscus*) are more aggressive and hold higher dominance rank than female chimpanzees (*Pan troglodytes*), and the male:female testosterone ratio is lower (i.e. closer to 1) in bonobos than in chimpanzees (Sannen *et al.* 2003). Similar

results have been found in comparisons of female-dominant spotted hyenas (*Crocuta crocuta*) to male-dominant brown hyenas (*Hyaena brunnae*) and striped hyenas (*Hyaena hyaena*; Racey and Skinner 1979; van Jaarsveld and Skinner 1987). Across mammals, males typically have androgen levels 2-6 times higher than females in species with relatively little female competition (Rasmussen *et al.* 1984; Holekamp and Talamantes 1991; Shargal *et al.* 2008). However, in mammals where female competition is intense females can have higher androgen levels than males (rock hyrax *Procavia capensis* Koren *et al.* 2006; spotted hyenas *Crocuta crocuta* Goymann *et al.* 2001).

Positive associations between androgens and female behavior are most likely to be seen in social systems where female intrasexual competition is intense. If higher dominance rank is associated with higher fitness, as is the case in many female mammals (Clutton-Brock *et al.* 1986; Ellis 1995; Woodroffe and MacDonald 1995), then mechanisms that increase competitive ability should have evolved in females. Androgens mediate competitive ability in males (Hirschenhauser and Oliveira 2006), and if androgens serve a similar function in females then natural selection might favor females with higher androgens and thus higher dominance in systems where female competition is intense. Resource defense polygyny may be one such system. In resource defense polygynous systems females congregate on areas with high quality resources, and males defend these areas to monopolize the females accessing those resources (Emlen and Oring 1977). Since females congregate at clumped nutrient resources, there is substantial opportunity for interference competition (Reiter *et al.* 1981). I expect female behavior in these systems to be punctuated by bouts of agonism, and for androgens to associate with dominance and agonism.

Although androgens have beneficial effects on competitive ability, in males androgens are associated with a number of factors that decrease long-term fitness. Similar associations may be prevalent in females. In males, testosterone is associated with increased energy expenditure, increased injury risk, and decreased immune function (reviewed in Wingfield *et al.* 2001). Testosterone might have similar consequences in females. One factor in particular that testosterone may influence is glucocorticoid secretion. Glucocorticoids are steroid hormones typically associated with stress and physical activity. When secreted at elevated levels for prolonged periods, they have a

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variety of detrimental effects on immune function, reproductive behavior, and stored energy reserves (Sapolsky *et al.* 2000). Field studies indicate that testosterone often positively associates with glucocorticoids (Duffy *et al.* 2000; Muehlenbein 2006; Zysling *et al.* 2006; Koren and Geffen 2009). Such an association might be a consequence of testosterone increasing energetically expensive behavior and physiological processes that in turn increase glucocorticoid secretion. However, the positive associations between testosterone and glucocorticoids contrast with laboratory studies indicating that testosterone inhibits glucocorticoid secretion in response to stressors (Viau 2002; Seale *et al.* 2004). Lab studies have generally not accounted for changes in behavior (such as aggression) that may result from testosterone – changes that could lead to increased activity and/or stress and thus stimulate glucocorticoid secretion.

Here I investigate relationships between behavior and testosterone in female Grant's gazelle (*Nanger granti*). Grant's gazelle exhibit classic resource defense polygyny, where females form groups that move between male territories (Walther *et al.* 1983). Female competitive ability has a strong effect on fitness in several ungulates (Clutton-Brock *et al.* 1986; Côté and Festa-Bianchet 2001). Competitive ability may be particularly important for Grant's gazelle since females congregate on patchy, high-quality nutrient resources that likely are monopolizable, potentially leading to high rates of agonistic behavior. I tested whether: 1) The male:female testosterone ratio is at the lower end of the reported range; 2) Females engaged in agonistic behavior; 3) Testosterone is positively correlated with female competitive behavior, specifically dominance and agonistic behavior; and 4) Testosterone is associated with higher glucocorticoids.

Methods

Study site and population

I studied Grant's gazelle at Mpala Research Center (MRC; $0°17'$N, $37°52'$ E, 1800m ASL) in the Laikipia District of central Kenya. Rainfall at MRC averages 500-600 mm/year and typically falls in a trimodal fashion with peaks occurring April-May, August, and October. Vegetation consists of semi-arid savannah with a mix of grassland

and *Acacia* bushland, and the area is interspersed with open glades of one to several hectares dominated by short grasses. *Acacia drepanolobium* and *Acacia mellifera* are the dominant tree species, and the 22,000ha area encompasses both black cotton vertisols and red rocky friable soils. MRC supports 21 species of native ungulates, several species of domestic livestock, and a full suite of native carnivores (Ezenwa 2003; Ogada *et al.* 2003). In 2008-2009, Laikipia experienced a 10-month drought from December 2008-September 2009. Rainfall during that 10-month span totaled 88mm, compared to 147mm in the two months October-November 2009 after the rains began (MRC unpublished data).

Grant's gazelle are selective browsers and grazers frequenting grassland, bushland, and open woodland (Spinage *et al.* 1980). Group size usually ranges from 6-20 animals, with larger groups generally seen in more open habitats. Group composition is relatively fluid, and females can freely join and leave groups (Walther 1972). Males defend high-quality areas to maximize reproduction. There is considerable sexual dimorphism, and males generally weigh about 50 percent more than females (Estes 1991). Breeding occurs year-round, but there are peaks during the rainy seasons (Walther *et al.* 1983). Grant's gazelle are arid-adapted and fairly drought tolerant (Estes 1991), but at the study site some individuals showed marked improvements in body condition from August 2009 (drought) to November 2009 (rainy season), suggesting that resource conditions improved with the onset of the rains (Ezenwa unpublished data). Data collected in August 2009 therefore reflect conditions during the drought when resources were more limited, while November 2009 samples reflect improved resource conditions.

Behavioral and fecal sampling

Grant's gazelle were captured using drive-nets in January-February 2009 and helicopter net-gunning in August 2009 (Kock *et al.* 1987). For drive-netting, 200-300m long drive-nets were set up along natural escape routes, and animals were driven into the nets by vehicle. Upon capture, each animal was tagged with a unique set of color ear-tags, standard body measurements and weight were recorded, and a fecal sample was collected. Age was estimated using a combination of tooth wear measurements and the relationship between horn size and age (Spinage 1976; Stelfox *et al.* 1985). To evaluate

tooth wear on live female gazelle, a subset of animals were sedated ($N = 9$) and an impression of the upper molar was taken using dental silicon. Tooth impressions were used to classify age according to criteria described in Stelfox *et al*. (1985). The relationship between tooth wear and mean horn length was used to estimate age for all additional females (Ezenwa, unpublished data). Pregnancy was assessed in all females by abdominal palpation, which reliably detected late-term pregnancies but likely missed earlier pregnancies (Pratt and Hopkins 1975). Lactation was assessed by manual milking of the teats. Handling time for each animal (females and males) averaged 23 minutes (range: 11-51 min). A total of 34 males and 26 females were captured in August 2009, and fecal samples from this period were used for male-female comparisons to avoid confounding seasonal effects.

Focal behavioral observations were collected on 12 tagged adult females during November 2009. At the beginning of each observation I recorded group size, group composition, other ungulate species present in the group, date and time, and a GPS location. I collected behavioral data using continuous focal sampling (Martin and Bateson 1993). Behavioral samples were targeted to last 60 minutes, but were ended early if the focal subject went out of sight for 10 minutes. To increase the number of individuals sampled, I ended samples after 30 minutes if I observed 2 or more bouts of agonism initiated by the focal animal during the first 30 minutes. Samples lasting less than 10 minutes were excluded from all analyses. Behavioral data were recorded using a hand-held digital voice recorder and subsequently scored into JWatcher (Blumstein and Daniel 2007). Behavior was scored as feeding, moving, agonistic, subordination, reproductive (e.g. copulating, receiving courtship displays, mounting), grooming, or vigilant. Agonism was scored to include chasing, fighting, vegetation horning, threats, and supplants. I stratified behavioral sampling by time of day including: early morning (0600-0900), late morning (0900-1200), early afternoon (1200-1500), and late afternoon (1500-1800). Focal observations were spaced evenly across these periods for each focal subject, and focal subjects were never sampled more than once in a given time period on any day. Females were sampled multiple times in November for both behavior and feces, and since these data were collected over a short time frame (~4 weeks), I averaged all fecal hormone values for each individual and used those averages in all statistical tests. The

rate at which each animal initiated agonism was calculated from the total number of agonistic events observed during focal samples divided by the total focal sampling time for that individual. To account for the effect of group size on agonistic behavior, for each focal animal I calculated mean group size by averaging the total number of adult and subadult females seen in the group in each focal sample. Analyses on the relationships between dominance, behavior, and hormone concentrations were done on November 2009 behavioral and fecal samples to match behavior to hormone concentrations.

To quantify agonism I recorded all instances of agonism involving tagged females during and outside of focal samples, noting which animal initiated the interaction and which animal 'won' the interaction. These observations included all 12 focal adult females in addition to 3 younger tagged females (2.5 year old). I used agonistic interactions between tagged adult females to assign dominance scores to each individual. Dominance was scored using the normalized David's rank, D_{ij}, which accounts both for the number of observations between each dyad and for the relative dominance score of each animal j that animal i dominates or subordinates to (de Vries *et al.* 2006). Larger numbers indicate higher dominance, negative numbers indicate low dominance, and animals at the 50$^{\text{th}}$ percentile of dominance have a score of 0. Individuals were considered high ranking if D_{ij} was greater than 0, and were considered low ranking if D_{ij} was below 0. A single tagged female who was never observed to engage in agonism with other tagged females was excluded from all rank analyses. Although D_{ij} places greater weight on dyads with a larger number of observed agonistic interactions, the number of animals that animal i dominates or subordinates to has a much larger effect on D_{ij} than the number of agonistic interactions observed between i and a given animal j. Dominance as measured by D_{ij} is therefore largely independent from the rate at which animal i engages in agonistic behavior.

Hormone analyses

Hormones circulating in the blood stream are in part cleared by the liver and excreted in feces. Hormone concentrations in feces thus reflect the cumulative amount of free hormone experienced by an animal over the course of several hours (Keay *et al.* 2006). In tandem with behavioral observations, fresh fecal samples were collected from

tagged individuals whenever defecations were observed. Immediately after being retrieved (<60min after defecation was observed), fecal samples were placed in a cooler with ice, and within 12 hours a subsample was homogenized, placed in a 15ml screw top tube, and stored at -20°C.

To extract hormones from feces, I dried a subsample of the frozen feces to calculate percent water. I used this figure to determine the wet feces equivalent of 0.2g dry feces, and placed this amount of feces ±10% in a test tube for hormone extraction. Fecal samples were then placed in 10ml 95% ethanol and boiled in a water bath at 78.5^{o}C for 30min to dissolve hormone into the ethanol. After boiling, tubes were centrifuged for 15min, and the ethanol was poured into a new screw cap test tube while the old test tube containing the wet fecal pellet (now devoid of hormone) was discarded. The new tube containing ethanol with dissolved hormone was placed in a $72\,^{o}$C water bath while under forced air to evaporate the ethanol, leaving hormone residue on the side of the tube. The screw cap was firmly tightened, and samples were refrigerated and transported from the field site to the lab, where they were reconstituted in 2ml of 95% ethanol upon arrival (total time before reconstitution = 5 days) and stored at $-20\,^{o}$C for one month until extraction was completed. To complete the extraction I evaporated the remaining 2ml ethanol, reconstituted samples in 1ml 100% methanol and stored at $-80\,^{o}$C.

Cortisol and testosterone metabolite analyses were performed with ELISA kits (Assay Designs, Ann Arbor, MI). Cortisol assays have low cross-reactivity (<4%) for other steroid hormones. Testosterone assays also have a very low cross-reactivity (<1%) for steroid hormones other than 19-hydroxytestosterone (14.6%) and androstendione (7.2%). Cortisol extracts were assayed at a 30-fold methanol dilution. For testosterone, sample dilutions ranged from 60-501:1 dilution, based on testosterone concentration in the sample. Samples were run in duplicate, and I used the average of the two samples in all analyses. Samples were re-analyzed if the coefficient of variation between the duplicates exceeded 15%. Interplate coefficient of variation was 9.4% for testosterone plates and 11.3% for cortisol plates. Intraplate coefficient of variation was 8.6% for testosterone plates and 6.9% for cortisol plates. Hormone concentrations are reported as immunoreactive fecal testosterone metabolites (fT) and immunoreactive fecal glucocorticoid metabolites (fGCM). Hormone concentrations were read as pg/ml from

the assay, which I divided by the amount of dry feces from which hormones were extracted to report fT and fGCM in ng/g$_{dry feces}$.

The cortisol assay has been validated for Grant's gazelle, with experimental injections of ACTH in captive Grant's gazelle indicating that fecal glucocorticoid concentrations reflect circulating hormones from 14-20 hours previously (Ezenwa & Creel unpublished data). To validate the testosterone assay for this species, I ran a serial dilution of a pooled sample of extracts and found that binding curves for the serial dilution of extract were parallel to those from testosterone standards in the range of 29-2000 pg/ml. For biological validation, I analyzed fT concentrations in fecal samples collected from 6 untagged juveniles <12 months old between June-August 2009 and compared these to fT values for adults. Contrary to expectation, there was no difference in fT between juveniles and adults when pregnant and lactating females were excluded from analyses (Permutation test, juveniles *n=6*, adults *n=52* (females *n=18*, males *n=34*), observed mean fT difference=-33.3, 95% CI: -94.4–78.6, *P=0.50*). These results therefore do not provide validation for the testosterone assay.

Statistical analyses

Pregnancy is associated with increases in both testosterone and glucocorticoids in mammals (Humphreys *et al.* 1985; Tsutsui 1992; Gudermuth *et al.* 1998; Keller-Wood and Wood 2001); controlling for reproductive status is thus important when analyzing testosterone and glucocorticoid data from female mammals. Since it was only possible to test directly for pregnancy during the capture (August 2009), I compared fT in pregnant females to non-pregnant females in this period and used the lowest testosterone value of a pregnant female from August (761 ng/g$_{dry feces}$) as a threshold for determining likely pregnancies for all other sampling periods. All females that had testosterone concentrations higher than this threshold in November (*n=5*) and August (*n=1*) were considered to be potentially pregnant and were excluded from analyses when necessary to avoid potentially confounding effects of pregnancy. Because females that had recently given birth may have had endocrine profiles similar to pregnant females, and parturition data for lactating females at capture was unknown, lactating females were excluded from analyses comparing pregnant and non-pregnant females.

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I used permutation tests to compare hormone differences between males and females and between pregnant and non-pregnant females. Permutation tests are non-parametric, distribution-free tests that test the probability of finding the observed differences in distributions between two groups if all data are randomly assigned to one group or the other (Hesterberg *et al.* 2005). Permutation tests were performed in R (R Development Core Team 2005; code in Appendix I).

I used the Pearson product-moment correlation (r) to examine associations between dominance rank D_{ij}, agonistic behavior, fT, fGCM, and age. Residuals were inspected for normality using Q-Q plots and histograms of the residuals, and I used Spearman's rank correlation (r_s) when residuals showed non-normal distributions. Multiple linear regressions were used to account for the effects on fT or fGCM of age, dominance, and agonism simultaneously. I treated age, D_{ij}, and agonistic bouts initiated per hour as explanatory variables; response variables were fT and fGCM.

I tested for effects of monthly differences using permutation tests on hormone values from all animals. I also used Wilcoxon rank sum tests to compare the distribution of fT and fGCM in August to those of fT and fGCM in November for females that were sampled in both August and November and who were likely not pregnant during either period (*n*=7). I used permutation tests to compare changes in fT and fGCM from August to November among high-ranking versus low-ranking females. For clarity I report the sample size for each statistical test in the text. Except permutation tests, all statistical tests were performed in SPSS (SPSS Inc, Chicago, IL). All statistical tests are 2-tailed, with an alpha value of 0.05.

Results

Sex, pregnancy, age, and testosterone

Pregnancy was strongly associated with immunoreactive fecal testosterone metabolite (fT) concentrations, and pregnant females had significantly higher fT than non-pregnant females (August capture samples, pregnant *n*=3, non-pregnant *n*=18, Permutation test, observed mean fT difference=1312, 95% CI: -318–755, *P*=0.0014). Comparing all females to males, fT reached 10-fold greater concentrations in females

(range: 58-4791 ng/g dry feces; $n=26$) than in males (range 78-453 ng/g$_{dry feces}$; $n=34$), and females had marginally higher mean fT concentrations than males (Permutation test, observed mean fT difference=-308, 95% CI: -322–274, $P=0.059$; Figure 1). However, after excluding pregnant and lactating females, I found no difference in mean fT between females and males (males $n=34$, range 78-453 ng/g$_{dry feces}$; females $n=18$, range: 58-447 ng/g$_{dry feces}$; Permutation test, observed mean fT difference=37, 95% CI: -53–53, $P=0.26$). Age was positively correlated with August fT in non-pregnant females ($n=18$, r=0.85, $P<0.001$). Overall, the male:female mean testosterone ratio was 0.4:1 including all females, and 1.2:1 when excluding pregnant and lactating females.

Dominance, agonism, and testosterone

     Females frequently engaged in agonistic behavior, initiating an average of 2.1±0.5 (SE) agonistic bouts per hour ($n=12$ females, range: 0-5.6 bouts per hour, 39.7 hours of total focal behavioral data in November). Higher-ranking animals initiated more agonistic bouts ($n=11$, r=0.81, $P=0.003$; Figure 2). Animals might be expected to engage in agonism more frequently when group size is larger and they have more potential females to dominate. However, I found no association between group size and number of agonistic bouts initiated per hour ($n=11$, r=0.09, $P=0.79$).

     fT was positively associated with dominance ($n=9$, r=0.86, $P=0.003$; Figure 3a) and number of agonistic bouts initiated per hour among non-pregnant females ($n=8$, r=0.91, $P<0.001$; Figure 3b). Including both agonism and dominance in a multiple linear regression indicated that agonism was marginally positively associated with fT while dominance was not associated with fT (Multiple linear regression, $n=8$, F=15.1, D_{ij} β=0.15 $P=0.67$, agonism β=0.80 $P=0.059$).

     Age was positively associated with dominance ($n=11$, r=0.73, $P=0.02$; Figure 4), but I found no association between age and agonistic bouts initiated per hour ($n=8$, r=0.40, $P=0.33$). Although age was positively associated with August fT, I found no association between age and November fT ($n=8$, r$_s$=0.38, $P=0.35$).

Testosterone, glucocorticoids and monthly variation

Testosterone-glucocorticoid associations

fT was positively associated with fGCM in August ($n=26$, $r_s=0.47$, $P=0.02$). However, this association disappeared when pregnant and lactating females were excluded ($n=18$, $r_s=0.27$, $P=0.28$). In November there was a positive correlation between fT and fGCM across all females ($n=14$, $r_s=0.53$, $P<0.001$), and unlike in August this relationship remained when females likely to be pregnant were excluded ($n=9$, $r_s=0.70$, $P=0.04$).

Dominance-glucocorticoid associations

Associations between fT and fGCM may have been mediated by dominance or agonistic behavior; however I found no associations between dominance and fGCM or agonism and fGCM. There was no association between dominance and November fGCM among non-pregnant females ($n=8$, $r=0.37$, $P=0.32$). Including age as a covariate did not change relationships between dominance and fGCM (Multiple linear regression, $n=8$, F=0.66, D_{ij} β=0.47 $P=0.38$, age β=-0.03 $P=0.95$). Even after including fT in the model dominance was only marginally negatively associated with fGCM, while fT remained positively associated with fGCM (Multiple linear regression, $n=9$, F=7.0, D_{ij} β=-0.90 $P=0.09$, fT β=1.5 $P=0.015$).

Number of agonistic bouts initiated per hour was marginally correlated to November fGCM among non-pregnant females ($n=8$, $r=0.63$, $P=0.095$). Including age as a covariate did not alter the relationship between agonism and fGCM (Multiple linear regression, $n=7$, F=0.69, agonism β=0.49 $P=0.32$, age β=0.04 $P=0.93$). After including fT in the model, neither agonism nor fT was associated with fGCM (Multiple linear regression, $n=8$, F=3.2, agonism β=-0.34 $P=0.68$, fT β=1.0 $P=0.23$).

Monthly differences

Month (August vs. November) had a strong effect on fT, but a weaker effect on fGCM. I found no differences in fT between August and November when including all females (Permutation test, August $n=26$, November $n=14$, observed mean fT difference = -289, 95% CI: -633–525, $P=0.39$). However, fT was significantly higher in November

than August when pregnant and lactating females were excluded (Permutation test, August $n=18$, November $n=9$, observed mean fT difference = -212, 95% CI: -153–144, $P=0.006$). Furthermore, of the 7 adult females that were sampled in both August and November and who were likely not pregnant during either period, all had higher fT in November than August (Wilcoxon signed rank test, $P=0.02$). fT rose significantly more from August to November in high-ranking than low-ranking females (Permutation test, high-ranking $n=5$, low-ranking $n=2$, observed mean fT change difference = 395, 95% CI: -322–395, $P<0.001$; Figure 5a).

fGCM was higher in November than in August when including all females (Permutation test, August $n=26$, November $n=14$, observed difference = -334, 95% CI: -332–304, $P=0.048$). However, there were no differences in fGCM between November and August when pregnant and lactating females were excluded (Permutation test, August $n=18$, November $n=9$, observed mean fGCM difference = -154, 95% CI: -222–202, $P=0.17$). fGCM was marginally higher in November than in August when restricting analysis to the 7 non-pregnant females sampled in both August and November (Wilcoxon signed rank test, $P=0.07$). Unlike with fT, fGCM did not change differently in high-ranking compared to low-ranking females from August to November (Permutation test, high-ranking $n=5$, low-ranking $n=2$, observed mean fGCM change difference = 142, 95% CI: -357–414, $P=0.48$; Figure 5b).

Discussion

Theory on resource defense polygyny suggests that female competition should be strong in these systems. Studying a classic resource defense polygynous species, I found significant support for this hypothesis in behavioral data, relationships between testosterone and behavior, and male:female testosterone ratios. Females frequently engaged in agonistic behavior, initiating more than two bouts per hour on average. Agonistic behavior was associated with dominance, and fT strongly correlated with agonistic behavior. Females and males also had similar fT concentrations.

Agonistic behavior between females was high relative to other ungulates, averaging 2.1 bouts per hour and ranging as high as 5.6 bouts per hour. Of the eight wild

female ungulate species studied to date, only two (mountain goats *Oreamnos americanus* and Roosevelt elk *Cervus elephaus roosevelti*) engaged in agonism more frequently (reviewed in Fournier and Festa-Bianchet 1995; Weckerly 1999). High-intensity agonistic behavior such as chases and fights were rare in Grant's gazelle, as all observed agonistic interactions (175 interactions between tagged females) were low-intensity and quickly resolved. This is similar to what has been found in other female ungulates (Fournier and Festa-Bianchet 1995; Holand *et al.* 2004; Heitor *et al.* 2006), and likely reflects females trying to maximize nutrient intake while minimizing energy expenditure. Dominance was positively associated with agonistic behavior, suggesting that high-ranking females may have taken advantage of their rank by frequently displacing other females; alternately more aggressive females may have attained higher rank.

Female Grant's gazelle exhibited an exceptionally tight linear association between agonistic behavior and fT. Testosterone is broadly associated with agonistic behavior in male vertebrates (Sapolsky 1998), and testosterone is likewise associated with agonistic behavior in a wide suite of mammalian females (Glickman *et al.* 1992; Albert *et al.* 1993; Plusquellec and Bouissou 2001; Beehner *et al.* 2005). Studies investigating associations between agonism and testosterone often find positive associations that fit threshold patterns rather than linear relationships. Such patterns often result from aggressive interactions causing massive increases in circulating testosterone (e.g. Wingfield *et al.* 1990). Fecal testosterone concentrations – which reflect aggregate circulating hormones over many hours – likely smooth out some of these spikes, making linear associations more likely (e.g. Beehner *et al.* 2005). Nonetheless, the correlation I found between agonism and fT in female Grant's gazelle is exceptionally tight, suggesting that agonistic behavior may be particularly closely linked to testosterone secretion in this system.

Dominance was also correlated to fT. However, accounting for agonism and dominance simultaneously indicated that dominance did not directly associate with fT; instead this relationship appears to have been driven by the positive associations between agonism and both fT and dominance. Testosterone at normal physiological concentrations usually does not activate aggression. Positive associations between aggression and testosterone more often result from aggression increasing testosterone secretion (reviewed in Sapolsky 1998). One possible explanation for fT having been

more strongly associated with agonism than dominance is that high rank may have increased agonism, which then increased testosterone secretion. Dominance rank is often associated with testosterone, including in four of the six female mammals in which it has been studied (Clarke and Faulkes 1997; Beehner *et al.* 2005; Dloniak *et al.* 2006; Shargal *et al.* 2008; but see Von Engelhardt *et al.* 2000; Koren and Geffen 2009). Notably, females are dominant to males in the two species where this relationship was not found (ring-tailed lemurs *Lemur catta* Von Engelhardt *et al.* 2000; rock hyrax *Procavia capensis* Koren and Geffen 2009). High-ranking females in these species thus do not experience being dominated by other animals, which could have some effect on androgen physiology. However, as of yet there is no clear explanation for interspecific variation in the relationship between female dominance rank and testosterone.

Mean fT levels were similar between males and non-pregnant females in August 2009. This seemingly contradicts expectations from investigations on female testosterone in other species (Longcope 1986). However, studies on male:female androgen ratios broadly indicate that this ratio may be lower in mammalian species where there is more intense female-female competition. For example, in cooperatively breeding rock hyrax, female intrasexual competition is high, females are dominant to males, and females have higher mean testosterone levels than males (M:F testosterone ratio<1; Koren *et al.* 2006). In female resource defense polygynous spotted hyenas, female intrasexual competition is high, females are dominant to males, and the mean androgen M:F ratio is 14:1 for testosterone but 0.7:1 for androstenedione (Goymann *et al.* 2001). Among species with social systems where female intrasexual competition is likely less intense, M:F testosterone ratios range from 2:1 to 6:1 (African elephants *Loxodonta africana* 6:1, Rasmussen *et al.* 1984; California ground squirrels *Spermophilus beecheyi* 3:1, Holekamp and Talamantes 1991; Nubian ibex *Capra nubiana* 2:1, Shargal *et al.* 2008). A low M:F testosterone ratio in Grant's gazelle therefore suggests that female competition may be high, a hypothesis my behavioral data also support, and which should perhaps be expected in a resource defense polygynous species where females congregate on clumped nutrient sources. Nonetheless, caution should be taken in interpreting these results. A small number of samples collected from territory-holding males in November 2009 indicated that male fT reached far greater concentrations in November (*n*=4, max fT:

2791ng/g$_{\text{dry feces}}$) than in August (*n*=34, max fT: 453 ng/g$_{\text{dry feces}}$). Although these samples were not representative of all males in November, they do suggest that there may be substantial temporal variability in M:F testosterone ratios. At this point it is unclear whether hormone values in August 2009 – at the height of a drought – are representative of other periods. Nonetheless, relationships between fT and female behavior were strong and independent of any temporal variability in male fT levels.

I found a positive association between fT and fGCM among females in November 2009. Glucocorticoids are commonly associated with energy mobilization and the stress response. Higher glucocorticoid concentrations can indicate higher energy expenditure, and chronic elevations of glucocorticoids have a variety of negative fitness consequences (Sapolsky 2002). Testosterone has been positively associated with glucocorticoid secretion in several field studies on birds and mammals (Duffy *et al.* 2000; Muehlenbein 2006; Zysling *et al.* 2006; Koren and Geffen 2009), perhaps because animals need to mobilize stored energy reserves in response to testosterone-induced increases in energy expenditure. This appears to be the case for female Grant's gazelle.

Notably, I failed to find an association between dominance rank and glucocorticoids – fT was the main variable associated with fGCM in November 2009. Dominance is expected to be associated with high glucocorticoids when maintaining dominance is costly (Creel 2001); conversely, low-ranking animals are expected to have higher glucocorticoids when being low-ranking is stressful and maintaining dominance is not costly (Sapolsky 2005). Since there was neither a positive nor a negative association between fGCM and rank, female Grant's gazelle do not fit cleanly into either of these hypotheses. Females very rarely engaged in high-intensity agonism to establish dominance, so maintaining dominance did not appear costly. The question then is why low-ranking animals were not stressed. One explanation is that dominance may not have strong effects on behavior or physiology in these animals. Another explanation is that open social groups might allow low-ranking females to avoid particularly aggressive females by moving into different groups. Theory on the relationship between glucocorticoids and dominance rank has largely been developed in primates and carnivores with closed social groups (Creel 2005; Sapolsky 2005); species with open social groups may function differently. Alternately low-ranking females may not be

stressed by receiving low-intensity agonism such as threats and supplants that rarely escalate into more severe confrontations. Whatever the explanation, dominance rank did not appear to play a strong role in shaping glucocorticoid physiology in female Grant's gazelle.
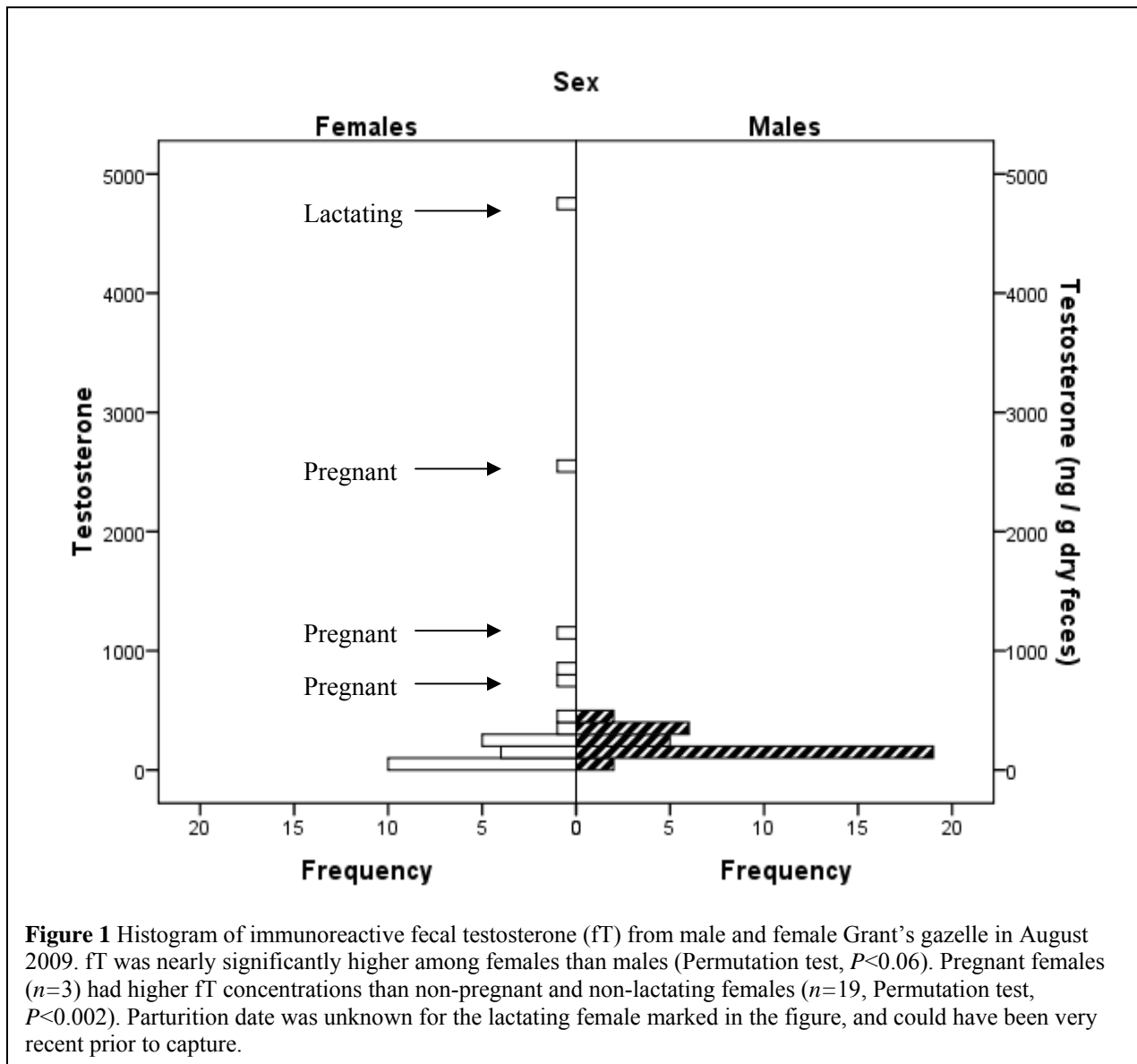
In addition to behavior, month seems to have played an important role in shaping testosterone physiology in female gazelle. fT was higher among non-pregnant females in November after the rains had begun than it was in August during a drought period. Furthermore, over this time period fT concentrations rose significantly more among high-ranking than low-ranking females. This is somewhat surprising since one might expect competition to be more intense, and thus fT levels to be higher, when fewer nutrients are available. One explanation is that females may have "trimmed the behavioral fat" and suppressed testosterone secretion and/or avoided agonistic behavior during the drought to conserve energy, particularly if there were no high-quality resources to compete for (Sapolsky 1986; Nelson 1993; Beehner *et al.* 2005). This might explain the higher fT values observed in November than August 2009. Alternately, there is a spike in breeding during rainy seasons in Grant's gazelle (Walther *et al.* 1983), and there may be greater competition for resources during this period if more females were attempting to reproduce. This could explain both the overall shift towards higher fT in November and the rank-differences in fT change from August to November. If low-ranking females are less likely to reproduce during optimal times, high-ranking females would have been more likely to engage in agonistic behavior during the rainy season (November) and would have experienced a larger increase in fT than low-ranking females. Under such a scenario low-ranking females would experience no change in fT while high-ranking females would have higher fT in November, causing an overall shift towards higher fT in November. Similar shifts were not seen in fGCM, reinforcing the notion that competition did not have a strong effect on glucocorticoids.

Results showing that fT increased during pregnancy are typical for mammals. Pregnancy is associated with increases in testosterone in a wide range of mammals (Tsutsui 1992; Gudermuth *et al.* 1998; Altmann *et al.* 2004). Increases in testosterone during pregnancy may be due to physiological reasons since testosterone is a precursor to estrogen (Millier *et al.* 1994; Simpson 2002), which increases during pregnancy (Siiteri

and Macdonald 1966). Associations between testosterone and pregnancy may also be linked to increased competition for resources. Pregnant females have higher resource requirements than other females, and as a result may be more forceful in acquiring nutrients. If so, they may engage in more frequent or more intense agonism (Wise 1974; Beehner *et al.* 2005), causing testosterone levels to rise. Alternately, increased testosterone levels due to physiological processes may promote agonistic behavior.

Since pregnancy was associated with increased fT, pregnancy was an important factor potentially confounding relationships between fT, agonism, and dominance in this study. Unfortunately I had no way to reliably detect pregnancies outside of the capture. fT provided some insight, but I may have misdiagnosed high-fT non-pregnant females as pregnant and thus discarded important data. Testosterone levels in male vertebrates are thought to rise dramatically in response to challenges (Wingfield *et al.* 1990). If similar changes in circulating testosterone occurred in female gazelle and were reflected in fT, I likely misdiagnosed such females as pregnant and discarded them in analyses. I may also have misdiagnosed females with high baseline testosterone as pregnant and discarded them. I expect females with high testosterone to have shown the strongest associations between fT and behavior, and since these females were dropped from analyses my results may actually be conservative.

Overall my results suggest that there is substantial female-female competition in Grant's gazelle, and this may reflect a more general trend among females in resource defense polygynous systems. Furthermore, testosterone may be an important factor mediating competitive ability among females in this species, leading to similar fT levels between males and females. Given the costs known to be associated with testosterone in males, and the associations observed here between fT and fGCM in female gazelle, testosterone may have important fitness effects (direct and indirect) in females. Further studies on females would be well served to investigate these effects and how they relate to female competition.

Figure 1 Histogram of immunoreactive fecal testosterone (fT) from male and female Grant's gazelle in August 2009. fT was nearly significantly higher among females than males (Permutation test, $P<0.06$). Pregnant females ($n=3$) had higher fT concentrations than non-pregnant and non-lactating females ($n=19$, Permutation test, $P<0.002$). Parturition date was unknown for the lactating female marked in the figure, and could have been very recent prior to capture.
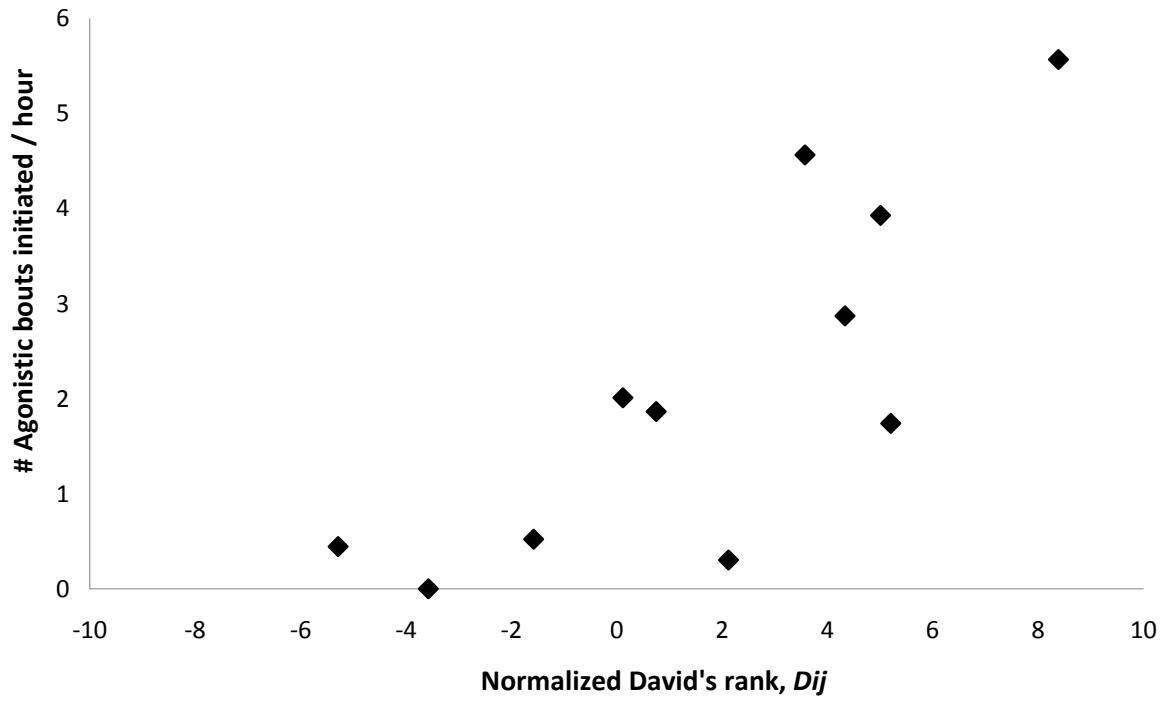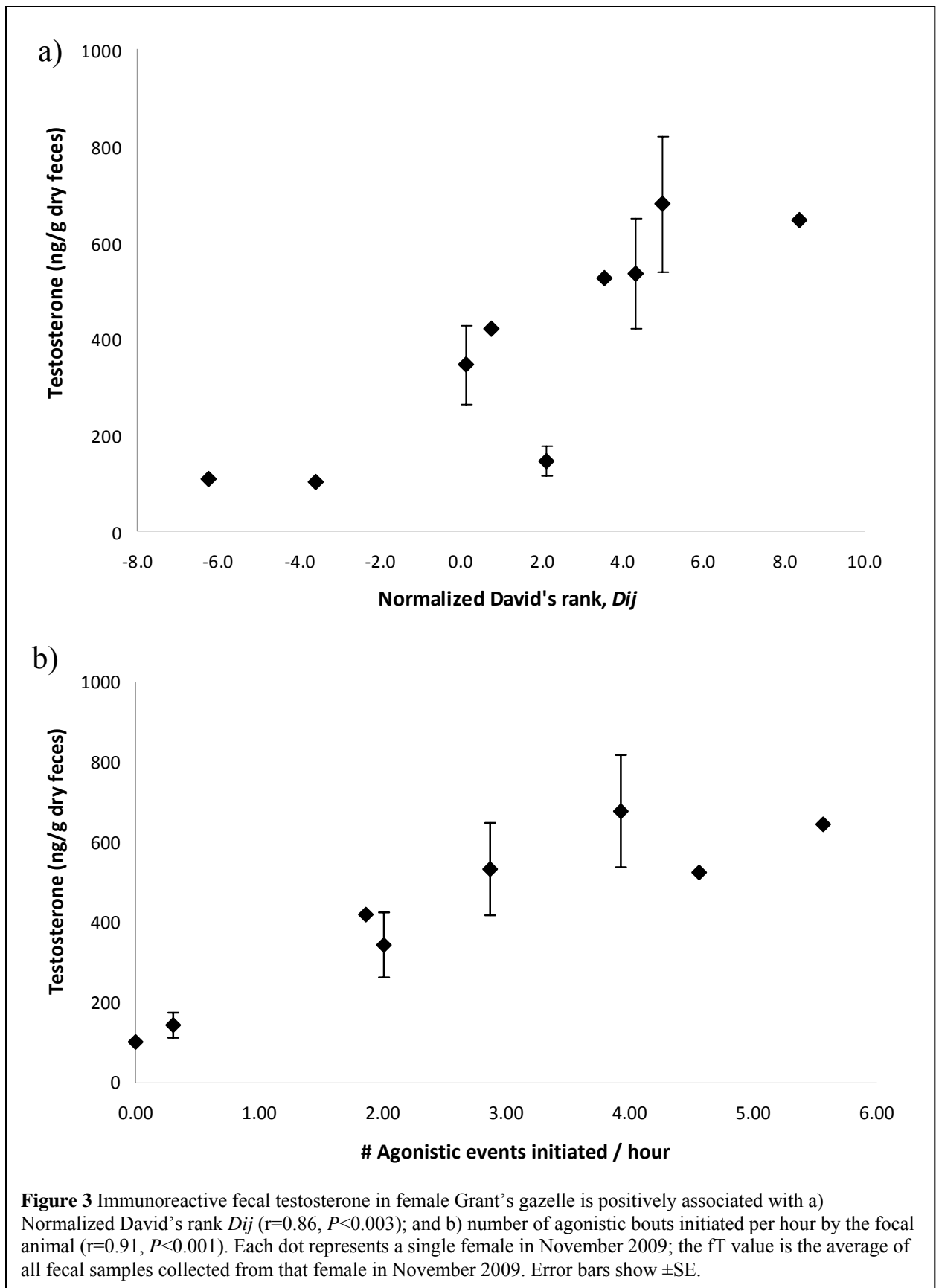
Figure 2 Dominance D_{ij} is positively correlated with number of agonistic bouts initiated per hour in female Grant's gazelle (r=0.81, *P*<0.01). Each dot represents a single female in November 2009.

Figure 3 Immunoreactive fecal testosterone in female Grant's gazelle is positively associated with a) Normalized David's rank *Dij* (r=0.86, *P*<0.003); and b) number of agonistic bouts initiated per hour by the focal animal (r=0.91, *P*<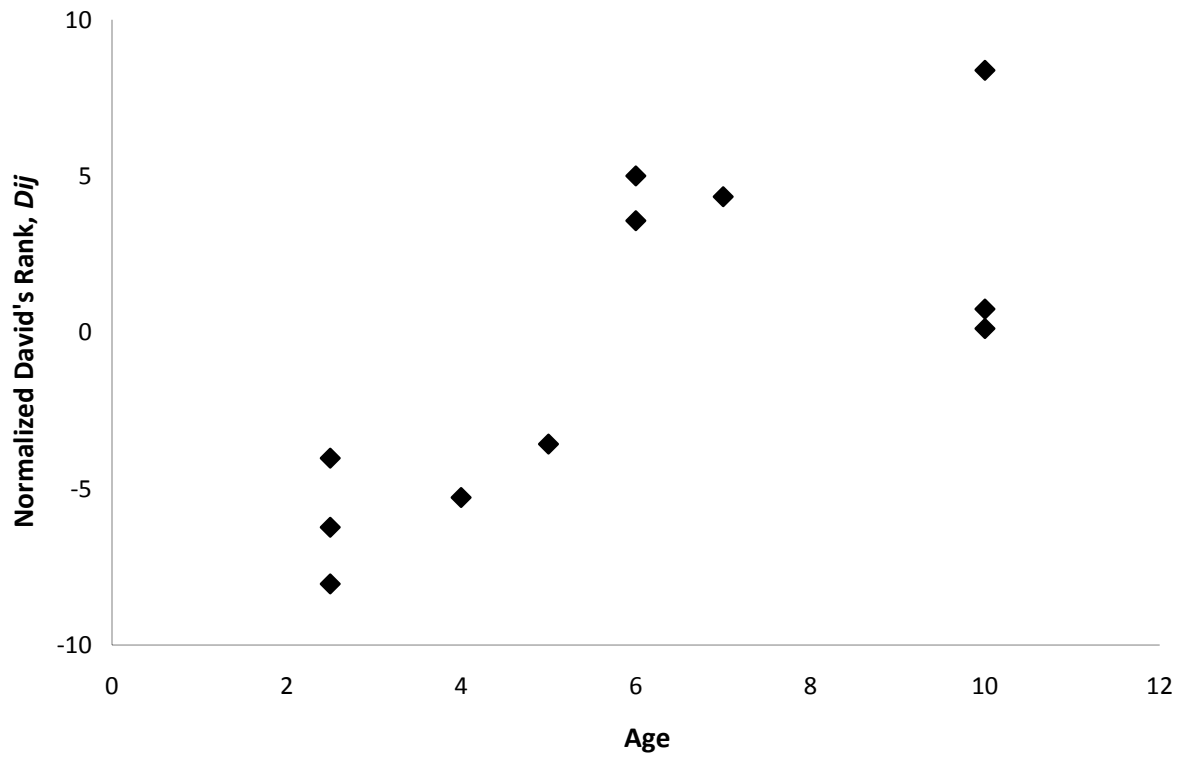0.001). Each dot represents a single female in November 2009; the fT value is the average of all fecal samples collected from that female in November 2009. Error bars show ±SE.

Figure 4 Age is positively associated with normalized David's rank, D_{ij}, in female Grant's gazelle (r=0.73, $P<0.02$). Each dot represents a single female in November 2009.
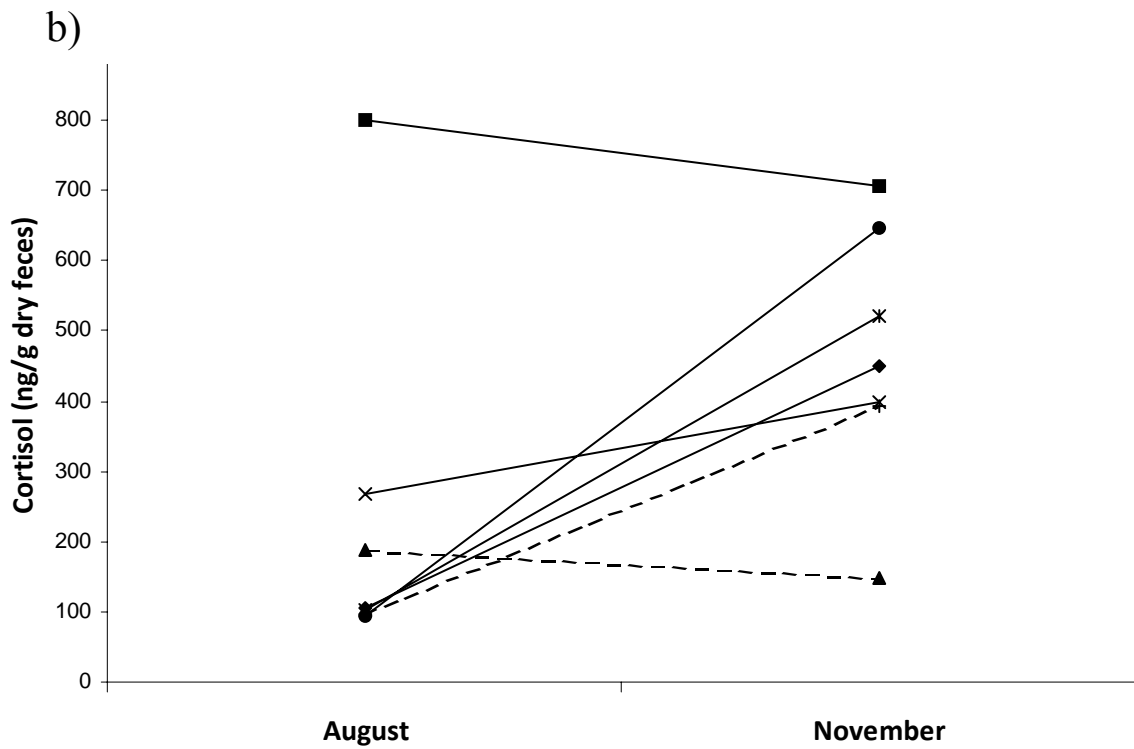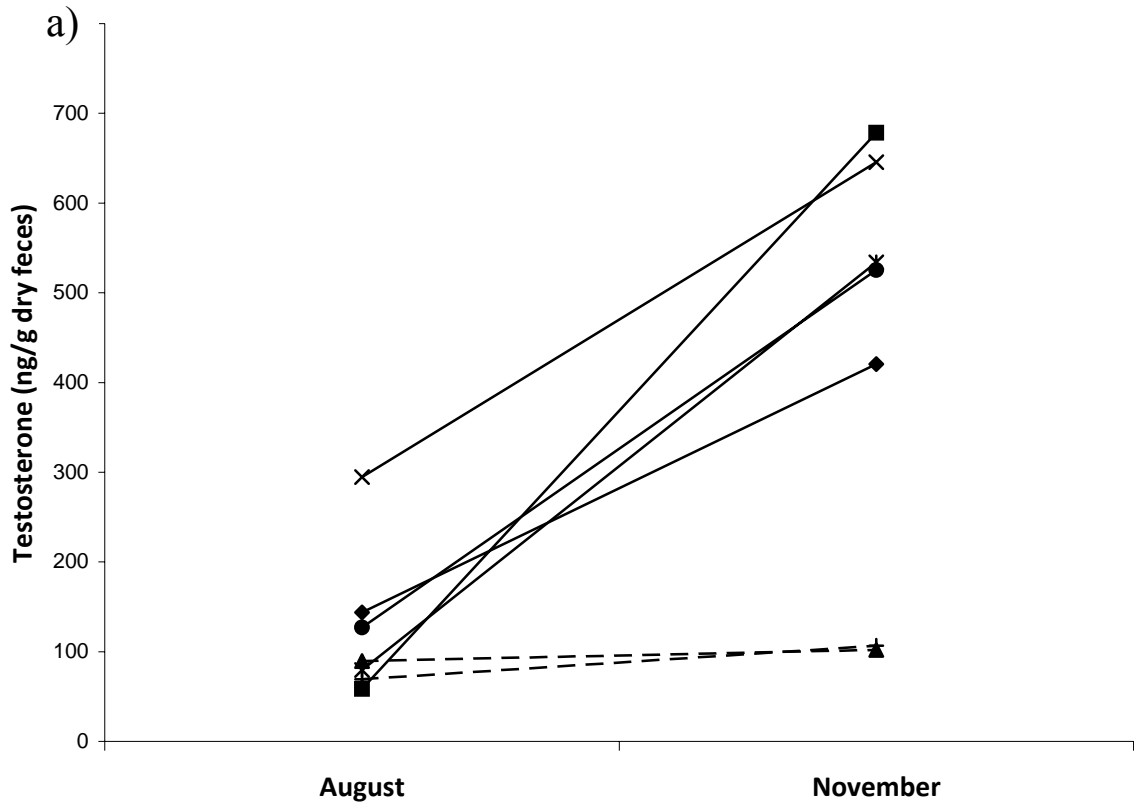
Figure 5 Seasonal changes from August (drought) to November (rainy season) for non-pregnant females in a) fT and b) fGCM. Each dot represents hormone concentrations for one female in a given month, and lines connect samples from the same individual. High-ranking females are shown with solid lines, and low-ranking females are shown with dotted lines.

References

Albert, DJ, Jonik, RH and Walsh, ML. 1992. "Hormone-dependent aggression in male and female rats - experiential, hormonal, and neural foundations." *Neuroscience and Biobehavioral Reviews* 16(2): 177-192.

Albert, DJ, Jonik, RH and Walsh, ML. 1993. "Influence of combined estradiol and testosterone implants on the aggressiveness of nonaggressive female rats." *Physiology & Behavior* 53(4): 709-713.

Altmann, J, Lynch, JW, Nguyen, N, Alberts, SC and Gesquiere, LR. 2004. "Life-history correlates of steroid concentrations in wild peripartum baboons." *American Journal of Primatology* 64(1): 95-106.

Beehner, JC, Phillips-Conroy, JE and Whitten, PL. 2005. "Female testosterone, dominance rank, and aggression in an Ethiopian population of hybrid baboons." *American Journal of Primatology* 67(1): 101-119.

Blumstein, DT and Daniel, JC. 2007. *Quantifying behavior the JWatcher way*, Sunderland, MA: Sinauer

Clarke, FM and Faulkes, CG. 1997. "Dominance and Queen Succession in Captive Colonies of the Eusocial Naked Mole-Rat, Heterocephalus glaber." *Proceedings: Biological Sciences* 264(1384): 993-1000.

Clutton-Brock, TH, Albon, SD and Guinness, FE. 1986. "Great expectations: dominance, breeding success and offspring sex ratios in red deer." *Animal Behaviour* 34(2): 460-471.

Côté, SD and Festa-Bianchet, M. 2001. "Reproductive success in female mountain goats: the influence of age and social rank." *Animal Behaviour* 62(1): 173-181.

Creel, S. 2001. "Social dominance and stress hormones." *Trends in Ecology & Evolution* 16(9): 491-497.

Creel, S. 2005. "Dominance, Aggression, and Glucocorticoid Levels in Social Carnivores." *Journal of Mammalogy* 86(2): 255-264.

de Vries, H, Stevens, JMG and Vervaecke, H. 2006. "Measuring and testing the steepness of dominance hierarchies." *Animal Behaviour* 71(3): 585-592.

Dloniak, SM, French, JA and Holekamp, KE. 2006. "Rank-related maternal effects of androgens on behaviour in wild spotted hyaenas." *Nature* 440(7088): 1190-1193.

Duffy, DL, Bentley, GE, Drazen, DL and Ball, GF. 2000. "Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings." *Behavioral Ecology* 11(6): 654-662.

Ellis, L. 1995. "Dominance and reproductive success among nonhuman animals: A cross-species comparison." *Ethology and Sociobiology* 16(4): 257-333.

Emlen, ST and Oring, LW. 1977. "Ecology, sexual selection, and the evolution of mating systems." *Science* 197(4300): 215-223.

Estes, R. 1991. *The behavior guide to African mammals*: University of California Press Berkeley.

Ezenwa, VO. 2003. "Habitat overlap and gastrointestinal parasitism in sympatric African bovids." *Parasitology* 126: 379-388.

Fournier, F and Festa-Bianchet, M. 1995. "Social dominance in adult female mountain goats." *Animal Behaviour* 49(6): 1449-1459.

Gill, SA, Alfson, ED and Hau, M. 2007. "Context matters: female aggression and testosterone in a year-round territorial neotropical songbird (Thryothorus leucotis)." *Proceedings of the Royal Society B: Biological Sciences* 274(1622): 2187.

Glickman, SE, Frank, LG, Pavgi, S and Licht, P. 1992. "Hormonal correlates of 'masculinization' in female spotted hyaenas (Crocuta crocuta). 1. Infancy to sexual maturity." *J Reprod Fertil* 95(2): 451-462.

Goymann, W, East, ML and Hofer, H. 2001. "Androgens and the Role of Female "Hyperaggressiveness" in Spotted Hyenas (Crocuta crocuta)." *Hormones and Behavior* 39(1): 83-92.

Gudermuth, DF, Concannon, PW, Daels, PF and Lasley, BL. 1998. "Pregnancy-specific elevations in fecal concentrations of estradiol, testosterone and progesterone in the domestic dog (Canis familiaris)." *Theriogenology* 50(2): 237-248.

Heitor, F, do Mar Oom, M and Vicente, L. 2006. "Social relationships in a herd of Sorraia horses: Part II. Factors affecting affiliative relationships and sexual behaviours." *Behavioural Processes* 73(3): 231-239.

Hesterberg, T, Monaghan, S, Moore, DS, Clipson, A and Epstein, R. 2005. "Bootstrap methods and permutation tests." In (Eds).*Introduction to the Practice of Statistics*.

Hirschenhauser, K and Oliveira, RF. 2006. "Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis." *Animal Behaviour* 71: 265-277.

Holand, Ø, Gjøstein, H, Losvar, A, Kumpula, J, Smith, ME, Røed, KH, Nieminen, M and Weladji, RB. 2004. "Social rank in female reindeer (Rangifer tarandus): effects of body mass, antler size and age." *Journal of Zoology* 263(04): 365-372.

Holekamp, KE and Talamantes, F. 1991. "Seasonal variation in circulating testosterone and oestrogens of wild-caught California ground squirrels (Spermophilus beecheyi)." *J Reprod Fertil* 93(2): 415-425.

Humphreys, EM, Ghione, R, Gosden, RG, Hobson, BM and Wide, L. 1985. "Relationship between corpora lutea or fetal number and plasma concentrations of progesterone and testosterone in mice." *Reproduction* 75(1): 7.

Jawor, JM, Young, R and Ketterson, ED. 2006. "Females competing to reproduce: dominance matters but testosterone may not." *Hormones and Behavior* 49(3): 362-368.

Keay, JM, Singh, J, Gaunt, MC and Kaur, T. 2006. "Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: A literature review." *Journal of Zoo and Wildlife Medicine* 37(3): 234-244.

Keller-Wood, M and Wood, CE. 2001. "Pituitary-Adrenal Physiology during Pregnancy." *The Endocrinologist* 11(3): 159-170.

Ketterson, ED, Nolan Jr, V and Sandell, M. 2005. "Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both?" *American Naturalist* 166: S85-S98.

Kock, MD, Jessup, DA, Clark, RK, Franti, CE and Weaver, RA. 1987. "Capture methods in five subspecies of free-ranging bighorn sheep: an evaluation of drop-net, drive-net, chemical immobilization and the net-gun." *Journal of Wildlife Diseases* 23(4): 634.

Koren, L and Geffen, E. 2009. "Androgens and social status in female rock hyraxes." *Animal Behaviour* 77(1): 233-238.

Koren, L, Mokady, O and Geffen, E. 2006. "Elevated testosterone levels and social ranks in female rock hyrax." *Hormones and Behavior* 49(4): 470-477.

Longcope, C. 1986. "Adrenal and gonadal androgen secretion in normal females." *Journal of Clinical Endocrinology and Metabolism* 15(2): 213-228.

Martin, P and Bateson, P. 1993. *Measuring Behaviour: An Introductory Guide*, 2nd Edition. Cambridge, UK: University Press.

Millier, SG, Whitelaw, PF and Smyth, CD. 1994. "Follicular oestrogen synthesis: the `two-cell, two-gonadotrophin' model revisited." *Molecular and Cellular Endocrinology* 100(1-2): 51-54.

Muehlenbein, M. 2006. "Intestinal parasite infections and fecal steroid levels in wild chimpanzees." *American Journal of Physical Anthropology* 130(4): 546-550.

Nelson, RJ. 1993. "Simulated drought affects male reproductive function in deer mice (Peromyscus maniculatus bairdii)." *Physiological Zoology*: 99-114.

Ogada, MO, Woodroffe, R, Oguge, NO and Frank, LG. 2003. "Limiting depredation by African carnivores: the role of livestock husbandry." *Conservation Biology* 17(6): 1521-1530.

Plusquellec, P and Bouissou, M-F. 2001. "Behavioural characteristics of two dairy breeds of cows selected (Hérens) or not (Brune des Alpes) for fighting and dominance ability." *Applied Animal Behaviour Science* 72(1): 1-21.

Pratt, MS and Hopkins, PS. 1975. "The diagnosis of pregnancy in sheep by abdominal palpation." *Australian veterinary journal* 51(8): 378.

Racey, PA and Skinner, JD. 1979. Endocrine aspects of sexual mimicry in spotted hyenas, *Crocuta crocuta*. *J. Zool*. 187: 315-326.

Rasmussen, LE, Buss, IO, Hess, DL and Schmidt, MJ. 1984. "Testosterone and dihydrotestosterone concentrations in elephant serum and temporal gland secretions." *Biology of reproduction* 30(2): 352.

Reiter, J, Panken, KJ and Le Boeuf, BJ. 1981. "Female competition and reproductive success in northern elephant seals." *Animal Behaviour* 29(3): 670-687.

Sannen, A, Heistermann, M, Elsacker, LV, Möhle, U and Eens, M. 2003. "Urinary Testosterone Metabolite Levels in Bonobos: A Comparison with Chimpanzees in Relation to Social System." *Behaviour* 140(5): 683-696.

Sapolsky, RM. 1986. "Endocrine and behavioral correlates of drought in wild olive baboons (Papio anubis)." *American Journal of Primatology* 11(3): 217-227.

Sapolsky, RM. 1998. *The Trouble with Testosterone*. New York, NY: Touchstone.

Sapolsky, RM. 2002. "Endocrinology of the stress response." In Becker, JB, Breedlove, SM, Crews, D and McCarthy, M (Eds).*Behavioral Endocrinology*. Cambridge, Massachusetts: MIT Press. 409-450.

Sapolsky, RM. 2005. "The Influence of Social Hierarchy on Primate Health." *Science* 308(5722): 648-652.

Sapolsky, RM, Romero, LM and Munck, AU. 2000. "How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions." *Endocrine Reviews* 21(1): 55-89.

Seale, JV, Wood, SA, Atkinson, HC, Bate, E, Lightman, SL, Ingram, CD, Jessop, DS and Harbuz, MS. 2004. "Gonadectomy reverses the sexually diergic patterns of

circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats." *Journal of Neuroendocrinology* 16(6): 516-524.

Shargal, D, Shore, L, Roteri, N, Terkel, A, Zorovsky, Y, Shemesh, M and Steinberger, Y. 2008. "Fecal testosterone is elevated in high ranking female ibexes (Capra nubiana) and associated with increased aggression and a preponderance of male offspring." *Theriogenology* 69(6): 673-680.

Siiteri, PK and Macdonald, PC. 1966. "Placental Estrogen Biosynthesis During Human Pregnancy." *J Clin Endocrinol Metab* 26(7): 751-761.

Simpson, ER. 2002. "Aromatization of androgens in women: current concepts and findings." *Fertility and Sterility* 77(Supplement 4): 6-10.

Spinage, C. 1976. "Age determination of the female Grant's gazelle." *African Journal of Ecology* 14(2): 121-134.

Spinage, CA, Ryan, C and Shedd, M. 1980. "Food selection by the Grant's gazelle." *African Journal of Ecology* 18: 19-25.

Staub, NL and De Beer, M. 1997. "The Role of Androgens in Female Vertebrates." *General and Comparative Endocrinology* 108(1): 1-24.

Stelfox, JB, Hudson, RJ and Groer, N. 1985. "Relationships among physical traits, age and social status in Thomson's and Grant's gazelles." *Applied Animal Behaviour Science* 13(4): 347-357.

Tsutsui, K. 1992. "Inhibitory role of sex steroid in the regulation of ovarian follicle-stimulating hormone receptors during pregnancy." *Journal of Experimental Zoology* 264: 167-167.

van Jaarsveld, AS and Skinner, JD. 1987. Spotted hyaena monomorphism: an adaptive phallusy'? *S. Afr. J. Sci.* 83: 612-615.

Viau, V. 2002. "Functional cross-talk between the hypothalamic-pituitary-gonadal and-adrenal axes." *Journal of Neuroendocrinology* 14(6): 506-513.

Von Engelhardt, N, Kappeler, PM and Heistermann, M. 2000. "Androgen levels and female social dominance in Lemur catta." *Proceedings of the Royal Society B: Biological Sciences* 267(1452): 1533.

Walther, FR. 1972. "Social grouping in Grant's gazelle (Gazella granti Brooke 1827) in the Serengeti National Park." *Zeitschrift für Tierpsychologie* 31(4): 348.

Walther, FR, Mungall, EC and Grau, GA. 1983. *Gazelles and Their Relatives*, Park Ridge, NJ: Noyes Publications.

Weckerly, FW. 1999. "Social bonding and aggression in female Roosevelt elk." *Canadian Journal of Zoology* 77(9): 1379-1384.

Wingfield, JC, Hegner, RE, Dufty, AM, Jr. and Ball, GF. 1990. "The "Challenge Hypothesis": Theoretical Implications for Patterns of Testosterone Secretion, Mating Systems, and Breeding Strategies." *The American Naturalist* 136(6): 829-846.

Wingfield, JC, Lynn, SE and Soma, KK. 2001. "Avoiding the 'costs' of testosterone: Ecological bases of hormone-behavior interactions." *Brain Behavior and Evolution* 57(5): 239-251.

Wise, DA. 1974. "Aggression in the female golden hamster: Effects of reproductive state and social isolation." *Hormones and Behavior* 5(3): 235-250.

Woodroffe, R and MacDonald, DW. 1995. "Female/Female Competition in European Badgers Meles meles: Effects on Breeding Success." *Journal of Animal Ecology* 64(1): 12-20.

Zysling, DA, Greives, TJ, Breuner, CW, Casto, JM, Demas, GE and Ketterson, ED. 2006. "Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (Junco hyemalis carolinensis)." *Hormones and Behavior* 50(2): 200-207.

Appendix A

Code used in R for permutation tests

```
# This tests for differences in testosterone between males and females. Other tests use similar
# code but with appropriate variables substituted in.

step=read.table(AugHormones.csv',header=T,sep=",")
names(step)

sex=step$Sex                    # Separates males from females

my_var = step$T                 # Creates vector with testosterone values

n = 100000                      # Number of permutations to run

observed_mean = mean(my_var[sex=="M"],na.rm=TRUE)-
        mean(my_var[sex=="F"],na.rm=TRUE)      # Observed mean difference in
                                               # testosterone between males and
                                               # females

meandiff = rep(NA,n)            # Initializes the array to store the mean differences in

# Permutations testing the difference in means when testosterone values are randomly
# assigned to males or females

for(i in 1:n) {
        status.samp = sample(sex,length(sex),replace=FALSE)
        meandiff[i] = mean(my_var[status.samp=="M"],na.rm=TRUE) -
mean(my_var[status.samp=="F"],na.rm=TRUE)
}

hist(meandiff)                  # Histogram of all permutations
perc_greater = sum(meandiff > observed_mean,na.rm=TRUE)/length(meandiff)
perc_less = 1-perc_greater
two_tailed = 2*min(perc_greater,perc_less)
two_tailed                      # 2-tailed p-value indicating where the observed mean falls
                                # in the distribution of means from the permutations

# 2-tailed pval, T, Males vs Females, p = 0.05912
```