Reproductive status and testosterone among females in cooperative mole-rat societies

*[1]Heike Lutermann, [2]Andrew J. Young and [1]Nigel C. Bennett

[1]*Department of Zoology and Entomology, Mammal Research Institute, University of Pretoria, Pretoria 0002, South Africa*
[2]*Centre for Ecology and Conservation, School of Bioscience, University of Exeter, UK*

*corresponding author: hlutermann@zoology.up.ac.za, Tel.: +27-12-420 4872, Fax: +27-12-362 5242

Abstract: Sexual selection acts on traits that increase reproductive success. Variation in reproductive success is often higher among males than females. Consequently, sexual selection has been studied extensively in males while its possible role in females has only recently attracted considerable attention. In some cooperatively breeding species females compete intensely for reproductive opportunities and may thereby have evolved 'male-like' traits such as increased intra-sexual aggression and exaggerated secondary sexual traits. The expression of the latter tends to be testosterone-dependent in male vertebrates but whether this is also the case among females remains poorly understood. Here, we compare two cooperatively breeding mole-rat species (Natal, *Cryptomys hottentotus natalensis*, and Damaraland mole-rats, *Fukomys damarensis*) in which a single female monopolises reproduction through behavioural and physiological suppression, respectively, to evaluate the effect of female intra-sexual competition. Consistent with the hypothesis that intra-sexual competition has shaped patterns of testosterone (T) secretion among females in these species, we show that (i) female T levels in both species are significantly higher among breeding (BFs) (who may face the highest degree of intra-sexual competition) compared to non-breeding females (NBFs), (ii) that T levels in both species are significantly higher when access to unrelated males can be assumed to be greatest (i.e. wet season), and (iii) that the average female T levels are a full order of magnitude higher in the absence of a physiological mechanism of reproductive suppression. Together, our results suggest a role for intra-sexual competition in shaping patterns of T secretion among females of the social mole-rats and raise the possibility of a modulatory role for the mode of reproductive suppression on competition-related traits in females.

Keywords: female-female competition, testosterone, *Cryptomys*, *Fukomys*, Bathyergidae, cooperative breeding, reproductive suppression

1. INTRODUCTION

Sexual selection acts on traits that affect the reproductive success of an individual and is thought to have led to elaborate traits such as increased body size or ornaments observed in males of many species [1]. The conspicuousness of such traits among males may account for the marked sex bias in studies of sexual selection [10,11]. Accordingly, studies on sexual selection in females often focus on species where females exhibit pronounced sexual secondary characters such as in species with reversed sex roles [29–31]. The comparative lack of studies of sexual selection in females can partly be attributed to the common assumption that females exhibit less variation in reproductive success and should thus be less affected by sexual selection [24,37]. However, females may compete intensely for mates in species without sex-role reversal and may exhibit a number of traits commonly associated with males, such as high intra-sexual aggression, sexual displays and elevated testosterone levels [13,31], suggesting that the role of sexual selection among females warrants closer attention [10,11]. Recent work has shown that variance in female reproductive success can be very high in social vertebrates, particularly in cooperative breeders with high reproductive skew (in which a small number of females monopolize the majority of reproduction) [11,24]. Indeed, in a number of cooperatively breeding vertebrates marked intra-sexual aggression among females is common, particularly in a reproductive context [9,13,14,18,28]. In addition, body mass or size is a key determinant in acquiring and/or maintaining a breeding position in females of various cooperatively breeding species [19,25,27]. Accordingly, the degree of sexual dimorphism in traits used in intra-sexual competition appears to be reduced in some cooperatively breeding species [38]. Thus, cooperatively breeding species may be particularly suitable to evaluate the effects of sexual selection on females.

In male vertebrates the expression of traits that enhance access to mates such as aggression, large body size and elaborate sexual signals is often mediated by the androgen testosterone (T) [12,23,42]. Male T levels frequently vary with social and environmental stimuli and tend to be elevated during breeding periods and in response to intra-sexual encounters or social instability (captured in the challenge hypothesis) [22,42]. The challenge hypothesis has found support in males across vertebrate taxa [26]. In contrast, the possible role of environmental and social stimuli on patterns of T in females has been largely neglected [34]. Evidence suggests that the expression of morphological and behavioural traits linked to intra-sexual competition in females can be affected by T similar to males [41]. In addition, female T levels may vary seasonally and in response to social interactions [3,8,16,31,39]. Despite the potential significance of T in competition among females, T is still considered to be of relatively minor importance in females and few studies have explored the effects of intra-sexual competition on circulating T levels in female vertebrates [41].

In the current study we investigated the extent to which intra-sexual competition may have shaped the patterns of T secretion among females in cooperatively breeding mole-rat societies. Social mole-rats offer a novel opportunity for studies of this kind as, while all species show high reproductive skew (only one female breeder per colony), closely-related species can differ markedly in the extent to which this skew arises from behavioural mechanisms (i.e. intra-sexual aggression) alone [20]. Reproductive suppression in mole-rats is thought to result from a combination of control by the breeding female (BF) or queen (e.g. aggression) and self-restraint in non-breeding females (NBFs) due to a lack of unrelated males [20]. Mole-rats exploit the subterranean niche and since the digging required for breeding dispersal is much less energetically costly in soft soils, access to unrelated males is assumed to be linked to rainfall patterns and consequently, the dispersal increases strongly with precipitation [6,7,20]. When new breeding opportunities arise, escalated aggression among females that can result in the death of competitors has been observed in several mole-rat species [14,19,32]. Here we exploit the variation in modes of reproductive suppression among two social mole-rat species to investigate not only how intra-sexual competition has shaped T levels among females in high skew species, but to consider the possibility that the strength of selection on androgen levels in such species may depend upon the mechanisms through which intra-sexual reproductive conflict is resolved. Where intra-sexual reproductive competition is resolved solely through behavioural means (i.e. NBFs show comparable reproductive physiology to BFs), selection may differentially favour the elevation of circulating T levels to promote those traits that may favour success in competition (e.g. aggression). Specifically, we compare and contrast the patterns of circulating T among females in two closely related mole-rat species that differ in their modes of suppression (the Natal mole-rat, *Cryptomys hottentotus natalensis*, and the Damaraland mole-rat, *Fukomys damarensis*, Table 1). While both species live in colonies in which a single female monopolizes reproduction, in Natal mole-rats NBFs are physiologically capable of breeding [35] whereas in Damaraland mole-rats NBFs experience a block to ovulation attributable to the down-regulation of the pituitary sensitivity to gonadotropin releasing hormone (GnRH) [5,33]. This may be linked to the variation in ecological constraints to dispersal and hence breeding opportunities (i.e. unrelated males entering a colony or NBFs leaving their natal colony) that both species experience as Natal mole-rats occur in more mesic habitats than Damaraland mole-rats [20]. Consequently, while sexual selection may have favoured 'androgenised' aggressive females in both species [14,19], selection for androgen-mediated traits may be substantially stronger in Natal mole-rat societies as intra-sexual traits could be the principal means through which their reproductive monopolies are maintained. We therefore tested (i) whether Natal mole-rat females exhibit higher T levels than Damaraland mole-rat females. Both species breed throughout the year, nevertheless, NBFs of both species exhibit an up-regulation of their pituitary in response to rainfall; the baseline and response levels of luteinizing hormone (LH) and pituitary sensitivity are elevated during this period in Natal and Damaraland mole-rats, respectively [35,46]. Our second aim was therefore to test (ii) whether circulating T levels would be elevated during the wet season, when NBFs may have access to reproductive opportunities and hence, challenges to the breeding monopoly of the BF are likely to occur. Lastly, we predicted that (iii) BFs would exhibit higher T levels than NBFs as a result of the intra-sexual challenges to their breeding monopoly that they may experience by NBFs. We also tested whether this is correlated with body mass as a proxy for age or linked with breeding status irrespective of size.

2. MATERIAL AND METHODS

2.1 Study sites and trapping methods

We caught Natal mole-rats on a bimonthly basis from March 2003 to January 2004 and in March and July 2006 on a golf course surrounded by montane grassland at Glengarry Park (1500 m altitude) in the Kamberg region of KwaZulu-Natal (25°58'S; 21°49'E). Damaraland mole-rats were trapped at the Tswalu Kalahari Reserve in the southern Kalahari (27°13'S, 22°28'E) during March and October 2004 and March 2005. Captures conducted from May to October coincided with the dry season in both locations while the remainder of the year received the majority of precipitation and was thus defined as the wet season [46]. In both locations captures were conducted by exposing mole-rat tunnels close or underneath fresh molehills and setting modified Hickman life-traps baited with sweet potato at the entrances of those tunnels. All animals were live-trapped. Colony members were housed together in plastic containers until the entire colony had been captured. They were provided with wood shavings or soil as nesting and were fed on sweet potato. Group sizes were similar for both species (Natal mole-rats: 8.8±3.7 individuals, n=22 colonies, Damaraland mole-rats: 8.2±4.8 individuals, n=17 colonies). On capture body mass was recorded for each individual. Animals were regarded as belonging to the same colony only if they were collected at the same trap site. The breeding status was determined for all adult females. BFs could be readily identified by their perforate vagina and prominent teats while the remaining females were regarded as non-breeders.

2.2 Plasma sample collection and hormone assays

Natal mole-rats were killed by inhalation of halothane and blood was immediately collected from the right ventricle. The reproductive condition of females was evaluated by dissection of their reproductive tract. For blood sampling in Damaraland mole-rats individuals were hand restrained while blood was taken from the saphenic vein in the foot. Blood samples were centrifuged, plasma collected and frozen at –40°C until hormone assays were conducted. For both species samples were collected no later than 2 weeks after capture. Plasma samples were collected for a total of 101 (72 non-breeders [39 dry, 33 wet season], 29 breeders [18 dry, 11 wet season]) Natal and 75 Damaraland mole-rat females (51 non-breeders [17 dry, 34 wet season], 24 breeders [8 dry, 16 wet season]). All protocols were approved by the University of Pretoria ethics committee and complied with regulations stipulated in the Guidelines for the use of Animals in Research.

Plasma samples were assayed for testosterone using commercially available coated tube assay kit (CoaT a-Count TKTT1, Diagnostic Products Corporation, Los Angeles, CA) that had previously been validated for the study species [35]. The antiserum is highly specific for testosterone and has a low cross-reactivity with other naturally occurring steroids except dihydrotestosterone, which is 5%. All samples were assayed in duplicate and the intra- and inter-assay coefficients of variation were 3.0% and 7.9% for Natal and 1.7% and 6.3% for Damaraland mole-rats, respectively.

2.3 Evaluation of the effects of pregnancy on T levels

As some of the dominant females in our study are likely to have been pregnant when sampled, we used two methods to investigate whether pregnancy could have impacted T levels. First, as the Natal mole-rat females were all euthanized and dissected (for another study), allowing pregnancy determination, we were able to directly confirm that the T levels of pregnant and non-pregnant BFs did not significantly differ (n=7, n=22, Z=-0.816, p=0.42). This was not possible for Damaraland mole-rats as they were not euthanized in the field, precluding direct pregnancy determination (and thereby the exclusion of pregnant BFs from our analyses). To therefore determine whether pregnancy impacts T levels in Damaraland mole-rat females, we paired seven NBFs with unrelated males in the laboratory and then collected blood samples from them for T determinations at bi-weekly intervals for up to 18 weeks after pairing or until parturition. Based on the known duration of pregnancies in this species of about 2 months [4] we identified samples collected as either being from a gravid or non-gravid female. Values were averaged across both periods for analysis. Only 3 of these females became pregnant during the collection period and plasma T levels during pregnancy were not significantly higher than those before they became pregnant (Wilcoxon-test, Z=6.00, p=0.109). However, the small sample size may have limited the probability of finding significant differences.

2.4 Statistical analyses

The hormone data failed to satisfy the criteria for parametric tests and transformations were not successful. Consequently, the relationship between body mass and T was explored with Spearman's correlations. The effects of intra-sexual competition were further assessed with a generalized linear mixed model (GLMM) with T levels as dependent variable assuming a gamma distribution and a log-link function [15] with colony identity as random factor. Species, breeding status and season were included as factors in the model. All 2 and 3-way interactions were included in the model. Initially, we also included body mass as co-variate. However, since body mass was not significant in the model we report the results without body mass here. Post-hoc comparisons were carried out using the least significant difference (LSD).

3. RESULTS

Circulating levels of T increased with increasing body mass in both Natal (R_S=0.272, n=92, p<0.01, Fig. 1A) and Damaraland mole-rats (R_S=0.331, n=75, p=0.004, Fig. 1B). However, as BFs in both species were significantly heavier than NBFs (p<0.0001 for both species), this relationship could be driven by higher T levels in dominants rather than with increasing body mass *per se*. Indeed, our full statistical model of the factors affecting T levels suggests that this is the case. The model reveals that independent of body mass BFs had significantly higher T titres (1.96±0.2nmols/l) than NBFs (1.02±0.12nmols/l, Wald-χ^2=11.755, df=1, p=0.001). Furthermore, female T levels were significantly higher during the wet (1.76±0.20 nmols/l) compared to the dry season (1.17±0.18nmols/l, Wald-χ^2=4.776, df=1, p=0.029). The model also revealed that females in Natal mole-rat (3.26±0.31nmols/l) societies show significantly and markedly higher T levels than Damaraland mole-rats (0.40±0.12nmols/l, Wald-χ^2=100.661, df=1, p<0.0001).

None of the 2-way interaction terms were significant (all p≥0.221). However, the 3-way interaction between species, season and breeding status was of borderline significance (Wald-χ^2=3.813, df=1, p=0.051, Fig. 2). The T levels of both Natal mole-rat NBFs and BFs were significantly greater than those of Damaraland NBFs and BFs during both seasons (p≤0.005). During the dry season, in Natal mole-rats, BFs had significantly higher T levels than NBFs (LSD: p=0.001, Fig. 2A), while in Damaraland mole-rats they did not (LSD: p=0.649, Fig. 2B). By contrast in the wet season the opposite was true: in Natal mole-rats, BFs had comparable T levels to NBFs (LSD: p=0.400, Fig. 2A), while in Damaraland mole-rats BFs had near-significantly higher T levels than NBFs (LSD: p=0.052). This contrast arose because, in Natal mole-rats, the T levels of NBFs were significantly higher in the wet than the dry season (LSD: p=0.013), while those of BFs were not (LSD: p=0.453). In contrast, in Damaraland mole-rats, the T levels of BFs were near-significantly higher in the wet season than the dry (LSD: p=0.052, Fig. 2B), while those of NBFs were not (LSD: p=0.208).

4. DISCUSSION

4.1 *Evidence that mechanism of reproductive skew modulates female T levels*

In the current study, Natal mole-rats had markedly higher T levels than Damaraland mole-rats irrespective of breeding status. In fact, Natal mole-rat female T levels were approximately an order of magnitude higher than those of Damaraland mole-rat females, and are comparable with those for Natal mole-rat males (5.05±0.82nmols/l, unpublished data).

This would be predicted if physiological suppression among NBFs eased selection for androgen-mediated intra-sexual competition in the latter species. In the absence of physiological suppression and possibly regular access to immigrating unrelated males Natal mole-rat BFs are likely to experience frequent challenges to their breeding monopoly [20]. If such challenges in Natal mole-rats are settled via T mediated aggression rather than physiological suppression as seen in Damaraland mole-rats this could lead to elevated T levels as proposed for males in the 'challenge hypothesis' [42]. Furthermore, although we did not assess intra-sexual aggression in our study, Natal mole-rats appear to be substantially more aggressive than Damaraland mole-rats and injuries as a result of intra-colony aggression when reuniting colony members after capture may occur in the former but not the latter species (H. Lutermann, personal observation). Further support for a role of T mediated aggression in intra-sexual competition among females in the absence of physiological mechanisms of reproductive suppression comes from other cooperatively breeding species. BFs of *Neolamprologus pulcher* respond with elevated T levels to simulated territory intrusions and exhibit the strongest level of defence in the group [16,17]. Similarly, elevations in female androgens and intra-sexual aggression have been reported for non-cooperative species and strongly affect female reproductive success [31,39].

4.2 *Evidence that ecological factors affect female T levels*

Season had a significant modulatory effect on the androgen levels in both study species, with females showing significantly higher T levels in the wet season than in the dry season. This observation lends support to the hypothesis that intra-sexual competition may be linked to female androgen levels in the study species. Intra-sexual competition can be expected to increase during the wet season when NBFs of both species show an upregulation of their pituitary sensitivity and opportunities for encounters with unrelated males may arise frequently [35,46]. However, the detection of a near-significant three way interaction between season, species and dominance status raises the possibility that the seasonal pattern is more complicated than this, and could differ markedly between the two species. While in Natal mole-rat T levels did not differ between BFs and NBFs during the wet season, in Damaraland mole-rats T levels of BFs and NBFs were comparable during the dry season. Interestingly, this resulted from significant increases of T levels in NBFs in the former species and in BFs in the latter. If the observed increases were a result of agonistic interactions among females, the lack of increase in Natal mole-rat BFs may be indicative of a restraint on the part of dominants regarding further elevations in T, given what may be prohibitive costs associated with increasing what are already remarkably high T levels. For example, elevated T levels have been shown to impair a females' reproductive success and offspring care in a number of species [21,36,39]. Damaraland mole-rat BFs did clearly not exercise a similar restraint as they showed clear increases in T levels in the wet compared to the dry season, but there may have been comparatively little cost entailed in doing so as their mean T levels were much lower. This wet season T increase in Damaraland mole-rat BFs may be a result of the reproductive activation of NBFs in response to rainfall in this species [46] , which may force the BF to assert her breeding monopoly by behavioural means. Similar elevations in T levels of BFs have been observed in meerkats (*Suricata suricatta*) where pregnant dominant females show increased T titres and targeted aggression against subordinate females that pose the strongest threat to their reproductive success [13,44]. Perhaps surprisingly however, we only found a non-significant increase of T levels during the wet season for Damaraland mole-rat NBFs. This may be linked to the increase in cortisol-levels in NBFs during the wet season [46] as increases in corticosteroids can disrupt female reproductive activity and suppress aggression [43,45]. The seasonal patterns of T found in Damaraland mole-rat females correspond to those reported for common mole-rats (*Cryptomys hottentotus hottentotus*) [40]. Although, like Natal mole-rats, common mole-rat NBFs are not physiologically suppressed, they breed seasonally. Consequently, challenges to the BFs breeding monopoly may only occur seasonally and require elevations in T levels in accordance with these seasonal challenges.

4.3 Effect of breeding status on female T levels

BFs of both species had higher T levels than NBFs. These findings mirror patterns observed in vertebrate males and assuming that elevated T levels in BFs are linked to the ability to attain and defend the breeding monopoly they support the hypothesis that female intra-sexual competition exerts selective pressures on T mediated traits thought to enhance reproductive success [12,23,42]. Our results are in accordance with studies in naked and common mole-rats (*Cryptomys hottentotus hottentotus*) that found elevated T levels in BFs compared to NBFs [9,40]. Comparable observations have been reported from other cooperatively breeding vertebrates. For example, BFs of the cooperatively breeding cichlid *Neolamprologus pulcher* exhibit a masculinisation in behaviour, T levels and brain gene expression compared to subordinate females [2,16]. These data suggest that elevations in circulating T levels could play a crucial role in the establishment and/or maintenance of the female breeding monopoly across cooperatively breeding vertebrates with high reproductive skew.

In conclusion, our study provides support for a role for intra-sexual competition in shaping the patterns of T secretion among females in high skew cooperative societies. Furthermore, our data raise the novel and hitherto unexplored possibility that the mode of reproductive suppression among subordinates (whether physiological or behavioural) may modify the strength of intra-sexual selection in a given species, and thereby the extent of elaboration of those traits associated with success in competition. In addition to social factors, we found evidence of seasonal elevations of female T levels during periods when intra-sexual reproductive competition among females is expected to be most acute. As such, our study also provides evidence consistent with the modulation of female T levels in response to both social and seasonal factors, as envisaged for males in the challenge hypothesis [42].

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Table 1. Key life-history, environmental and physiological traits of Natal and Damaraland mole-rats.

	Natal mole-rat	Damaraland mole-rat
Reproductive skew	1 breeding female	1 breeding female
Group size	8.8±3.7	8.2±4.8
Habitat	mesic	arid
Mode of suppression	behavioural	physiological
Reproduction	aseasonal	aseasonal
HPG-axis in NBFs	Seasonal upregulation	Seasonal upregulation

HPG: hypothalamic-pituitary-gonadal, NBF: non-breeding female

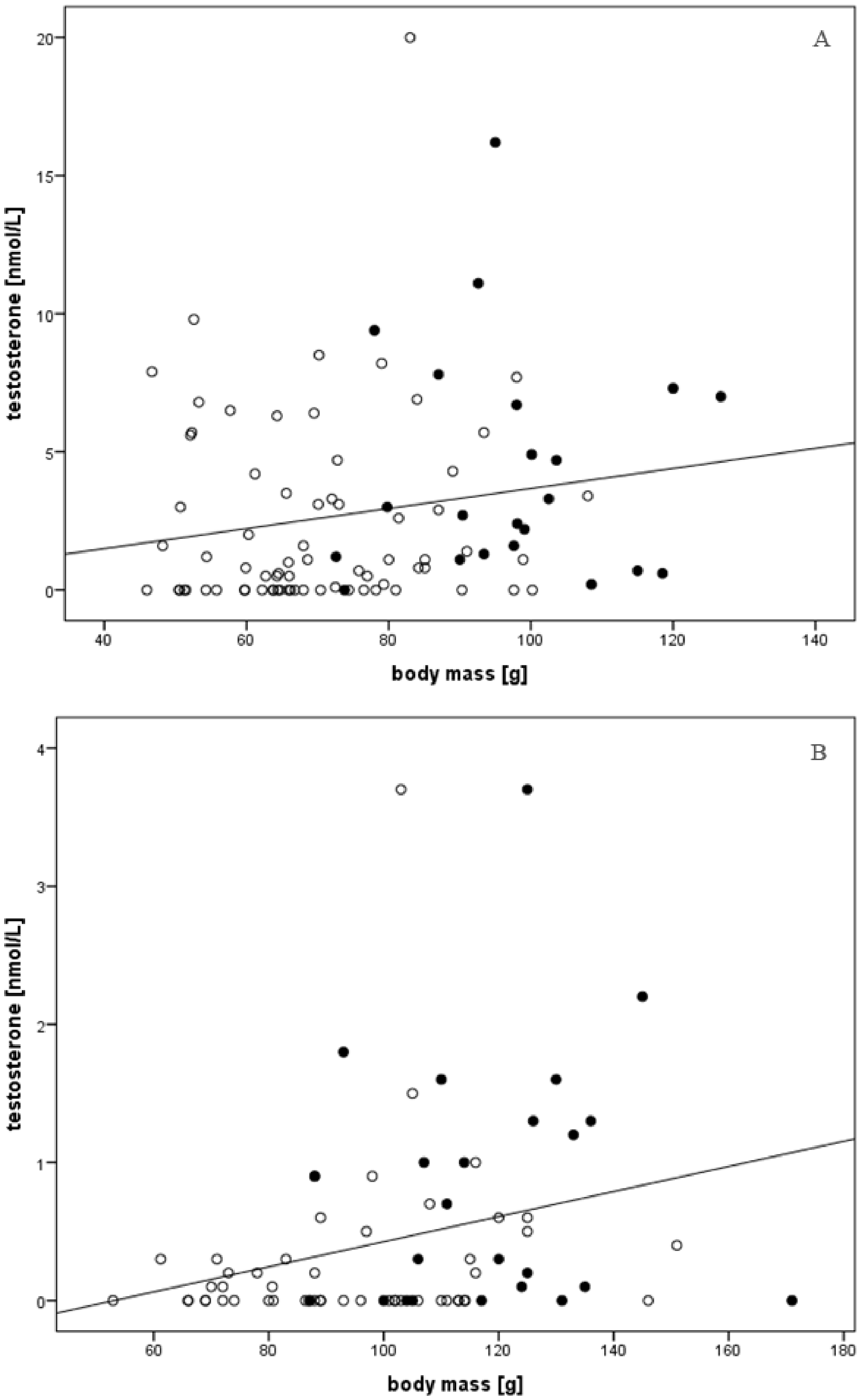

Fig 1. Correlation between body mass and plasma testosterone concentrations for a) Natal mole-rats and b) Damaraland mole-rats. Open circles indicate NBFs and closed circles indicate BFs**.**

Figure 2

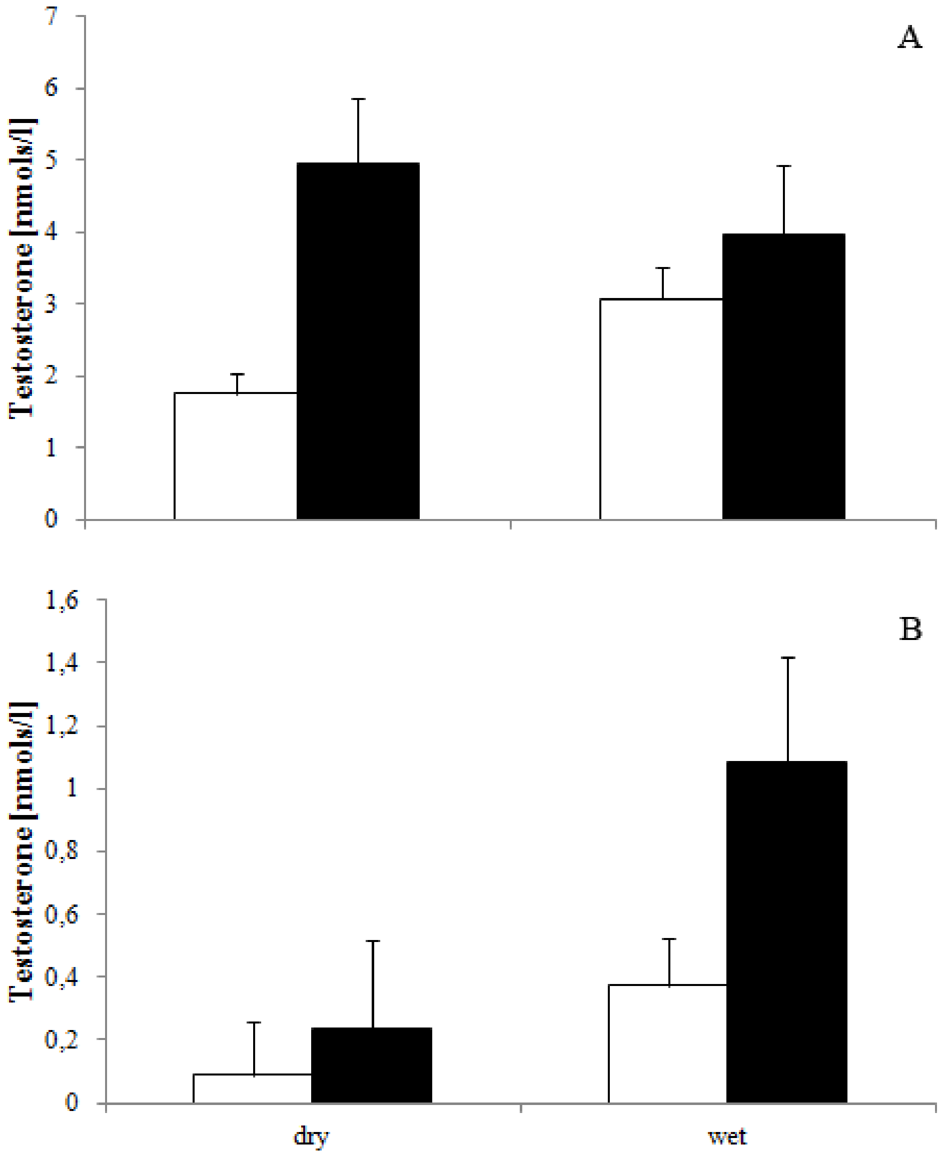

Fig 2. Season and status-dependent differences in plasma testosterone concentrations of female a) Natal and b) Damaraland mole-rats. Open bars indicate non-breeders, solid bars breeders. Displayed are means±SE.