

## Female choice, male dominance and condition-related traits in the polygynous subterranean rodent *Ctenomys talarum*



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### ABSTRACT

*Ctenomys talarum* is a solitary and highly territorial species in which dominant males aggressively deter other males and monopolize reproductive activity. Female preference for dominant males is not easy to assess due to coercive mating by males. Hence, we aimed to answer if behavioural dominance and several condition-related traits like testosterone and cortisol levels, endoparasite load, and hematocrit volume may affect female preference when they have the opportunity to exert it. We designed a laboratory experiment using wild-caught *C. talarum* and employed a combined approach involving behavioural observations and the measurements of parameters of physical condition. We staged dyadic encounters between males to determine dominance index and then analyzed female preference towards tethered males ( $n = 15$ ) or their odours ( $n = 18$ ). Male dominance did not affect female preferences when odours were presented. When two tethered males were offered, females preferred those with higher dominance index. Preference of females for dominant males would mainly represent indirect benefits. Females did not show preference for males in relation to any physiological trait evaluated. Dominance was found negatively related to cortisol levels, probably avoiding the glucocorticoids-related costs, and positively related to parasite diversity, since they could tolerate it without impairing their health.

### 1. Introduction

Female selectivity in their choice of mate is widely recognized in many species, but it is enhanced in mammals due to the cost of pregnancy and lactation (Andersson, 1994; Kokko et al., 2003). Benefits result clearer when females receive direct benefits from males in the form of better food and territorial resources, assistance in parental care, or a safe mating if potential partners do not carry transmittable parasites (Andersson, 1994; Beltran-Bech and Richard, 2014). In the case of males providing only sperm, females may benefit by selecting those characterized by high genetic quality – good genes or compatible genes – enhancing viability and attractiveness of progeny (Folstad and Karter, 1992; Drickamer et al., 2003; Neff and Pitcher, 2005). To assess male quality, odour is a condition-dependent trait that convey a wide array of information, e.g. individual identity, physical condition, competitive ability, hormonal levels, and reproductive status (Blaustein, 1981; Tang-Halpin, 1986; Penn and Potts, 1998; Zinkevich and Vasilieva, 2001; Hurst, 2009).

Male-male competition for mating opportunities is common in mammals, and success in a contest is usually related to costly traits like

body size, aggressiveness and fighting ability (Qvarnström and Forsgren, 1998). To afford these metabolic requirements, along with the increased risk of injury and rate of disease, males need to be in good physical condition (Andersson, 1994; Qvarnström and Forsgren, 1998). Therefore, behavioural dominance and physical condition-associated traits result good indicators of male quality that serve females to assess potential partners (Maynard Smith and Harper, 2003; Qvarnström and Forsgren, 1998). Definitely, preference for dominant males has been found in many rodent species (e.g. *Rattus rattus*, Carr et al., 1982; *Clethrionomys glareolus*, Horne and Ylonen, 1996). Despite dominance is not a simple function of aggressiveness, the acquisition and maintenance of certain status often requires different degrees of aggression. Androgenic steroid hormones mediate aggressive behaviour. Testosterone levels were related to the chance of winning a contest and dominance, a dynamic outlined as part of the challenge hypothesis (Wingfield et al., 1990; Nelson, 2005). Moreover, in species that aggressively defends territory, testosterone pulse after winning a contest was proved to increase the chances of a future victory (winner effect, Oyegbile and Marler, 2006). High testosterone levels were also related to male attractiveness, extra pair fertilizations, and higher mating

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success (reviewed in Clutton-Brock and McAuliffe, 2009). However, there are potential costs of holding elevated testosterone levels, such as increased metabolic rate and depressed immune response (Wingfield et al., 2001). These imposed costs should guarantee the honesty of the resulting signals of mate quality (Immunocompetence Handicap Hypothesis; Folstad and Karter, 1992; Roberts et al., 2004).

Social interactions are an important source of stress. Male-male competition for access to potential partners can be harsh, especially when few males dominate the access to multiple females (Setchell et al., 2010). In vertebrates, stress is mediated by the rise of plasmatic levels of glucocorticoid hormones (Nelson, 2005) which help to mobilize energy, increase cardiovascular tone and are involved in the regulation of immunity, while inhibiting costly anabolic processes (Sapolsky, 2011). Hence, stress response may be used as a measure of attractiveness during mate choice, since it may represent a complex of traits that signal mate quality (Bauer et al., 2008; Husak and Moore, 2008; Moore, 2012).

Ability to resist parasites and pathogens constitutes another major aspect of male quality (Hamilton and Zuk, 1982; Getty, 2002), conferring both direct (safe mating) and indirect (good genes for the progeny) benefits for females. Nonetheless, whether dominance is related to overall health, impairment of immunity performance, or immunosuppressant effects through testosterone increments, it is clear that it does not conform to a single pattern (Adelman et al., 2014).

The subterranean rodent *Ctenomys talarum* (Talas tuco-tuco Thomas, 1898) provides an excellent opportunity to evaluate female sexual preference in relation to male behavioural dominance and traits associated to physiological condition. This is an herbivorous species that live solitarily in permanently sealed burrow systems (Busch et al., 2000) along coastal grasslands in Argentina, South America (Reig et al., 1990; Woods, 1993). Most of their activities occur inside their burrows but they emerge from underground during short and fast trips to collect food (Busch et al., 2000). Both sexes usually store soil heavily mixed with urine, feces, and small bits of grass next to aboveground burrow openings which serves neighbouring tuco-tucos to obtain information about the individual (Zenuto and Fanjul, 2002), gender (Fanjul et al., 2003), and reproductive condition (Zenuto et al., 2004) of the occupant from the outside of the burrow. DNA fingerprinting analyses suggest that *C. talarum* is polygynous (Zenuto et al., 1999a). Males are bigger than females (Zenuto et al., 1999b) and do not provide parental care (Zenuto et al., 2002). Although both sexes are territorial, only males utter a typical territorial vocalization that informs potential intruders about the owner's presence in a territory (Schleich and Busch, 2002). Evidence of scars along the neck in males (R.R.Z., unpublished data) accounts for strong aggressive contests in the wild. Laboratory studies using seminatural enclosures (Zenuto et al., 2001) showed that males aggressively fight each other, and the winner (dominant) vocalize their territorial call ("tuc-tuc") and scent mark the burrow's tunnels of the subordinate male (using urine, feces and anogenital rubbing) (Zenuto et al., 2002). As a result, dominant males monopolize reproductive activity, aggressively impeding other males to access mature females that they later mate coercively (Zenuto et al., 2002). Marked inter-individual variance in testosterone levels would be associated to those dominant/subordinate relationships (Vera et al., 2013).

Courtship is complex in *C. talarum*, starting with agonistic approaches and followed by recognition behaviors, chases, and neck bites previously to mating. Once the couple reaches close contact, the male intent to coercively mount the female. The copulation occurs if the female expose the back area, but frequently evasive movements impede it, showing resistance (Fanjul and Zenuto 2008). Female choice seems to be very important for *C. talarum* mating (Zenuto et al., 2007). Female familiarization with male scent affects the outcome of a reproductive encounter. Moreover, females show low aggression during courtship when encountering familiar males (Zenuto et al., 2007). Nonetheless, they prefer novel males when confronted simultaneously with familiar ones (Fanjul and Zenuto, 2013). Studies on MHC-associated mate

choice provide evidence of females preferring heterozygous males and those carrying specific alleles (Cutrera et al., 2012). Certainly, male size and aggression could limit the expression of female preferences in the wild, but being females the sex that more energy invests in reproduction (Zenuto et al., 2002), female choice is expected, especially when coercion is prevented.

The aim of this work is to assess female preference for males in relation to their performance during male-male contests, and to their physical condition. Two levels of partner assessment were considered: chemical signals and full contact with males that were not able to coerce female response. We also explored the relationship between male behavioural dominance and condition-related traits, and the relationship between dominance rank and hormonal condition after male-male interaction. To address these subjects we designed a laboratory experiment using wild-caught individuals and employed a combined approach involving behavioural observations and the measurement of physiological parameters indicating physical condition. We first staged dyadic encounters between males and determined dominance indexes. Later, we analyzed female preference towards males and their odours according to their dominance status. We also analyzed the relationship with particular condition-related male traits as hematocrit volume, testosterone and cortisol levels, and endoparasite load, recorded before each behavioural trial (both dominance and female preference trials). Preference test allowed us to evaluate if females differentially devote their interest over males according to dominance status, using visual, auditive and chemical signals/cues from individuals or its odours (a source of metabolites such as cortisol, and testosterone that allow mate quality assessment). We predicted that males in good condition are likely to perform more successfully during male-male contests and females would show preference for high quality males, specifically those with higher dominance rank and characterized by good physical condition. If only males of a relatively high quality can bear the cost of dominance, females could benefit mating dominant males.

## 2. Material and methods

### 2.1. Animal capture and housing conditions

We used live traps to collect mature *C. talarum* at Mar de Cobo, Buenos Aires province, Argentina, (37°45'S, 57°26'W). Plastic tube traps were inserted into animal's burrow systems showing fresh surface mounds. Females were captured during their non-breeding season (March–May 2007–2009) to avoid the influence of previous reproductive activity, while males were captured during the reproductive season (June–December 2007–2009). We minimize the chance of animals being neighbours or partners at field since we captured each sex in different areas. All animals were taken to laboratory, where each tuco-tuco was individually housed in a plastic cage measuring 42 × 34 × 26 cm with a wire-mesh lid. We provided wood shavings for bedding, and animals were fed every day with fresh food (carrots, sweet potatoes, catalogna chicory, corn, mixed grasses, and sunflower seeds) *ad libitum* to secure water provision since *C. talarum* does not drink free water. Temperature and photoperiod were automatically controlled (25 ± 1 °C; non-breeding 12L: 12D; breeding 14L: 10D). We performed experiments during the breeding season, and at the end of the experiments, animals were returned to their site of capture. We wore disposable gloves in all instances: sample collection and during the experimental trials. All equipment used during the study was washed with tap water and odourless glassware cleaner, wiped with alcohol, and allowed to air dry to ensure that no trace odours from previous trials remained. We employed a total of 35 females (average time of residence in the lab was 3 months), and 50 males (average time of residence in the lab was 2 weeks) to conduct a total of 35 trials. Only trials in which females showed active and displayed reproductive behaviours were included in this study (Zenuto et al., 2007).

## 2.2. Measures of male condition-related traits

On the 3th and 8th day after males were brought into captive conditions, we carefully collected a blood sample from each individual suborbital sinus as described by Vera et al. (2011a). Next, blood samples were centrifuged (3000 rpm, 15 min) for isolation of plasma, which was stored at  $-20^{\circ}\text{C}$  until analysis. On the same days, fresh fecal pellets of each male were collected from cage beddings. Pellets were fixed in 4% formalin and stored until analysis of endoparasite load.

Hematocrit volume. As a measure of overall health condition, we determined the percentage of erythrocytes circulating in the peripheral blood (e.g. Hellgren et al., 1989). Following standard protocols, a heparinized capillary tube was filled with whole blood to within 1–2 cm of one end, and the unfilled end was sealed with clay. After that, the tube was centrifuged at 12000 rpm during 15 min using a micro-hematocrit centrifuge Giumelli® Z-12-D. Hematocrit was then measured as the proportion of the capillary tube containing packed red blood cells relative to the length occupied by all blood components using a Giumelli® Abaco.

Parasite quantification. Fecal eggs and oocyst for the protozoan *Eimeria* sp.- shedding was assessed using the MacMaster flotation technique, which had previously been employed in the analysis of endoparasite load in free-living tuco-tucos (Cutrera et al., 2011). One gram of wet feces (fixed in 4% formalin) was ground up and suspended in 15 ml of modified Sheather's flotation solution (Sheather, 1923). Fecal suspension was mixed to homogenate before a subsample was taken to fill each of the two MacMaster chambers. We let the chamber stand for 5 min to allow eggs to float to the surface and debris to go to the bottom of the chamber. For each individual, fecal egg counts were estimated per milligram of wet feces (eggs/mg). We found four taxa of gastrointestinal nematodes (*Thrichuris pampeana*, *Paraspidodera uncinata*, *Graphidioides subterraneus* and *Pudica ctenomydis*) and one intestinal protozoan (*Eimeria* sp.), in the evaluated samples. Parasitological status was assessed as: (1) parasite load, as total fecal egg count by counting the total number of parasite eggs per mg of feces (FEC); (2) parasite diversity, as Shannon index (Shannon and Weaver, 1949).

Hormone analyses. Cortisol and testosterone were measured using the Coat-A-Count procedures (Siemens Medical Solutions Diagnostics, USA). Both the cortisol and testosterone kits have been extensively validated for their use with plasma samples of *C. talarum* (Vera et al., 2011a,b). Plasma samples were used directly for cortisol dosages, but for testosterone determination, plasma samples were previously heated ( $56^{\circ}\text{C}$ , 30 min) and then diluted (1:6) using phosphate buffered saline buffer (pH = 7) to eliminate interference of plasma components and measure testosterone levels near the optimal range of the evaluation (i.e. 50% binding) following the methodology of Vera et al. (2011b). Intra-assay coefficients of variation (CVs) were 8 and 8.5% for cortisol and testosterone, respectively (Vera et al., 2011b). Given that all plasma samples were run in a single assay, the inter-assay CVs were not calculated.

## 2.3. Male-male contest: estimating dominance

Dominance rank of each pair of males was established by means of male-male contests. These contests were performed on the 5th day after animals arrived at the lab. Pairs of males were randomly arranged but their body mass did not differ by more than 5%. The experimental apparatus consisted of three Perspex cages ( $45 \times 30 \times 30$  cm) connected to each other by an acrylic tube (10 cm diameter  $\times$  20 cm length). To distinguish each contestant, one randomly chosen male was marked with a nontoxic temporary dye (Creameches™, L'Oréal) on the back. Each test animal was individually confined for 60 min (acclimation time) in one of the cages, which contained shavings from each one's housing cage. At the start of the trial, both animals were allowed to enter the central cage, provided with clean shavings, to interact

**Table 1**

Agonistic behaviors displayed by male *Ctenomys talarum* during male-male contests. A weight score was assigned to each behavior as an ordinal scale of aggressiveness.

Behaviour	Description	Weight
Fight	Fighting includes boxing, bites and wrestling	
Winning	Characterized by forward movements during wrestling	3
Losing	Characterized by backwards movements during wrestling	2
Bite	Male bites or attempts to bite the other	2
Show teeth forward	Male raises his head and show his incisors while moving forward	2
Show teeth backwards	Male raises his head and show his incisors while moving backwards	1
Chasing	One male chase the other	1
Mount	One male mounts the other	1
Scent mark	A male scent mark with urine, feces or anogenital rubbing	1
Present back	A male turn his back towards the contestant male	-1
Avoid	A male walk away when the contestant male is approaching	-2
Try to escape	Males attempt to leave the test apparatus, scratching anxiously the walls and floor	-2
Abandon their box	Males stayed on the neutral arena as a result of being displaced of its own box	-3

freely for 20 min along the test apparatus. Trials were recorded with a HD camcorder (Sony™ HDR-XR100). Later, we observed the videos and recorded agonistic behaviours (Table 1). According to their motivational considerations, a weight score from  $-3$  to  $+3$  was assigned to each animal's behaviour as an ordinal scale of aggressiveness (Table 1). For each individual, Dominance Index (DI, *sensu* Barki et al., 1992) was then calculated as  $DI = \Sigma (\text{behaviour frequency} \times \text{behaviour weight}) / \Sigma (\text{behaviour frequency})$ .

## 2.4. Preference trials: evaluating female preferences

Preference tests were carried out on the 10th day after arrival of males to the lab and conducted according to Fanjul and Zenuto (2013). Female preference was assessed at two levels: male odours and full contact with tethered males. Females participated in one preference trial while males could participate once as stimuli male in a full contact trial, and once as donor of odour sample. The experimental apparatus consisted of Perspex boxes ( $45 \times 30 \times 30$  cm) and a Y maze (10 cm diameter and 25 cm length each arm). Prior to preference tests, each female and/or males were placed in their respective box for at least an hour to familiarize with it before the test begun (Zenuto and Fanjul, 2002). To induce behavioural estrous, 24 hs before preference test females were injected IP with oestradiol benzoate ( $10 \mu\text{g}/100$  g live animal weight; Estradiol 10, Laboratorios Rio de Janeiro) as suggested for induced ovulators (Bakker and Baum, 2000) like *C. talarum* (Weir, 1974; Fanjul and Zenuto, 2008).

During the odour preference test ( $n = 20$ ), the female box was connected to the Y maze. At the end of each maze arm, a sample of each male odour was placed as an end-cap. Trials lasted for 7 min starting when the test female entered the maze (Fanjul et al., 2003; Zenuto et al., 2004). We recorded the preference of a female in each sample as the total time devoted to sniffing each sample. This investigatory bias was reported to correspond to their actual mating preference for many species (e.g. Egid and Brown, 1989; Hurst and Rich, 1999) including tuco tucos (Fanjul and Zenuto, 2013).

In preference tests involving full contact with tethered males ( $n = 15$ ), the stem of the Y maze was connected to the female cage while the arms were connected to cages, containing a male individual each one. Male cages were provided with a transversal rod and a movable line of stainless steel fishing leader ending in a snap swivel that could be attached to a self-locking nylon tie collar worn by the male.

Males were individually tethered to their cage before familiarization and collars were removed immediately after trials (see Fanjul and Zenuto, 2013). Trials lasted 30 min starting when females entered the Y pipe. The essays were also recorded in HD videos. We determined the preference of a female for each male by recording both the total time the female stayed active moving around the box of a male, and the mating events that occurred.

We defined the 'preferred male' based on the time that the choosing female spent towards the male and/or its odours in relation to the total time devoted to both males combined (*sensu* Cutrera et al., 2012). In full contact assessment, when a female mated with a male, that male was defined as the preferred one even if the specified criteria were not met. When considering mating females, only one out of 8 animals mated both males; in that case, the preferred one was defined based on the time devoted to investigate each male and/or moving around in the cage.

## 2.5. Data analysis

If assumptions (i.e. normality and homoscedasticity) were not met, index and condition-related traits in preferred vs non preferred males or odours were tested by the Paired-sample *t*-test or by its non-parametric analogue, Wilcoxon matched-pair test (Zar, 2010). To evaluate female preference in relation to male condition, traits were measured from blood or feces samples obtained on day 8th, that is, 48 hs before preference trial was conducted. Accordingly, to assess the relationship between dominance index (DI) and male condition, Spearman correlations were performed, and condition traits were measured over samples taken 48 hs before the male-male contests (i.e. 5th day). Furthermore, to analyze the relationship between male performances during the contest and changes in hormonal traits (winner effect), we correlated dominance index with variation in cortisol and testosterone, before and after the contest.

Results were reported as means  $\pm$  SD. In all cases, the critical significance level was set at  $p \leq 0.05$ . Along with the exact *p* values, the observed effect sizes according to the suggestions of Nakagawa (2004) and Stoehr (1999) for behavioural sciences were reported. Cohen (1992) defined effect sizes as 'a scale-free value that measures, in terms appropriate to it, the discrepancy between  $H_0$  and the  $H_1$ '. Cohen (1992) proposed the benchmark values for what are to be considered 'small', 'medium' and 'large' effects, which are 0.2, 0.5 and 0.8, respectively. We calculated effect size, as standardized Cohen's  $d_z$ , for paired samples using the program GPower 3.1.1 (by F. Faul, Universität Kiel, Germany; <http://www.psych.uni-duesseldorf.de/abteilungen/aap/gpower3/>) (McDonald 2014).

## 2.6. Ethical note

We adhere to the International Guiding Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences (CIOMS) and the International Council for Laboratory Animal Science (ICLAS). In the present study, none of the animals died during or directly after blood extraction. We were ready to interrupt the trial if a threat of animal health or wellbeing was observed. Such threats never occurred during male-male contests. The use of collars did not affect the exploring behaviour of males, which interacted with the choosing females and even mated with them.

## 3. Results

### 3.1. Female preference

Preferred and non-preferred male odours corresponded to individuals showing similar mean values of dominance index (Fig. 1A; Paired *t*-test  $t = -0.630$ ,  $df = 17$ ,  $p = 0.537$ ,  $d_z = 0.148$ ). The same pattern was observed for the condition-related traits: hematocrit

(Fig. 1B; Wilcoxon matched-pairs test  $t = -77.500$ ,  $df = 16$ ,  $p = 0.963$ ,  $d_z = 0.547$ ), parasite load (Fig. 1C;  $t = -0.037$ ,  $df = 12$ ,  $p = 0.971$ ,  $d_z = 0.010$ ), parasite diversity (Fig. 1D;  $t = -1.089$ ,  $df = 7$ ,  $p = 0.312$ ,  $d_z = 0.384$ ), testosterone levels (Fig. 1E;  $t = -0.774$ ,  $df = 14$ ,  $p = 0.452$ ,  $d_z = 0.199$ ), and cortisol levels (Fig. 1F;  $t = 0.619$ ,  $df = 13$ ,  $p = 0.547$ ,  $d_z = 0.175$ ). However, when females assessed males, preferred ones showed a higher dominance index (Fig. 2A;  $t = 2.449$ ,  $df = 14$ ,  $p = 0.028$ ,  $d_z = 0.631$ ). The remaining traits presented no differences between preferred and non preferred males: hematocrit (Fig. 2B;  $t = -0.654$ ,  $df = 10$ ,  $p = 0.528$ ,  $d_z = 0.197$ ), parasite load (Fig. 2C;  $T = 27$ ,  $df = 11$ ,  $p = 0.638$ ,  $d_z = 0.361$ ), parasite diversity (Fig. 2D;  $t = 0.403$ ,  $df = 7$ ,  $p = 0.699$ ,  $d_z = 0.142$ ), testosterone levels (Fig. 2E;  $t = -1.022$ ,  $df = 10$ ,  $p = 0.331$ ,  $d_z = 0.308$ ), and cortisol levels (Fig. 2F;  $t = 0.872$ ,  $df = 10$ ,  $p = 0.404$ ,  $d_z = 0.263$ ).

### 3.2. Dominance, condition-related traits and contest effect

Male dominance index was not related to hematocrit (Fig. 3A). In relation to parasite load, total FEC did not correlate with dominance (Fig. 3B) but parasite diversity (Fig. 3C) did. Hormone levels showed a dissimilar pattern since plasmatic testosterone was found unrelated to dominance index (Fig. 3D) while cortisol presented a negative association (Fig. 3E). Testosterone remained unchanged with the dominance status attained in the male-male contest, but cortisol increased in those males that attained a higher level of dominance (Fig. 4).

## 4. Discussion

Despite odours play an important role in the life of *C. talarum* (Zenuto and Fanjul, 2002; Fanjul et al., 2003; Zenuto et al., 2004; Zenuto, 2010; Fanjul and Zenuto, 2013) we failed to find any preference to male odours in relation to dominance or any of the related traits evaluated by chemical cues. There are two ways by which dominance can be advertised by scents: the composition of the scent itself (e.g. Jones and Nowell, 1973; Novotny et al., 1990) and/or the spatial and temporal pattern of scent deposition (e.g. Johnston et al., 1997; Rich and Hurst, 1998). In the present study, preference tests allowed us to evaluate the first one since only the composition of the scent can be assessed. Several reports indicate female preference for odours of dominant males (e.g. *Mus domesticus*, Mossman and Drickamer, 1996; *Clethrionomys glareolus*, Hoffmeyer, 1982). In some species, such preferences were related to numerous androgen-dependent urine chemicals that females use to assess male social status and competitive ability (Malone et al., 2001). Thus, the lack of relationship between dominance and testosterone levels in our study system may account for no preferences for dominant male odours in *C. talarum*. Indeed, tuco tuco may use scent marking pattern to convey dominance. Chemical assessment of dominance and competitive ability appear more strongly linked to the frequency of scent marking, spatio-temporal pattern of deposition, and the presence of a competitor's scent mark (Wong and Candolin, 2005). Ongoing research would indicate that female of *C. talarum* employs and responds to scent marked territories, and territories plus owner presence, but that it does not respond to olfactory signals alone (Fanjul, Varas and Zenuto unpublished manuscript).

When females assessed males directly, they clearly preferred dominant ones. Since dominant males are expected to be of superior quality, it would be on the best interest of females to choose those individuals as mates (Qvarnström and Forsgren, 1998). There is plenty of literature reporting female preference for dominant males: e.g. *Mus musculus* (Parmigiani et al., 1982) *Microtus orchogaster* (Shapiro and Dewsbury, 1986), *Mesocricetus auratus* (Place et al., 2014), *Clethrionomys auratus* (Hoffmeyer, 1982). In some cases, the competitive advantage of the male to establish and monopolize a high quality territory provides some benefits to the progeny like parental care, food resources, and proximity to mates. In other cases, it would result in

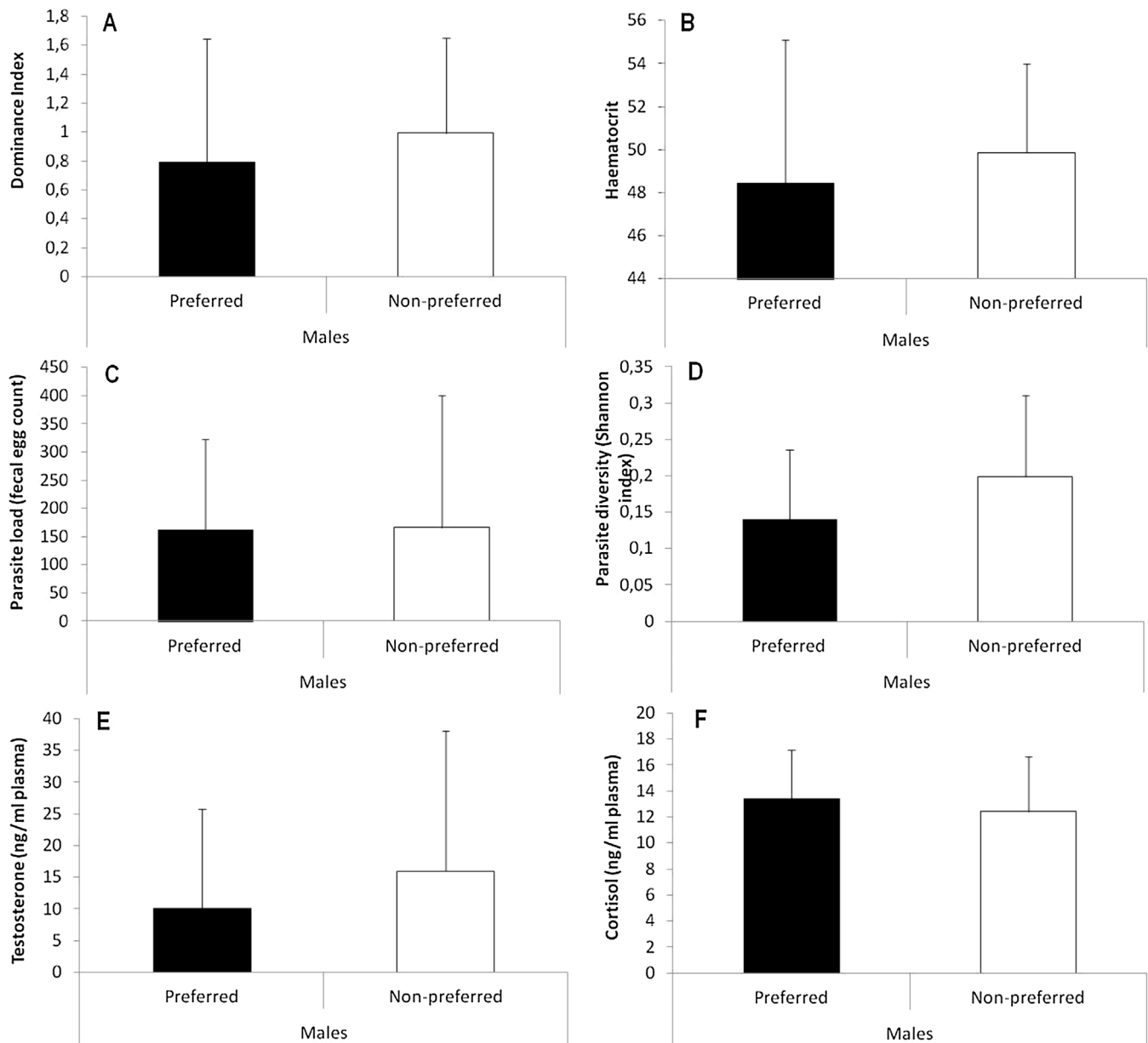
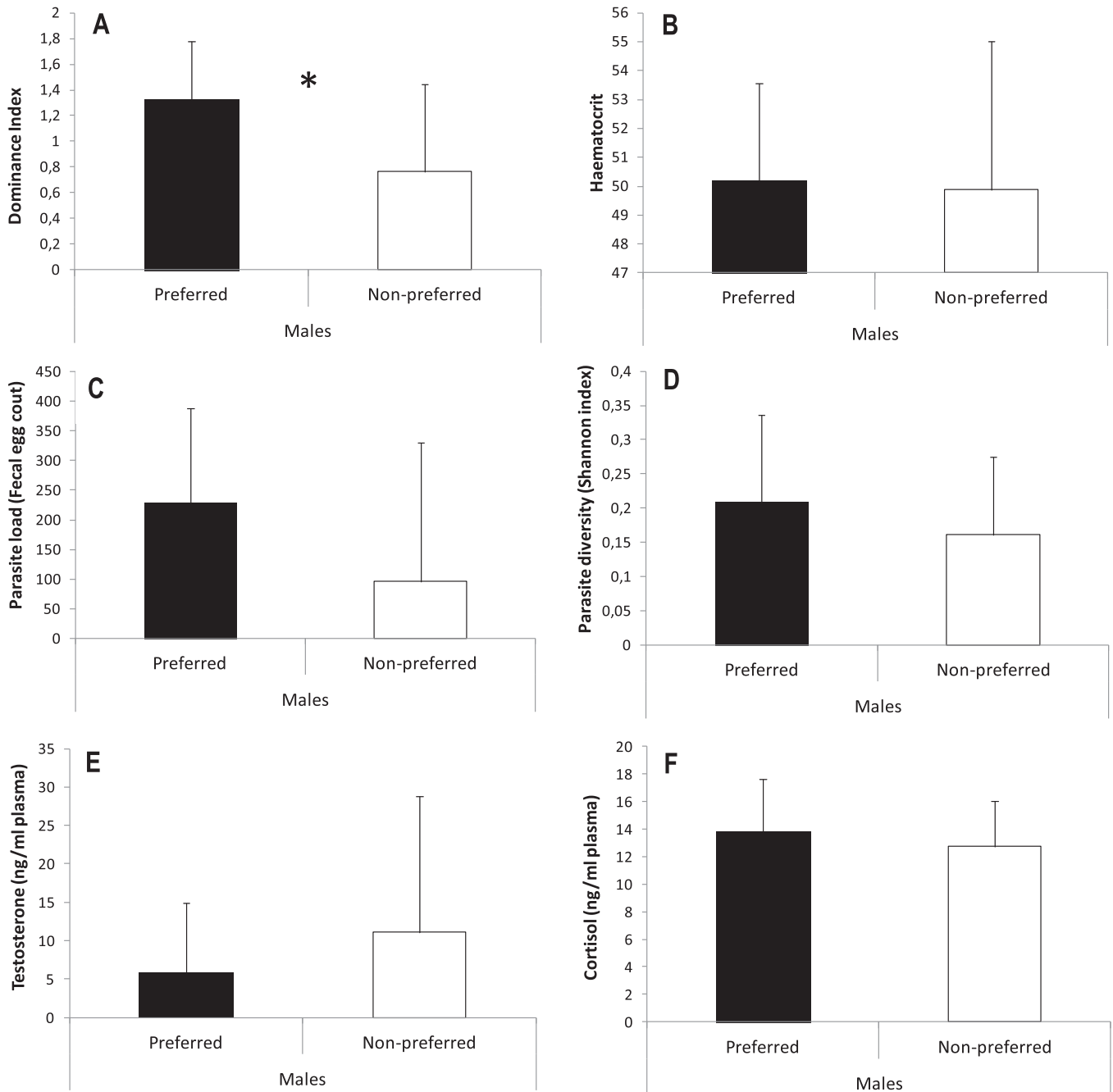


Fig. 1. Mean ( $\pm$  SE) dominance index (A), haematocrit (B), parasite load (C), parasite diversity (D), testosterone levels (E), and cortisol levels (F) of preferred and non-preferred donor males based on time spent by tuco-tuco females during odour preference trials. Statistically significant differences (Paired *t*-tests or its non-parametric equivalent) are noted as \* when  $p \leq 0.05$ .

genetic benefits if sons inherit father's competitive skills, ensuing in dominant males siring successful sons (Wong and Candolin, 2005). This last scenario corresponds to tuco-tucos: a polygynous rodent with no paternal care. Moreover, the territorial establishment and its defense are crucial to males in order to successfully monopolize females. The performance in such critical task is affected by male fighting ability, strength (Zenuto et al., 2002; Becerra et al., 2012), and territorial scent marking (Zenuto, 2010). Experimental evidence using enclosures in captivity, but imitating the social environment in the wild (see Zenuto et al., 2001, 2002), showed that females of *C. talarum* mate exclusively with the dominant male (Zenuto et al., 2002). This occurred after males established their dominance status by fighting and threatening other males in the enclosure. Then, the dominant male imposes and precludes other males access to neighbouring females (Zenuto et al., 2002). As it occurs with other species (Reichard et al., 2005), such observations could lead us to suspect a very limited gap in which females can exert their own preference. We could then wonder what a female would

choose, if they had the opportunity. Our study shows that when female tuco-tucos were allowed to freely display their preference – as it occurred when tethered males were impeded to coerce potential mates – they chose dominant males. So, given the central role that territorial defense has for both sexes, but especially for males, good genes for dominance is a key trait for female preference due to the clear benefits that it represents for the progeny.

Females showed no preference for the remaining dominance-related traits evaluated. Since male dominance is essentially related to an individual's overall performance, competitive ability, and health (Qvarnström and Forsgren, 1998), it would constitute a complex cue comprehending several aspects associated to its quality as a potential mate (Horne and Ylönen, 1996). Despite the multiple information conveyed in dominance, the decision-making process of female choice may also rely on the use of multiple cues. Thus, composite traits plus multiple communication channels should reduce the cost of a given choice as well as facilitate mate assessment by increasing the amount of



**Fig. 2.** Mean ( $\pm$  SE) dominance index (A), haematocrit (B), parasite load (C), parasite diversity (D), testosterone levels (E), and cortisol levels (F) of preferred and non-preferred males based on time and mating of tuco-tuco females during full contact preference trials. Statistically significant differences (Paired *t*-tests or its non-parametric equivalent) are noted as \* when  $p \leq 0.05$ .

information gained (Candolin, 2003). In our study, chemical signals seemed not to be enough to convey dominance status. Female preference towards dominant males may be related to behavioural cues together with acoustic signals – an important communication channel for subterranean rodents- (Francescoli, 2000; Schleich and Busch, 2002); other dominance related traits that could be relevant for females (e.g. body size, fat reserves) would require further research (Arnott and Elwood, 2009).

#### 4.1. Dominance, condition related traits and contest effect

A remarkably large inter-individual variation in male testosterone levels characterized wild populations of tuco-tucos (Vera et al., 2013).

It may reasonably be assumed that such pattern could be associated to dominance-subordinate relationships in a context of high variation in male mating success (Zenuto et al., 1999a; Vera et al., 2013). However, we were not able to find a supporting testosterone/dominance relationship in tuco-tucos. We consider that the range of variation of testosterone titers found in the present study is notably narrower than that found for the species in the field, a fact which was recognized as part of the chronic response to captive conditions in our study species (Vera et al., 2011b) and in many others (Calisi and Bentley, 2009), and which could influence our ability to detect such relationship. Males bank voles with naturally high testosterone levels showed higher social status (laboratory dominance) than low testosterone males (*Myodes glareolus*, Mills et al., 2009). Reports on diffuse or lack of relationship

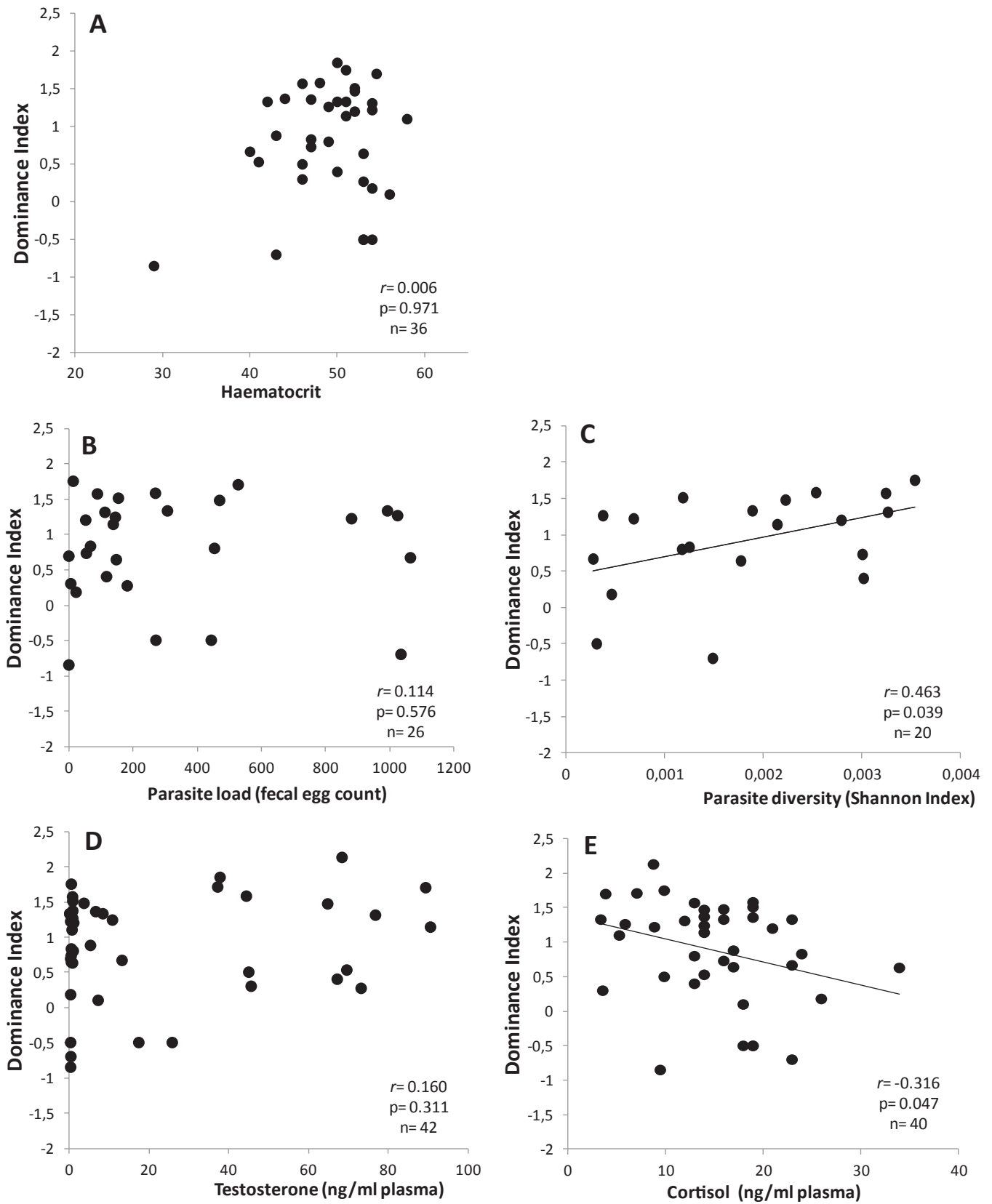


Fig. 3. Relationships (Spearman correlations) between dominance index and haematocrit (A), parasite load (B), parasite diversity (C), testosterone levels (D), and cortisol levels (E) in male tuco-tuco (*Ctenomys talarum*). In each panel Correlation coefficient  $r$ ,  $p$  value and sample size are displayed. Tendency lines are displayed only for significant relationships.

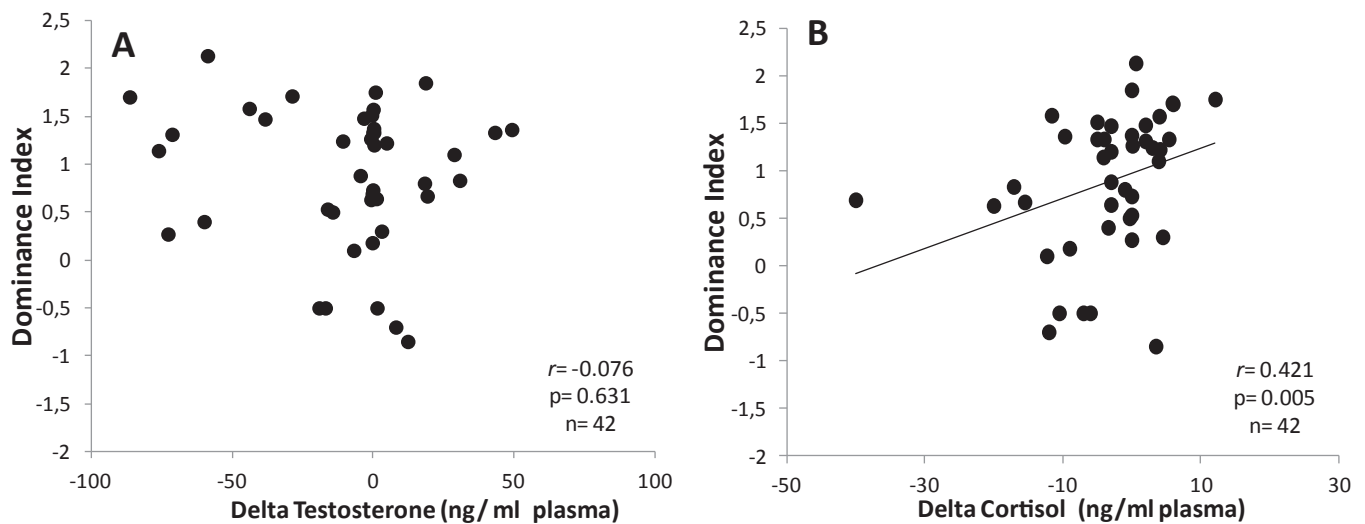


Fig. 4. Effect of male-male contest. Spearman correlations between dominance index and the variation (delta: measures previous- after the contest) of testosterone levels (A), and cortisol levels (B) in male tuco-tuco (*Ctenomys talarum*). In each panel correlation coefficient  $r$ ,  $p$  value and sample size are displayed. Tendency lines are displayed only for significant relationships.

between testosterone and dominant behaviour are getting increasing attention in research (e. g. *Mimus polyglottos*, Logan and Wingfield, 1990; *Homo sapiens*, Archer, 1991; *Lemur catta*, Cavigelli and Pereira, 2000; *Peromyscus californicus*, Trainor and Marler, 2001; *Microtus oeconomus*, Borowski et al., 2014). Among others, mating system and incidence of male competition under operational sex ratios deviated to males or females may account for part of these results. It has also been proposed that achieving a given level of dominance would only require a threshold level of plasmatic testosterone, and the additional concentrations would indeed generate a critical extra cost on fitness (Borowski et al., 2014). In view of the recognized cost of bearing high testosterone levels, such as immunosuppression (Wingfield and Romero, 2001), higher predatory risk associated to increased mobility (Perrot-Sinal et al., 1998), and higher metabolic rate (Buchanan et al., 2001), it has been proposed that a multifactor trade-off underlie testosterone levels and fitness-related traits (Borowski et al., 2014).

Considering stress and health, it is now clear that a generalized pattern is not easy to propose since dominance means different things to different species and social organizations (Sapolsky, 2005). Cortisol levels were lower in tuco-tucos showing higher dominance index. For polygynous species, like *C. talarum*, the dominant male monopolizes mates through male-male aggression, and it is assumed they are also those suffering more physiological stress (Sapolsky, 2005). Even though stress hormones are known to suppress gonadal axis and then reproductive output (Sapolsky, 1992), endocrine mechanisms have evolved to moderate these suppressive effects or the release of stress hormones (Wingfield and Sapolsky, 2003), explaining the lower cortisol levels shown by dominant males in this study. Definitely, dominant males displayed a higher release of cortisol levels after an aggressive encounter. This would indicate a short term role of cortisol on dominance acquisition, probably related to the mobilization of energy to cope with a stressful situation (Sapolsky, 1992). Furthermore, the outcome of male-male competition would reflect disease status because infection will preclude a male from becoming dominant; in consequence, females would benefit mating with dominant males because it is assumed that they do not carry parasites (Hamilton and Zuk, 1982). However, if high androgen levels suppress the immune system, dominant males are expected to be more susceptible to parasites. Then females would benefit from this type of mating because these males would cope with the immunocompetence handicap and their offspring would inherit that trait (Folstad and Karter, 1992; Qvarnström and Forsgren, 1998). In this regard, two paradigms were proposed to explain the underlying relationship between parasites and dominance:

one of them suggests that chronic stress is associated to dominance status while the other highlights the cost of dominance (Habig and Archie, 2015). Our results could not be explained under the first proposition since dominant tucos did not present higher levels of immunosuppressant hormones such as testosterone or cortisol (Lee, 2006). In addition, parasite incidence can be related to particular behavioural traits that characterize dominant individuals since these animals are frequently exposed to parasitized conspecifics (both competitor males and female partners, Ezenwa, 2004; Altizer et al., 2003). They also suffer higher energetic requirements to maintain dominance (i.e. assessing, patrolling and fighting) that deviate energy from immune defense (Dhabhar, 2009). Finally, more than resistance to parasites, tolerance is another component of defense that must be considered (Råberg et al., 2009; Råberg, 2014). Dominant males may also be more tolerant to parasites than subordinates, implying that individuals can bear parasite burden without substantial impairment of their health (Getty, 2002; Medzhitov et al., 2012; Hayward et al., 2014).

In conclusion, scents of *C. talarum* are not enough to recognize dominant males, but behavioural signals seem to convey such information to females. Preference of females for dominant males was not related to any condition-related trait considered in this study. It appears that dominance itself constitutes a complex signal of diverse aspects of male quality and represents mainly indirect benefits (good genes for the progeny) for females. In nature, dominant males mate coercively with females, but it is important to know whether females prefer them if they have the opportunity to choose between potential partners. Hence it is very difficult to differentiate an unwanted copulation from mate evaluation. Females can selectively resist mating to select most vigorous males or most persistent males who are able to overcome such resistance (Bisazza et al., 2001). This may explain why females do not simply cooperate with males during mating. Behavioural observations of reproduction are extremely limited in wild subterranean rodents. Studies of *C. talarum* in captivity showed that females are choosy; they are able to discriminate and prefer potential partners according to their familiarity, MHC profile, and dominance status. Such abilities are noticeable in a context of polygyny with high male-male competition for females. Given the importance of territoriality when accessing females, the ability of male tuco-tucos to maintain an exclusive territory needs to be evaluated as part of female preference.

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