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Females prefer good genes: MHC-associated mate choice in wild and captive tuco-tucos

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The major histocompatibility complex (MHC) genes are one of the most suitable candidates for elucidating the genetic bases of mate choice in vertebrates, given the potential benefits in terms of immunocompetence that can be passed to the offspring through MHC-associated mate choice. Female mate choice may favour males that possess particular MHC alleles or those with diverse MHC genotypes (good genes hypothesis), or males that possess MHC genotypes that differ from that of the female (compatibility hypothesis). Our goal was to evaluate mate choice in relation to MHC genotype in the subterranean rodent *Ctenomys talarum*. Using both laboratory and field analyses, we investigated whether the (1) number of shared MHC alleles between males and females, (2) number of amino acid differences between female and male MHC alleles, (3) MHC heterozygosity of males, (4) number of amino acid differences between male MHC alleles, and (5) frequency of MHC alleles of males differ between preferred and nonpreferred males in the laboratory and between potential sires and random males from the population in the field. In the laboratory, our results indicate that MHC alleles of preferred males differ in fewer amino acids compared to MHC alleles of nonpreferred males. Concomitantly, in the field, MHC alleles of possible sires differed in fewer amino acids than those of random males in the population. Plus, possible sires were more heterozygous and carried distinct MHC alleles compared with random males, thus providing more support to the 'good genes' hypothesis. We discuss the possible reasons why MHC-based mate choice was more evident in the field and the implications of such a female mating strategy in the subterranean environment.

A female's reproductive success usually depends on the quality and quantity of offspring produced (Bateman 1948; Trivers 1972). Hence, if males differ in their effects on the number and quality of offspring, then females should be able to recognize and mate with the male that provides more benefits to her, exerting mate choice selection (Alcock 1989).

Benefits from mating preference can be direct (recently named 'dilutable', see Tazzyman et al. 2012), such as improved parental care, or getting more or high-quality food, or a better territory in which to rear offspring (reviewed in Andersson 1994), or indirect ('fixed' sensu Tazzyman et al. 2012), such as the inheritance of genes for viability (Zahavi 1975) or attractiveness (Fisher 1930). There are many mating systems in which females receive few or no direct resource benefits from the male (reviewed in Alcock 1989); therefore, in these systems indirect genetic benefits play a major role in female mate choice. These benefits may include increased offspring growth rate, increased dominance of sons, larger number of pups weaned and/or also improved nest construction or predator evasion skills in the progeny (Drickamer et al. 2003).

Other proposed indirect benefits derived from mate choice include increased offspring immunocompetence and parasite resistance (Hamilton & Zuk 1982; Folstad & Karter 1992). In this sense, the major histocompatibility complex (MHC) genes are one of the most suitable candidates for elucidating the genetic bases of mate choice in vertebrates (Penn & Potts 1999), given the potential benefits of immunocompetence that can be passed to the offspring through MHC-associated mate choice. MHC genes code for glycoproteins involved in the recognition and binding of foreign antigens, with presentation of bound antigens to T lymphocytes eliciting the associated immune response (Klein 1986). Female mate choice may favour males that possess particular MHC alleles or those with diverse MHC genotypes (good genes hypothesis, Penn & Potts 1999), or males that possess MHC genotypes that differ from that of the female (compatibility hypothesis, Neff & Pitcher 2005). The good genes hypothesis predicts that all females should agree in their mating preferences (Eizaguirre et al. 2009; Ekblom et al. 2010), choosing males with more diverse MHC genotypes or

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those with particular MHC alleles, thus providing offspring with alleles/genotypes that confer resistance to particular pathogens (Penn & Potts 1999). The genetic compatibility hypothesis posits that each female will benefit from mating with a different male, depending on how good a fit the male's genotype is with that of the female. Thus, females should choose mates with a MHC genotype that differs from their own (Olsson et al. 2003; Schwensow et al. 2008a). Importantly, the choice of MHC-compatible partners might be associated with providing offspring with a more diverse MHC genotype that may resist a broader range of pathogens (Apanius et al. 1997; Fromhage et al. 2009) and/or avoiding inbreeding (Grob et al. 1998), with MHC genes functioning as kinship markers (Penn & Potts 1998a). Furthermore, recent evidence suggests that females may choose mates with whom they share an intermediate MHC diversity, which may contribute to avoidance of (1) negative selection on T cell repertoire size and reduce the amount of detectable pathogens (Wegner et al. 2003) or (2) the costs associated with the disruption of coadapted genes (Bonneaud et al. 2006; Eizaguirre et al. 2009).

Studies conducted in laboratory rodents indicate that MHC genes influence an individual's urine odour (reviewed in: Penn & Potts 1998b; Yamazaki et al. 1998; Eggert et al. 1999), and several hypotheses have been proposed about how this influence on volatile components of the urine is exerted (reviewed in Penn 2002). Evidence of MHC-associated mate choice comes from studies conducted either in the laboratory (mice, *Mus musculus*: Yamazaki et al. 1976; but see Penn & Potts 1998b; humans: Wedekind et al. 1995; but see Hedrick & Black 1997; sticklebacks, *Gasterosteus aculeatus*: Reusch et al. 2001; Milinski et al. 2005; bank voles, *Myodes glareolus*: Radwan et al. 2008; house sparrows, *Passer domesticus*: Griggio et al. 2011) or, more recently, in the field (brown trout, *Salmo trutta* L.: Forsberg et al. 2007; primates: Schwensow et al. 2008a, b; Setchell et al. 2010; great frigatebirds, *Fregata minor*: Juola & Dearborn 2012). Other studies have failed to find evidence in support of MHC-associated mate choice (reviewed in Milinski 2006). Here, we attempt to elucidate the role of MHC in patterns of mate choice in the subterranean rodent *Ctenomys talarum*, combining analyses conducted both in the laboratory and in the field.

Rodents in the genus *Ctenomys* (tuco-tucos) provide an important opportunity to assess the role of MHC-associated mate choice in naturally occurring populations of vertebrates. These subterranean mammals, which occur from southern Peru to Tierra del Fuego and from the Andes to southeastern Brazil, are characterized by extensive sharing of MHC allele lineages among species (trans-species polymorphism, Klein 1987), suggesting that balancing selection has played a significant role in shaping MHC diversity in these animals (Cutrera & Lacey 2007). Within species, selection on MHC genes appears to vary predictably with behavioural and demographic attributes such as social structure and population density (Lacey & Cutrera 2007). Specifically, comparisons of the group-living colonial tuco-tuco *C. sociabilis* and the solitary Patagonian tuco-tuco *C. haigi* have revealed that selection on the class II DQB locus is stronger in the social species (Hambuch & Lacey 2002), while comparative studies of two demographically distinct populations of *C. talarum* (talas tuco-tuco) have demonstrated that number of alleles, heterozygosity and intensity of selection at two class II loci (DRB and DQA) are greater at higher population density (Cutrera & Lacey 2006). Because both greater social contact and greater population density may increase exposure to pathogens (Anderson & May 1979; Coté & Poulin 1995; Arneberg 2002), these findings are consistent with a possible role for parasite-driven selection in maintaining MHC polymorphism in tuco-tucos. Furthermore, a recent study demonstrated that specific DRB alleles were associated with parasite resistance and the intensity of the humoral response in the talas tuco-tuco (Cutrera et al. 2011), suggesting that the MHC sequences are capable of providing resistance/susceptibility to pathogens in this species, and thus, might be the foundation of MHC-associated mate choice.

Talas tuco-tucos are solitary and highly territorial (Busch et al. 1989), and DNA fingerprinting suggests that they are polygynous (Zenuto et al. 1999), with a spatial distribution characterized by the presence of one dominant male surrounded by other burrow systems individually occupied by females (Busch et al. 1989). Courtship and copulation are thought to occur in the female burrow, based on semi-natural observations, and males do not provide parental care (Zenuto et al. 2001). Both sexes usually scent-mark burrow openings, providing odour signals to neighbours during their aboveground excursions, which may provide information about the individual (Zenuto & Fanjul 2002), its gender (Fanjul et al. 2003) and reproductive condition (Zenuto et al. 2004). Interestingly, in the laboratory, females familiarized with male odours showed reduced aggression during courtship but preferred unfamiliar males as mates, copulating repeatedly with them (Zenuto et al. 2007).

The goal of this study was to evaluate mate choice in relation to MHC genotype in the talas tuco-tuco. Specifically, using both laboratory and field analyses, we investigated whether (1) the number of shared MHC alleles between male and female, (2) the number of amino acid differences between male and female MHC alleles, (3) MHC heterozygosity and (4) the number of amino acid differences between male MHC alleles differ between preferred males and nonpreferred males in the laboratory and between potential sires and random males from the population in the field. We also evaluated whether the distribution of MHC allele frequencies differ between preferred males and nonpreferred males in the laboratory and between potential sires and random males from the population in the field. According to the 'good-genes hypothesis' described above, preferred males (laboratory) and possible sires (field) should carry MHC alleles with a higher average number of amino acid differences between them, have higher average MHC heterozygosity and distinctive distributions of MHC allele frequencies compared to nonpreferred males (laboratory) and a sample of randomly assigned males (field). Furthermore, according to the genetic compatibility hypothesis, preferred males (laboratory) and possible sires (field) should share on average fewer MHC alleles with the choosing female (laboratory) or mother (field) and have higher amino acid differences with them.

METHODS

Animal Capture and Housing

The study population was located at Mar de Cobo, Buenos Aires Province, Argentina (37°46′S, 57°27′W), in coastal dune habitat characterized by sandy soils and dominated by *Panicum racemosum, Ambrosia tenuifolia* and *Distichlis scoparia*; a detailed description of the study location is provided by Comparatore et al. (1991). For laboratory analyses of mate choice, a total of 129 tuco-tucos were trapped at the study site using plastic tube traps inserted into each animal's burrow system (see Vera et al. 2011 for details). Females were captured during the nonbreeding season to avoid the influence of previous reproductive activity; males were captured during the breeding season. Specifically, 86 adult males were captured during the breeding seasons (June–early December) of 2006, 2007 and 2008, and 43 nonpregnant adult females were captured during the nonbreeding seasons (February–May) of those years. After capture, tuco-tucos were all transported to the Laboratorio de Ecofisiología of the Universidad Nacional de Mar del Plata. For the field analyses of mate choice, a total of 112 tuco-tucos were trapped at the study site using the same trapping system. Specifically, 77 adult males and 35 adult females were captured

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during the breeding seasons of 2007 and 2008. Captured males were weighed and a nondestructive tissue sample for genetic analyses was obtained prior to release at their point of capture. All of the females captured were transported to the Laboratorio de Ecofisiología of the Universidad Nacional de Mar del Plata. Of these, 24 gave birth in captivity to an average ± SD of 2.88 ± 1.57 pups.

In captivity, the animals were housed individually in plastic cages (25 × 32 × 42 cm), the bottoms of which were lined with wood shavings. The animals were fed ad libitum quantities of a mixture of grasses, alfalfa, lettuce, corn, sweet potatoes, carrots and sunflower seeds. Temperature and photoperiod in the room housing the animals were strictly controlled (25 ± 1 °C; nonbreeding: 12:12 h light:dark; breeding: 14:10 h LD). The animals were held in captivity until mate choice trials were completed or pups were born, after which they were returned to the field and released at the point of capture. Immediately prior to release, a nondestructive tissue sample for genetic analyses was obtained from each animal by removing the distal 1–2 mm of the outer digit of the left hindfoot (Cutrera et al. 2005). All field and laboratory procedures conformed to institutional and national guidelines (Argentine National Council for Scientific and Technological Research: PIP 5670, Argentine Agency for Scientific Promotion: PICT 1992, 2102) as well as the guidelines of the American Society of Mammalogists (Gannon et al. 2007) for the capture, handling and use of mammals.

Genetic Analyses

Variability at the MHC class II DRB locus was assessed for all individuals in this study. Exon 2 of this locus was selected for analysis because it contains the peptide-binding regions of the associated MHC molecules, which are the portions of these genes that are typically most subject to balancing selection (Hughes & Hughes 1995). High molecular weight genomic DNA was extracted from all tissue samples using the DNeasy tissue extraction kit (QIAGEN, Inc., Crawley, West Sussex, U.K.). A 270 base pair (bp) fragment of exon 2 was amplified using primers GH46 and GH50 (Scharf et al. 1988). PCR master mixes and reaction conditions were prepared according to Cutrera & Lacey (2006). To characterize allelic diversity at the DRB locus, all MHC amplicons were cloned and sequenced following the protocol of Cutrera et al. (2011).

DRB sequences were edited and aligned manually using Sequencher 4.0 (Applied Biosystems, Inc., Foster City, CA, U.S.A.). Sequences that differed by only a single base pair substitution were considered to be distinct alleles if each variant occurred in (1) multiple individuals and/or (2) multiple cloning products per individual (obtained from at least two different PCR reactions and rounds of cloning). To assess whether population genetic structure differed between study years for laboratory and field data, we conducted global and pairwise population differentiation tests based on allele frequencies using 10 000 simulation steps (Raymond & Rousset 1995), as implemented in Arlequin v. 3.1 (Excoffier et al. 2005). Allelic and genotypic variability were assessed and Hardy–Weinberg equilibrium tests were performed using Arlequin v. 3.1 (Excoffier et al. 2005). Specifically, the method of Guo & Thompson (1992) was used to determine whether observed heterozygosity at DRB exon 2 was significantly in excess of that expected based on allele frequencies. Pairwise differences between DRB sequences were assessed at the amino acid level using MEGA v. 4 (Tamura et al. 2007).

Laboratory Analyses of MHC-disassortative Mating

Female choice trials

Experiments were carried out during the breeding season (2006–2008). Males were allowed to adapt to captivity for at least 5 days before the beginning of the mating trials. Animals were randomly assigned to each treatment. Only trials in which females displayed investigative interest in male odours and reproductive behaviours such as exposing the genitalia to the male, sniffing male genitalia, contacting the male's body and mounting the male were included. Animals participated only once as test animals or odour donors. Preference tests were used to determine whether females displayed a sexual preference at three levels of male assessment: (1) male odours ($N = 16$ trials), (2) confined males ($N = 15$ trials) and (3) full contact with tethered males ($N = 12$ trials) (Fig. 1). As MHC genes influence individual odours that affect volatile components of the urine, urine cues were present for females to use

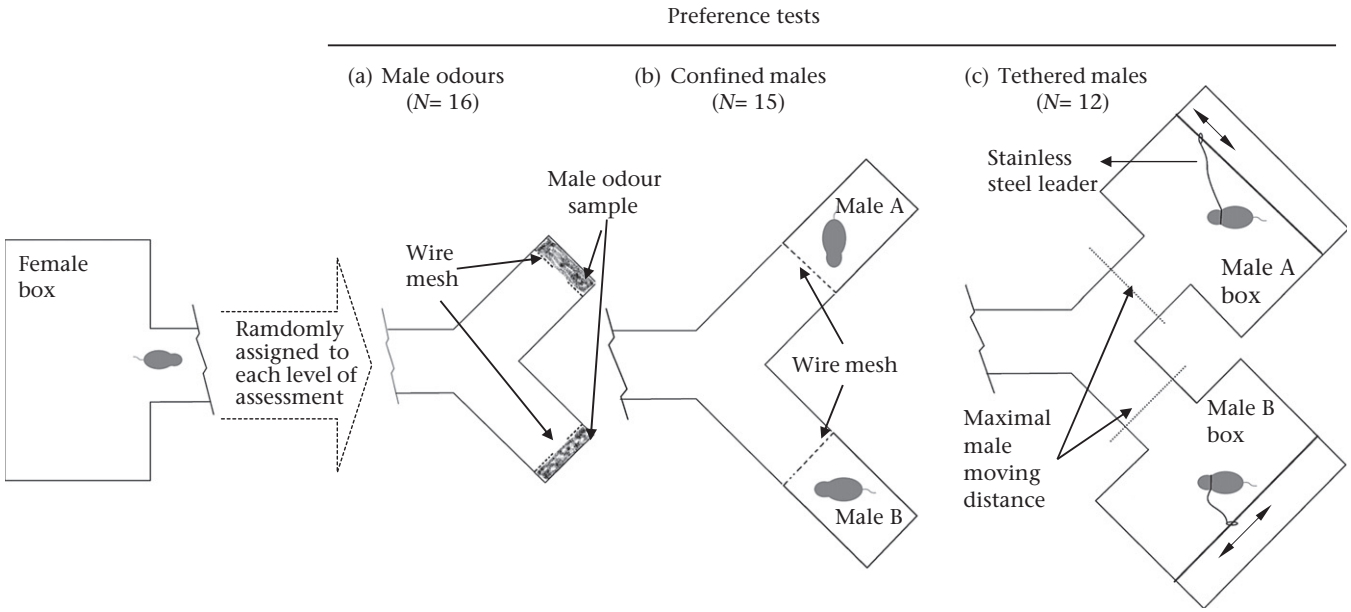

Figure 1. Schematic representation of the Y-mazes used for talas tuco-tuco mate choice trials that involved (a) male odours, (b) confined males and (c) tethered males. See Methods for details.

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in male assessment in all three types of treatments. Nevertheless, additional signals that could enhance the interest of females were also included. Thus, for females in the male odours group, chemical information was the only cue available to assess males' characteristics, whereas in the confined males group, females could sniff, hear and see the males, although body contact was prevented. In the last group (tethered males group), females were able to have full contact with the males as well as access to olfactory and acoustic cues. However, because of tethering, males' aggression towards the choosing female or their attempts to coerce her into mating were limited; therefore, we predicted the strongest results for this treatment.

Pairs of males that were randomly assigned to each female did not differ in body mass by more than 5%. The experimental devices consisted of Perspex boxes (45 × 30 × 30 cm) and a Perspex Y maze (10 cm diameter and 25 cm length for each arm). Prior to preference tests, each female and/or males were placed individually in their respective Perspex box and left to habituate for 1 h before the test began (Zenuto & Fanjul 2002). Disposable gloves were used in all instances of sample collection and during the experimental trials to prevent transfer of human odours. 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.

During the odour preference test ('male odours'), the female box was connected to the Y maze (Fig. 1a). A sample of odour from each male was placed by the cap at the respective arm of the maze. Odour samples consisted of soiled shavings collected from the corners of the animals' cages, which had not been changed for 7 days. The samples were covered with wire mesh, but a hole through the mesh allowed the female to lick or touch the shavings, hence allowing female tuco-tucos to use not only the main olfactory epithelium but also the vomeronasal organ to assess the odour samples. Trials started when the test female entered the maze and lasted for 7 min (Zenuto & Fanjul 2002). We recorded the female's relative interest in each odour sample, as the total time devoted to investigate and sniff a sample. In preference tests using confined males, the box containing a female was connected to a Y maze and each arm of the maze was connected to one male's tube (Fig. 1b). The entrances to both male tubes were covered with wire mesh, which prevented the male and the female from being in full contact, but allowed them to see, sniff and hear each other. Shavings from the respective male housing cage were placed next to the mesh at each arm of the maze in a way that allowed the female to contact the shavings. Trials started when the females entered the Y tube and lasted 30 min. We determined the female's relative interest in each male by recording the total amount of time she stayed active or resting in each arm tube. In preference tests involving full contact with tethered males, the stem of the Y maze was connected to the female's cage and the arms were connected to two cages, each containing an individual male (Fig. 1c). These cages were provided with a rod running across the width of the cage and a movable line of stainless steel fishing leader, which ended in a ball bearing snap swivel that was attached to a cable tie worn as a collar by the male. Males were individually tethered to their respective cage before the habituation phase. Trials started when the females entered the Y tube and lasted 30 min. We determined the female's relative interest in each male by recording the total amount of time she stayed active moving around the box and/or displayed reproductive and/or courtship behaviours: odour sniffing (soiled shavings, male hindquarters or genitalia), rump presentation, tail raising, pushing, spinning and mounting (Zenuto et al. 2007, see Appendix, Table A1, for details). Collars were put on males 24–48 h before the trial began and were removed as soon as the trial was ended.

The male (or male odours) that the choosing female spent more time with and/or displayed more reproductive behaviours towards in relation to the time devoted to both test males combined (Shapiro et al. 1986; Spritzer et al. 2005) was defined as the 'preferred male' (see Appendix). In the case of the tethered male treatment, when a female mated with a male, that male was classified as 'preferred' even if the previously mentioned criteria were not met (only one female presented a discrepancy in the number of behaviours, displaying 53.6% of total reproductive behaviours to the male that she did not mate with).

Ethical note

We adhered to the Guidelines for the Use of Animals in Research and Teaching (ASAB/ABS 2003). Collars and fishing leaders did not cause significant stress for males since they walked normally while exploring the entire box, interacted with the choosing female and/or mated with the female.

Data analysis

First, two factors of genetic compatibility were considered in the analyses of MHC disassortative mating, following Juola & Dearborn (2012). We compared the average degree of sharing of identical MHC DRB exon 2 alleles between the female and the preferred male and between the female and the nonpreferred male using a paired *t* test. In addition, we also considered the degree of sequence dissimilarity between MHC alleles, by calculating the magnitude of amino acid differences between MHC alleles of the female and the preferred male and between the female and the nonpreferred male (see Juola & Dearborn 2012) using a paired *t* test. Second, we considered three factors to compare the MHC characteristics of preferred versus nonpreferred males: (1) number of heterozygotes present in the sample of preferred and nonpreferred males, using a chi-square test; (2) average number of amino acid differences between the two DRB alleles carried by each individual male using a paired *t* test, to analyse the magnitude of sequence dissimilarity in MHC genotypes at the individual level between preferred and nonpreferred males; and (3) the distribution of allele frequencies in the samples of preferred and nonpreferred males, using a chi-square test. Because animals used in laboratory mate choice trials were not genotyped a priori, there were instances in which both males participating in the same trial had the same MHC genotype or were both heterozygotes. Therefore, we also analysed our data using only trials in which the MHC genotype of the two males differed and using only those trials involving a heterozygous and homozygous male. In all cases but one, we obtained the same results. When differences were detected between the two data sets, we report both results (see Results, Table 1). Otherwise, we report the results obtained using trials involving all males. All statistical analyses of this section, including power analysis for each test, were performed using Statistica 6.0 (Statsoft, Tulsa, OK, U.S.A.). When parametric test assumptions were not met, the equivalent nonparametric test was used. Throughout the paper, results are expressed as means ± SD.

Field Analyses of MHC-disassortative Mating

Data analysis

Using the genotype of each mother and her pups, we were able to infer the genotype/s of the potential sires for 22 litters, based on previous findings of a lack of multiple paternity of litters in talas tuco-tucos (Zenuto et al. 1999). To elucidate whether each female and the potential sire of her pups (henceforth 'mated pairs') were more dissimilar than would be expected under random female choice (genetic compatibility hypothesis), or whether potential sires showed particular MHC DRB exon 2 characteristics (good

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genes hypothesis), we generated a random model of female choice where we let each female choose randomly for 10 000 times between all males of the respective year to generate a null distribution. Subsequently, the observed values were compared to the simulated values of random female choice using the Simulation (Monte Carlo analysis) and Resample tools available at PopTools v. 3.0 (Hood 2008). Specifically, we evaluated whether (1) mated pairs shared fewer MHC DRB exon 2 alleles than would be expected under random female choice; (2) mated pairs shared fewer MHC DRB exon 2 amino acids than would be expected under random female choice; (3) the number of heterozygotes was higher in the sample of potential sires than in the sample of randomly chosen males; and (4) potential sires carried DRB alleles that differed in more amino acids than those of the randomly chosen males. To analyse these factors, we calculated the sum of (1) shared MHC alleles, (2) shared amino acids between males and females from pairwise combinations of alleles, (3) number of heterozygotes and (4) shared amino acids between male alleles. This observed sum was then compared with the distribution of scores generated from 10 000 simulations of 22 random pairings selected from the 77 males and the 22 females captured in the population. Ninety-five per cent confidence intervals were generated from the simulated data distribution. If the observed value was contained within the 95% confidence interval, the difference between observed and simulated values was considered statistically nonsignificant. Finally, the distribution of allele frequencies of the sample of potential sires was compared to that of the total males captured in the population using a chi-square test.

RESULTS

DRB Variability

As in previous studies of MHC variation in the talas tuco-tuco (Cutrera & Lacey 2006, 2007; Cutrera et al. 2011), all DNA samples analysed produced a single, clearly resolved PCR product. No evidence of chimeric amplification products was detected. After cloning, no more than two sequences per individual were obtained, suggesting that only a single copy of the DRB locus was amplified. Additionally, comparisons of mother–pup genotypes revealed no evidence of amplification of more than one copy of DRB locus. Inspection of the resulting sequences revealed no insertions or deletions and, when translated, no stop codons were evident within the DRB alleles obtained.

Nine DRB alleles were detected in the 241 adult tuco-tucos genotyped during this study. Of these, seven alleles (Ctta_DRB01-Ctta_DRB07, Genbank accession numbers: JF799108-JF799114) had been described in Cutrera et al. (2011). The sequences of the two new alleles found were published in Genbank under accession numbers JQ317129 and JQ317130. We detected no significant difference in genetic structure among study years in the laboratory data (global: exact $P = 0.82 \pm 0.03$; pairwise: $P_{2006-2007} = 0.29$, $P_{2006-2008} = 0.78$, $P_{2007-2008} = 0.36$) or in the field data (exact $P_{2007-2008} = 0.26 \pm 0.02$), which allowed us to pool data from several years for subsequent analyses. Observed heterozygosity (0.81) at this locus was significantly higher than that expected under Hardy–Weinberg equilibrium ($He = 0.74$, $P < 0.001$). The mean ± SD number of pairwise nucleotide differences between alleles was 4.78 ± 1.31. When translated, the mean number of pairwise amino acid differences between alleles was 1.61 ± 0.81.

MHC Allele Similarity between Males and Females

In mate choice trials, there was no significant difference in the number of shared MHC alleles between the female and the preferred or nonpreferred male (Table 1). Similarly, no differences were detected in the number of shared MHC alleles between mated pairs in the field versus the simulated values of randomly assigned males (observed value = 20, simulated 95% confidence interval = 17–33; Fig. 2a).

MHC Amino Acid Similarity between Males and Females

In mate choice trials, there was no significant difference in the number of shared amino acids between MHC alleles of the female and the preferred or nonpreferred male (Table 1). Similarly, no differences were detected in the number of amino acid differences between MHC alleles of the mated pairs in the field versus those of the simulated values of randomly assigned males (observed value = 80, simulated 95% confidence interval = 74–102; Fig. 2b).

MHC Heterozygosity in Males

In mate choice trials, the number of MHC heterozygotes present did not differ significantly in samples of preferred and nonpreferred males (Table 1). In contrast, the number of MHC heterozygotes in the sample of potential sires was significantly higher than that in the sample of randomly assigned males in the field population (observed value = 18, simulated 95% confidence interval = 2–10; Fig. 2c).

Table 1
Number of shared MHC alleles and MHC amino acid differences between females and their preferred and nonpreferred males, number of heterozygotes in samples of preferred and nonpreferred males, and number of amino acid differences between MHC alleles of preferred and nonpreferred males in tuco-tuco mate choice trials

Mate choice trial	Male	Mean±SD or number	Statistics
Female–male shared alleles			
Odour	Preferred	0.94±0.85	$T=-20$, $N=16$, $P=0.07$
	Nonpreferred	0.56±0.63	(0.29)
Confined male	Preferred	0.53±0.83	$T=0$, $N=15$, $P=1$
	Nonpreferred	0.87±0.74	(0.30)
Tethered male	Preferred	0.58±0.79	$T=18$, $N=12$, $P=0.25$
	Nonpreferred	0.83±0.94	(0.52)
Amino acid difference in female–male MHC alleles			
Odour	Preferred	1.46±0.55	$T=-6$, $N=16$, $P=0.25$
	Nonpreferred	1.34±0.61	(0.11)
Confined male	Preferred	1.38±0.59	$T=-11$, $N=15$, $P=0.38$
	Nonpreferred	1.20±0.56	(0.16)
Tethered male	Preferred	1.17±0.49	$t_{11}=0.49$, $P=0.63$
	Nonpreferred	1.04±0.83	(0.18)
Number of heterozygote males			
Odour	Preferred	14	$\chi^2_1=0$, $P=1$
	Nonpreferred	13	(0.45)
Confined male	Preferred	13	$\chi^2_1=0.54$, $P=0.46$
	Nonpreferred	15	(0.57)
Tethered male	Preferred	10	$\chi^2_1=0$, $P=1$
	Nonpreferred	9	(0.33)
Amino acid difference in male–male MHC alleles			
Odour	Preferred	1.88±2.70	$t_{15}=0.62$, $P=0.50$
	Nonpreferred	1.56±1.31	(0.17)
Confined male 1*	Preferred	2.07±1.16	$\mathbf{t_{14}=0.97, P=0.04}$
	Nonpreferred	3.20±1.61	(0.58)
Confined male 2*	Preferred	2.10±1.20	$t_9=-1.61$, $P=0.14$
	Nonpreferred	3.30±2.00	(0.33)
Tethered male	Preferred	1.42±1.31	$t_{11}=0.77$, $P=0.29$
	Nonpreferred	2.00±1.35	(0.18)

Odour trials ($N = 16$); confined male trials ($N = 15$); tethered male trials ($N = 12$). For each test, results of power analysis are reported in parentheses. Results are shown for Wilcoxon signed-ranks tests (T), Student's t tests (t) or chi-square tests (χ^2). Significant values ($P < 0.05$) are shown in bold.

* Amino acid differences between male MHC alleles in the confined male trial are presented for (1) all trials and (2) only trials involving males with different MHC genotypes.

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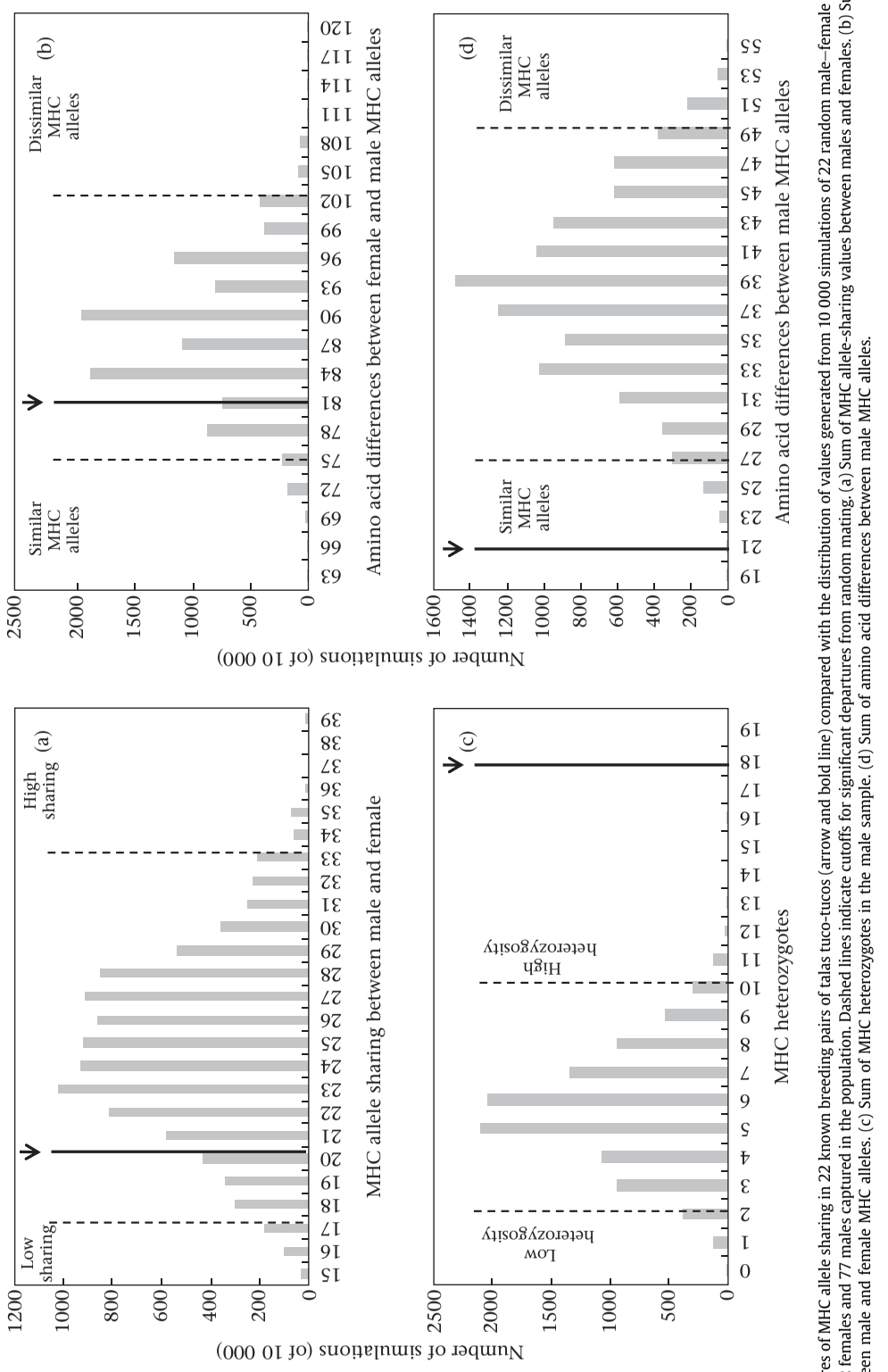

Figure 2. Measures of MHC allele sharing in 22 known breeding pairs of talas tuco-tucos (arrow and bold line) compared with the distribution of values generated from 10 000 simulations of 22 random male–female pairings selected from the same 22 females and 77 males captured in the population. Dashed lines indicate cutoffs for significant departures from random mating. (a) Sum of MHC allele-sharing values between males and females. (b) Sum of amino acid differences between male and female MHC alleles. (c) Sum of MHC heterozygotes in the male sample. (d) Sum of amino acid differences between male MHC alleles.

MHC Amino Acid Similarity between Male Alleles

In captivity, females in the 'confined males' trials, preferred males that had significantly fewer amino acid differences in their MHC alleles relative to those of nonpreferred males (Table 1). For the two remaining types of mate choice trials, no significant differences were detected between preferred and nonpreferred males (Table 1). In the field, similar to the results of the 'confined male' mate choice trials conducted in the laboratory, potential sires had fewer amino acid differences in their MHC alleles compared to amino acid differences in the sample of randomly assigned males (observed value = 20, simulated 95% confidence interval = 26–50; Fig. 2d).

MHC-specific Alleles

No significant differences were detected in the MHC allele frequency distributions of preferred and nonpreferred males ($\chi^2_8 = 6.54$, $P = 0.58$). In contrast, the MHC allele frequency distributions of potential sires and randomly assigned males from the population differed significantly ($\chi^2_6 = 14.03$, $P = 0.03$).

DISCUSSION

Our findings support a role for MHC-associated mate choice in shaping MHC diversity in natural populations of the talas tuco-tuco. In particular, female tuco-tucos in the laboratory preferred males that carried MHC alleles that differed from each other in fewer amino acids compared to nonpreferred males; concomitantly, possible sires in the field carried MHC alleles that differed in fewer amino acids from each other, that were more heterozygous and that carried specific MHC alleles in comparison with random males in the population.

What Do Female Tuco-tucos Choose with Respect to MHC: Good Genes or Genetic Compatibility?

Evidence from previous mate choice assays of talas tuco-tucos in the laboratory showed that when potential partners were randomly assigned, there was a predominance of avoidance and indifference behaviours and little reproductive activity (Zenuto et al. 2007). In contrast, when female tuco-tucos were allowed to interact with the preferred male, there was an increase in mating success (Zenuto et al. 2007). Several studies have shown that both free female and/or free male mate preferences can have significant consequences for offspring viability and performance (Drickamer et al. 2000, 2003; Gowaty et al. 2003). In this sense, if indirect benefits of mate choice (i.e. increased pathogen resistance in the progeny) are a critical component of fitness affecting mating preferences in tuco-tucos, as expected in most mating systems with no paternal care (Neff & Pitcher 2005), then female tuco-tucos should identify and choose males that carry advantageous genetic combinations (i.e. MHC alleles or genotypes).

In the context of MHC-associated mate choice, what aspects of genetic quality are female tuco-tucos selecting in their mates? Females may choose males that carry 'good genes', such as specific MHC alleles that confer disease resistance and/or MHC heterozygosity that provides resistance to a broader array of pathogens (Penn & Potts 1999). Alternatively, females may select males that are 'genetically compatible' (dissimilar), so as to increase MHC variation in the progeny and/or avoid inbreeding (Neff & Pitcher 2005). Our results provide support for the good genes hypothesis: MHC alleles carried by preferred males and possible sires differed in fewer amino acids than did MHC alleles carried by nonpreferred males and random males, respectively, which suggests that females are selecting specific MHC alleles or allele groups. Accordingly, a recent study revealed significant associations between specific MHC allele groups and both parasite load and intensity of humoral immune response against a novel antigen in the talas tuco-tuco (Cutrera et al. 2011), supporting the idea that certain MHC alleles can provide resistance/susceptibility to pathogens in this species. In line with this, the MHC allele frequency distribution of possible sires in the field differed significantly from that of random males in the population. Specifically, MHC allele Ctta_DRB06 (Genbank accession number JF799113) was absent from the sample of possible sires, while MHC allele Ctta_DRB03 (Genbank accession number JF799110) was present in this sample, but was absent from the group of random males. The same pattern was observed in the laboratory for the same MHC alleles, although differences were not statistically significant. Interestingly, Cutrera et al. (2011) found that Ctta_DRB06, which was absent from our samples of possible sires and preferred males, is associated with tuco-tucos' susceptibility to *Eimeria* sp., an intestinal protozoan known to cause haemorrhagic enteritis in other mammals (Hill et al. 1985). Finally, as expected under the 'good genes as heterozygosity hypothesis' (Brown 1997, 1999), possible sires in the field had higher levels of MHC heterozygosity compared to random males in the population. Choice for a male heterozygous for MHC may be advantageous because heterozygotes carry rare alleles more often than homozygotes, which may confer resistance to novel pathogens to the offspring. Besides, MHC heterozygous males are less likely to share alleles with the female (but see Roberts et al. 2006 for a correlation between heterozygosity and genetic similarity in humans and peafowls), leading to more diverse offspring that can respond to a broader array of pathogens (Apanius et al. 1997; Fromhage et al. 2009). Also, mating with an MHC heterozygous male may represent a direct benefit to the female. If MHC heterozygotes are more resistant to infections (Doherty & Zinkernagel 1975), then females would benefit from mating with heterozygous males by lowering the chances of contracting contagious infections from interactions during courtship and copulation (as seen in humans; Roberts et al. 2005).

Contrary to expected under the genetic compatibility hypothesis (Neff & Pitcher 2005), preferred males or possible sires did not share fewer MHC alleles with the choosing female or mother, respectively. Similarly, MHC alleles of preferred males or possible sires were not more dissimilar in their amino acid sequence than MHC alleles of the choosing female or mother, respectively. Together, our findings suggest that female tuco-tucos mate preferentially with males that carry specific MHC alleles and/or those that are MHC heterozygous, possibly increasing the offsprings' chances of resisting infections. However, we found no evidence that female tuco-tucos choose MHC-dissimilar mates, and thus, no support for the hypothesis that MHC-associated mate choice is associated with inbreeding avoidance in tuco-tucos.

Combining Two Approaches: Laboratory and Field Analyses of MHC-associated Mate Choice

When given the chance, females are expected to show interest in males carrying novel genetic combinations (Tregenza & Wedell 2000), even if the costs of mating are elevated by higher aggression during courtship (Zenuto et al. 2007) and the need to repeat copulations to achieve oestrus and/or ovulation, as in copula-mediated-induced ovulators such as tuco-tucos (Fanjul & Zenuto 2008, 2011). Under this scenario, we expected to find more evidence of MHC-associated mate choice in the laboratory, where females were able to exercise their mating choice fully, compared to the field.

In contrast to our expectation, however, our analyses revealed less evidence of MHC-associated mate choice in female tuco-tucos

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in the laboratory than in the field. Several factors may have contributed to these findings. Rodent urine includes a wide array of cues that influence mating decisions, providing information regarding MHC genotype, major urinary proteins (MUPs) profile, diet quality, health and vigour, and/or testosterone levels (Ferkin et al. 1997; Candolin 2003; Milinski 2006; Thom et al. 2008; Hurst 2009). Although most of these elements are available for female assessment of possible mates, their relative importance in mating decisions will depend, among other factors, on female mating opportunities, female genotype, emphasis on direct and/or indirect benefits, and ecological conditions (e.g. predation pressure, incidence of parasitism; Jennions & Petrie 1997; Candolin 2003). In this sense, the outcomes of preference tests in captivity may differ from those in a natural situation. First, all animals in our study were provided with the same diet of mixed grasses and vegetables; thus, assessment of male quality via differential access to high-quality food was not available for females. Also, male tuco-tucos show a significant drop in testosterone levels after a few days of captivity compared with levels observed in the field (Vera et al. 2011), which could reduce or eliminate female interest in male odours (Ferkin et al. 1994; Gottreich et al. 2000). As mentioned previously, in our laboratory assays, female tuco-tucos were able to use the main olfactory epithelium and the vomeronasal organ, a key requisite to assess both the volatile and nonvolatile products of MHC in urine (Spher et al. 2006), to assess odour samples. Nevertheless, females in the laboratory showed no preference for odours from males with different MHC genotypes. This may be due, at least in part, to the fact that urine samples presented to the choosing female in the laboratory were not fresh, but consisted of soiled shavings collected from the male's cage, which had not been changed for 7 days. MHC peptide ligands, the more informative fraction of MHC identity in scents compared to their volatile components, are also more susceptible to endo- and exoproteolytic attack, severely compromising their usefulness as individual identity cues over time (Sherborne et al. 2007). Finally, unlike previous laboratory studies that have explored female MHC preferences in model species (*Mus musculus*: Yamazaki et al. 1979), we did not know the MHC genotype of the animals before conducting mate choice trials. Tuco-tucos are wild animals, and conducting studies on inbred strains differing only in their MHC haplotypes, such as those in mice, was not possible. This may have contributed to our limited capacity to detect, for example, the effect of MHC heterozygosity, given that most male pairs offered to the choosing female were composed of two heterozygous males, because of the high frequency of MHC heterozygotes observed in the study population (0.81). However, given that the male tuco-tucos offered to the choosing female were a random sample of those present in the field, our laboratory mate choice trials closely resembled the choices of males available to free-living females in a natural situation. Finally, one additional factor that may have contributed to apparent differences between laboratory and field results is the greater sample size we had to conduct field analyses. As a result, the power to detect a pattern of MHC-associated mate choice was probably greater for the field data. In particular, our negative findings in the laboratory should be treated with caution, since statistical power for these trials was low. Additionally, one important factor contributing to the observed discrepancy between laboratory and field results might be related to the design of our laboratory mate choice trials, which evaluated female but not male choice. In contrast, in the field, we looked at the outcome of the possible interplay between both female and male choice, which together may have more significant consequences for reproductive success and offspring performance (Drickamer et al. 2003). In addition, females may be choosier when the number of available mates is higher (Eizaguirre et al. 2009), which may have also contributed to the observed differences. Finally, field data provided information on both pre- and post-copulatory female choice, while laboratory assays focused on mechanisms of precopulatory mate choice. Although post-copulatory female choice is expected to occur more frequently in promiscuous mating systems (Krebs & Davies 1998), the extent of this process has yet to be explored in tuco-tucos.

In spite of these possible pitfalls, we still found evidence of MHC-associated mate choice in captive tuco-tucos, although it was limited to a specific type of trial. Similar to results from the field, preferred males in the laboratory had MHC alleles that were more similar in their amino acid sequences, compared to those of non-preferred males. Interestingly, this female preference was only evident in the trial in which males were confined. It is likely that both fresh odours and the presence of the male played a critical role in eliciting female interest and enhancing the female's ability to assess male quality. However, females may actively avoid aggressive males, regardless of their dominance rank (Pereira & Weiss 1991) or spatial ability (confined versus free ranging) (Spritzer et al. 2005), because females also have to trade any benefits gained from mating with a particular male against the potential risk of harm to themselves or their offspring (Ophir & Galef 2003). This could explain why females in the tethered males treatment also showed no evidence of MHC-associated preference. Nevertheless, free-living females interacted with males in the wild, and potential sires had fewer amino acid differences in MHC alleles than those in the sample of randomly assigned males. Therefore, these results and the fact that, globally, MHC-associated mate choice was more evident in the field were both interesting and, to some degree, unexpected. Previous studies have explored MHC-associated mate choice using either laboratory or field data (reviewed by Milinski et al. 2005); to the best of our knowledge, ours is the first attempt to combine both perspectives. Ongoing work on the determinants of female mating preferences in tuco-tucos will help us further our understanding of the role of MHC profile in mate choice in relation to other male characteristics, such as dominance or health status.

Conclusions

Our findings support a scenario of MHC-associated mate choice in tuco-tucos, providing evidence in favour of the good genes hypothesis. MHC-heterozygous males and those carrying specific MHC alleles seemed to be preferred by females. However, female tuco-tucos seem not to be choosing dissimilar mates, as expected under the genetic compatibility hypothesis.

Such a female mating strategy may be related to particular features of the subterranean environment in which tuco-tucos live. Talas tuco-tucos are characterized by a relatively low richness of gastrointestinal helminths (Rossin & Malizia 2002), a pattern also evident in other subterranean rodents that has been linked to their typically solitary existence (Nevo 1999). This solitary lifestyle may result in patterns of infection that differ from those observed in species in which conspecifics frequently interact with one another (Rossin et al. 2010). In this context, female preference for unfamiliar males verified in the laboratory (Zenuto et al. 2007) could represent a first strategy, since mating with non-neighbours would provide new allelic variants for the progeny. Following this first assessment, female tuco-tucos may benefit from choosing males that carry locally adapted MHC alleles that provide resistance to a few highly prevalent parasites, such as *Eimeria* sp. (Cutrera et al. 2011). Plus, as discussed above, choosing MHC-heterozygous males could represent both indirect (rare alleles) and direct (disease transmission avoidance) benefits to female tuco-tucos. Therefore, as shown in other studies (i.e. Bonneaud et al. 2006; Eizaguirre et al. 2009), choosing the most dissimilar male compared to themselves may

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not be the most rewarding mating strategy for female tuco-tucos. Future studies will help elucidate what impact female mating preferences have on the relative immunity of the progeny.

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Appendix

Table A1
Mean ± SD percentage of total time that female tuco-tucos spent sniffing odours or displaying reproductive and/or courtship behaviours, and the relative frequencies of reproductive and/or courtship behaviours devoted to each male and to both males combined

Mate choice trial	Male	%Time	Frequency
Odour (N=16)	Preferred	77.60±15.46	—
	Nonpreferred	22.40±15.46	—
Confined males (N=15)	Preferred	69.93±14.33	68.32±9.93
	Nonpreferred	30.06±14.33	31.67±9.93
Tethered males (N=12)	Preferred*	66.15±12.35	80.72±15.52
	Nonpreferred	33.84±12.35	19.27±15.52

Behaviours recorded during female encounters with male odours (odour sniffing: female sniffs the male's soiled shavings), confined males (odour sniffing; rump area sniffing: female sniffs the male hindquarters or genitalia; mesh scratching: female scratches and bites the wire mesh that separates her from the male) and tethered males (rump presentation: female shows her rump when she encounters the male; tail raising: female raises her tail exposing her genitalia to the male; pushing: female pushes the flank of the male, promoting close contact; female mount: female mounts the male; spinning: male and female sniff each other's anogenital area and/or try to mount each other at the same time, which results in circling movements).
* Four females mated with the preferred male.

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