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Masculinized females produce heavier offspring in a group living rodent

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Summary

1. Alternative morphotypes have been reported less frequently in females than in males. An exception to this rule is the gradient of phenotypical masculinization reported in some female mammals, in which feminized and masculinized females represent two opposite ends along this gradient. These phenotypical differences originate during prenatal development as the consequence of maternal effects. Feminized and masculinized females differ in several traits, including morphological, physiological, behavioural and reproductive traits.

2. Differences previously reported in reproductive traits between feminized and masculinized females come mostly from mechanistic studies performed in the laboratory, and not necessarily on social species. As a result, it is unclear to what extent these reported differences between female alternative morphotypes materialize in wild, natural populations.

3. We quantified the effect of female alternative morphotype on female reproductive traits in a natural population of *Octodon degus*, a highly social rodent. We assessed female alternative morphotype through a continuous gradient of anogenital distance. Thus, feminized females were close to the short end of anogenital distance, while masculinized females were close to the long end of this gradient. We also tested the hypothesis that the social environment interacts with female morphotype to influence female reproductive traits.

4. In female degus, only body weight affected litter size, where heavier females weaned more offspring. Masculinized females delivered male-biased litters and weaned heavier offspring. Lastly, masculinized females gave birth later in the breeding season compared to feminized females.

5. Contrary to previous claims, our findings do not support that masculinized females are less fertile than feminized females. Moreover, masculinized females produced heavier, potentially higher quality offspring compared with feminized females.

Key-words: female alternative morphotype, female masculinization, maternal effects, reproductive traits, social context

Introduction

Intrasexual phenotypic variation has attracted the interest of researchers for decades (Gross 1996; Rhen & Crews 2002; Knapp 2004; Taborsky, Oliveira & Brockmann 2008). This phenotypical variation may determine the existence of alternative morphotypes within a sex, where each morphotype is characterized by a suite of morphological, physiological, behavioural and reproductive traits (Gross 1996; Taborsky, Oliveira & Brockmann 2008; Vercken, Clobert & Sinervo 2010). Alternative morphotypes can be of genetic or environmental origin. Environmentally originated morphotypes arise as the consequence of changes in developmental pathways during early ontogeny, which are mediated by hormones (Gross 1996; Rhen & Crews 2002; Knapp 2004). In contrast to genetically originated morphotypes, an environmentally determined morphotype cannot be eliminated from populations by natural selection, even if it results in lower fitness compared with alternative morphotypes under some environmental conditions (Brockmann 2001; Taborsky, Oliveira & Brockmann 2008; Vercken, Clobert & Sinervo 2010).

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Same-sex alternative morphotypes may exist for males and females, yet a wider diversity in male morphotypes has been reported (Engqvist & Taborsky 2016). In females, morphotypes have generally been linked to different levels of female masculinization and aggressiveness and have been reported in insects, fish, reptiles and mammals (vom Saal *et al*. 1999; Van Gossum, Stokes & De Bruyn 2005; Aubin-Horth *et al*. 2007; Vercken, Clobert & Sinervo 2010). In mammals, female masculinization is caused via maternal effects. During prenatal development, the uterine environment plays a decisive role in modelling the phenotype of undifferentiated female embryos. In particular, three proximal mechanisms mediated by androgens have been attributed to female embryo masculinization. The first mechanism is maternal allostasis in which testosterone precursors are produced by maternal adrenal glands in response to stressful stimuli (Sachser & Kaiser 1996; Drea 2009). The other two mechanisms, the intrauterine position phenomenon (IUP) and the 'the horn effect', modify the female embryo phenotype via testosterone produced by male siblings. During the IUP phenomenon, males neighbouring female siblings transfer testosterone to undifferentiated female embryos. The 'horn effect' depends on the total number of males in the same uterine horn, and where non-neighbouring males can also increase female embryo exposure to testosterone (Gandelman, vom Saal & Reinisch 1977; Clemens, Glaude & Coniglio 1978; Hotchkiss & Vandenbergh 2005). Taken all together, prenatal exposure to androgens caused by these three mechanisms may result in litter and population gradients of female offspring masculinization that persist through adulthood (Clark, Karpluk & Galef 1993; Vandenbergh & Hugget 1994; Clark & Galef 1998; Bánszegi, Altbäcker & Bilkó 2009).

Available evidence indicates that feminized and masculinized females, two alternative morphotypes representing the extremes of a continuous gradient of masculinization, differ from each other in a series of morphological, physiological, behavioural and reproductive traits (vom Saal 1989a; Sachser & Kaiser 1996; vom Saal *et al*. 1999; Zher, Gans & McClintock 2001; Kaiser & Sachser 2005; Hackländer & Arnold 2012). Typically, female alternative morphotypes differ in anogenital distance. Anogenital distance is a morphometric trait that allows for the external and non-invasive assessment of female masculinization level. Utility of this measure comes from the fact that prenatal exposure to androgens affects the development of perineal tissue (Clemens, Glaude & Coniglio 1978; vom Saal & Bronson 1978; vom Saal 1981; Hotchkiss & Vandenbergh 2005; Bánszegi, Altbäcker & Bilkó 2009). Thus, distance between the genitalia and the anus is longer in males than in females, and longer in females that have been exposed to more androgens *in utero* (Clemens, Glaude & Coniglio 1978; Dušek, Bartoš & Sedláček 2010; Fouqueray *et al*. 2014).

Females of different morphotypes show differences in reproductive traits, including fertility and the proportion of male/female offspring (vom Saal 1981; Clark & Galef 1998; vom Saal *et al*. 1999). For example, feminized females of European rabbits (*Oryctolagus cuniculus*) and Mongolian gerbils (*Meriones unguiculatus*) deliver larger litters than masculinized females (Clark & Galef 1998; Bánszegi *et al*. 2012), while no such differences have been reported in house mice (*Mus musculus*), mound-building mice (*Mus spicilegus*) or Alpine marmots (*Marmota marmota*) (Vandenbergh & Hugget 1994; Hackländer & Arnold 2012; Szenczi *et al*. 2013), implying that fitness effects of female masculinization may be species- or context-dependent (Sachser, Kaiser & Hennessy 2013). Feminized females have also been found to deliver female-biased litters, while masculinized females deliver male-biased litters (vom Saal *et al*. 1999; Ryan & Vandenbergh 2002). Male-biased litters, via the IUP and horn effect, are more likely to result in female offspring to develop into the masculinized morphotype (Clark, Karpluk & Galef 1993; vom Saal *et al*. 1999; Ryan & Vandenbergh 2002). While the effects of female masculinization on litter size and male/female offspring proportion have been well studied, other reproductive traits linked to offspring survival need to be examined, such as date of female parturition and offspring body weight at weaning.

The effect of female morphotype on reproductive success may also be influenced by social context (vom Saal & Bronson 1978; Zher, Gans & McClintock 2001). For example, co-nesting feminized females of house mice delay timing of breeding compared with solitary feminized females (Zher, Gans & McClintock 2001), implying that the size of social groups may influence reproductive success. In addition, group composition may also have an effect on reproductive success, as feminized females co-nesting with masculinized females delay timing of breeding compared with feminized females co-nesting with other feminized females in house mice (vom Saal & Bronson 1978; vom Saal 1989b). However, we lack studies on whether social context modifies the effects of female morphotype on reproductive success in wild, free-living animals. Thus, the aim of this study was to determine whether social context modulates the effects of female morphotype on reproductive traits including litter size, proportion of male offspring per litter, offspring body weight at weaning and date of parturition of wild, free-living degus (*Octodon degus*).

Degus are small to medium-sized (170–300 g) diurnal rodents that inhabit shrubland areas of arid central Chile (Ebensperger *et al*. 2004; Veloso & Kenagy 2005). Degus live in social groups consisting of anywhere from zero to three adult males and one to nine adult females (Ebensperger *et al*. 2004; Hayes *et al*. 2009). Members of social groups share a common underground burrow system where females rear their offspring communally (Ebensperger *et al*. 2004). Sexual maturity occurs ~6 months after birth (Mahoney *et al*. 2011), with the primary mating season taking place during the late, austral fall (June)

(Quirici *et al.* 2011a). After a gestation period of 87 ± 3 days (Rojas, Montenegro & Morales 1982), females give birth to an average of 6 ± 1 precocial offspring (14 g, Long & Ebensperger 2010). The lactation period is relatively short, lasting ~30 days. More than the 95% of degu females successfully breed each year (Hayes *et al.* 2009; Ebensperger *et al.* 2011a), although most adult degus (85–90%) do not survive to their second year of age, which means that reproductive success during their first (and likely only) breeding event has a major impact on lifetime fitness (Ebensperger *et al.* 2011a).

A previous study on captive degus (L. Correa, unpublished data) demonstrated that proximal mechanisms such as maternal allostasis, the IUP phenomenon and the horn effect all contribute to modify the female phenotype, thus producing a female masculinization gradient (Correa 2012). In degus, this gradient probably originates via differences in the testosterone concentration in amniotic fluid during the last third of pregnancy. Thus, female offspring experience high, medium or low levels of testosterone *in utero* depending on whether they neighbour two, one or zero male siblings during prenatal development, respectively. Additionally, the total number of male offspring per horn affects amniotic testosterone concentration (Correa 2012). The same study found that masculinized females produce larger and male-biased litters compared with feminized females, thus supporting the hypothesis that female morphotype significantly affects reproductive success (Correa 2012). However, how effects materialize and have consequences among free-living degus remained unknown.

Our general objective was to assess the effects of female morphotype on female reproductive performance in a natural population of degus, including the modulator role of social environment. We hypothesized that (1) social context modulates the effect of female morphotype on female reproductive success (measured as litter size at weaning). Specifically, we predicted (i) feminized females would exhibit greater reproductive success in social groups with fewer females and/or groups where feminized females are the most frequent morphotype. We further expected (ii) reproductive success of masculinized females to be insensitive to variation in the number of females and social group composition. Additionally, we also hypothesized that (2) female morphotype affects the proportion of male offspring per litter. Thus, we predicted that (iii) feminized females would have female-biased litters, while masculinized females would have male-biased litters. Regarding the potential effect of social context on the proportion of male offspring per litter, we tested whether (iv) social groups with a higher proportion of feminized females wean more female offspring, and if social groups with more masculinized females wean more male offspring. Finally, we examined the extent to which social context modulates the effects of female morphotype on other reproductive traits, including date of parturition and offspring weight at weaning.

Materials and methods

STUDY POPULATION

This study was conducted between 2009 and 2013 on a natural degu population located at the Estación Experimental Rinconada de Maipú (33°23′S, 70°31′W, altitude 495 m), a field station of the Universidad de Chile. This study area is characterized by a Mediterranean climate with cold, wet winters and warm, dry summers (di Castri & Hajek 1976). The site consisted of open areas with scattered shrubs (*Proustia pungens, Acacia caven,* and *Baccharis* spp.) that on average covered 14·5% of the field site (Ebensperger & Hurtado 2005).

LIVE TRAPPING AND TELEMETRY

Live trapping and telemetry were conducted between September and October (a time encompassing parturition, lactation and offspring weaning) of each year. Degus are diurnally active and remain in underground burrows overnight (Ebensperger *et al.* 2004).

A burrow system was defined as a group of burrow openings surrounding a central location spanning one to three metres in diameter where individuals were repeatedly found during night-time telemetry (Fulk 1976; Hayes, Chesh & Ebensperger 2007). Ten traps (Tomahawk model 201; Tomahawk Live Trap Company, Tomahawk, WI, USA) were used at each burrow system daily. Traps were set prior to the emergence of adults during morning hours (06:00 h). After 1·5 h, traps were closed until the next trapping day. The identity, location, sex, anogenital distance, body mass (weighed to the nearest 0·1 g) and reproductive condition (perforated, pregnant or lactating) were determined for all captured degus. At first capture, each degu received ID coded tags on each ear (Monel 1005-1; National Band and Tag Co., Newport, KY, USA). Adults weighing greater than 170 g were fitted with six to seven gram radiocollars (AVM Instrument Co., Colfax, CA, USA) with unique pulse frequencies. Analyses based on 2008–2011 data from the Rinconada population indicate that radiocollars do not influence physical condition or survival (L.A. Ebensperger, unpublished data). The total area examined at Rinconada was nearly 2 ha and did not vary across years of study. During night-time telemetry, females were tracked to their home burrows via radiotelemetry. Previous studies at Rinconada confirm that night-time locations represent underground nest sites (Ebensperger *et al.* 2004). Locations were determined once per night approximately 1 h before sunrise using LA 12-Q receivers (for radiocollars tuned to 150·000–151·999 MHz frequency; AVM Instrument Co., Auburn, CA, USA) and hand-held, three-element Yagi antennas (AVM instrument Co.). Previous studies have verified these radiotelemetry methods as sufficient for determining group membership (Hayes *et al.* 2009; Ebensperger *et al.* 2011a,b). The number of burrow systems monitored, the number of days that each burrow system was trapped, the number of radiocollared degus and the number of night-time telemetry locations per radiocollared degu and per year of study is given in Table S1 (Supporting Information).

INDIVIDUAL ATTRIBUTES: FEMALE MORPHOTYPE, FEMALE BODY WEIGHT AND FEMALE AGE

Female morphotype in terms of masculinization level was assessed through anogenital distance analyses. Females that were close to the short end of the anogenital distance distribution were classified

as *feminized females*, while females that were close to the long end of this gradient were classified as *masculinized females*. Anogenital distance was measured as the distance from the ventral anus commissure to the base of the genital papilla, as defined by Vandenbergh & Hugget (1994). We measured the anogenital distance of each adult female with a digital calliper (precision 0·1 mm) at every capture event. Anogenital distance measures were recorded exclusively for females exhibiting a non-perforated vagina. All anogenital distance measurements were taken by the same observer across all five years of the study. Anogenital distance measures were averaged per female degu to obtain a single measure per subject (Bánszegi *et al*. 2012). The number of anogenital measures per degu averaged 17·8 ± 8·6 (range: 2–42). Repeatability of anogenital distance and its correlation with female body weight were calculated according to the methods of Dušek, Bartoš & Sedláček (2010). For all statistical analyses, anogenital distance was utilized as a continuous predictor variable. From trapping data collected in the field, we calculated the mean female body mass during the lactation period. The number of body mass measures per female during the lactation period averaged 17·01 ± 9·47 (range: 2–42). The age of the females included two age cohorts: females of one and two years old.

SOCIAL GROUP DETERMINATION

The main criterion used to assign individuals to social groups was the sharing of burrow systems at night. The sharing of burrow systems was determined by (i) burrow trapping during early morning activity and (ii) night-time telemetry. To determine group composition, we first compiled a symmetric similarity matrix of pairwise association of burrow locations of all adult degus during trapping and telemetry (Whitehead 2008). The association (overlap) between any two individuals was determined by dividing the number of early mornings that these individuals were captured at or tracked with radiotelemetry to the same burrow system, by the number of early mornings that both individuals were trapped or tracked with radiotelemetry on the same day (Ebensperger *et al*. 2004; Hayes *et al*. 2009). To determine social group composition, a hierarchical cluster analysis of the association matrix was conducted using SOCPROG software (Whitehead 2009). The fit of the data was analysed using cophenetic correlation coefficients, correlations between the actual association indices and the levels of clustering in the diagram. In this procedure, values above 0·8 indicate that hierarchical cluster analysis has provided an effective representation of the data (Whitehead 2008). The maximum modularity criterion (Newman 2004) was used to cut off the dendrogram and define social groups. Social group membership was analysed as an individual trait. Females that did not belong to any social group (i.e. adult females that were alone) were excluded from all analyses.

SOCIAL GROUP ATTRIBUTES

For our analyses, we included two variables related to social group attributes. These variables were (i) the number of females, representing the total number of adult females in the social group; and (ii) mean group anogenital distance, measured as the mean anogenital distance of all female group members, a variable that measures predominance of some female morphotype within a social group (i.e. whether females with short or long anogenital distance predominates).

GENETIC METHODS AND MATERNITY ANALYSES

We genotyped a total of 907 individuals captured between 2009 and 2013 (Table S2). Tissue samples (a 1 × 5 mm ear snip) were taken from each individual. Tissue samples were stored in ethanol 99% at 5–6 °C until analysis. DNA was extracted using the ReliaPrep DNA animal tissue miniprep system kit (Promega) mouse tail protocol. DNA was eluted in 200 μL of free nuclease water and stored at −20 °C. We worked with 12 microsatellite loci, 11 from *O. degus* (Quan *et al*. 2009) and one from S. *cyanus* (Schroeder *et al*. 2000). These loci were amplified via polymerase chain reaction (PCR), with the following protocol: 15 min at 94°C for DNA denaturation, 30 cycles of a 1 min denaturation step at 94°C, followed by 1 min of locus-specific annealing temperature (Table S3), 1 min at 72 °C for elongation and a final elongation step of 10 min at 72 °C. For fragment analysis, the PCR products were mixed in three combinations of four loci. Each of these mixes was contrasted with an internal size standard and analysed using an ABI Prism 3130Xl genetic analyser, and allele sizes were determined using the GENEMAPPER software (Applied Biosystems, Foster City, CA, USA). All loci amplified successfully and were polymorphic (Table S3). Genotypes for all individuals across years were complete with no missing data. We tested the Hardy–Weinberg observed and expected heterozygosity for each study year with CERVUS 3.0 software (Marshall *et al*. 1998). Deviations from Hardy–Weinberg expectations were detected in four out of five years (Table S4) and were not the consequence of null allele presence (all markers were checked for null alleles with Microchecker software, van Oosterhout *et al*. 2004). This finding was not surprising for us because our population is finite, open and non-panmitic and possesses a relatively high level of genetic relatedness (Quirici *et al*. 2011a).

To conduct maternity analyses, we used CERVUS 3.0 software (Marshall *et al*. 1998), and all offspring were checked against all potential mothers in the population. Confidence calculations were made using the LOD score option in CERVUS 3.0. Simulations were run for 10 000 cycles using allele frequency data from the entire population, with a genotyping error rate of 1% and under the assumption that 90% of the population was sampled. Maternity assignment analyses were made using strict (95%) confidence levels. Maternity was assigned when the following conditions were met: (1) the LOD score for the pair mother–offspring tested was positive, (2) the mother–offspring pair confidence level was significant, and (3) there were no mismatches. We had several cases in which only criteria one and two were met, yet the offspring–mother pair exhibited one mismatch. We examined these cases to determine which loci were involved. Whenever single mismatches involved two repeat motif and contiguous loci (i.e. separated by two base pairs), we verified (i) spatial concordance between the putative mother and offspring based on the burrows they used and (ii) developmental concordance between body weights of doubtful offspring and their potential siblings during a particular time in the breeding season. Any single mismatches between offspring–mother found in a four repeat motif loci or that involved non-contiguous alleles in two repeat motif loci were discarded. Any maternity assignments with two or more mismatches were also discarded as our degu population exhibit a relatively high level of genetic relatedness (Ebensperger *et al*. 2004; Quirici *et al*. 2011a). Table S5 displays the number of offspring that were and were not assigned to a mother.

DETERMINATION OF REPRODUCTIVE TRAITS

Using maternity analysis data, we next determined litter size (total number of offspring produced) and the proportion of male offspring per litter for each adult female. These variables were determined at weaning, when offspring become trappable. Annual trapping ended when the daily capture rate of new offspring was <5% of all offspring captured. Using trapping data collected in the field, we next determined the date of parturition and offspring body weight at weaning. Date of parturition was defined as the day between the last day of pregnancy and the first day of nursing, and was also verified via the presence of colostrum, vaginal perforation and bloody vaginal secretions. Date of parturition was analysed as a time interval that extends from the first date where some parturition was recorded to the last date in where some parturition was recorded. Each date of parturition was assigned an ordinal number according to the order in which the parturition occurred. The number one was assigned to the first parturition recorded. The following numbers were assigned in order as parturitions occurred. If two females gave birth the same day, these females share the same ordinal number. All females where we could not accurately determine the day of parturition were excluded from the analysis. Litter size or offspring quantity at weaning was analysed as a measure of reproductive success. The proportion of male offspring per litter, the offspring body weight at weaning and the date of parturition, were analysed as reproductive traits.

STATISTICAL ANALYSES

We used PERMANCOVA to examine relevant models for each hypothesis prediction.

All probability values (P_{perm}) of statistically significant models were derived from a pseudo-F distribution calculated after 10 000 permutations of the original data set. When the simulated permutations were <1000, probability values were obtained by Monte Carlo simulations (P_{MC}). Regressions models between selected predictor variables and dependent variables were used throughout as *post hoc* analyses. The linearity of the relationships between variables was analysed through GAM models. All analyses were performed using the PERMANOVA+ for the PRIMER statistical package (Clarke & Gorley 2006; Anderson, Gorley & Clarke 2008).

For hypothesis 1, we tested whether social context factors modulate the effects of female morphotype on litter size at weaning. Predictions (i) and (ii) from hypothesis 1 were tested through *model 1:* log-transformed litter size = year + female anogenital distance + female mean body weight during the lactation period + female age + social group membership (nested in year) + the number of females + mean group anogenital distance. In this model, year was a fixed factor and social group membership, an individual level trait, was a random factor (Anderson 2001). In addition, we added factor interaction terms involving individual female anogenital distance and social group factors (female anogenital distance by the number of females, female anogenital distance by mean group anogenital distance).

For hypothesis 2, we tested whether social context factors modulate the effects of female morphotype on the proportion of male offspring per litter at weaning. Predictions (iii) and (iv) from hypothesis 2 were tested through *model 2:* log-transformed proportion of male offspring per litter = year + female anogenital distance + female mean body weight during the lactation period + female age + social group membership (nested in year) + the number of females + mean group anogenital distance. In this model, year was a fixed factor and social group membership was a random factor. In addition, we added factor interaction terms involving individual female anogenital distance and social group factors (anogenital distance by the number of females, anogenital distance by mean group anogenital distance).

We analysed the effect of female morphotype and social context on offspring body weight at weaning. To test for these effects, we considered *model 3:* log-transformed offspring body weight at weaning = year + female anogenital distance + female mean body weight during the lactation period + social group membership (nested in year) + the number of females + mean group anogenital distance. Offspring sex and litter size were included as a covariates. In addition, we added an offspring sex by year interaction term. In this model, year was a fixed factor and social group membership was a random factor.

To examine how female anogenital distance influenced date of parturition, we built *model 4:* log-transformed date of parturition = year + female anogenital distance. Litter size and the proportion of male offspring per litter were included as covariates, and year was a fixed factor. The relationship between female anogenital distance and female average body weight during the lactation period was assessed through Pearson correlation (r).

ETHICAL NOTE

Animal handling techniques and all protocols used in this study were approved and supervised by the Ethics Committee of the Pontificia Universidad Católica of Chile (CBB-155, 2012 resolution, revised 03/03/2015), and followed the Chilean Ethical Legislation (Permits 1-31/2009 [1956], 3881/2012, and 2826/2013 by the Servicio Agricola y Ganadero). Blood and tissue sampling were performed by well-trained veterinarians and biologists (CL and JR-E).

Results

FEMALE ANOGENITAL DISTANCE

Across all five study years, mean female anogenital distance was 2·1 ± 0·5 mm (SD) (range: 1·12 mm to 3·86 mm, n = 3569 measurements from 89 females). Intraclass correlation coefficient (ICC) or intraseason repeatability of female anogenital distance was 0·72 (95% confidence interval: 0·66–0·79, n = 918 measurements from 89 females), a relatively high value. Intraclass correlation coefficient (ICC) or interyear repeatability of female anogenital distance was 0·69 (95% confidence interval: 0·38–0·97, n = 79 measurements from four females), also a relatively high value. We verified that female body weight and anogenital distance were not correlated (r = 0·098; t = 0·976; P = 0·331).

SOCIAL GROUP CHARACTERISTICS

A total of 89 adult females were assigned to 35 different social groups. The number of social groups with multiple females varied from 0 in 2010 to 13 in 2013 (Table 1).

Table 1. Group size of multifemale social groups recorded from 2009 to 2013

	Year				
	2009	2010	2011	2012	2013
Group size					
Number of groups (*n*)	11	0	6	5	13
Average	4·03	–	2·93	3·5	3·26
SD	0·8	–	0·92	0·52	0·86
Range	(3–5)	–	(2–4)	(3–4)	(2–4)
No. of females					
Average	3·07	–	2·43	3	2·74
SD	1·11	–	0·51	0·78	0·68
Range	(2–5)	–	(2–3)	(2–4)	(2–4)
No. of males					
Average	0·97	–	0·5	0·5	0·52
SD	0·96	–	0·76	0·52	0·51
Range	(0–3)	–	(0–2)	(0–1)	(0–1)

Thus, social groups from 2010 were not included in the subsequent analyses. Mean group anogenital distance averaged 2·19 ± 0·48 mm (range: 1·5–3·23 mm).

FEMALE REPRODUCTIVE SUCCESS (LITTER SIZE) AT WEANING

Regarding individual attributes of females, female anogenital distance ($_{pseudo}F(1, 46) = 2·192$; $P_{perm} = 0·150$), female age ($_{pseudo}F(1, 46) = 3·421$; $P_{perm} = 0·069$) and social group membership ($_{pseudo}F(28, 46) = 1·497$; $P_{perm} = 0·114$) did not have a statistically significant relationship with female litter size at weaning. However, female mean body weight during the lactation period ($_{pseudo}F(1, 46) = 10·223$; $P_{perm} = 0·003$) had a significant and positive effect on female litter size at weaning (Fig. 1). Female mean body weight during the lactation period explained 9·15% of litter size variance. Considering social group attributes, the number of females ($_{pseudo}F(1, 46) = 2·133$; $P_{perm} = 0·133$) and mean group anogenital distance ($_{pseudo}F(1, 46) = 0·015$; $P_{perm} = 0·901$) did not influence female litter size at weaning. Likewise, two-way interaction involving individual and social attributes influenced female litter size at weaning: female anogenital distance by the number of females ($_{pseudo}F(1, 46) = 0·004$; $P_{perm} = 0·946$); female anogenital distance by mean group anogenital distance ($_{pseudo}F(1, 46) = 1·396$; $P_{perm} = 0·249$). Female litter size at weaning did not vary across years ($_{pseudo}F(3, 46) = 1·398$; $P_{perm} = 0·274$). Details of statistical analyses are provided in Table S6.

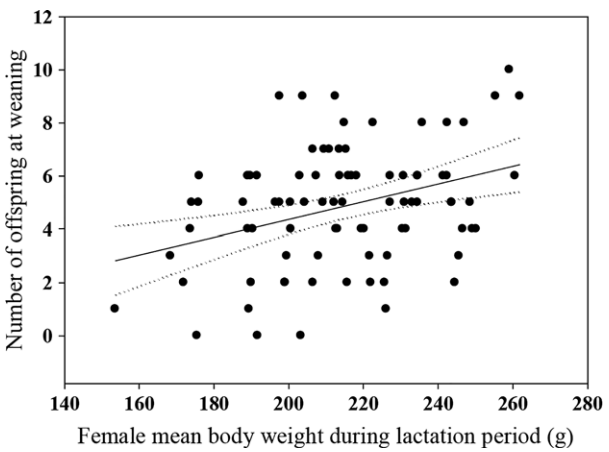

Fig. 1. Simple regression between female mean body weight (g) during the lactation period and the number of offspring at weaning. Relationship between female mean body weight during the lactation period and the number of offspring at weaning was positive and linear. Female mean body weight during the lactation period explained 9·15% of variance. Dashed lines indicate 95% confidence intervals.

PROPORTION OF MALE OFFSPRING PER LITTER AT WEANING

For individual female attributes, only female anogenital distance ($_{pseudo}F(1, 46) = 6·541$; $P_{perm} = 0·014$) had a detectable and positive effect on the proportion of male offspring per litter (Fig. 2). Female anogenital distance explained 5·38% of the variance of the proportion of male offspring per litter. Female mean body weight during the lactation period ($_{pseudo}F(1, 46) = 1·042$; $P_{perm} = 0·311$), female age ($_{pseudo}F(1, 46) = 3·224$; $P_{perm} = 0·080$) and social group membership ($_{pseudo}F(28, 46) = 1·062$; $P_{perm} = 0·421$) did not influence the proportion of male offspring per litter. Considering social group attributes, the number of females ($_{pseudo}F(1, 46) = 1·533$; $P_{perm} = 0·204$) and mean group anogenital distance ($_{pseudo}F(1, 46) = 0·010$; $P_{perm} = 0·923$) did not have detectable effects on the proportion of male offspring per litter at weaning. No factor interactions influenced the proportion of male offspring per litter at weaning: female anogenital distance by the number of females ($_{pseudo}F(1, 46) = 1·246$; $P_{perm} = 0·269$); female anogenital distance by mean group anogenital distance

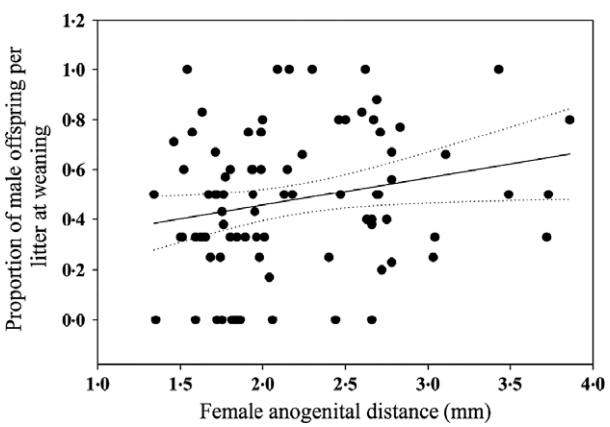

Fig. 2. Simple regression between female anogenital distance (mm) and the proportion of male offspring per litter at weaning. Relationship between female anogenital distance and the proportion of male offspring per litter at weaning was positive and linear. Female anogenital distance explained 5·38% of the variance. Dashed lines indicate 95% confidence intervals.

($_{pseudo}F(1, 46) = 0{\cdot}049$; $P_{perm} = 0{\cdot}833$). The proportion of male offspring per litter at weaning did not vary with year ($_{pseudo}F(3, 46) = 0{\cdot}432$; $P_{perm} = 0{\cdot}730$). Details of statistical analyses are provided in Table S7.

BODY WEIGHT OF OFFSPRING AT WEANING

Regarding individual attributes, female anogenital distance ($_{pseudo}F(1, 128) = 23{\cdot}338$; $P_{perm} < 0{\cdot}001$) had a positive effect on offspring body weight at weaning (Fig. 3). Female anogenital distance explained 10·21% of the variance of offspring body weight at weaning. Similarly, female mean body weight during the lactation period ($_{pseudo}F(1, 128) = 13{\cdot}786$; $P_{perm} = 0{\cdot}002$) had a positive effect on offspring body weight at weaning and explained 5·17% of the variance of offspring body weight at weaning. Social group membership also had an effect on offspring body weight at weaning ($_{pseudo}F(12, 128) = 5{\cdot}299$; $P_{perm} < 0{\cdot}001$). Social group membership explained 7·44% of the variance of offspring body weight at weaning. Regarding social group factors, the number of females ($_{pseudo}F(1, 128) = 0{\cdot}318$; $P_{perm} = 0{\cdot}974$) and mean group anogenital distance ($_{pseudo}F(1, 128) = 1{\cdot}576$; $P_{perm} = 0{\cdot}216$) did have not detectable effects on offspring body weight at weaning. Year influenced the offspring body weight at weaning ($_{pseudo}F(3, 128) = 13{\cdot}125$; $P_{perm} = 0{\cdot}001$) and accounted for 62·09% of the variance of offspring body weight at weaning. Offspring sex ($_{pseudo}F(3, 128) = 1{\cdot}685$; $P_{perm} = 0{\cdot}168$) did not influence offspring body weight at weaning, but litter size did ($_{pseudo}F(1, 128) = 4{\cdot}647$; $P_{perm} = 0{\cdot}047$), explaining 1·54% of the variance. Details of statistical analyses are provided in Table S8.

DATE OF PARTURITION

Female anogenital distance ($_{pseudo}F(1, 27) = 5{\cdot}436$; $P_{perm} = 0{\cdot}027$) had a positive effect on date of parturition, and where females with longer anogenital distance delivered offspring later in the breeding season (Fig. 4). Female anogenital distance explained 3·77% of the variance in parturition date. Likewise, date of parturition differed across study years ($_{pseudo}F(3, 27) = 9{\cdot}111$; $P_{perm} < 0{\cdot}001$). Year explained 64·95% of the variance in date of parturition. Litter size ($_{pseudo}F(1, 27) = 0{\cdot}220$; $P_{perm} = 0{\cdot}644$) and proportion of male offspring per litter ($_{pseudo}F(1, 27) = 3{\cdot}377$; $P_{perm} = 0{\cdot}078$) did not influence the date of parturition. Details of statistical analyses are provided in Table S9.

Discussion

We assessed the effects of female morphotype, social group attributes and their interactions on female reproductive performance. We found that individual female attributes including female masculinization level (anogenital distance), female body weight during lactation and the membership to a social group, all influenced female reproductive performance and fertility. We further verified that female body weight and anogenital distance had independent effects on reproductive traits. Social factors such as group size and mean group anogenital distance, and the interaction of these variables with individual attributes, did not influence female reproductive performance. Previous studies have compared the reproductive performance of masculinized and feminized females, focusing on differences in fertility (vom Saal 1981, 1989a; Clark & Galef 1998; Bánszegi *et al*. 2012). Previous evidence supports that feminized females are more fertile than masculinized females (vom Saal 1981, 1989a; Clark & Galef 1998; Bánszegi *et al*. 2012; Monclús & Blumstein 2012). Likewise, masculinized females were thought to be at a reproductive disadvantage compared with feminized females due to irregular oestral cycles, undetectable oestrus,

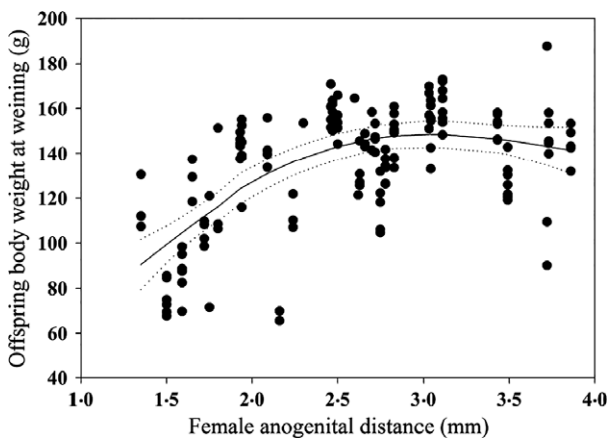

Fig. 3. Relationship between female anogenital distance (mm) and offspring body weight (g) at weaning. Relationship between female anogenital distance and the offspring body weight at weaning was positive and no linear. Female anogenital distance explained 10·21% of the variance. Dashed lines indicate 95% confidence intervals.

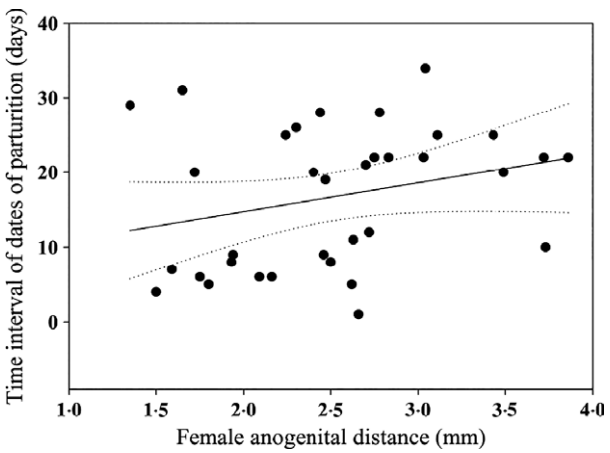

Fig. 4. Simple regression between female anogenital distance (mm) and the time interval of dates of parturition expressed in number of days after first litter born. Relationship between female anogenital distance and date of parturition was positive and linear. Female anogenital distance explained 3·77% of the variance. Dashed lines indicate 95% confidence intervals.

shorter reproductive life span, delayed onset of breeding or through decreased attractiveness to males (vom Saal 1981; Clark, Karpluk & Galef 1993; Zher, Gans & McClintock 2001; Ryan & Vandenbergh 2002; Monclús *et al*. 2014). Thus, masculinized females were suggested to represent a 'pathological' female morphotype (Kaiser & Sachser 2005). However, several studies have failed to support these claims. Fertility did not differ between feminized and masculinized females in a variety of rodent species, including the house mouse, the mound-building mouse, the Alpine marmot (Vandenbergh & Hugget 1994; Hackländer & Arnold 2012; Szenczi *et al*. 2013) and the degu (this study). Findings from our current study confirmed previous observations on captive degus where masculinized females were found to deliver larger and/or male-biased litters, while feminized females were found to deliver smaller and/or female-biased litters (Correa 2012), implying that female masculinization does not represent a pathological morphotype in the degu.

Regarding the effects of female morphotype on litter sex ratio biases, we found that feminized females delivered female-biased litters, while masculinized females delivered male-biased litters. Similar biases according to female morphotype have been reported in several other species of litter-bearing mammals (vom Saal *et al*. 1999; Ryan & Vandenbergh 2002). From the perspective of adaptive explanations, sex ratio biases could be adaptive when the cost (Clutton-Brock, Albon & Guinness 1984) and/or benefits (Trivers & Willard 1973) of producing a male or a female offspring differ between the sexes, as this occurs in mammals with sexual dimorphism and higher male reproductive variance such as ungulates and primates (Gomendio *et al*. 1990). In degus, there is no firm evidence of sexual size dimorphism (Correa 2012). In addition, our long-term study in a wild population indicates that reproductive variance between males and females does not differ (unpublished data). Thus, adaptive manipulation of sex ratio by the females seems implausible in degus (Trivers & Willard 1973; Gomendio *et al*. 1990). The proximal mechanisms controlling sex ratio biases have been well studied in other mammals (Hedricks & McClintock 1990). These mechanisms can act at different stages of the breeding process, from the pre-conception to pre-weaning stages, differentially affecting Y/X spermatozoon, or male/female embryos, foetuses or offspring survivorship (Roberts 1972; Myres 1978; Gosling 1986; James 1986; Hedricks & McClintock 1990; Davison & Ward 1998; Cameron 2004; Grant 2007). Some of these mechanisms are related to variation in hormone profiles during the female oestral cycle (James 1986; Cameron 2004; Grant 2007), and there is evidence that in rodents, masculinized and feminized females differ in several features of their oestral cycles (vom Saal 1981; vom Saal *et al*. 1999; Ryan & Vandenbergh 2002). Thus, subsequent studies should assess whether some of these proximal mechanisms may explain how female morphotype affect litter sex ratios.

From an adaptive perspective, offspring body weight at weaning generally has a positive effect on post-weaning survival (Rieger 1996), adult body weight (Clutton-Brock 1991) and subsequent female adult fertility (Campbell & Slade 1995). Our study has shown how female body weight at the adult stage is positively related to female fertility, with heavier degu females having higher fertility. However, we do not know if heavier sons and daughters of masculinized females will be heavier as adults. If this is true, this would suggest an adaptive maternal effect on offspring fertility because increased maternal allocation during lactation could increase offspring's future adult fertility. Further studies are needed to determine whether heavier offspring attain higher survival to adulthood in degus. Proximally, offspring body weight at weaning has been positively related to female parental care (Clutton-Brock 1991). We found that masculinized females weaned heavier offspring than feminized females, a finding consistent with previous laboratory studies where compared with feminized females, masculinized female degus lost more body weight during pregnancy and lactation, and weaned heavier male and female offspring (Correa 2012). Taken together, previous laboratory observations and results from this study are consistent with the hypothesis that masculinized degu females exhibit greater parental care than feminized females, as reported in Mongolian gerbils (Clark & Galef 1998). In degus, this higher maternal care likely does not decrease future reproductive success (Clutton-Brock 1991), as degus are a semelparous species where only 10% of individuals experience a second reproductive event (Ebensperger *et al*. 2011a). Thus, the higher maternal allocation of masculinized females could be adaptive because this would not jeopardize future reproductive events or maternal survival, and would improve offspring fitness (Hamel *et al*. 2010; Fisher & Blomberg 2011).

Early offspring delivery in seasonally varying habitats has been suggested to enhance subsequent offspring survival because offspring would have more time to increase energetic reserves in advance of periods of resource scarcity (Monclús *et al*. 2014). However, masculinized female degus gave birth to heavier offspring later during the breeding season than feminized females. Similar findings have been reported in the Uinta ground squirrel (*Spermophilus armatus*), where females produce larger litters of lighter offspring early in the breeding season, but change to producing smaller litters of heavier offspring late in the breeding season (Rieger 1996). Under laboratory conditions (with artificial photoperiod), we found that masculinized females deliver large litters, while under wild conditions, we found that masculinized and feminized females do not differ in litter size (Correa 2012). However, we found that in the wild, masculinized females produce heavier offspring than feminized females. Future studies should examine if heavier offspring are of higher quality, as this would support that masculinized females maintain their maternal allocation, but change their reproductive tactic by increasing offspring quality rather

than quantity, as reported in Uinta ground squirrels (Rieger 1996).

Overall, our field-based study found that fertility did not differ between masculinized and feminized females, and that masculinized females made a higher maternal allocation during lactation, implying that masculinization does not impair female reproductive performance as has been previously suggested (Vandenbergh 2003; Kaiser & Sachser 2005; Hackländer & Arnold 2012; Monclús *et al*. 2014). While we cannot currently posit that masculinized females are a superior morphotype, we can at least hypothesize that masculinized females are not a 'pathological/suboptimum' morphotype. Our study indicated that social context (in terms of female number and within group anogenital distance) did not influence reproductive success of masculinized or feminized females in degus. However, future studies should examine whether female masculinization could act as an adaptive mechanism to increase female reproductive success under varying conditions of density or other ecological factors (vom Saal 1981; Ryan & Vandenbergh 2002; Kaiser & Sachser 2005; Sachser, Kaiser & Hennessy 2013). In particular, we need to establish how enhanced maternal allocation of masculinized females increases offspring survival during relatively challenging conditions in terms of food availability and population density. We have shown previously how reproductive success in female degus increases in larger social groups, but mostly during years with lower food abundance, lower mean precipitation and lower density (Ebensperger *et al*. 2014).

Author contributions

All authors have contributed equally to the preparation of this document.

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Data accessibility

Data available from the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.ph8s1 (Correa *et al*. 2016). Data files: DATA 1. Litter size and Male offspring proportion. DATA 2. Offspring body weight at weaning (65 days). DATA 3. Date of parturition.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Number of burrow systems, number of days that each burrow system was trapped, number of radiocollared degus, and mean (± SD) number of night-time telemetry locations per radiocollared degu during spring of each year of the study (2009, 2010, 2011, 2012, and 2013) at Rinconada de Maipú.

Table S2. Number of genotyped adult and offspring degus from 2009 to 2013 (study total = 907 degus).

Table S3. Sequence, annealing temperature (Ta), size in bp (bp = pair bases), and number of alleles (Na) of 12 microsatellite loci used to genotype degu adults and offspring.

Table S4. Analysis of Hardy–Weinberg expectations for each locus within each study year (2009–2013).

Table S5. Number of genotyped offspring assigned to a candidate mother and number of genotyped offspring not assigned to a candidate mother (2009–2013).

Table S6. Details of statistical analysis for model 1.

Table S7. Details of statistical analysis for model 2.

Table S8. Details of statistical analysis for model 3.

Table S9. Details of statistical analysis for model 4.