Early fitness consequences and hormonal correlates of parental behaviour in the social rodent, *Octodon degus*

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ABSTRACT

Males are expected to assist their mates whenever this behaviour raises survival of offspring with little expense in terms of mating opportunities. At a more proximate level, cortisol and testosterone hormones seem involved in the expression of parental care in mammals. We examined the consequences to postnatal offspring development and survival of the males' presence in the social rodent, *Octodon degus*. Offspring quality and quantity, and maternal condition of females were contrasted among females rearing their litters in the presence of the sire, females breeding in the presence of a non-breeding female, and females breeding solitarily. We related these differences to variation in parental behaviour and plasma levels of testosterone and cortisol. Twenty two females and their litters were studied under constant conditions of adult density, nest availability, food availability, and breeding experience. Males huddled over and groomed offspring. However, neither the number nor the mass of pups from dams that nested with the sire differed from those recorded to breeding females that nested with a non-breeding female and females that nested solitarily. Body weight loss and associated levels of plasma cortisol in dams nesting with the sire were similar to those of solitary females, but higher than mothers nesting with a non-breeding female. Thus, male care had no consequences to offspring, and seemed detrimental to breeding females. Circulating levels of cortisol and total testosterone were either poor (mothers) or no (fathers, non-breeding females) predictors of parental care.

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1. Introduction

The scattered distribution of parental behaviour across vertebrates indicates that this trait has evolved multiple times [1]. In mammals most species exhibit uniparental care where the females typically become the parental sex [2,3]. While paternal care is generally rare across mammals, it occurs more often in species of carnivores, primates and perissodactyls, in which biparental care is the rule [3]. A greater frequency of female uniparental care is expected given that mammalian females are more associated with offspring than males through pregnancy and lactation, two processes that are energetically costly [4].

Males are expected to assist their mates whenever the young that survive under biparental care exceeds survival under uniparental care times the mating success of a nonparental male [3]. Unsurprisingly, experimental studies that examine the consequences of a male's presence on quantity and quality of offspring in mammals have relied on rodent models amenable to experimentation. These studies support that the importance of a father's presence is contingent upon animals facing environmentally challenging conditions. Thus, the sire's presence enhances infant survivorship under cold (i.e., energetically stressful) but not under warm ambient conditions in the California mouse, *Peromyscus californicus* [5], and Djungarian hamsters, *Phodopus campbelli* [6]. Low temperatures also seem linked with male-increased offspring development in the striped mouse, *Rhabdomys pumilio* [7]. Challenging conditions related to a need to forage for food were recorded to interact with a male's presence to increase pup survival (but not pup growth) in *P. californicus* [8] and in the house mouse (*Mus musculus*) [9]. The presence of sires in Mongolian gerbils (*Meriones unguiculatus*), prairie voles (*Microtus ochrogaster*), and red-backed voles (*Clethrionomys gapperi*) has no direct effect on offspring growth and survival under less challenging rearing conditions [10–12].

A male might be expected to provide brood care under conditions where its presence has marginal consequences to offspring survival and growth provided that expectations for additional breeding attempts are relatively low [3]. Factors that may decrease probability of multiple mating in males include a spatially over dispersed distribution of females and high synchrony of female oestrus [13–16]. Expectations for

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subsequent breeding could be further reduced in males of species that breed annually and in which annual mortality of adults is high.

To a more proximate level of causation, evidence is generally consistent with the hypothesis that hormones are implied in the expression of maternal and paternal behaviours in mammals [17,18]. Regarding paternal behaviour, the role played by different hormones is variable across species. Thus, while paternal behaviour takes place in association with relatively high levels of prolactin in male rodents and primates [but see Ref. [19]] with biparental care [20–23], this hormone seems unimportant to the expression of care in male rodents and carnivores of more polygynous species [24–26]. In some of these species, male parental and alloparental care is instead more associated with high circulating levels of cortisol [19,25] or corticosterone [27], a pattern that contrasts with other rodents and primates in which cortisol decreases upon delivery of offspring [17]. Likewise, variation across species characterizes the role of other hormones such as testosterone. Thus, a reduction in testosterone seems necessary at the onset of paternal and alloparental care in males of polygynous Mongolian gerbils, socially monogamous prairie voles, and black tufted-ear marmosets, *Callithrix kuhlii* [11,28–30]. In contrast, testosterone seems critical to the expression of male care in other biparental species [31,32], but see Ref. [20]. Taken together, a hormonal influence for paternal care in mammals remains indisputable. However, variation linked to roles played by different hormones across species indicates that underlying mechanisms cannot be assumed to be homologous [1,18]. Thus, studies on other, phylogenetically unrelated species may serve the double purpose of contributing to these patterns of variation and providing additional independent replicates to subsequent comparative analyses.

Based on the above, our study had two major objectives. First, we examined the consequences of the males' presence on maternal condition and early offspring growth and survival in the social rodent, *Octodon degus*. We chose degus because several aspects of the reproductive biology and demography of these rodents indicate that a cost in terms of mating opportunities to male degus is probably nil. Degus typically breed once per year [33]: mating at the time of litter delivery (i.e., 3 months after the main mating episode) as a result of postpartum oestrous is rare (L.A. Ebensperger unpublished observations). In addition, most adults (and males in particular) die before two years of age [34–36], suggesting that success during first breeding event has a major impact on lifetime fitness. We predicted these conditions would favour males of this species providing paternal care to some extent. Degus live in social groups where a variable number of females (1–4) share an underground burrow system and a foraging area with one or two males [37,38]. While communal nesting by lactating females under semi-natural and natural conditions has been well demonstrated [37,39], the role played by males remains unknown. During lactation, males also share underground burrows with the females.

A second major objective of our study was to examine hormonal correlates of parental behaviour in these rodents. Most endocrinological evidence on parental care in rodents comes from murid species (mice, rats) where prolactin, cortisol, and testosterone seem to play roles. In contrast, proximate correlates of paternal care among hystricognath species lag behind despite the prevalence of reports of paternal behaviour in these rodents [40]. While no endocrinological studies have examined the parental behaviour of degus, glucocorticoids (mainly cortisol) and androgens (testosterone) mirror major breeding events in wild populations [41–43], implying that these hormones may play roles.

2. Methods

2.1. Study subjects, animal housing and experimental setting

The study subjects were one year old, laboratory raised *O. degus* (Pontificia Universidad Católica de Chile), representing a first generation of wild pregnant female descendants captured in Rinconada de Maipú (33°23′ S, 70°31′ W; 30 km west of Santiago), central Chile. Upon weaning (ca. 30 days of age), degu subjects were kept in same sex-sibling pairs inside 45 by 23 by 21 cm clear polycarbonate rat cages with a bedding of hardwood chips, and water and food (rabbit commercial pellet) provided ad lib. Animals were kept in a ventilated room exposed to a 12:12 h light:dark cycle with temperatures controlled between ca. 15–25 °C. Available data for 2003 through 2008 recorded at the Pudahuel weather station (Dirección Meteorológica de Chile), distant 15 km from Rinconada de Maipú, indicated that minimum air temperature averages 5.5 and 7.4 °C during September and October (time during which lactation and offspring weaning takes place), respectively. Maximum air temperature during September and October averages 19.2 and 22.5 °C.

When 3 months of age, female siblings were marked with an ear tag and transferred to a cage with either a full sister (related) or a completely unrelated female. Pairs of related (n = 15) and unrelated (n = 18) female pairs were then allowed to mate with a same age, unrelated male when 10 months of age. Upon 60 days of the introduction of males, time at which pregnant females were easy to recognize, animals were assigned to one of three conditions and transferred to experimental cages and housed in a different room.

Three experimental conditions were compared. Two of these consisted of (1) a female raising her litter in the presence of the sire (n = 7 female–male pairs), and (2) a female raising her litter in the presence of a non-breeding female (i.e., the former female cage mate that did not became pregnant during the previous stage) (n = 7 female–female pairs). Thus, sires and non-breeding females were socially familiar to the breeding female, a realistic condition for degus. Breeding females were randomly assigned to nest with a full sib or an unrelated non-breeding female. Previously, we demonstrated that kinship between a breeding female that nested with a non-breeding female does not influence offspring development, survival and body condition of dams [44]. These two social conditions allowed us to separate the potential effect of maleness care from that due to the presence of an additional adult. With the aim of comparing our results to previous studies, we also included (3) a condition in which a female raised her offspring solitarily (n = 8 females). All three experimental conditions to which female subjects were assigned (solitary, breeding with male, breeding with female) were established from previous trios in which only one of the two females became pregnant. It has been argued that pregnancy of dams that mate during postpartum estrus may potentially overshadow the effects due to the presence of fathers on female behaviour [10]. While postpartum estrus has been suggested in degus [45], its occurrence is rare [46]. Most critical to this study, none of the experimental females that nested with the male through lactation became pregnant.

Approximately 7–15 days before estimated delivery, breeding females and their cage mates (experimental conditions 1 and 2) were transferred into squared-shaped (61.5 by 62.0 by 40.5 cm) clear acrylic aquaria, and housed in a different (experimental) room. The rear section of these cages was provided with two clear nest boxes to facilitate observation (18 by 29 by 15 cm) lined next to each other. Solitary nesting females were housed into rectangular-shaped (61.5 by 31.5 by 40.5 cm) clear acrylic aquaria. The rear section of these aquaria housed a single clear nest box made of clear glass (18 by 29 by 15 cm). These cage dimensions allowed us to keep adult density constant through the experiments. Cage size (and the resulting space per animal) may influence food intake and metabolic expenditure in captive rodents [47]. While in these cages, degu subjects were supplied with a 1–2 cm layer of saw dust as bedding material, and water ad lib. Food consisted of rabbit commercial pellet and was provided in such a way that females obtained no more than their empirically determined energy requirements for adult lactating and non-lactating females [48]. Photoperiod of room housing the animals inside the experimental room was controlled and set according to conditions of temperature and photoperiod of a natural population of

degus near Santiago (Rinconada de Maipú). While lights were turned on always at 07:00 h, the length of the light phase was extended every 3 days to track the natural length of day at the time of experiments. Minimum and maximum ambient temperatures of the experimental room during this time averaged 18.3 ± 0.3 and 21.8 ± 0.2 °C, respectively. While these experimental temperatures were closer to the maximum rather than to the minimum temperatures that degus are likely to experience naturally, these thermal conditions were still well below the thermoneutral zone of the animals [49] and energetically challenging.

2.2. Morphological data collection and pup survival

Upon delivery, pups were individually marked. Adults were fitted a plastic 5 mm thick color collar to distinguish individual degus during behavioural observations (see below). Because litter size may influence subsequent offspring quantity and quality in rodents [50–53], we standardized all litters to 6 pups at this time [i.e. the mean litter size for degus, [48]]. Of the 22 litters examined, we reduced pups to 13 and added pups to 5. All pups removed were adopted by other lactating females in our breeding colony — we have demonstrated that lactating degus do not discriminate against unrelated offspring even in the presence of own offspring [54]. Pups added and pups of recipient litters were of similar age. Pups added were also genetically related to the recipient mothers. Mothers and pups were weighed to 0.05 g at birth (day 1) and then every other day until day 31, always during morning hours. We used these data to compare the mass attained by pups through lactation. In the case of dams, we calculated the percentage of body mass lost through lactation as an estimate of body condition. Body mass is a predictor of body condition in rodents [55]. At day 31, we tallied surviving pups to record survivorship of weanlings.

2.3. Blood sampling and hormonal determinations

Blood samples (500 μL per sample) were obtained from the orbital sinus for all adults at days 5 and 15 after pup delivery during morning hours. Every time, subjects were introduced into a 5 L jar and anesthetized through adding a piece of tissue paper impregnated with isoflurane. The total handling time measured from the initial restraint of an animal to the completion of the blood collection did not surpass 2 min. Blood samples were consistently taken by the same experienced observer (Cecilia Leon) who holds a professional degree in Veterinary Medicine (Universidad de Chile), and a 4-year experience history handling and monitoring these rodents. Blood samples were centrifuged at 7000 rpm for 10 min, plasma separated from blood cells, and stored at −20 °C until subsequent hormone assays [43].

We used blood samples collected to determine testosterone (total and free testosterone) and cortisol. Testosterone and cortisol concentrations were measured by enzyme immunoassay with reagents supplied by the World Health Organization program for the Provision of Matched Reagents for RIA of Hormone in Reproductive Physiology, and according to their recommended procedures [56]. For total testosterone, we extracted the hormone with diethyl-ether, evaporating 50 μL of plasma, and resuspending in RIA assay buffer. For cortisol, we diluted 25 μL of plasma sample in 500 μL of RIA buffer solution, and took 20 μL of this solution for protein denaturation to 60 °C. These methods had resolution limits for total testosterone of 0.12 ng mL^{-1} and 17.8 ng mL^{-1} for cortisol. All samples were analyzed in duplicate, and the precision of the assay was evaluated through the intra- and interassay coefficients of variation (CV). Intra- and interassay CV for testosterone were 5.3% and 9.3%. For cortisol we recorded intra- and interassay CV of 8.3% and 11.7%, respectively. We assayed free testosterone with the Coat-A-Cont® kit of hormone determination, followed by a I^{125} radioimmunoassay procedure (Soto-Gamboa 2005). Intra- and interassay coefficients of variation were 15 and 19%, respectively.

2.4. Behavioural data collection

The behaviour of experimental degus was recorded with a video camera (Sony Corporation, Tokyo, Japan), mounted on a tripod at a height of ca. 1.5 m above floor level and located in front of each cage at a distance of 1 m. The camera was connected to a video recorder and to a TV monitor in an adjacent room. Video recordings were made during 6 h, between 08:30 and 14:30 h, on days 5 (early lactation) and 15 after delivery date. This period represents the most energy demanding stage of lactation to female degus [57], and the stage at which precocial degu pups are especially dependent on maternal nourishment [58,59]. Degu pups begin eating solid food after 6–7 days of age [[58], L.A. Ebensperger personal observations]. To compare the postnatal care of adults we used the videotapes to record elements of degu direct parental behaviour. From the adults' perspective, we recorded (i) the amount of time (% s) that each adult spent huddling over the pups or away (typically outside the nest box) from the pups, (ii) the rate at which adults licked and groomed pups (events h^{-1}), and (iii) the rate of pup retrieval (events h^{-1}). These activities are thought to be relevant in the context of parental investment as they may have immediate and more long-term fitness consequences [60–62]. In addition, we recorded (iv) the number of agonistic interactions among the adult females, which always consisted of short vocalizations followed by short chases. From the offspring perspective, we recorded (iv) the amount of time that litters were covered by at least one adult, (v) the total rate at which any pup was licked or groomed, (vi) the total rate at which any pup was retrieved, and (vii) the total rate at which any pup received agonistic chases from the adults.

2.5. Data analysis

We first examined the potential effects of nesting condition on the mass attained by offspring and survival at weaning (day 30). We ran two separate ANCOVA with nesting condition (solitary vs. female breeding with sire vs. female breeding with non-breeding female) as a fixed main factor. During these analyses the mean mass of mothers through the 30-day lactation period and the original litter size (i.e., before litter adjustment) were entered as covariates.

We then used repeated measures ANCOVA to examine the effect of nesting condition on the parental behaviour of mothers (i.e., % time with the litter, rate of grooming, rate of retrieval, and rate of aggression). During these analyses, nesting condition was again entered as a fixed main factor. Behavioural measures recorded at days 5 and 15 in early lactation were entered as a repeated measure, random factor. The mean mass of mothers through the 30-day lactation period and the original litter size were entered as covariates. We used repeated measures ANOVA to examine the influence of nesting condition on the parental behaviour of the sires and non-breeding females (i.e., % time with the litter, rate of grooming, rate of retrieval, and rate of aggression). Behavioural measures recorded at days 5 and 15 were again entered as a repeated measure, random factor, and the mean mass of fathers and non-breeding females entered as a covariate.

From the offspring perspective, we used repeated measures ANCOVA to examine the effect of nesting condition on % of time that litters were covered, the total rate pup of grooming, the total rate of pup retrieval, and the total rate of agonistic chases received. Nesting condition was entered as a fixed main factor, behavioural measures recorded at days 5 and 15 entered as a repeated measure, random factor, and mean mass of mothers through lactation and the original litter size entered as covariates.

Procedures used to examine the effect of nesting condition on plasma levels of total testosterone, free testosterone and cortisol were planned to mirror those used on the behaviour of mothers and nest mates. We used ANCOVA to examine how varying levels of the hormones examined predicted any variation in parental behaviour of mothers across nesting conditions, and of non-breeding females and males. We verified any correlation between plasma circulating levels of hormones with either ANCOVA (mothers) or simple regression (nest

mates). All these statistical procedures were conducted with the general lineal model (GLM) module of Statistica 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA). We used the Kolmogorov–Smirnov and Cochrans's test to monitor the assumptions of normal distribution and homogeneity of variances. As a result, % time with the litter was arsine squared root transformed, and total testosterone squared root transformed. Given that the rate of offspring retrieval could not be normalized despite the multiple data transformations attempted, its analyses should be viewed with caution. Data are reported as means ± SE. Differences were considered as statistically significant at $P < 0.05$. We reported the size of statistical effects as η^2-values, the percentage of variance accounted for by a variable [63].

2.6. Bioethical note

We have used clear nest boxes before [39,44] and never had problems with lactating females behaving abnormally (e.g., not being maternal) or cases where most pups do not reach weaning age. Regarding our bleeding of the animals, the orbital sinus is an accepted technique when relatively larger quantities of blood are needed (Animal Care and Use Committee 1998). This procedure was conducted always by one of us (Cecilia León) who holds a professional degree in Veterinary Medicine. Discomfort to the animals was minimized by using light doses of anaesthesia (isoflurane). All research conducted as part of this study adhered to institutional guidelines and Chilean laws for research on live mammals (permit no. 1-58.2005 [2711] by the Servicio Agrícola y Ganadero).

3. Results

Out of 66 females that were caged with one other female and a male, 36 exhibited pregnancy (55%). These animals allowed us to establish 8 breeding females nesting solitarily, 7 breeding females nesting with a non-breeding female, and 7 more breeding females that nested with the sire. Mean litter size before pup adjustment (6.7 ± 0.4 pups) did not differ across nesting condition ($F_{2,21} = 0.11$, $P = 0.900$).

3.1. Short-term fitness consequence of nesting condition

On average, dams weighed 235.9 ± 5.3 g at day 1 of lactation and lost 17 ± 2% of their body mass upon day 31. The GLM procedure revealed a statistically significant effect of nesting condition on dams' mass loss from day 1 through day 31 ($F_{2,17} = 4.76$, $P = 0.023$, $\eta^2 = 0.35$), in which breeding females that were nesting with a non-breeding female lost less weight than females nesting solitarily or with the sires (Student–Newman–Keuls test, P-values < 0.03, Fig. 1A). Neither the original litter size ($F_{1,17} = 0.55$, $P = 0.468$, $\eta^2 = 0.02$) nor the mean mass ($F_{1,17} = 0.03$, $P = 0.855$, $\eta^2 < 0.01$) of breeding females influenced their body weight loss.

Most (99%) offspring survived to weaning age, and weighed 48.8 ± 1.1 g. Nesting condition did not influence the body mass attained ($F_{2,17} = 2.92$, $P = 0.081$, $\eta^2 = 0.24$; Fig. 1B) or the survival ($F_{2,17} = 1.41$, $P = 0.272$, $\eta^2 = 0.11$; Fig. 1C) of offspring weaned. Neither of the covariates, namely the original litter size ($F_{1,17} = 0.35$, $P = 0.563$, $\eta^2 = 0.01$) and the mean body weight ($F_{1,17} = 0.86$, $P = 0.367$, $\eta^2 = 0.04$) of breeding females, had an effect on the body weight attained by the weaned offspring. Likewise, offspring survival was not significantly influenced by original litter size ($F_{1,17} = 4.46$, $P = 0.050$, $\eta^2 = 0.18$, $\beta = 0.42$) or mean body weight of breeding females ($F_{1,17} = 0.36$, $P = 0.556$, $\eta^2 = 0.01$).

To summarize, nesting condition influenced the lost of body weight by the breeding females but not the quality or quantity of offspring weaned. Breeding females nesting with a non-breeding female lost less weight than females nesting solitarily or with the sire.
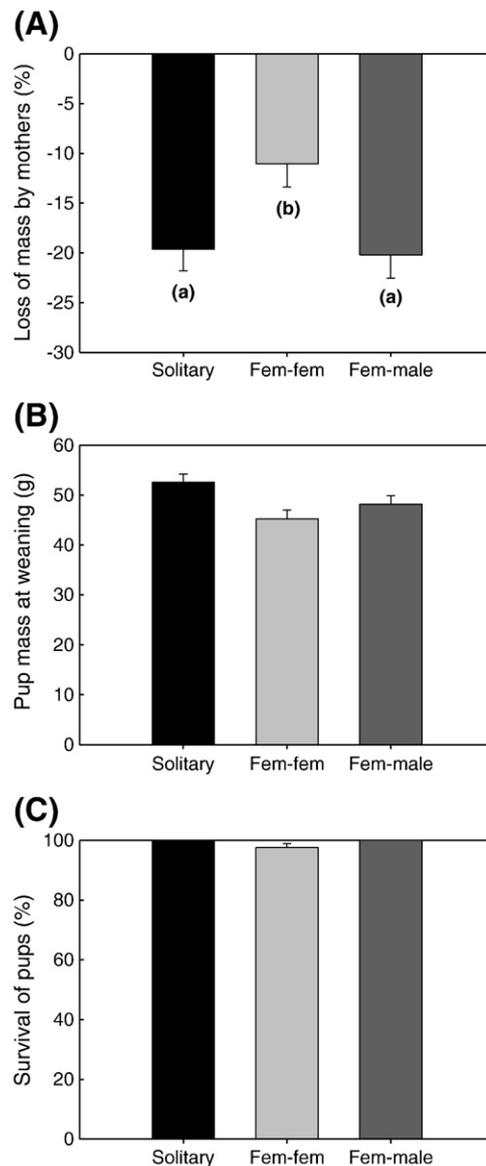
Fig. 1. (A) Means (± SE) of body mass loss (%) of mothers rearing their litters solitarily (solitary), in the presence of a non-breeding female (fem–fem), and in the presence of the sire (fem–male). (B) Means (± SE) of mass attained (g) and (C) survival of pups under these three nesting conditions. Different letters are used to indicate statistically significant differences, but only if previous ANCOVA (see text) revealed a significant effect of nesting condition.

3.2. Effects of nesting condition on the parental behaviour of dams

From the perspective of mothers, the time that mothers huddled over their litter was heavily affected by nesting condition ($F_{2,17} = 29.01$, $P < 0.001$, $\eta^2 = 0.76$). In particular, females in the company of the sire spent similar amounts of time huddling over the pups compared with females in the company of a non-breeding female, but less so than solitary nesting females (Student–Newman–Keuls test, P-values < 0.001, Fig. 2A). Time huddling over the pups by mothers was not influenced by day of lactation (day 5 vs. 15; $F_{1,17} = 0.85$, $P = 0.370$, $\eta^2 = 0.03$), original litter size ($F_{1,17} = 0.56$, $P = 0.464$, $\eta^2 < 0.01$), mean body weight of mothers ($F_{1,17} = 0.70$, $P = 0.414$, $\eta^2 < 0.01$), or factor interactions (all P-values > 0.07, all η^2-values < 0.25).

Females in the company of fathers groomed pups at a similar rate compared with females in the company of a non-breeding female, but significantly less than solitary nesting females ($F_{2,17} = 7.14$, $P = 0.006$, $\eta^2 = 0.38$; Student–Newman–Keuls test, P-values < 0.024, Fig. 2B).
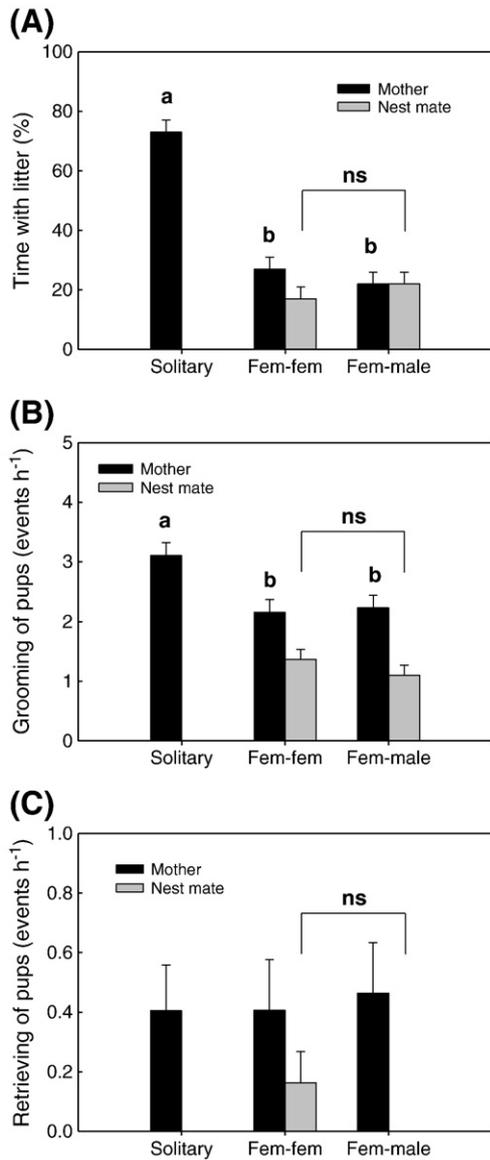
Fig. 2. Means (±SE) of time spent over the pups (A), grooming directed at the pups (B), and retrieval of pups (C) of a breeding female (black bars) and nest mates (gray bars), when the mothers were rearing their litters solitarily (solitary), in the presence of a non-breeding female (fem–fem), and in the presence of the sire (fem–male). Statistically significant differences between dams subjected to different rearing conditions are highlighted with the use of different letters, but only if previous ANCOVA (see text) revealed a significant effect of nesting condition. Statistically non-significant differences between sires and non-breeding females are highlighted with the use of lines headed with "NS" initials.

Grooming of pups was not influenced by day of lactation ($F_{1,17} = 1.30$, $P = 0.270$, $\eta^2 = 0.05$), original litter size ($F_{1,17} = 2.98$, $P = 0.102$, $\eta^2 = 0.08$), or mean body weight of mothers ($F_{1,17} = 2.85$, $P = 0.110$, $\eta^2 = 0.08$). Similarly, no interaction between nesting condition, day of lactation and the covariates was detected (all P-values >0.159, all η^2-values <0.18).

Independently, neither nesting condition ($F_{2,17} = 0.07$, $P = 0.935$, $\eta^2 < 0.01$), day of lactation, original litter size ($F_{1,17} = 1.48$, $P = 0.240$, $\eta^2 = 0.08$), nor body weight of mothers ($F_{1,17} < 0.01$, $P = 0.950$, $\eta^2 < 0.01$) influenced the rate of offspring retrieval by the dams (Fig. 2C). However, a statistically significant interaction between nesting condition and day of lactation ($F_{2,17} = 4.76$, $P = 0.023$, $\eta^2 = 0.33$) revealed that breeding females nesting with the sire retrieved offspring significantly less at day 15 compared with day 5 (Student–Newman–Keuls test, $P < 0.036$, Fig. 3). This effect was not

observed in the breeding females that nested with a non-breeding female or females that nested solitarily (Student–Newman–Keuls test, $P > 0.05$). No further factor interactions were statistically significant (all P-values >0.360, all η^2-values <0.03).

Only a single female was recorded to chase offspring, once at day 5 and on another occasion at day 15 of lactation. This finding precluded further analysis.

To summarize, breeding females in the company of the sire spent similar amounts of time huddling over and grooming the pups compared with females in the company of a non-breeding female, but less so than solitary nesting females. Females nesting with the sire (but not females nesting with a non-breeding female or nesting solitarily) retrieved offspring significantly less at day 15 compared with day 5 during early lactation.

3.3. Parental behaviour of males and non-breeding females

All sires and non-breeding females were observed to co-nest with the dams and their litter during the study. Sires spent $22 \pm 4\%$ of their time huddling over the litter, a figure similar to that recorded from non-breeding females ($F_{1,11} = 0.65$, $P = 0.439$, $\eta^2 = 0.06$; Fig. 2A). These times were not influenced by the day of lactation ($F_{1,11} = 3.89$, $P = 0.074$, $\eta^2 = 0.20$), the mean body weight of sires and non-breeding females ($F_{1,11} = 0.06$, $P = 0.810$, $\eta^2 = 0.06$), or factor interactions (all P-values >0.06, all η^2-values <0.22).

Sires also groomed offspring to a similar extent compared with non-breeding females ($F_{1,11} = 1.92$, $P = 0.193$, $\eta^2 = 0.14$; Fig. 2B). This grooming did not vary with the day of lactation ($F_{1,11} < 0.01$, $P = 0.991$, $\eta^2 < 0.01$), the mean body weight of sires or non-breeding females ($F_{1,11} = 0.64$, $P = 0.440$, $\eta^2 = 0.05$), or with factor interactions (all P-values >0.35, all η^2-values <0.08).

Fathers of litters never retrieved their offspring and non-breeding females did it only occasionally (Fig. 2C). This retrieval activity did not vary with the day of lactation ($F_{1,11} = 0.16$, $P = 0.695$, $\eta^2 = 0.01$), the mean body weight of sires or non-breeding females ($F_{1,11} = 0.64$, $P = 0.440$, $\eta^2 < 0.01$), or with factor interactions (all P-values >0.66, all η^2-values <0.02).

No fathers or non-breeding females were ever recorded to direct any aggression against offspring.

To sum up, sires exhibited two out of three measures of parental behaviour examined. The extent to which males exhibited these behaviours, namely time in physical contact with the litter and grooming of offspring was similar to that exhibited by non-breeding females.

3.4. Overall care received by the offspring

From the perspective of offspring, the time that developing young were huddled by at least one adult did not vary with nesting condition
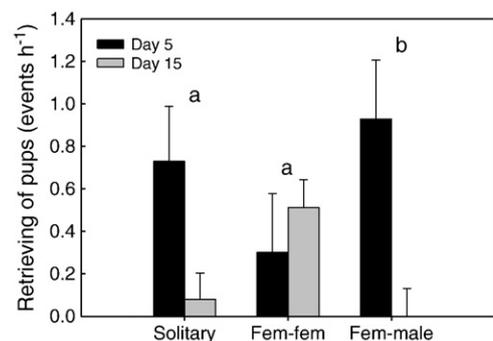
Fig. 3. Means (±SE) of retrieval of pups at days 5 (black bars) and 15 (gray bars) of early lactation by a breeding female that reared their litters solitarily (solitary), in the presence of a non-breeding female (fem–fem), and in the presence of the sire (fem–male).

($F_{2,17} = 0.99$, $P = 0.391$, $\eta^2 = 0.09$; Fig. 4A). Time over the pups by adults was not influenced by day of lactation ($F_{1,17} = 0.17$, $P = 0.685$, $\eta^2 < 0.01$), original litter size ($F_{1,17} = 0.36$, $P = 0.559$, $\eta^2 = 0.02$), mean mass of mothers ($F_{1,17} = 2.22$, $P = 0.155$, $\eta^2 = 0.10$), or factor interactions (all P-values > 0.20, all η^2-values < 0.09).

The total rate of grooming received by litters from any adult did not differ across nesting conditions ($F_{2,17} = 0.34$, $P = 0.720$, $\eta^2 = 0.03$; Fig. 4B). Total grooming was not influenced by day of lactation ($F_{1,17} = 3.25$, $P = 0.089$, $\eta^2 = 0.10$), mean body weight of dams ($F_{1,17} = 3.29$, $P = 0.087$, $\eta^2 = 0.12$), or factor interactions influenced (all P-values > 0.07, all η^2-values < 0.13). However, litters of dams whose original litter was larger, received more grooming events than litters of dams whose original litter was smaller ($F_{1,17} = 5.48$, $P = 0.032$, $\eta^2 = 0.21$).

The total rate of retrieval received by the offspring was not affected by nesting condition ($F_{2,17} = 0.18$, $P = 0.835$, $\eta^2 = 0.02$), day of lactation ($F_{1,17} = 0.67$, $P = 0.424$, $\eta^2 = 0.02$), original litter size ($F_{1,17} = 1.76$, $P = 0.203$, $\eta^2 = 0.09$), or body weight of dams ($F_{1,17} = 0.04$, $P = 0.838$, $\eta^2 < 0.01$) (Fig. 4C). A statistically significant interaction between nesting condition and day of lactation ($F_{2,17} = 5.21$, $P = 0.017$, $\eta^2 = 0.36$) suggested that offspring of females that nested with the sire were retrieved less at day 15 compared with day 5, a marginally significant difference after post-hoc multiple comparisons (Student–Newman–Keuls test, $P > 0.083$). This effect was not observed in the breeding females that nested in the company of a non-breeding female or females nesting solitarily (Student–Newman–Keuls test, $P > 0.05$). No other factor interactions were statistically significant (all P-values > 0.470, all η^2-values < 0.02).

In short, offspring experienced similar amounts of time being huddled and groomed by the adults. Offspring that developed in the presence of fathers experienced less retrieval, but only during late lactation.

3.5. Circulating levels of hormones and behaviour of mothers

The GLM procedure revealed a statistically significant effect of nesting condition on dams' circulating plasma cortisol ($F_{2,17} = 5.24$, $P = 0.017$, $\eta^2 = 0.37$). In particular, mothers that nested with a non-breeding female exhibited lower levels of circulating cortisol compared with mothers that nested solitarily or with the sire (Student–Newman–Keuls test, P-values < 0.02, Fig. 5A). Cortisol levels were not affected by day of lactation ($F_{1,17} = 0.60$, $P = 0.449$, $\eta^2 = 0.03$), the original litter size ($F_{1,17} < 0.01$, $P = 0.992$, $\eta^2 < 0.01$), or mean body weight ($F_{1,17} = 0.59$, $P = 0.451$, $\eta^2 = 0.02$) of breeding females. Similarly, no factor interactions explained any variation in cortisol of mothers (all P-values > 0.40, all η^2-values < 0.06).

Nesting condition did not influence circulating levels of total testosterone in mothers ($F_{2,17} = 0.02$, $P = 0.981$, $\eta^2 < 0.01$; Fig. 5B).
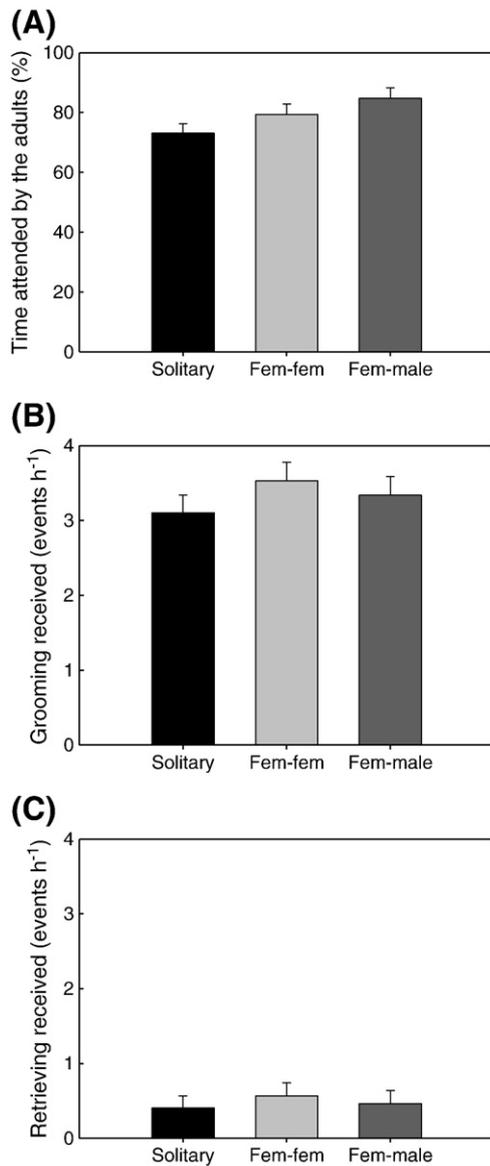
Fig. 4. Means ($\pm$ SE) of (A) total time that pups were covered, (B) total grooming received, and (C) total retrieval events experienced while reared by mothers nesting solitarily, in the presence of a non-breeding female (fem–fem), and in the presence of the sire (fem–male).
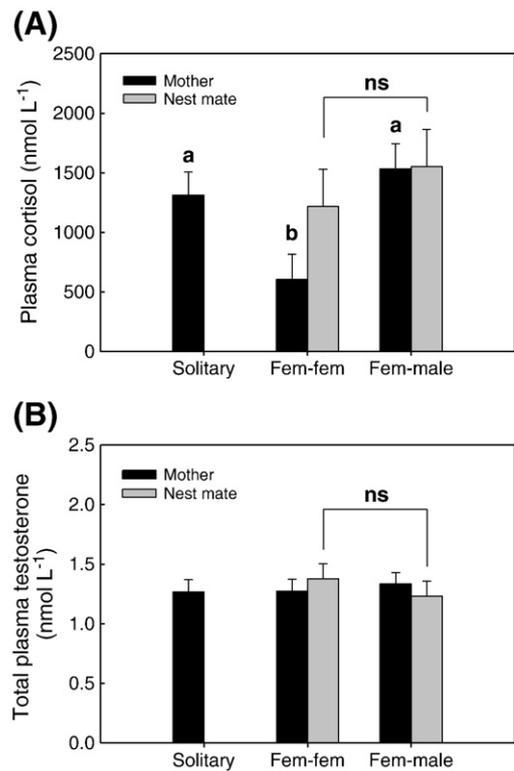
Fig. 5. Means ($\pm$ SE) of cortisol (A) and total testosterone (B) plasma levels of breeding females (black bars) and nest mates (gray bars), when the mothers were rearing their litters solitarily (solitary), in the presence of a non-breeding female (fem–fem), and in the presence of the sire (fem–male). Different letters are used to indicate statistically significant differences. Statistically significant differences between dams subjected to different rearing conditions are highlighted with the use of different letters, but only if previous ANCOVA (see text) revealed a significant effect of nesting condition. Statistically non-significant differences between sires and non-breeding females are highlighted with the use of lines headed with "NS" initials.

Neither day of lactation ($F_{1,17} = 2.51$, $P = 0.131$, $\eta^2 = 0.10$) or the covariates, namely original litter size ($F_{1,17} = 0.72$, $P = 0.408$, $\eta^2 = 0.04$) and mean body weight ($F_{1,17} = 0.17$, $P = 0.687$, $\eta^2 < 0.01$) of breeding females, had an effect on total testosterone. Again, no factor interactions explained any variation in total testosterone of mothers (all P-values >0.10, all η^2-values <0.13).

Neither circulating cortisol ($F_{1,17} = 0.26$, $P = 0.614$, $\eta^2 < 0.01$) or total testosterone ($F_{1,17} = 0.42$, $P = 0.528$, $\eta^2 < 0.01$) seemed related to the time that mothers huddled over offspring across nesting conditions. Variation in grooming activity of breeding females was unrelated to varying cortisol ($F_{1,17} = 1.60$, $P = 0.224$, $\eta^2 = 0.04$). However, mothers with lower circulating levels of total testosterone groomed offspring at a higher rate than mothers with relatively higher testosterone levels across nesting conditions ($F_{1,17} = 9.46$, $P = 0.007$, $\eta^2 = 0.22$, $\beta = -0.52$). No links between cortisol ($F_{1,17} = 2.61$, $P = 0.124$, $\eta^2 = 0.12$), or total testosterone ($F_{1,17} = 0.47$, $P = 0.504$, $\eta^2 = 0.02$) and retrieval of the offspring by the mothers were recorded across nesting conditions.

Plasma circulating levels of cortisol and total testosterone in mothers were not correlated across nesting condition (ANCOVA, $F_{1,18} = 2.58$, $P = 0.120$, $\beta = 0.28$, $\eta^2 = 0.08$).

The observation that only two dams and a single male exhibited detectable levels of free testosterone precluded any subsequent statistical analysis.

To summarize, mothers nesting with a non-breeding female experienced low levels of cortisol compared with dams nesting solitarily or with the males. Only circulating levels of testosterone predict one (grooming) out of three measures of parental behaviour examined.

3.6. Circulating levels of hormones and behaviour of males and non-breeding females

Plasma circulating levels of cortisol measured to the males were similar to those recorded to non-breeding females ($F_{1,11} = 0.36$, $P = 0.562$, $\eta^2 = 0.02$; Fig. 4A). These levels were not influenced by the day of lactation ($F_{1,11} = 0.71$, $P = 0.416$, $\eta^2 = 0.06$), or an interaction between nesting condition and day of lactation, or with the mean body weight of males or non-breeding females (all P-values >0.063, all η^2-values <0.23).

Circulating levels of total testosterone in males were statistically similar to those recorded in non-breeding females ($F_{1,11} = 0.63$, $P = 0.446$, $\eta^2 = 0.05$; Fig. 4B). These levels were not influenced by the day of lactation ($F_{1,11} < 0.01$, $P = 0.941$, $\eta^2 < 0.01$), the mean body weight of males or non-breeding females ($F_{1,11} = 0.03$, $P = 0.868$, $\eta^2 < 0.01$), or factor interactions (all P-values >0.14, all η^2-values <0.18).

Neither circulating cortisol ($F_{1,10} = 0.04$, $P = 0.839$, $\eta^2 < 0.01$) nor total testosterone ($F_{1,10} = 0.71$, $P = 0.419$, $\eta^2 = 0.06$) explained any statistically significant variation in time that non-breeding females and fathers huddled over offspring. Likewise, variation in grooming activity was unrelated to varying levels of cortisol ($F_{1,10} = 0.50$, $P = 0.495$, $\eta^2 = 0.04$) and total testosterone ($F_{1,10} < 0.01$, $P = 0.929$, $\eta^2 < 0.01$).

Plasma circulating levels of cortisol and total testosterone in males and non-breeding females were not correlated across nesting condition (simple regression, $F_{1,12} = 0.89$, $P = 0.364$, $\beta = 0.26$, $\eta^2 = 0.07$).

To summarize, fathers of litters exhibited similar plasma circulating levels of cortisol and total testosterone compared with non-breeding females, and circulating levels of these hormones did not predict any aspect of parental behaviour examined.

4. Discussion

4.1. Male care in degus

Our study revealed that under controlled conditions of adult density, nest availability, food availability, and breeding experience male degus behaved parentally to offspring of females with which

these males have mated and co-nested through lactation. Two observations indicate that these findings are not laboratory artefacts as suggested in other species [64]. Under natural conditions male degus share underground nest burrows with communally rearing females [65], and participate in the transportation of material for nest insulation (V. Quirici unpublished observations). During this study, male direct care was expressed in the form of time spent in physical contact (i.e., huddling) and grooming of the offspring. Moreover, males never exhibited aggression to the young. Qualitatively, male degus seemed parentally intermediate between more paternal males of biparental and cooperatively breeding rodents [66–68], and the less paternal males of more promiscuous or less social species [10,67,69,70]. Quantitatively, care by male degus did not occur only occasionally. Dams that nested with a non-breeding female or the sire similarly reduced their care to offspring when compared with solitary nesting females. Despite this quantitative reduction in maternal care, measures of parental care recorded to non-breeding females and males occurred at a rate high enough so that offspring were similarly attended, groomed and retrieved across nesting conditions.

4.2. Hormonal influences on parental care

While glucocorticoids (cortisol) and androgens (testosterone) often mirror major breeding events in natural populations [41–43], we did not find evidence that variation in these metabolites predicts variation in the expression of parental care in male or female degus. Only grooming activity in breeding females seemed inversely related to circulating levels of total testosterone. These findings contrast with previous evidence that circulating cortisol either increases [19,25] or decreases [17] during the expression of parental care. Likewise, testosterone has been recorded to decrease [11,28–30] or remain elevated [31,32] during the onset of paternal and alloparental care in males of other rodents and primates. To what extent differences between degus and previous species examined (murid rodents and primates) reflect different endocrine mechanisms remains an open question. The observation that cortisol decreased in females rearing their litters in the presence of a non-lactating female compared with females rearing their litters solitarily or with the sire is intriguing. This finding suggests that the social environment played a more active role than lactation per se in causing these differences.

4.3. Causes of male care

Direct care provided by the males in degus did not enhance short-term fitness in terms of postnatal growth and survival of young in captivity. To some extent, these findings are consistent with the hypothesis that males exhibit offspring care even if this activity has marginal fitness consequences to offspring [3]. Paternal care might be expected if the probability for future breeding attempts is low in males [69]. The reproductive biology of degus supports that this might be the case. Since breeding in degus takes place once per year and postpartum estrus occurs rarely after a ca. 3-month pregnancy [46], males can hardly increase breeding success through searching for recently parturient females. Evidence from natural populations also indicates that most adults (males in particular) do not survive to a second breeding event [34–36]. Under these conditions males might be expected to care for offspring produced during their (likely) sole lifetime breeding attempt.

Alternatively, and based on previous arguments ([71,72]), parental behaviour exhibited by male degus could be a by-product of the cohabitation of the male and the pups, i.e., the consequence of males communally nesting with breeding females. Communal nesting by the males in turn may be the consequence of burrow shortage or a defence against predation. The observations of no early fitness benefits to offspring or breeding females, and the complete lack of offspring

retrieval activity in the males (i.e., an indication of responsiveness toward the young [68]) are consistent with this interpretation.

4.4. Fitness consequences of male care

Previous evidence indicates that fitness consequences of the males' presence may be contingent upon animals facing environmentally challenging conditions. This effect has been recorded not only to biparental [5,6,8], but also to more polygynous species [7,9]. Given that degus of this study faced controlled conditions of food supply and moderately challenging thermal conditions, we cannot rule out that the male's presence may have a greater impact on offspring quality and quantity under more ecologically demanding conditions. For instance, degu offspring under natural conditions may face lower minimum temperatures than those faced by offspring in our study. Males may also contribute with attendance to litters whenever females are foraging outside their underground burrows. However, the positive effects of harsh environmental conditions recorded in previous studies need to be faced against at least two other considerations. In particular, a male contribution to offspring care in social species such as degus might become diluted as the number of breeding females within social groups increases. Second, and obviously, the extent of male care and its associated fitness consequences would depend on how breeding success is shared in multi-male degu groups [65].

Our current study indicated that, if any, the presence of fathers in degu social groups has potentially detrimental fitness effects on the breeding females. Breeding females that nested with the males lost a similar amount of body weight through lactation compared with that lost by females nesting solitarily, but more so than breeding females that nested with a non-breeding female. Given that body weight is a predictor of body condition in rodents [55], female degus that bred in the presence of males ended lactation in poorer physical condition. The observation that cortisol in these females was similarly elevated to that of solitary females suggests that the presence of sires represented a psychosocial stressor quantitatively similar to solitary nesting, and the likely mechanism that caused differences in physical condition. The possibility that females in the presence of the sire lost body weight and became physiologically stressed due to sexual or other social harassment by the males seemed unlikely. Sexual harassment was not recorded during the experiments. Instead, dams in the presence of sires may have experienced decreased social support compared with females in the presence of a non-breeding female. Social support occurs whenever the stress response is ameliorated by the establishment of social bonds, e.g., mother–infant bonds [73]. As social groups in degus are composed mostly of multiple females that share nest sites [34,65], social bonds between the females may be stronger than female–male bonds, resulting in greater social support. While high physiological stress does not necessarily mean lower lifetime reproductive success, it does indicate metabolic costs imposed by current environmental stressors, which may lower lifetime reproductive success if sustained. Accordingly, individuals subjected to periodic stressors may have long-lasting effects on reproductive success of future generations [74], and stress hormones can predict mortality risk and survival of individuals under natural conditions [75–77].

Clearly, our findings in degus need to be replicated under natural conditions. Fitness consequences of the male's presence may involve not only short-term [6–8,78,79], but also long-term effects, including an acceleration of the timing to sexual maturation in offspring [80]. While our study was restricted to the examination of short-term effects, several years of data may be needed to quantify other potentially long-term fitness consequences in a seasonally breeding species such as the degu.

To sum up, we recorded that males provide parental care to their offspring. However, this activity has no detectable consequences to offspring, and is potentially detrimental to breeding females. These results complemented a previous study [44] and suggest that neither the presence of females or males enhances short-term fitness of breeding females in the communally rearing degu.

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