Mating sequence, dominance and paternity success in captive male tammar wallabies

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Abstract

The tammar wallaby (*Macropus eugenii*) is a small, promiscuous, macropodid marsupial. Females usually produce a single young each year and there is a clear dominance hierarchy between adult males. The dominant male usually mates first and then guards the female to prevent access to her by other males. In this study, agonistic encounters and mating behaviour were observed to determine male dominance hierarchies in six groups of captive tammars consisting of a total of 23 males and 50 females. Mating behaviour was observed immediately post-partum when females were in oestrus and was correlated with plasma testosterone concentrations. Male mating sequences were recorded, and the paternity of offspring was determined by using seven macropodid marsupial microsatellites. Rates of sexual checking and aggression by males housed with females in oestrus in the non-breeding season were lower than in the breeding season. These males also had lower concentrations of testosterone, but were still able to sire young. High testosterone concentrations neither ensured dominance nor appeared to control directly the level of sexual activity. Females usually mated with more than one male. The dominant male most often secured the initial copulation (60%), but the first-mating male did not always secure parentage, with second and third matings resulting in as many young as first matings. Using these data, we were unable to discount first sire, last sire or equal chance models of paternity in this species. Half the young (50%) were sired by the dominant α male, but of the remaining progeny, the β male sired more (35%) than γ and δ males (15%). Dominance therefore is only a moderately effective predictor of paternity in the tammar. Although the dominant males gained most first matings and individually sired half of the offspring, the subordinate males still contributed significantly to the population, at least in captivity.

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Introduction

The tammar wallaby is a highly synchronous, seasonally breeding, macropodid marsupial with a multimale mating system (Flint & Renfree 1982, Tyndale-Biscoe & Renfree 1987). During the breeding season in late January or early February, 83% of females give birth and enter oestrus (Renfree & Tyndale-Biscoe 1973). The tammar has a post-partum oestrus and normally mates about 1 h after birth (Rudd 1994). Ovulation occurs about 40 h later (Renfree & Lewis 1996) and the resulting conceptus develops to the blastocyst stage and enters an 11-month period of embryonic diapause while the young is reared in the pouch (Tyndale-Biscoe & Renfree 1987). Seventeen per cent of females are non-parous (Renfree & Tyndale-Biscoe 1973). These females will cycle, enter oestrus and mate in January, and then give birth and undergo post-partum oestrus in February. Between February and June, any female

that loses her young will reactivate the diapausing blastocyst, and after a pregnancy of about 1 month will give birth and enter oestrus again.

In contrast to seasonally breeding eutherians such as the sheep, which shows seasonal regression of the testes (Legan *et al.* 1977, Lincoln & Davidson 1977), the male tammar wallaby produces spermatozoa throughout the year (Inns 1982). In the wild, male tammar wallabies show a rise in serum testosterone in late January coinciding with the onset of the breeding season, but testosterone concentrations are low for most of the year (Catling & Sutherland 1980, Inns 1982). There is a second small increase in late October when juvenile females leave their mother's pouches and enter their first oestrus. The weight of the prostate and Cowper's glands fluctuate seasonally in response to testosterone (Inns 1982, Williams *et al.* 1998). In the major breeding season (January/February), the male reproductive tract is heaviest,

ejaculation volume and coagulation index are the greatest, and sperm motility is highest (Paris 2004).

Access to tammar females by males is essential for the seasonal increase in testosterone concentrations. Males that are isolated from females in the breeding season do not show a rise in plasma testosterone (Catling & Sutherland 1980). Olfactory stimuli are clearly important in signaling the reproductive condition of females, and males monitor the reproductive status of females by sniffing their urogenital region or pouch (sexual checking) (Renfree *et al.* 1989, Rudd 1994). Castrated males show a substantial decrease in male-typical sexual behaviour, such as sexual checking and copulatory behaviour, while females implanted with testosterone have the same level of sexual behaviour as intact males (Rudd *et al.* 1996). These testosterone-implanted females all showed increased aggression toward copulating males, suggesting that male-like levels of testosterone can activate copulation-induced aggression (Rudd *et al.* 1996).

In species where females mate with multiple males, reproductive success results from both precopulatory and postcopulatory strategies (Ginsberg & Huck 1989, Preston *et al.* 2003). Sperm competition occurs when an oestrous female mates with more than one male in a single reproductive cycle (Ginsberg & Huck 1989, Møller & Birkhead 1989, Parker 1990). Male tammar wallabies attempt to reduce the chance of sperm competition by various mechanisms, including extensive pre- and postcopulatory mate guarding behaviour with frequent mating (Ewen *et al.* 1993, Rudd 1994, Rudd *et al.* 1996), relatively large ejaculate volumes and epididymal sperm stores, large testicular size and ejaculated sperm concentrations, and production of large, firm copulatory plugs (Tyndale-Biscoe & Renfree 1987, Paris 2004, Paris *et al.* 2004). These plugs may enhance reproductive success by increasing sperm retention in the female tract, by locating spermatozoa in close apposition to the cervix and improving sperm transport into the cervix and uterus, and/or by acting as temporary barriers (chastity enhancement) to sperm deposition by other males (Murie & McLean 1980, Michener 1984, Toner *et al.* 1987, Cukierski *et al.* 1991a, 1991b, Taggart & Shimmin 1999, Jia *et al.* 2002), although evidence in some species does not support chastity enhancement (Koprowski 1992, Moreira & Birkhead 2004). Success of these measures usually depend on a large ratio of testis to body weight, which in the tammar is around 0.5% (unpublished data of R V Short in Tyndale-Biscoe & Renfree 1987, Rose 1997, Taggart *et al.* 1998), and is similar to that found in 31 multimating mammals (Kenagy & Trombulak 1986).

The α male tammar wallaby usually controls initial access to oestrous females by mating first and guarding the female against advances by subordinate males for up to 8 h (Rudd 1994), although mate guarding by the α male prevents ejaculations by subordinates in only half the matings (Rudd 1994). In a group of four captive tammar males allowed to mate with 13 females, there was no significant difference in the number of young sired by

each male (Ewen *et al.* 1993). However, no behavioural observations were made in that study to verify social structure or record mating sequences.

Recent genetic analyses show that paternity cannot accurately be inferred from male dominance hierarchies or even from observed matings. In some cases, dominance or copulation frequency reliably reflects parentage (Cowlshaw & Dunbar 1991, Altmann *et al.* 1996, Roed *et al.* 2002), but in others there is minimal correlation (Coltman *et al.* 1999, Soltis *et al.* 2001). A dominant male can achieve a higher proportion of paternity in two main ways: by decreasing the ability of subordinates to mate with and fertilize females, such as by suppression of gonadotrophin or testosterone, as occurs in subordinate males of lesser mouse lemurs (Perret 1992) and marsupial sugar gliders (Stoddart *et al.* 1994, Bradley & Stoddart 1997), and by the superior ability of dominant males to compete for and guard oestrous females against matings by subordinate males.

The aim of this study was to examine further the paternity success of males in several groups of captive tammar wallabies in both the major breeding season and in the non-breeding season by recording the mating sequence and paternity success of each male in relation to its dominance rank and its sexual and aggressive behaviour.

Materials and Methods

Animals

Tammar wallabies of Kangaroo Island (South Australia) origin were housed in grassy paddocks in our breeding colony in Victoria, Australia. The grass was supplemented with oats, lucerne cubes, fresh vegetables and water *ad libitum*. Care and treatment of animals conformed to the National Health and Medical Research Council of Australia guidelines (1997), and all experimental work was approved by Animal Experimentation Ethics Committees at Monash University and the University of Melbourne.

Blood sampling

Blood was drawn from the lateral tail vein into a 5 ml syringe coated with either sodium heparin (50 μ l of 125 IU/ml) (CSL, Parkville, Australia), for testosterone assay, or EDTA (1.5 g/ml blood) (Sigma), for DNA extraction, and stored at -20°C in plastic 5 ml tubes (Sarstadt, Australia, Pty Ltd) until assayed. When required for DNA extraction, the buffy coat was aspirated and stored at -20°C .

Blood was taken from adult males in the colony every 1–4 weeks from February 2002 until February 2003 to determine the annual cycle of change in testosterone concentrations. These males were housed with seven females with both male and female pouch young that were born in March 2002. A total of 17 males (different from those used for the behavioural study) were sampled over the year, with 4–6 of these males sampled in each month. Blood was assayed for testosterone.

Testosterone radioimmunoassay

Plasma testosterone concentrations were measured by a radioimmunoassay validated for the tammar (Williamson *et al.* 1990). The antiserum (no. 457) (Bioquest Ltd, Artamon, NSW, Australia) has a cross-reactivity of 100% with testosterone, 98% with 5 α -dihydrotestosterone, 47% with 4-androsten-3 β ,17 β -diol, 4.7% with androstendione and 3.6% with 4-androsten-17 β ,19-diol-3-one. The sensitivity of the assay was 10 pg/tube. Double extractions gave an efficiency of $87 \pm 4.5\%$. Plasma (0.2 ml) was extracted twice with hexane:toluene (2:1) (Merck), freshly mixed before each assay. The extract was redissolved in 0.7 ml assay buffer, and duplicate 0.3 ml aliquots were assayed. Testosterone added to blank plasma was recovered quantitatively ($y = 0.9558x - 6.4192$, $r = 0.9994$, $P = 0.0017$). Plasma pools with high and low testosterone concentration were included in each assay as quality controls. The intra-assay coefficient of variation was 9.2% ($n = 10$). The interassay coefficient of variation was 14.6% ($n = 5$).

Behavioural observations

A total of five groups were observed during the breeding season. In January/February 2003, two groups were observed (group 1: 3 males, 15 females; group 2: 4 males, 13 females). In March 1995, three groups were observed (group 4: 4 males, 9 females; group 5: 4 males, 8 females; group 6: 4 males, 5 females). A single further group was observed in September/October 2003 during the non-breeding season (group 3: 4 males, 13 females).

Pregnancies were initiated between late January and mid-April in both 1995 and 2003 by removal of pouch young (RPY) from lactating females. This reactivates the diapausing blastocyst (Tyndale-Biscoe & Renfree 1987), birth occurs 26.5 days later (Renfree *et al.* 1989) and females enter a post-partum oestrus within 1 h of birth (Rudd 1994). Blood samples were collected each week from males in groups 1 and 2 at the peak of the breeding season between 20 January and 11 February 2003, and assayed for testosterone.

Out-of-season births and matings were induced in September in the 13 group 3 females, housed with 4 males, by 50 mg melatonin implanted under the pouch skin to terminate seasonal quiescence (Renfree & Short 1984, McConnell *et al.* 1986). Blood samples were taken from the males from the day the implants were inserted (day 0) and then on days 6, 13, 20, 27, 31, 41 and 71, and assayed for testosterone. Blood samples were also collected daily from 14–18 October in the non-breeding season from group 3 males and assayed for testosterone.

Wallabies were identified by colour-coded collars and tape on the tail. During observation periods, animals were kept in yards either 12 $\times$ 15 or 25 $\times$ 15 m. Floodlights were used for night viewing. Observations were made with the assistance of 7 $\times$ 35 binoculars from an observation platform 2 m above ground level. Every 5 min, the enclosure was scanned visually to locate all animals and note their

current behaviour. Any female showing signs of parturition (Renfree *et al.* 1989) was monitored until mating was complete ('complete mating sequence'). The time and duration of copulations and mounts were recorded. During undisturbed mating, there is an initial period of about 30–60 s of mounting, intromission and pelvic thrusting, followed by about 6–8 min *in copula* without thrusting (Rudd 1994). Ejaculation presumably occurs at the time when thrusting ceases, as uncoagulated semen is often observed exuding from the urogenital region of the female soon after completion of thrusting. A copulating male was considered to have ejaculated when he stopped thrusting. Behavioural observations were made from dusk to dawn, and some further observation periods were made during the day, opportunistically, if any reproductive behaviour was noted.

Dominance hierarchies were determined by noting behaviour during male–male interactions over resources such as food, shelter, space and females. Interactions were either scored as a win, a loss or a draw. A win was defined as an occasion where one male displaces another, while a loss was when a male was displaced. A draw required both males retreating equal distances from each other, or neither retreating. A dominance score was calculated for each male by the method designed by Jameson *et al.* (1999). This method is based on a mathematical model of paired comparisons and takes into account the percentage of wins as well as the competitive ability of opponents. A dominance hierarchy for each group was determined on the basis of these scores.

A scoring system was used to analyse sexual activity. Sexual checking was defined as the male sniffing and/or nosing the pouch or cloacal area of the female (1 point). After checking a female, the male often cuffed the female, or pawed her tail or back (2 points). The female frequently moved away in response to this behaviour. If the male followed, 3 points were allocated and 4 points for blocking the female's path. A copulation attempt was allocated 5 points.

Paternity analysis

Seven microsatellite markers developed for the tammar by Taylor and Cooper (1998) were used to assign parentage to offspring. Microsatellite PCR primers were modified with Oligo 4.0 for Macintosh. DNA was extracted from blood of adults and the tails of pouch young. Most extractions were carried out by the standard salting-out procedure (Miller *et al.* 1998). The remaining DNA was extracted with the DNeasy Tissue Kit (Qiagen) and methods outlined in the DNeasy Tissue Handbook (May 2002). Blood and buffy coat samples (100 μ l) were processed according to the 'isolation of DNA from whole non-nucleated blood' protocol and pouch young tails (6 mm) were extracted according to the 'DNeasy protocol for rodent tails'. These were both modified by performing two 100 μ l elutions of EA buffer instead of one 200 μ l elution, to improve the DNA yield. The concentration of DNA in extracts was determined by loading 1 and 2 μ l

aliquots of sample into a 0.8% agarose gel (Scientifix, Cheltenham, Australia) stained with ethidium bromide (Sigma). Samples were run on a gel at 110V for 30 min. DNA concentration was estimated visually by comparing density of bands of unknown DNA with samples of known concentrations (25 mg/10 μ l, 50 mg/10 μ l, 75 mg/10 μ l and 100 mg/10 μ l). DNA from 2002/2003 experiments was then diluted to approximately 2.5 ng/ml and delivered to the Australian Genome Research Facility for PCR and microsatellite analysis.

The conditions for microsatellite analysis were optimized, as described in Taylor and Cooper (1998). Paternity was assigned by exclusion principles because allele frequencies for the population were not available. Of the two known alleles, one was assumed to be inherited from the mother and the other from the father. Males that had an allele that did not match that of the young were excluded. A male with matching alleles for known microsatellites was assumed to be the father, provided that all remaining group males had been excluded.

Microsatellite primers were [γ 32 P] incorporated (Amersham Pharmacia Biotech, Castle Hill, NSW, Australia) with T kinase (New England Biolabs, Beverly, MA, USA) before amplification. PCR reactions of 10 μ l contained 100–120 ng DNA, 2.5 mM MgCl₂, 1 $\times$ reaction buffer (Life Technologies Invitrogen, Mt Waverley, Victoria, Australia), 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 0.5 U Taq polymerase (Life Technologies), 200 μ M of dNTPs, 0.3 μ M radioactively labeled reverse primer and 0.2 μ M forward primer with mineral oil overlay. PCR amplifications were carried out in a Corbett Thermocycler (Corbett Research Australia, Mortlake, NSW) and consisted of an initial denaturation at 94 °C for 5 min and then 30 cycles of 30 s at 94 °C, 30 s at Ta optimised for each primer 47–54 °C and 1 min at 72 °C. The final elongation step was 72 °C for 10 min. PCR products were run on 6% denaturing polyacrylamide gels and visualized by autoradiography. Size of fragments was determined by comparison with a standard sequencing reaction of pUC18 DNA (Promega).

Statistical analysis

Changes in testosterone concentration were analysed by either one-way ANOVA or Student's *t*-test (Prism, Graphpad, Graphpad Software Pty Ltd, San Diego, CA, USA). Correlations between testosterone and sexual behaviour were tested by Spearman correlations (Prism, Graphpad). Student's *t*-test was used to compare rates of agonistic encounters in the breeding season and the non-breeding season.

The observed mating and paternity successes for the complete mating sequences were compared by log likelihood analysis with three different sperm competition models: first mating paternity success, last mating paternity success and equal chance of success for all males that successfully mated with a female.

Results

Males housed with females in the colony had a clear annual cycle in plasma testosterone concentrations, reaching a peak of 9.68 ± 1.26 ng/ml in late January (Fig. 1). Concentrations were relatively low during the period of shortest days in May and June (0.38 ± 0.08 ng/ml). Plasma testosterone increased in January, coinciding with the beginning of the breeding season (ANOVA: $P = 0.0002$) Testosterone decreased during February, as the number of females in oestrus diminished. Testosterone concentrations increased again toward the end of February as those females that mated but did not give birth in January entered oestrus post-partum.

Plasma testosterone concentrations increased slightly from 0.56 ± 0.19 (7 Oct.) to 1.44 ± 0.53 ng/ml (18 Oct) in three out of four males housed with melatonin-implanted group 3 females at about the time the females were in midpregnancy after reactivation, and fell gradually to basal levels by November. The peak testosterone concentrations in these males (2.7 ± 2.6 ng/ml) were well below the 10–15 ng/ml recorded in all males in the breeding season groups (Fig. 1). This difference was highly significant (*t*-test: $P = 0.0001$).

Males in the non-breeding season group were involved in fewer agonistic interactions than males in the breeding season groups (0.79 ± 0.21 vs 0.28 ± 0.08 interactions/h, $P = 0.03$). Dominance rank was significantly correlated with body weight ($P < 0.01$): the heaviest males had the highest rank and the lightest males the lowest (α males averaged 7.5 ± 0.2 kg; β , 7.1 ± 0.3 kg; γ , 6.3 ± 0.1 kg; δ , 6.1 ± 0.4 kg). Plasma testosterone concentration was not clearly correlated with dominance rank in either breeding or non-breeding season (Fig. 2).

The average rates of sexual behaviour and checking were significantly higher in the breeding season than in the non-breeding season (ANOVA $P = 0.008$). Rates of checking

Figure 1 Plasma testosterone concentration (ng/ml, mean $\pm$ S.E.M., $n = 4-7$ in each month) in males bled 1–3 times/month between February 2002 and February 2003. Testosterone concentrations rose significantly in colony males soon after reactivation of the blastocysts, reaching highest concentrations from late January to early February when females give birth and enter post-partum oestrus.

Figure 2 Plasma testosterone (ng/ml $\pm$ S.E.M.) of males in Groups 1 & 2 in the breeding season (Jan–Feb; solid bars) and one Group 3 (open bars) in the non-breeding season (Oct.). Testosterone concentrations are significantly higher in the breeding season, but there is no clear correlation between dominance rank at either time of the year.

for individual males were correlated with both peak testosterone (Spearman correlation: $r = 0.671$, $P = 0.028$) and average testosterone (Spearman correlation $r = 0.679$, $P = 0.025$) over the observation period. However, the total amount of sexual activity (as determined by sexual score per day) did not correlate with peak (Spearman correlation; $r = 0.465$, $P = 0.15$) or average testosterone (Spearman correlation; $r = 0.340$, $P = 0.30$).

Matings were observed for 40 females (Figs 3 and 4), but in only 24 of these was it possible to conduct observations for long enough to be certain that all matings to the last had been recorded ('complete mating sequences'). In this group, there were 7 single matings, 8 double matings, 5 females mated with 3 different males, and 4 females mated with 4 different males. From the 40 observed matings, the dominant male secured the first copulation on 60.0% of occasions. All single, male post-partum matings ($n = 7$) were by α males. However, α males were unable to monopolise initial copulations, and the lower ranking males ejaculated first in some cases (β males: 8/40 (20%), γ males: 4/40 (10%), δ males: 4/40 (10%) (Fig. 3a). Timed observations of the behaviour of males from group 2 in relation to four different females and one female from group 4 confirmed that the first mating did not always result in successful fertilization, nor did the α male always sire the pouch young (Fig. 4).

Of 36 pouch young born during this study, it was possible to confirm paternity by genotyping for 20 young, while for the remaining 16, genotyping did not result in unambiguous assignment of paternity. Of the 20 where paternity was assigned, α males sired 10 young (50%) (Fig. 3b) β males sired 7 (35%) and γ males sired 3 pouch young (15%) respectively (Table 1). No δ males secured paternity. We obtained data on paternity for only 9/24 females for which we had complete mating sequences. Of these, the male that mated first secured parentage in 4 sequences (44.4%), but in

Figure 3 (A) Number of copulations by males of different ranks. The higher ranked males achieved a greater number of first copulations. (B) Number of young sired by males of different ranks. The higher ranked males achieved a greater number of copulations. (C) Number of young sired in relation to first copulation by the father. More young resulted from early than later copulations.

3 (33.3%) and 2 (22.2%) sequences respectively the second and third copulations achieved paternity (Fig. 3c).

Using the mating sequences of the 24 females for which the full mating sequence was observed, we generated predictions of paternity based on three models. In the 'first sire' or 'last sire' models, the first or last mating male was predicted to be the sire respectively, and in the 'equal chance' model, any male that mated with the female was considered to have the same chance of siring the young as any other male in that mating sequence. These predictions were compared by a log linear model, with the observed frequency of siring by dominance ranking in the 20 animals for which paternity was determined (Fig. 3b). None of the three models were significantly different from

Figure 4 Examples of mating and mounting sequences of females, showing duration of mounts and matings. Paternity is known for females 0557 and 0820. In both, the α male is the father and was the first and second copulating male respectively. In one example (top), the γ male secured the first mating. Grey bars, copulation; black bars, mounting; boxes assign Paternity.

the observed ($P > 0.1$ in all comparisons) (Table 1). None of these models could therefore be discounted in this study.

Discussion

Male tammar wallabies show a marked increase in testosterone in January at the peak of the breeding and birth season. In these males and those housed with the melatonin-implanted females, this rise in testosterone coincided with the middle of active gestation, suggesting that the increase may result from pheromones induced by the rise in progesterone (or other pregnancy hormones) at this time. This rise in testosterone has been assumed to be important in establishing sexual behaviour, but the role of testosterone in establishing dominance hierarchies is less clear because the dominant male does not necessarily

have higher circulating testosterone concentrations than subordinates in his hierarchy. Although rates of sexual checking and aggression were lower in the non-breeding season when testosterone concentrations are lower, in the breeding season when testosterone concentration was significantly elevated, it did not necessarily increase the level of sexual activity or ensure dominance. In contrast, the heaviest (largest) males showed dominance over all other males in the group. Dominance confers some advantages for paternity success, and the α male was able to secure the initial copulation most frequently. However, mating first does not appear to be the only mechanism by which the dominant male achieved the greatest paternity. Other physiological or behavioural attributes of dominant males may be of equal or greater importance for successful paternity in the tammar. Dominant males may have larger epididymal sperm reserves and may, therefore, be able to ejaculate greater numbers of spermatozoa during copulation than subordinate males, or be able to produce a larger number of effective ejaculates, or to displace semen of subordinate males that mate before them. They may also, by their behavioural dominance, be able to reduce the copulatory success of subordinate males by mate guarding and fighting, as observed by Rudd (1994).

The lack of relationship in the tammar between dominance rank and testosterone indicates that the testosterone (and thus fertilizing ability) of subordinates is not

Table 1 Observed and predicted paternity success based on mating first, last or in any sequence.

Sire	Observed	Prediction from model		
		First ¹	Last ²	Equal chance ³
α	10	16	10	12.7
β	7	4	6	5.3
γ	3	2	4	3.2
δ	0	2	4	2.8

¹ $P = 0.112$; ² $P = 0.219$; ³ $P = 0.269$.

suppressed by rank. In another marsupial, the sugar glider, the dominant male has a significantly higher testosterone concentration than subordinates. The potential advantages of this testosterone-linked dominance in sugar gliders is reflected in the higher paternity achieved by the α male (over 75% of conceptions annually) (Mallick *et al.* 1994, Stoddart *et al.* 1994) and assisted by the smaller numbers of males (2–3) in male dominance hierarchies of this species.

Dominance in the tammar appears to be a function of body size and aggression and is usually associated with a greater competitive ability. Testosterone has been linked with aggression in many species, including the tammar, and acts of aggression in this study were more prevalent in the breeding season when testosterone concentrations were greater (Rudd *et al.* 1996). Dominance is a moderate predictor of paternity success in the captive tammar wallaby, yet paternity success does not always result from mating first. This is surprising, as the tammar has some characteristics often associated with a first-mating advantage, including mate guarding and the formation of a copulatory plug (Rudd 1994). Dominance correlates positively with larger size in male tammars and many other species of macropodids (Kaufmann 1974, Croft 1981, Rudd 1994, Fisher 1999). In the tammar, larger size is associated with heavier reproductive organs (Inns 1982, Paris 2004), suggesting that the dominant male has a greater fertilizing capability via greater ejaculate volume, higher coagulation index of ejaculates and higher proportion of motile sperm (Paris 2004). Thus, a contributing mechanism by which α males secure paternity may be by enhanced sperm competition.

In the first study of paternity in tammars, no male sired significantly more young than any other in a small group of 4 males and 13 females that produced 22 young (progeny sired per male were 8, 6, 4 and 4) (Ewen *et al.* 1993). This may have been influenced by the synchronous oestrus of the females, since one male cannot guard two or more females simultaneously, but the two largest males sired the most young. In a group of captive male red-necked wallabies (*Macropus rufogriseus*), at least 20% of young were not fathered by the dominant male, showing that the dominant male was unable to monopolise paternity (Watson *et al.* 1992). In that study, exact parentage was not able to be determined; thus, the number not sired by the dominant male is likely to have been much higher.

In most observed mating sequences from our study, the α male was unable to prevent other males from mating with the female. This is not purely an artefact of captivity, as tammars in the wild on Kangaroo Island in January mate with up to 5–7 males in a single oestrus (M B Renfree & G Shaw; unpublished observations). The inability of the dominant male to guard mates successfully and prevent copulation by subordinates is well documented for the tammar (Rudd 1994). The guarding activity of male tammars tends to be thwarted by the female's engaging in behaviour, such as mating chases, that draws the attention of other males to her oestrous state (Rudd 1994). Cavimorph rodents have

similar behaviour (Sachser *et al.* 1999). This advertisement of oestrus through conspicuous activity, such as calling and chasing, induces male–male competition so that females can mate with the most competitive male (Cox & Le Boueff 1977). Current theory suggests that the selective pressures from this male–male conflict have led to the evolution of the sexual size dimorphism seen in many species, including the tammar (Harcourt *et al.* 1981, Rudd 1994).

In the male tammar wallaby, there is intense competition for oestrous females. Females mate with more than one male; and the α male secures more than half, but not all, initial copulations. However, since dominant males mate first on only 60% of occasions, and only 50% of the young are sired by the dominant male, neither the mating sequence nor dominance is a reliable predictor of paternity success in the tammar.

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