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Onset of plural cooperative breeding in common marmoset families following replacement of the breeding male

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Common marmosets, *Callithrix jacchus*, are usually characterized as singular cooperative breeders, with only a single, dominant female reproducing in each group. Anecdotal reports, however, have described two females breeding concurrently when an unrelated male joins their group. We tested the hypothesis that incorporation of an unrelated adult male into a family systematically leads to the onset of reproductive activity in a daughter and investigated the underlying mechanisms. We collected hormonal and behavioural data from mothers and their eldest daughters before and after the father was removed from the family and either replaced by an unrelated male ($N = 11$) or immediately returned to the family ($N = 7$). Variation between daughters in the occurrence of ovulatory cyclicity was not associated with the presence of an unrelated male but was closely linked to daughters' relationships with their mothers: only anovulatory daughters behaved submissively towards their mothers. Daughters never engaged in sexual behaviour with their fathers, but most did so with unrelated males. Similarly, daughters never conceived in intact natal families but did so in eight of 11 families containing an unrelated male. In six of these families, the mother and daughter bred concurrently. Thus, incorporation of an unrelated adult male into a marmoset family may frequently lead to the onset of plural breeding by activating sexual behaviour in a daughter; however, daughters ovulate only if they are not behaviourally subordinate to their mothers. Therefore, both inbreeding avoidance and rank-related reproductive suppression may constrain reproduction in marmoset daughters and contribute to maintenance of singular breeding.

Among singular cooperative breeders, reproduction is usually monopolized by a single, dominant female within each social group. Subordinate females undergo suppression of reproductive physiology and/or inhibition of sexual behaviour, fail to breed, and may instead provide alloparental care for the offspring of the dominant female (Solomon & French 1997). In a number of these species, however, singular breeding may occasionally give way to plural breeding, with two or more females reproducing concurrently (e.g. naked mole-rat, *Heterocephalus glaber*: Jarvis 1991; African wild dog, *Lycaon pictus*: Fuller et al. 1992; golden lion tamarin, *Leontopithecus rosalia*: Dietz & Baker 1993; dwarf mongoose, *Helogale parvula*: Keane et al. 1994; slender-tailed meerkat, *Suricata suricatta*: Doolan & Macdonald 1997; Mongolian gerbil, *Meriones unguiculatus*: Clark & Galef 2001). While considerable attention has been given to the ultimate factors that may promote plural rather than singular breeding in cooperative species (e.g. Vehrencamp 1983; Creel & Waser 1991; Reeve et al. 1998; Johnstone 2000), the proximate mechanisms that regulate this transition are not well understood. Investigation of these mechanisms is likely to elucidate both the factors that normally constrain reproduction in nonbreeders and the functional significance of plural and singular cooperative breeding (Creel & Waser 1997; Solomon & French 1997).

Common marmosets, *Callithrix jacchus*, and other callitrichines (marmosets and tamarins) are small, New World monkeys that are typically characterized as singular cooperative breeders (Abbott et al. 1997; French 1997). Both field and laboratory studies have usually reported that only a single, behaviourally dominant female breeds in each social group (reviewed by French 1997; Saltzman 2003). Individuals of both sexes remain with their natal family into adulthood, helping to rear the offspring of the dominant, breeding pair (Stevenson & Rylands 1988). These nonreproductive helpers may undergo inhibition of sexual behaviour and, at least among females, social

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suppression of reproductive physiology (Abbott 1984; Saltzman 2003). Recently, however, a number of investigators have described groups of wild or captive common marmosets and other callitrichines in which two females bred concurrently (reviewed in: French 1997; Smith et al. 2001; Baker et al. 2002; Saltzman 2003). Among wild common marmosets (Digby & Ferrari 1994) and golden lion tamarins (Dietz & Baker 1993; Baker et al. 2002), groups may contain two reproductively active females for many months or years. These findings suggest that plural breeding may be a stable variant in the cooperative breeding system of callitrichines and may occur systematically under specific ecological, demographic, or social conditions.

The proximate factors promoting plural rather than singular cooperative breeding in callitrichines are unknown. In at least three species, however, plural breeding has been associated most reliably with replacement of a group's original breeding male by an unrelated male (golden lion tamarin: Dietz & Baker 1993; Baker et al. 2002; cottontop tamarin, *Saguinus oedipus*: Price & McGrew 1991; Savage et al. 1996; common marmoset: Abbott 1984; Rothe & Koenig 1991; Kirkpatrick-Tanner et al. 1996; Saltzman et al. 1997a, b; reviewed in: French 1997; Saltzman 2003). Anecdotal evidence from both wild and captive groups suggests that incorporation of an unrelated adult male into a group can result in the onset of breeding by a second female, usually a daughter of the original breeding female, with the two breeding females behaviourally dominating all other females in the group (Digby 1995b; Saltzman et al. 1997b, c; A. J. Baker, personal communication). Notably, though, the occurrence of more than two breeding females in a single group has rarely been reported despite the frequent presence of additional mature females (but see Kirkpatrick-Tanner et al. 1996; De Vleeschouwer et al. 2001).

The hypothesis that incorporation of an unrelated male into a family leads to plural breeding has not, to our knowledge, been tested experimentally. Moreover, the mechanisms by which plural breeding might emerge under these conditions are unknown. Marmosets typically avoid mating with close relatives, and inbreeding avoidance has been implicated in reproductive failure among marmosets housed with their natal families (Abbott 1984; König et al. 1988; Saltzman et al. 1997b, c; Baker et al. 1999). For females living with their natal families, immigration of an unrelated male may provide the first opportunity to engage in intragroup mating attempts with an unrelated partner. Alternatively, incorporation of an unrelated male into a family might lead to cessation of rank-related reproductive suppression in subordinate females. Many socially subordinate female common marmosets undergo suppression of ovulation and, possibly, of sexual behaviour in response to cues from a dominant female (reviewed by Abbott 1984, 1993; Saltzman 2003). If immigration of an unrelated male alters social dynamics and dominance relationships between females, as has been reported in other cooperative breeders (Brant et al. 1998; Cooney & Bennett 2000; O'Riain et al. 2000), then intrasexually determined, rank-related suppression might be disrupted, leading to the onset of reproductive activity in a previously subordinate female. In a small pilot study, we found that replacement of the breeding male by an unrelated male in families of common marmosets was sometimes followed by the onset of ovulatory cyclicity and sexual behaviour in previously anovulatory daughters, and that these daughters occasionally became behaviourally dominant to their mothers (Saltzman et al. 1997b, unpublished data).

In the present study, we experimentally tested the hypothesis that incorporation of an unrelated male into a marmoset family reliably induces the onset of plural breeding. We collected behavioural and hormonal data from mothers and daughters, both in families in which the father was replaced by an unrelated male and in control families, in which the father was removed and immediately returned, to determine the effects of unrelated males on females' ovulatory cyclicity, reproductive endocrine function, behaviour, dominance relationships and reproductive outcomes. In this initial paper, we describe females' ovarian function, conception rates, sexual interactions and intrasexual dominance relationships. Social dynamics, infant outcomes and parental behaviour will be presented elsewhere.

METHODS

Animals

We used a total of 35 captive-born, female common marmosets: 13 mothers and 22 of their young adult daughters. Five of the mothers were used twice: once in the unrelated male condition and once in the control condition (see below). All other females were used in only a single condition. At the time of unrelated male or father introduction, mothers were 72.0 ± 3.7 months of age ($\overline{X} \pm$ SE; range 53.0–102.8 months) and daughters were 18.4 ± 0.1 months of age (range 17.9–19.8 months). Age did not differ significantly between mothers ($t_{16} = 0.42$, NS) or between daughters ($t_{20} = -0.88$, NS) used in the unrelated male and control conditions. Female common marmosets undergo puberty by approximately 13–17 months of age (Abbott & Hearn 1978).

Initially, most animals were housed in families comprising a breeding pair (mother and father) and one to six (4.3 ± 0.5) of their nonreproductive offspring. In three families, however, the father had been euthanized due to chronic illness 3.0–5.4 months prior to unrelated male introduction, so that the family initially comprised only a female and three to six of her offspring. Focal daughters were limited to the eldest daughter currently housed in each family. In four cases, we collected data concurrently from two daughters, comprising a female–female twin pair. We used a total of 11 mothers and 13 daughters (including two female–female twin pairs) in the unrelated male condition, and seven mothers and nine daughters (including two female–female twin pairs) in the control condition. Animals were housed and maintained as previously reported (Saltzman et al. 1998), except that lights were on during 0700–1900 hours.

Design

Each family underwent a 'male introduction' (MI) procedure. For the unrelated male condition, the father

(and male co-twin, if present: $N = 5$ families) of the eldest daughter was removed from the family and permanently housed in a cage in a different room than that in which the family was housed. Approximately 10 min later, another adult male, which was unrelated to and had never lived with the family members, was released into the family's home cage. Unrelated males were 75.2 ± 7.5 months of age, and each had previously sired live offspring. They had been singly housed or pair-housed with a son for at least 1 week before introduction into the family. In addition to male co-twins of focal daughters, all younger sons that were at least 12 months of age (one in one family, two in another family) had to be removed from their families within several hours after unrelated male introduction because they engaged in persistent agonism with the unrelated male. These sons were subsequently housed with their fathers. For the control condition, we captured the father, removed him from his family, and released him back into his own family approximately 10 min later. Behavioural data were recorded on audio tape by a trained observer for 1 h following unrelated male or father introduction; these data will be presented elsewhere.

We collected focal animal behavioural data (see below) from the mother and eldest daughter(s) in each family, five mornings per week, beginning 2 weeks before MI and ending 4 weeks after MI. During the same 6-week period, we also conducted separation–reunion tests (see below) one afternoon per week to characterize dominance relationships between mothers and daughters. To monitor ovarian function and pregnancies, we collected blood samples (Saltzman et al. 1994) twice weekly from each mother and eldest daughter, beginning 8 weeks before MI until at least 8 weeks (for pregnant females) or 16 weeks (for nonpregnant females) after MI. This procedure is readily accommodated by marmosets in our colony and has not been found to elevate plasma cortisol levels (Saltzman et al. 1994). Blood samples were immediately placed on ice and centrifuged at 2000 revolutions/min for 10 min, and the plasma fraction was extracted and frozen at −20°C until assayed for plasma progesterone (P; see below). During the 6-week period of behavioural data collection, we additionally collected daily urine samples (see below) for analysis of oestradiol sulphate (E_2S) and luteinizing hormone-chorionic gonadotrophin (LH-CG) levels. Urinary E_2S and LH levels were expected to serve as an index of increasing ovarian and pituitary activity, respectively, in previously anovulatory daughters. In addition, measurement of the urinary preovulatory LH surge provides accurate timing of the day of ovulation, sustained elevations of urinary E_2S levels confirm that ovulation has occurred from the presence of endocrinologically active corpora lutea (Eastman et al. 1984), and elevations of urinary CG levels confirm the presence of an endocrinologically active placenta in a postimplantation pregnancy, beginning approximately day 15–18 of gestation (Webley & Hearn 1994). To further identify and monitor possible pregnancies, we performed uterine ultrasonography (see below) 20–27 days following each ovulation, unless the luteal phase ended spontaneously before this time.

Focal Animal Observations

We conducted focal animal observations on focal daughters and their mothers for 15 min per animal per day, 5 days per week, at 0900–1130 hours. Each female was observed at least once in any 2-day period. We always observed mothers and their daughters consecutively, with the order of observations balanced.

Behavioural data were collected by trained observers to whom the animals had been previously habituated, sitting quietly in full view of the animals. A variety of behaviours, including sexual, agonistic, affiliative and display behaviours, were recorded on a laptop computer each time the focal female performed them to or received them from any cagemate or performed them towards animals in other cages. We analysed the following sexual behaviours for this paper (see Baker et al. 1999 for definitions): sexual solicit (tufts back or freeze: Baker et al. 1999; a proceptive or receptive behaviour performed by females), tongue in–out (a proceptive behaviour performed by females or males), mount, attempted mount, intromission, ejaculation and pelvic thrusting. We also analysed the following agonistic behaviours: vocal submit (ngä), vocal threat (erh-erh), fight, attack, cuff, snap bite, ear-tufts flick and genital present (see Baker et al. 1999). Interobserver reliability scores were determined as a composite for all behaviours scored and averaged $89.7 \pm 1.5\%$.

We coloured marmosets' ear tufts (Redken Deco Color, Canoga Park, California, U.S.A.) to facilitate rapid and reliable identification of individuals. This procedure does not appear to alter the animals' behaviour or endocrine function (Abbott 1979). We locked nestboxes shut during observations to ensure that the animals remained in view of the observers. Ad libitum observations (unpublished) indicate that marmosets in our colony normally spend very little time in their nestboxes at this time of day.

Separation–Reunion Tests

Because agonism typically occurs infrequently in undisturbed families of common marmosets (Abbott 1984; Sutcliffe & Poole 1984b; Saltzman et al. 1997c), and because we consider submissive behaviour to be the most reliable indicator of dominance relationships in this species (Saltzman et al. 1994, 1996, 1998), we conducted separation–reunion tests to increase our likelihood of observing submissive behaviour among adult females. At approximately 1300–1400 hours 1 day per week, we ushered all family members into a nestbox and removed them from their home cage. The mother and eldest daughter(s) were each placed individually in a small cage ($61 \times 46 \times 61$ cm) with which they had been familiarized previously, in separate rooms containing no other marmosets; the remaining family members were released together into a standard cage ($61 \times 91 \times 183$ cm) in a room housing numerous conspecifics. During the separation period, all animals had access to food and water. After 1 h, we released the mother and eldest daughter(s) into the empty home cage in rapid succession, with the order of release balanced across tests, and collected behavioural data for 15 min on both/all females simultaneously, using

the methods described above for focal animal observations. At the end of this 15-min reunion period, we released the father or unrelated male into the home cage and collected behavioural data for an additional 15 min, after which the remaining family members were returned to the home cage. Nestboxes were locked shut during all behavioural observations. We considered a female to be subordinate to another female (i.e. her mother, daughter, or sister) if she performed vocal submission to her at least once, but did not receive vocal submission from her, during the six weekly separation–reunion tests.

Urine Sample Collection

For collection of daily urine samples, we manually captured animals from their nestboxes at approximately 0645 hours, prior to lights-on, and immediately placed each mother and focal daughter in an individual urine-collection chamber, which had been attached to a cage wall inside the family's home cage the previous afternoon. Urine-collection chambers comprised a stainless steel mesh inner cage (15 × 20 × 15 cm) surrounded by a Plexiglas outer cage (25 × 20 × 24 cm) through which the female could maintain visual, auditory and olfactory contact with other family members. Upon elimination, urine (and, to a limited extent, faeces) dropped through the floor of the mesh inner cage into a stainless steel tray positioned directly under the animal, from which we removed the urine by a sterile syringe, transferred it to a polypropylene vial, and immediately placed it on ice. Females were offered approximately 1 ml Ensure, a liquid nutritional supplement (Abbott Laboratories, Columbus, Ohio, U.S.A.), while in the urine-collection chambers and were released after at least 1 ml of urine had been collected from each focal female in the family or after 1 h had elapsed. Within each family, we released the mother and daughter(s) from urine-collection chambers simultaneously to avoid biasing their interactions with other family members. We centrifuged urine samples at 5000 revolutions/min for 5 min, transferred them to a clean vial, added glycerol (0.52 M; 50 μl per ml of urine) to stabilize protein hormones (Livesey et al. 1983), and froze samples at −20°C until assay.

Hormone Assays, Monitoring and Control of Ovarian Function

We assayed plasma P from twice-weekly blood samples using enzymeimmunoassay as previously described (Saltzman et al. 1994). Assay sensitivity was 3.6 pg/tube, and inter- and intra-assay coefficients of variation (CVs) of a marmoset plasma pool assayed in duplicate in each assay were 15.47 and 4.57%, respectively.

We assayed plasma LH-CG in several blood samples to determine whether conception had occurred (see below). We assayed these samples in duplicate by radioimmunoassay (RIA) as described by Saltzman et al. (1998). Assay sensitivity was 0.1 ng/tube, and inter- and intra-assay CVs were 5.17 and 4.66%, respectively.

We assayed urinary E_2S in duplicate using RIA as described by Converse et al. (1995). We first validated the assay for use with common marmoset urine. Serial dilutions ($N = 7$) of a marmoset urine pool yielded a displacement curve parallel to a standard curve obtained with E_2S standards (difference between slopes: NS). The recovery of E_2S standards ($N = 9$) added to a marmoset urine pool was 108.5 ± 2.7%. Assay sensitivity was 10 pg/tube, and inter- and intra-assay CVs were 12.13 and 5.15%, respectively.

We assayed urinary LH-CG in duplicate by RIA as described by Ziegler et al. (1993), following validation of the assay for use with common marmoset urine. Serial dilutions ($N = 5$) of a marmoset urine pool yielded a displacement curve parallel to a standard curve obtained with CG standards (difference between slopes: NS). The recovery of CG standards ($N = 5$) added to a marmoset urine pool was 101.3 ± 4.1%. Assay sensitivity was 0.059 ng/tube, and inter- and intra-assay CVs were 5.70 and 4.23%, respectively.

To correct for differences in concentration of urine, urinary E_2S and LH-CG concentrations were divided by creatinine concentrations in the same sample. We assayed creatinine in duplicate as described by Ziegler et al. (1995). Inter- and intra-assay CVs were 13.71 and 3.01%, respectively.

We considered animals to have ovulated on the day prior to a sustained (≥2 consecutive blood samples) increase in plasma P levels above 10 ng/ml (Harlow et al. 1983). Following ovulation, we considered them to be in the luteal phase of an ovulatory cycle, or pregnant, until plasma P levels fell below 10 ng/ml, and in the follicular phase until P concentrations again rose above 10 ng/ml (Harlow et al. 1983). Thus, we operationally defined the typically short (approximately 6–10 days: Harlow et al. 1983; Saltzman et al. 1997a, 1998) follicular phase as the time during which plasma P levels remained below 10 ng/ml in between two successive luteal phases. In some cases, this included an extended period of anovulation lasting up to approximately 2 months.

To ensure that females did not sustain term pregnancies prior to MI (and in some cases, following MI), we used cloprostenol (Estrumate, Mobay Corp., Shawnee, Kansas, U.S.A.), a prostaglandin F2α analogue, to terminate ovulatory cycles and early pregnancies during these times (Summers et al. 1985). Cloprostenol (0.75–1.0 μg) was injected intramuscularly 11–45 days following ovulation. To ensure that females would be in the early follicular phase at the time of unrelated male or father introduction, we treated them with cloprostenol 2 days prior to MI, if they were in the luteal phase or early pregnancy and at least 11 days postovulation. Three mothers and one daughter in the unrelated male condition, and one mother and one daughter in the control condition, ovulated fewer than 11 days before MI and were therefore in the luteal phase or early pregnancy at the time of MI. As an exception to the protocol described above, we permitted the mother in the control condition to carry her pregnancy to term because she had previously shown adverse reactions to cloprostenol treatment. We omitted this mother from analyses of ovulatory cycles, conceptions and sexual behaviours following MI.

To confirm pregnancies, we performed uterine ultrasonagraphy 20–27 days after each ovulation, if plasma P levels remained elevated (>10 ng/ml). We performed ultrasonagraphy using an Aloka SSD-650 real-time scanner equipped with an interoperative linear array probe (UST-5522L-7.5) operating at a frequency of 10 MHz. We considered ovulatory cycles to be nonconceptive if ultrasonagraphy revealed a single, linear, echogenic interface between adjacent endometrial layers, with no visible uterine lumen (Oerke et al. 1995; Saltzman et al. 1997c), or if the luteal phase ended spontaneously prior to ultrasonagraphy. Individual cycles of two mothers and two daughters were terminated by cloprostenol without uterine ultrasonagraphy. In these cases, we assayed one to two blood samples, collected 18–30 days after ovulation, for CG. Based on results from samples collected at similar time points in cycles confirmed to be conceptive or nonconceptive, we considered the cycles of these four animals to be conceptive if plasma CG concentrations exceeded 6.0 ng/ml.

Analysis

To compare urinary LH concentrations between daughters, we determined LH values during each preovulatory LH surge (defined as a peak value, followed 0–3 days later by a sustained elevation of plasma P above 10 ng/ml), luteal phase (plasma P>10 ng/ml, excluding the day after LH surge and excluding conceptive cycles), and follicular phase (P≤10 ng/ml between two successive luteal phases, excluding the day before LH surge). For each daughter undergoing ovulatory cycles, we determined a mean luteal phase, follicular phase and preovulatory surge urinary LH value in the pre- and post-MI conditions. Similar analyses were performed for urinary E_2S values. For daughters that were anovulatory throughout the experiment, we calculated mean concentrations of each hormone before and after MI.

Concentrations of plasma P, urinary LH-CG and urinary E_2S, and the durations of follicular and luteal phases, were analysed parametrically using *t* tests and ANOVAs. Other measures, including the numbers of females that ovulated, conceived and engaged in sexual behaviours, were analysed nonparametrically using Mann–Whitney tests and Fisher's exact probability tests. For analyses of sexual behaviours, we used the number of females performing or receiving each behaviour, rather than behavioural frequencies, because of high variance in rates of behaviours. Because we performed multiple comparisons on sexual behaviours, we evaluated these analyses using the Bonferroni correction (Sokal & Rohlf 1995). For most analyses, we compared groups cross-sectionally following MI, rather than longitudinally before and after MI, because (1) fewer behavioural and endocrine data were available before MI than after, and (2) ovarian cycle parameters have been found to change with maturation over the age range of daughters in this study (Saltzman et al. 1997a).

In one family in the unrelated male condition, the focal (eldest) daughter was behaviourally subordinate to a younger (by 5 months) sister, and the younger, but not the elder, sister engaged in sexual behaviour with the unrelated male and eventually conceived. Therefore, we began to collect blood samples for P analysis (but not urine samples or behavioural data) from the younger sister 3 days after MI. Data from both sisters are included in analyses of ovarian cyclicity and conceptions following MI.

Ethical Note

We maintained animals and conducted all procedures in accordance with the recommendations of the *Guidelines for the Use of Animals in Research*, *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act and its subsequent amendments. All procedures were reviewed and approved by the Graduate School Animal Care and Use Committee of the University of Wisconsin, Madison. To minimize novelty and stressfulness of our experimental procedures (e.g. urine collection, separation–reunion tests, behavioural observations), we adapted animals to the procedures and apparatuses before testing. Because introduction of an animal into an established group can lead to fighting and injuries, we monitored families closely following introduction of unrelated males and removed family members (sons ≥1 year of age) as necessary if fighting occurred.

RESULTS

Reproductive Hormones

Daughters showed considerable variability in ovulatory function (Fig. 1). In the 8 weeks prior to introduction of the father or unrelated male, plasma progesterone levels in twice-weekly blood samples indicated that three of the nine daughters (33%) in the control condition and five of the 13 daughters (38%) in the unrelated male condition ovulated at least once (Table 1). Introduction of an unrelated male into the family did not specifically increase the likelihood that daughters would ovulate. All the daughters that ovulated prior to male introduction (MI) continued to ovulate afterwards. Of the daughters that were initially anovulatory, three of six (50%) in the control condition and three of eight (37.5%) in the unrelated male condition ovulated after MI (Fisher's exact test: $N = 14$, $P = 0.844$). Latency from MI to first ovulation in these animals averaged 55.3 ± 16.7 days in the unrelated male condition and 52.0 ± 18.5 days in the control condition (unpaired *t* test: $t_4 = -0.134$, $P = 0.900$). In each of the four female–female twin pairs (two unrelated male and two control), one twin remained anovulatory throughout the study, whereas the other ovulated either after ($N = 2$) or both before and after ($N = 2$) MI.

Introduction of an unrelated male also did not influence the likelihood that mothers would ovulate. All mothers ovulated at least once in the 8 weeks prior to introduction of the father or unrelated male, and all but one ovulated at least once in the subsequent 16 weeks (Table 1). The only exception was the one control mother that was permitted to continue a pregnancy that had begun immediately before MI.

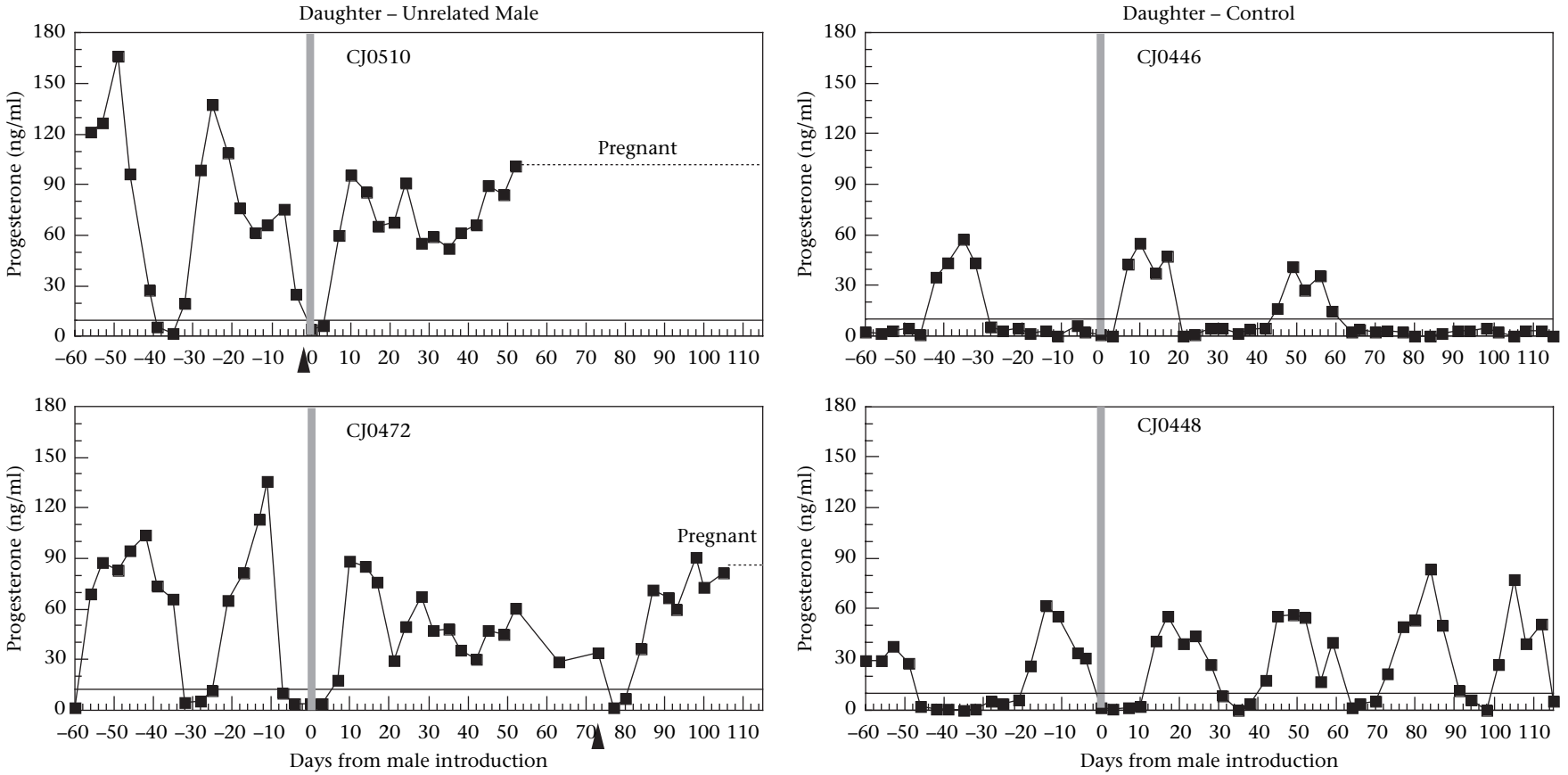

Figure 1. Plasma progesterone (P) concentrations in twice-weekly blood samples taken during the 8-week period before and the 16-week period after male introduction (vertical bars), in four representative daughters undergoing ovulatory cycles. Sustained elevations of P above 10 ng/ml (solid horizontal lines) indicate the luteal phase of the ovarian cycle or early pregnancy. Left panels: P profiles of two females in the unrelated male condition. Each of these two females conceived in her last ovulatory cycle depicted; blood sampling was discontinued following ultrasonagraphic confirmation of pregnancy. Arrowheads indicate treatment with prostaglandin F2α analogue to terminate cycles. CJ0472 was one of two daughters that had an extended luteal phase in a nonconceptive cycle following introduction of an unrelated male. Right panels: P profiles of two daughters in the control condition. Note the extended follicular phases/anovulatory periods in CJ0446.

Table 1. Numbers of ovulations and conceptions detected in mothers and daughters during the 8-week period before and the 16-week period after introduction of an unrelated male (UM) or father (control)

Group—Condition	Male introduction	Number of females that		Number of conceptions/ ovulations*
		Ovulated/total *N*	Conceived/ovulated*	
Mothers—Control	Pre	7/7	5/7	7/11
	Post	6/6†	6/6†	9/12
Mothers—UM	Pre	11/11	8/8‡	12/15‡
	Post	11/11	10/11§	12/15
Daughters—Control	Pre	3/9 (3/7)**	0/3	0/3
	Post	6/9 (6/7)**	0/6	0/14
Daughters—UM	Pre	5/13 (5/11)**	0/4‡	0/9‡
	Post	9/14†† (9/11)**	6/9††	6/12††

*Includes only those ovulations for which conception could be determined (based on uterine ultrasonagraphy and/or absence of spontaneous decline in plasma progesterone levels to below 10 ng/ml, or CG levels in blood samples collected 18—30 days after ovulation).
†Excludes one mother that was permitted to continue a pregnancy that began immediately before father introduction.
‡Excludes mothers and daughters from three families in which the original breeding male died prior to the beginning of data collection.
§The mother that did not conceive was ill and had to be euthanized approximately 12 weeks after male introduction.
**Numbers in parentheses exclude the anovulatory member of each sister pair.
††Includes a younger, dominant sister of one focal daughter.

Although introduction of an unrelated male did not significantly affect the probability that daughters would ovulate, we examined ovarian cycle parameters as well as plasma and urinary hormone concentrations to determine whether unrelated males triggered more subtle changes in daughters' reproductive function. Following MI, daughters housed with unrelated males tended to have shorter follicular phases than daughters in control families, but this tendency did not reach statistical significance (unrelated male: 7 ± 2 days, $N = 5$; control: 20 ± 6 days, $N = 5$; unpaired t test: $t_8 = 2.059$, $P = 0.074$). Retrospective power analysis (JMP v. 3.1, Cary, North Carolina, U.S.A.) indicated that the lack of significance may have been due to our small sample sizes, which provided statistical power of only 0.59. No differences or trends were found, following MI, in the length of the luteal phase in nonconceptive cycles (Mann—Whitney U test: $U = 15.500$, $N_1 = N_2 = 6$, $P = 0.688$) or in mean P values across the entire luteal phase (unpaired t test: $t_9 = 0.540$, $P = 0.602$).

Urinary LH-CG concentrations typically remained low (<20 ng/mg Cr) except during the preovulatory surge, when LH rose above baseline levels for 1—3 days, and during pregnancy, when CG levels began to rise approximately 16 days after ovulation (Fig. 2). Urinary E_2S concentrations, in contrast, increased during the luteal phase of the ovarian cycle, typically lagging behind plasma P levels by several days (Fig. 2). Comparison of urinary LH-CG concentrations between daughters in the unrelated male and control conditions following MI revealed no differences during the follicular phase (unpaired t test: $t_8 = -0.546$, $P = 0.600$), preovulatory surge ($t_7 = 0.795$, $P = 0.453$), or luteal phase ($t_5 = 0.937$, $P = 0.392$). Similarly, no differences were found in urinary E_2S concentrations during the follicular phase ($t_6 = -1.233$, $P = 0.264$) or preovulatory LH surge ($t_7 = -0.730$, $P = 0.489$). Insufficient data were available for comparisons of luteal phase E_2S levels.

Among daughters that never ovulated (five unrelated male and three control), LH concentrations remained low (typically $\leq$10 ng/mg Cr) and acyclic, whereas E_2S levels showed greater inter- and intra-animal variability (Fig. 2). We compared mean concentrations of each hormone in acyclic daughters in the control and unrelated male conditions, both (1) from the 2 weeks before to the 4 weeks after MI, and (2) more acutely, from the week before to the week after MI, using two-way (groups*conditions) ANOVAs. Neither hormone differed between groups or changed over time (LH-CG, weeks −2 through +4: ANOVA: groups: $F_{1,6} = 0.015$, $P = 0.906$; conditions: $F_{1,6} = 0.165$, $P = 0.698$; groups*conditions: $F_{1,6} = 0.480$, $P = 0.514$; LH-CG, weeks −1 through +1: groups: $F_{1,6} = 0.044$, $P = 0.841$; conditions: $F_{1,6} = 0.016$, $P = 0.904$; groups*conditions: $F_{1,6} = 0.059$, $P = 0.816$; E_2S, weeks −2 through +4: groups: $F_{1,6} = 0.001$, $P = 0.974$; conditions: $F_{1,6} = 2.025$, $P = 0.205$; groups*conditions: $F_{1,6} = 0.162$, $P = 0.701$; E_2S, weeks −1 through +1: groups: $F_{1,6} = 0.392$, $P = 0.554$; conditions: $F_{1,6} = 1.673$, $P = 0.243$; groups*conditions: $F_{1,6} = 1.319$, $P = 0.295$).

Conceptions

Prior to MI, we did not detect pregnancy in any of the focal daughters during the 12 ovulatory cycles that could be assessed for conception (Table 1). The nine daughters in the control condition further failed to conceive following father introduction. Of the eight focal daughters that ovulated following introduction of an unrelated male, however, five conceived within the first 16 weeks, and two

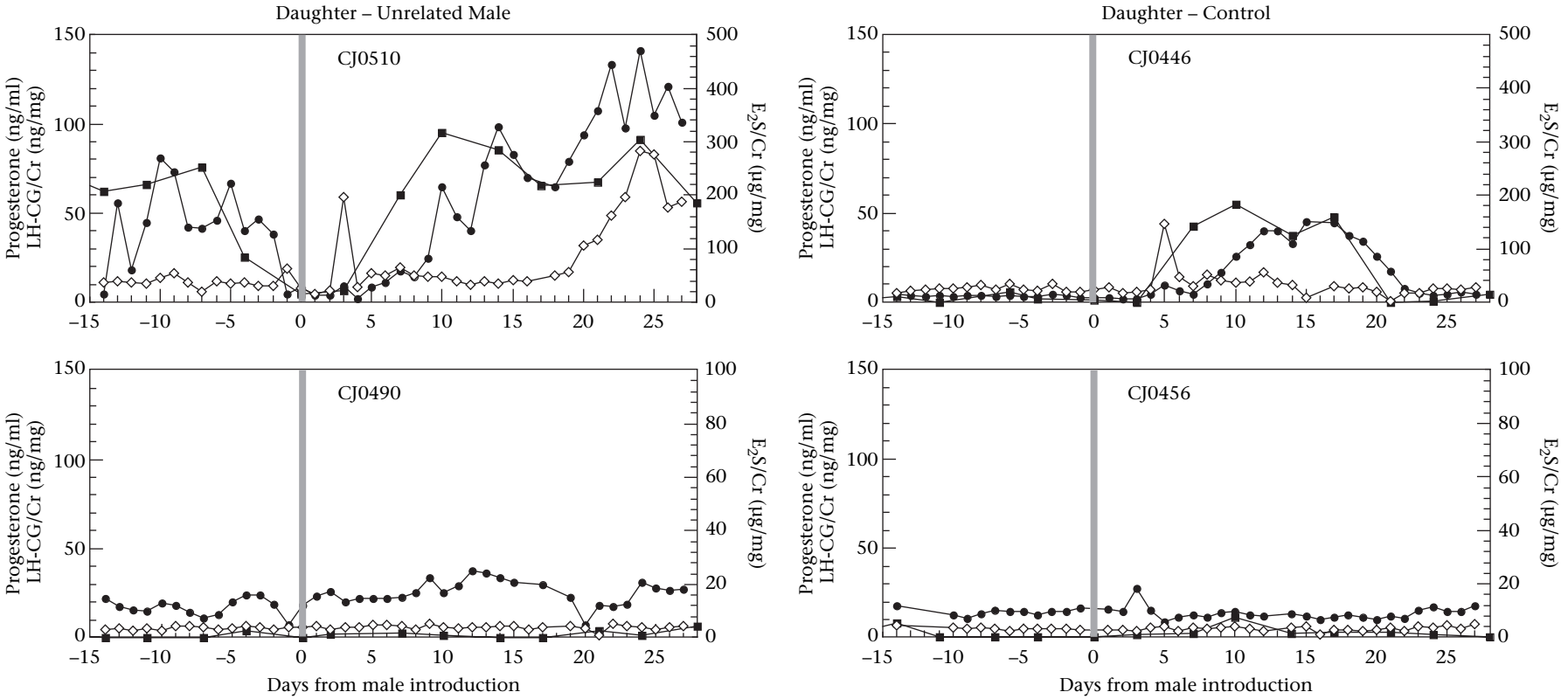

Figure 2. Hormonal profiles of four representative daughters during the 2-week period before and the 4-week period after male introduction (vertical bars), showing plasma progesterone (P, ■) concentrations in twice-weekly blood samples, and luteinizing hormone-chorionic gonadotrophin (LH-CG, ◇) and oestradiol sulphate (E_2S, ●) concentrations, corrected for creatinine concentrations, in daily urine samples. Sustained elevations of plasma P above 10 ng/ml indicate the luteal phase of the ovarian cycle or early pregnancy. Note that the *Y* axis scale for E_2S differs between the upper and lower graphs. Top panels: hormonal profiles of two representative daughters undergoing ovulatory cycles (the long-term plasma P profiles of these two animals are shown in Fig. 1). CJ0510's last cycle was conceptive, with CG levels rising beginning approximately 16 days after ovulation, characteristic of postimplantation pregnancy. Bottom panels: hormonal profiles of two daughters that remained anovulatory throughout the entire experiment.

conceived 17–22 weeks after MI. In addition, one female that was not a focal daughter but was a younger (by 5 months) sister of an anovulatory focal daughter (to which she was behaviourally dominant) conceived approximately 6 weeks after introduction of an unrelated male. Overall, in the 16 weeks following MI, a daughter conceived in six of the 11 families containing an unrelated male (Fisher's exact test: $P = 0.05$ compared to control families). During this time, these six daughters conceived during $50.0 \pm 21.7\%$ of their ovulatory cycles, a rate that was marginally lower than that of mothers in the same families ($83.3 \pm 11.5\%$ of cycles; Mann–Whitney U test: $U = 27.000$, $N_1 = 11$, $N_2 = 9$, $P = 0.059$). Each of the eight daughters that conceived carried the pregnancy to term and delivered at least two live-born infants.

No daughter conceived during her first detected ovulatory cycle. Of the five daughters that ovulated prior to introduction of an unrelated male, two conceived on their first ovulation following MI. Overall, mean latency from unrelated male introduction to conception was 31.8 ± 13.5 days in the five previously cycling daughters and 135.5 days in the two daughters that underwent their first ovulation following MI. Family size following introduction of the unrelated male did not differ reliably between families in which a daughter did or did not conceive (unpaired t test: $t_9 = 1.165$, $P = 0.274$).

Conception rates of mothers, in contrast to daughters, were not altered by introduction of an unrelated male. As indicated in Table 1, most mothers conceived both before and after introduction of the unrelated male or father. Among control mothers, each of the five pregnancies not terminated by cloprostenol injection was carried to term and resulted in at least two live-born infants. In the unrelated male condition, 10 of 11 mothers conceived in the 16 weeks after MI, of which nine delivered at least one live-born infant and one aborted. The remaining mother was euthanized due to illness 11.6 weeks after MI. Overall, neither the proportion of mothers that conceived following MI nor the proportion of cycles in which they did so differed between the unrelated male and control conditions (Table 1).

Sexual Behaviour

Of the eight sexual behaviours scored, three (intromission, ejaculation, pelvic thrusting) were observed too infrequently for analysis. The numbers of mothers and daughters that engaged in the remaining sexual behaviours after introduction of an unrelated male or father are presented in Table 2. Introduction of an unrelated male into the family altered the sexual behaviour of both mothers and daughters. No daughters were observed performing sexual behaviours to, or receiving them from, the father, either before or after MI. During the 4 weeks following introduction of an unrelated male, in contrast, eight of 13 daughters (in seven of 11 families) engaged in sexual behaviours with the unrelated male. As shown in Table 2, daughters in families containing unrelated males were significantly more likely to perform sexual solicitations to the breeding male, and to receive tongue in–out from him, than daughters in intact natal families. Similarly, mothers that were housed with unrelated males were significantly more likely to direct sexual solicitations and tongue in–out to the male than were mothers that were housed with their original mates; however, the two groups of mothers did not differ reliably in the likelihood of receiving any sexual behaviours from the male. Mothers and daughters in the unrelated male condition showed comparable levels of sexual behaviour, except that mothers were more likely than daughters to perform tongue in–out; however, this tendency was not significant at the modified α level (0.017) determined by the Bonferroni correction. Daughters living with unrelated males were more likely both to perform and to receive sexual behaviour if they were undergoing ovulatory cycles

Table 2. Numbers of mothers and daughters that engaged in sexual behaviours with the father (control) or an unrelated male (UM) during the 4 weeks following male introduction

	Number of						
Behaviour	Control mothers*	UM mothers	Control daughters†	UM daughters†	Control mothers vs UM mothers‡	Control daughters vs UM daughters‡§	UM mothers vs UM daughters‡§
Sexual solicit to male	0/6	10/11	0/9 (0/7)	8/13 (7/11)	$P<0.005$	$P=0.01$	NS
Tongue in–out to male	0/6	9/11	0/9 (0/7)	4/13 (4/11)	$P<0.005$	NS	$0.025<P<0.05$
Tongue in–out from male	2/6	7/11	0/9 (0/7)	7/13 (7/11)	NS	$P=0.01$	NS
Attempted mount from male	1/6	4/11	0/9 (0/7)	4/13 (4/11)	NS	NS	NS
Mount from male	4/6	5/11	0/9 (0/7)	6/13 (5/11)	NS	NS	NS

*Excludes one mother that was permitted to continue a pregnancy that began immediately before father introduction.
†First set of numbers in each cell includes all focal daughters. Numbers in parentheses exclude the anovulatory member of each female twin pair.
‡Comparisons were performed using Fisher's exact tests and assessed using a modified α of 0.017 (Bonferroni correction).
§Analyses exclude the anovulatory member of each female twin pair, so that each father's or unrelated male's behavioural interactions with daughters are represented only once.

than if they had never ovulated prior to or during the period of behavioural observations (six of six cycling versus two of seven anovulatory daughters; Fisher's exact test: $N = 13$, $P = 0.033$).

Female Dominance Relationships

In the 2 weeks prior to MI, eight daughters (six unrelated male and two control) performed vocal submission to their mothers in separation–reunion tests (number of vocal submits performed by these daughters: 173.6 ± 131.3, range 6–1089; number of tests in which individual daughters submitted: 4.6 ± 0.7, range 1–6) and were therefore considered to be subordinate to their mothers. One mother submitted to her daughter, and in no family did both mother and daughter submit to each other. MI procedures did not appear to alter dominance relationships between mothers and daughters. Of the 22 daughters, 21 showed the same pattern of submissive behaviour towards the mother in separation–reunion tests before and after MI. The only mother that submitted to a daughter did so both before and after MI. Daughters with female co-twins were less likely to submit to their mother than daughters without female co-twins, although this effect was significant only after MI (Fisher's exact test: $N = 22$, $P = 0.040$; Table 3). This difference between daughters might have reflected a difference in testing procedures: daughters with female co-twins were reunited simultaneously with the co-twin and the mother, whereas other daughters were reunited with only the mother. Daughters that did and did not submit to their mothers did not differ in age at the time of MI (unpaired t test: $t_{20} = -1.353$, $P = 0.191$).

To determine whether daughters that underwent ovulation suppression were more likely to be behaviourally subordinate to their mothers than daughters that ovulated, we classified each daughter as anovulatory or ovulatory (ovulated at least once before or during the period of separation–reunion tests), and subordinate or not subordinate to her mother, for the 2 weeks before and 4 weeks after MI. For all daughters considered together, Fisher's exact tests indicated that ovulatory and anovulatory daughters did not differ in their likelihood of submitting to their mothers. When we excluded daughters with female co-twins, however, we found a significant relationship between subordinate status and ovulatory function. Both before and after MI, daughters either were anovulatory and submissive to their mothers, or underwent ovulatory cycles and did not behave submissively (Fisher's exact test: pre-MI: $N = 14$, $P = 0.004$; post-MI: $N = 14$, $P = 0.00058$; Table 3). Patterns of spontaneous submissive behaviour observed during focal animal observations were consistent with results of separation–reunion tests: in the 4 weeks after MI, anovulatory daughters performed significantly more vocal submission to their mothers than did ovulatory daughters ($\overline{X} \pm$ SE: 11.6 ± 11.0/h versus 0.03 ± 0.01/h; Mann–Whitney U test: $U = 17.000$, $N_1 = 12$, $N_2 = 11$, $P = 0.002$). Neither aggression received from the mother (0.8 ± 0.1/h; $U = 81.500$, $N_1 = 12$, $N_2 = 11$, $P = 0.338$) nor aggression performed to the mother (0.2 ± 0.06/h; $U = 62.500$, $N_1 = 12$, $N_2 = 11$, $P = 0.818$) during focal animal observations, however, differed between ovulatory and anovulatory daughters. Aggression between mothers and daughters was mild and infrequent: only two fights (in a single mother–daughter pair, before MI) were observed.

Table 3. Number of daughters that were ovulatory or anovulatory, and that did or did not perform vocal submission to their mothers in separation–reunion tests, in the 2 weeks before and the 4 weeks after male introduction. Daughters in female–female twin pairs are shown separately, in parentheses, because they performed vocal submission at lower rates than those without a female co-twin

Reproductive status of daughters	Submitted to mother?	
	Yes	No
Before male introduction		
Ovulatory	0 (0)	6 (2)
Anovulatory	7 (1)	1* (5)
After male introduction		
Ovulatory	0 (0)	7 (3)
Anovulatory	7 (0)	0 (5)

*This daughter was anovulatory by our criterion of two consecutive plasma progesterone values greater than 10 ng/ml. However, plasma progesterone was elevated in the first blood sample collected from this animal, indicating that she was terminating a luteal phase at that time and had therefore ovulated prior to data collection.

DISCUSSION

The results of this controlled, experimental study confirm that replacement of the breeding male (biological father) by an unrelated male in captive families of common marmosets may frequently lead to the onset of breeding by a previously nonreproductive daughter, and hence, to the emergence of plural cooperative breeding. Introduction of an unrelated male led to conception in a daughter in eight of 11 families, and to production of live infants by both mother and daughter in six of these families. In contrast, conceptions were never detected in any daughter that remained with both biological parents. Moreover, in 12 years' time, during which approximately 85 marmoset daughters in our colony have been housed with both biological parents through at least 18 months of age, we have never confirmed a pregnancy in such a daughter (Saltzman et al. 1997a, unpublished data). Thus, while plural cooperative breeding might be relatively uncommon in this species, it occurs frequently in captive groups containing an unrelated adult male. Our results indicate, however, that this outcome does not occur invariably. The quality of the pre-existing relationship between females is the key determining factor: only those daughters that are not behaviourally subordinate to their mothers undergo ovulatory cycles, and thus, only these daughters can contribute to the onset of plural breeding.

The emergence of plural breeding was mediated by the activation of sexual behaviour in daughters undergoing ovulatory cycles. Daughters were never observed engaging in sexual behaviours with their fathers, but most did so with an unrelated male. These results are consistent with previous findings by us and others that common

marmosets usually avoid mating with familiar, closely related individuals (Abbott 1984; König et al. 1988; Saltzman et al. 1997b, c; Baker et al. 1999; but see Epple 1970; Anzenberger & Simmen 1987; Crook 1988; Adler & Jämmrich 1991) but that nonbreeding daughters will often mate readily with an unfamiliar, unrelated male that is either introduced into the family (Kirkpatrick-Tanner et al. 1996; Saltzman et al. 1997b, c) or paired briefly with the daughter (Anzenberger 1985; Hubrecht 1989). Inbreeding avoidance, therefore, is a key determinant of reproductive failure among marmosets living with their natal families, especially among daughters that are not clearly subordinate to their mothers and that are undergoing potentially fertile ovulatory cycles.

Although unrelated males clearly altered the likelihood that daughters would engage in sexual behaviour, we found little evidence that they triggered the onset of ovulatory cyclicity or stimulated pituitary or ovarian endocrine function in daughters. More daughters ovulated in the 16 weeks following introduction of unrelated males than in the 8 preceding weeks, but the same pattern occurred among control daughters. We have shown previously that the incidence of ovulatory cyclicity among captive female common marmosets housed with their natal families increases gradually from 12 to at least 20 months of age (Saltzman et al. 1997a). In our previous study (Saltzman et al. 1997a), the largest rise in incidence of ovulation among daughters occurred around 18 months of age, comparable to the age of daughters in the present study at the time of male introduction. Thus, the increasing incidence of ovulation among daughters in the present study appears to reflect maturational effects, rather than stimulation from an unrelated male. Nevertheless, all daughters in this study were likely to be fully mature and physiologically capable of ovulating at least by the end of data collection, which occurred, on average, at 22 months of age. First ovulation in female common marmosets can occur at approximately 11–13 months of age, although the onset of ovarian cyclicity may be delayed by social suppression (Abbott & Hearn 1978; Saltzman et al. 1997a). Among 136 daughters in our colony that could be classified as reproductively mature (i.e. ovulated at least once) or possibly immature (i.e. anovulatory but not housed with a dominant female) by 22 months of age, 130 (95.6%) were reproductively mature. Thus, anovulation in the behaviourally subordinate daughters in the present study was probably caused by social suppression rather than by immaturity.

Among daughters that remained anovulatory throughout the present study, introduction of an unrelated male did not lead to even transient changes in urinary concentrations of LH or oestrogen metabolites. Furthermore, among daughters that began to ovulate only after male introduction, those in the unrelated male and control conditions did not differ reliably in the latency from male introduction to first ovulation. Finally, we found only one (marginally) significant effect of unrelated males on daughters' ovarian cycle parameters: shortening of the follicular phase. Other cycle parameters, such as duration of the luteal phase and concentrations of circulating P, urinary LH and urinary oestrogen metabolites, showed no trends towards differences between groups. These results must be interpreted cautiously, as sample sizes were small (three to six per group). None the less, introduction of an unrelated male into a family clearly failed to increase the likelihood of ovulation and appeared to have only minimal effects on reproductive endocrine function in daughters.

These results contrast with our previous findings that marmoset daughters were more likely to ovulate in families containing an unrelated male than in intact natal families, and that introduction of an unrelated male into a family could apparently lead to the rapid onset of ovulatory cyclicity in previously anovulatory daughters (Saltzman et al. 1997a, b). This disparity probably reflects the fact that the two earlier studies used much smaller numbers of daughters in families containing unrelated males. Our results also differ from findings in cottontop tamarins, which require stimulation by unfamiliar males in order to initiate ovulatory cyclicity (Widowski et al. 1990, 1992). In common marmosets, exposure to unrelated males appears to be neither necessary nor sufficient to activate ovulatory function in females. It may be relevant, however, that in our colony, all daughters have visual, auditory and olfactory access to unrelated males in other cages. Therefore, we cannot rule out the possibility that distal cues from unrelated males contribute to the onset of ovulatory function in marmoset daughters. Nevertheless, the key social determinant of ovulatory function in adult daughters appears to be the mother–daughter dominance relationship.

In contrast to the presence of an unrelated male, the quality of a daughter's relationship with her mother was strongly associated with the daughter's ovarian status. Only 36% of daughters (50%, excluding female twin pairs) submitted to their mothers in separation–reunion tests, and these daughters were significantly less likely to ovulate than those that did not behave submissively. Clearly, we cannot infer causation from these correlational data alone. However, other studies involving a number of experimental approaches, including introduction of previously cycling females into social groups in which they became subordinate, removal of subordinate females from groups and use of exogenous hormones to stimulate ovulation in subordinates, have demonstrated that social subordination determines anovulation in this species, and not vice versa (Abbott & George 1991; Abbott et al. 1997).

Our findings challenge the common assumption that marmoset daughters are consistently subordinate to their mothers. Furthermore, although it has long been established that anovulation results from social subordination in female common marmosets (Abbott & George 1991), our findings demonstrate that variation in the quality of the behavioural relationship between females is associated with variation in the occurrence of ovulation suppression. Similar results have recently been obtained by Alencar and colleagues (Alencar et al. 1995; A. I. Alencar, M. B. C. Sousa & M. E. Yamamoto, unpublished data), who observed submissive behaviour and asymmetric agonistic relationships in pairs of female marmosets in which only one individual ovulated, but not in pairs in which both females (or neither) ovulated. The sources of this variation

in female–female relationships are unknown but probably primarily involve differences in the presumptive subordinates' perceptions of themselves as subordinate to another female, rather than differences in agonistic behaviours performed by the presumptive dominants: we found no evidence that ovulatory and anovulatory daughters received different frequencies or intensities of aggression from their mothers (see also Saltzman 2003).

In some cooperatively breeding species, replacement of the original breeding male by an unrelated, unfamiliar male is followed by intensified aggression among females, which can be associated with usurpation of the dominant position by a daughter or eviction of subordinates by the dominant female (pine vole, *Microtus pinetorum*: Brant et al. 1998; Damaraland mole-rat, *Cryptomys damarensis*: Cooney & Bennett 2000; meerkat: O'Riain et al. 2000). In contrast, we found no evidence that unrelated males altered dominance relationships among female common marmosets. All but one daughter showed the same pattern of submissive behaviour (i.e. did or did not submit) to the mother before and after introduction of an unrelated male. Furthermore, agonism between females did not appear to intensify during the 4 weeks following male introduction, and males were not observed participating in agonistic interactions between females (unpublished data). Thus, the onset of breeding by daughters following unrelated male introduction was not associated with weakening, intensification or reversal of mother–daughter dominance relationships or termination of rank-related reproductive suppression.

Anovulatory daughters were significantly less likely to engage in sexual interactions with unrelated males than were daughters undergoing ovulatory cycles. This finding is perhaps not surprising, but the cause is unclear. Anovulatory daughters might have been behaviourally inhibited by the presence of their mothers, to whom they were subordinate. Anzenberger (1985) found that sexual and affiliative interactions between nonbreeding adult female marmosets and unfamiliar breeding males in brief pair tests were inhibited by visual contact with or presence of either animal's family. However, behavioural inhibition of daughters by their mothers (or other family members) is unlikely to explain the low frequencies of sexual behaviours received by subordinate, anovulatory daughters from unrelated males, as compared to cycling daughters. A second possibility is that the failure of most anovulatory daughters to perform or receive sexual behaviours might have been a direct result of their suppressed reproductive hormones. In contrast to many nonprimate species, ovarian hormones are not essential for the expression of sexual behaviour in marmosets and other anthropoid primates (Dixson 2001). None the less, when observed under conditions of species-appropriate social complexity, marmosets and other primates show clear hormonal influences on sexual behaviour, with proceptivity and attractivity peaking around the time of ovulation (Dixson 2001; Wallen 2001). Thus, the failure of most anovulatory daughters in this study to either receive sexual behaviours from or perform them to unrelated males is likely to have resulted from reduced attractivity and proceptivity, secondary to rank-related suppression of ovarian hormones.

Unexpectedly, replacement of the breeding male by an unrelated male not only led to the onset of sexual behaviour in many daughters but also increased the number of mothers performing sexual behaviours. In the 4 weeks following male introduction, no mothers performed tongue in–out or sexual solicitation to their original mates, but 10 of 11 did so to new mates; half of these latter females performed courtship behaviours on the first day following unrelated male introduction. Mated female marmosets have often been found to show mate fidelity, responding antagonistically to unfamiliar adult males and abstaining from sexual interactions with them (Evans 1983; Sutcliffe & Poole 1984a; Anzenberger 1985; see also Lazaro-Perea 2001). Consistent with the present findings, however, recent evidence indicates that female common marmosets may behave promiscuously under both laboratory and field conditions. For example, we previously found that adult females in the early follicular phase of the ovarian cycle readily engaged in sexual behaviour with unfamiliar males, following overnight removal from their own families, and performed more proceptive and receptive behaviours to unfamiliar males than to their own mates (Baker et al. 1999). Moreover, in a field study of plurally breeding marmoset groups, both breeding and nonbreeding females copulated with males from neighbouring groups (Digby 1999). The reasons for these disparities between studies are not clear. In the present experiment, mothers' sexual behaviour might have been influenced by the presence of a potential female competitor (i.e. adult daughter). However, most mothers performed proceptive behaviours towards unrelated males, even if their daughters were subordinate and anovulatory and were therefore unlikely to represent reproductive competition. Alternatively, the finding that mothers with new mates were more likely to perform sexual behaviour, but were not more likely to receive sexual behaviour, than mothers housed with their original mates might indicate that females initiate a greater proportion of sexual interactions in new pairs than in established pairs.

Several functional explanations can be offered for the finding that marmoset daughters are more likely to breed in families containing an unrelated male than in intact natal families (see also Saltzman et al. 1997a; Saltzman 2003). First, daughters that mate with an unrelated male may be more likely to produce viable offspring than those that mate with their fathers or brothers. Studies of both captive (Ralls & Ballou 1982) and wild (Dietz & Baker 1993) callitrichines have shown that inbreeding can significantly increase infant mortality. Second, replacement of the father by an unrelated male significantly reduces a daughter's genetic payoffs for serving as a nonreproductive alloparent. Daughters in intact families with both biological parents present can assist in rearing their own full siblings, to which they are as closely related, on average, as they would be to their own offspring (coefficient of relatedness: $r = 0.50$). In contrast, a daughter whose father has been replaced by an unrelated male can help to rear only half-siblings ($r = 0.25$), to which she is less closely related, on average, than she would be to her own offspring (Saltzman et al. 1997a; Saltzman 2003; see

also Vehrencamp 1983). Thus, replacement of the father by an unrelated male both enhances a daughter's opportunities for accruing direct fitness and reduces her opportunities for accruing indirect fitness. Why, then, do some (i.e. behaviourally subordinate) adult daughters in these families continue to forgo direct reproduction? One possible answer is that these subordinate daughters may be less likely to successfully rear infants than non-subordinate daughters. Infanticide, apparently committed by breeding females on one another's infants, may be a significant cause of infant mortality in both wild and captive, plurally breeding groups of common marmosets (reviewed in Saltzman 2003). Daughters that are subordinate to their mothers may be less able to defend their infants against attack by the mother. Alternatively, subordinate daughters and/or their infants may be less likely to compete successfully with their mothers (or dominant sisters) and/or their infants for access to critical resources such as food or alloparents (Digby 1995a).

The findings of this experiment confirm and extend anecdotal reports from other laboratories and from the field that plural breeding in callitrichines may be associated with the presence of an unrelated adult male (reviewed in: French 1997; Saltzman 2003). Rothe & Koenig (1991) conducted a retrospective survey of departures from monogamy in captive colonies of common marmosets and concluded that 'the occurrence of polygyny was related to the integration of a male newcomer and the replacement of the former breeding male, which terminated sexual and reproductive suppression of the highest-ranking daughter' (page 31). Among wild callitrichines, immigration of an adult male has been associated with plural breeding in common marmosets (Nievergelt et al. 2000), golden lion tamarins (Dietz & Baker 1993), cottontop tamarins (Savage et al. 1996), and moustached tamarins, *Saguinus mystax* (Smith et al. 2001), and with usurpation of the breeding position by a previously nonreproductive daughter in buffy-headed marmosets, *Callithrix flaviceps* (Ferrari & Diego 1992). The most compelling evidence from wild callitrichines comes from an 8-year field study of golden lion tamarins, which showed that young adult (2-year-old) females living with their mothers became pregnant only in groups containing an unrelated immigrant male; however, no such relationship between likelihood of conceiving and the presence of an unrelated male in the group was found among older daughters (Baker et al. 2002). Interestingly, recent mitochondrial DNA analyses of two populations of wild common marmosets provide support for our conclusions that both inbreeding avoidance and rank-related reproductive suppression constrain breeding in adult females. Faulkes et al. (2003) found that seven of nine breeding females, as well as several nonbreeding adult females, were from different matrilines than their group's breeding male. They interpreted these data as evidence that inbreeding avoidance is important in structuring both social and reproductive dynamics but that intrasexual dominance relationships may also inhibit reproduction in some adult females.

In conclusion, the results of the present study confirm that at least two social factors can contribute to reproductive inactivity of marmoset daughters living with their natal families, and therefore, to maintenance of singular breeding: ovulation suppression in daughters that are behaviourally subordinate to their mothers, and inbreeding avoidance among daughters that are not behaviourally subordinate to their mothers but do not have access to an unrelated male. Thus, the onset of breeding by a previously nonreproductive female marmoset, and hence, the transition from singular to plural cooperative breeding, may depend upon both the absence of a clear dominance relationship between females and the immigration of an unrelated male into the group. Recent studies of other cooperatively breeding species have similarly highlighted the importance of both intersexual (inbreeding avoidance) and intrasexual (dominance) interactions in causing reproductive failure in nonbreeders (Damaraland mole-rat: Bennett et al. 1996; prairie vole, *Microtus ochrogaster*: Carter & Roberts 1997; acorn woodpecker, *Melanerpes formicivorus*: Koenig et al. 1998; meerkat: O'Riain et al. 2000; Mongolian gerbil: Clark & Galef 2001, 2002), although the relative importance of these two factors may differ among species.

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References

Abbott, D.H. 1979. The sexual development of the common marmoset monkey *Callithrix jacchus jacchus*. Ph.D. thesis, University of Edinburgh.

Abbott, D. H. 1984. Behavioral and physiological suppression of fertility in subordinate marmoset monkeys. *American Journal of Primatology*, **6**, 169–186.

Abbott, D. H. 1993. Social conflict and reproductive suppression in marmoset and tamarin monkeys. In: *Primate Social Conflict* (Ed. by W. A. Mason & S. P. Mendoza), pp. 331–372. Albany: State University of New York Press.

Abbott, D. H. & George, L. M. 1991. Reproductive consequences of changing social status in female common marmosets. In: *Primate*

Responses to Environmental Change (Ed. by H. O. Box), pp. 295–309. London: Chapman & Hall.

Abbott, D. H. & Hearn, J. P. 1978. Physical, hormonal and behavioural aspects of sexual development in the marmoset monkey, *Callithrix jacchus*. *Journal of Reproduction and Fertility*, **53,** 155–166.

Abbott, D. H., Saltzman, W., Schultz-Darken, N. J. & Smith, T. E. 1997. Specific neuroendocrine mechanisms not involving generalized stress mediate social regulation of female reproduction in cooperatively breeding marmoset monkeys. *Annals of the New York Academy of Sciences*, **807,** 219–238.

Adler, H. J. & Jämmrich, S. 1991. Breeding success in a polygynous group of *Callithrix jacchus* (L. 1758) in the Allwetterzoo Münster: a short report. *Primate Report*, **31,** 105–107.

Alencar, A. I., Yamamoto, M. E., Oliveira, M. S., Lopes, F. A., Sousa, M. B. C. & Silva, N. G. 1995. Behavior and progesterone levels in *Callithrix jacchus* females. *Brazilian Journal of Medical and Biological Research*, **28,** 591–595.

Anzenberger, G. 1985. How stranger encounters of common marmosets (*Callithrix jacchus jacchus*) are influenced by family members: the quality of behavior. *Folia Primatologica*, **45,** 204–224.

Anzenberger, G. & Simmen, C. 1987. Father–daughter incest in a family of common marmosets (*Callithrix jacchus*) (abstract). *International Journal of Primatology*, **8,** 524.

Baker, A. J., Bales, K. & Dietz, J. M. 2002. Mating system and group dynamics in lion tamarins. In: *Lion Tamarins: Biology and Conservation* (Ed. by D. G. Kleiman & A. B. Rylands), pp. 188–212. Washington, D.C.: Smithsonian Institution Press.

Baker, J. V., Abbott, D. H. & Saltzman, W. 1999. Social determinants of reproductive failure in male common marmosets housed with their natal family. *Animal Behaviour*, **58,** 501–513.

Bennett, N. C., Faulkes, C. G. & Molteno, A. J. 1996. Reproductive suppression in subordinate, non-breeding female Damaraland mole-rats: two components to a lifetime of socially induced infertility. *Proceedings of the Royal Society of London, Series B*, **263,** 1599–1603.

Brant, C. L., Schwab, T. M., Vandenbergh, J. G., Schaefer, R. L. & Solomon, N. G. 1998. Behavioural suppression of female pine voles after replacement of the breeding male. *Animal Behaviour*, **55,** 615–627.

Carter, C. S. & Roberts, R. L. 1997. The psychobiological basis of cooperative breeding in rodents. In: *Cooperative Breeding in Mammals* (Ed. by N. G. Solomon & J. A. French), pp. 231–266. Cambridge: Cambridge University Press.

Clark, M. M. & Galef, B. G., Jr. 2001. Socially induced infertility: familial effects on reproductive development of female Mongolian gerbils. *Animal Behaviour*, **62,** 897–903.

Clark, M. M. & Galef, B. G., Jr. 2002. Socially induced delayed reproduction in female Mongolian gerbils (*Meriones unguiculatus*): is there anything special about dominant females? *Journal of Comparative Psychology*, **116,** 363–368.

Converse, L. J., Carlson, A. A., Ziegler, T. E. & Snowdon, C. T. 1995. Communication of ovulatory state to mates by female pygmy marmosets, *Cebuella pygmaea*. *Animal Behaviour*, **49,** 615–621.

Cooney, R. & Bennett, N. C. 2000. Inbreeding avoidance and reproductive skew in a cooperative mammal. *Proceedings of the Royal Society of London, Series B*, **267,** 801–806.

Creel, S. R. & Waser, P. M. 1991. Failures of reproductive suppression in dwarf mongooses (*Helogale parvula*): accident or adaptation? *Behavioral Ecology*, **2,** 7–15.

Creel, S. R. & Waser, P. M. 1997. Variation in reproductive suppression among dwarf mongooses: interplay between mechanisms and evolution. In: *Cooperative Breeding in Mammals* (Ed. by N. G. Solomon & J. A. French), pp. 150–170. Cambridge: Cambridge University Press.

Crook, G. A. 1988. An incidence of the breakdown of incest taboo in the common marmoset. *Australian Primatology*, **3,** 23.

De Vleeschouwer, K., Van Elsacker, L. & Leus, K. 2001. Multiple breeding females in captive groups of golden-headed lion tamarins (*Leontopithecus chrysomelas*): causes and consequences. *Folia Primatologica*, **72,** 1–10.

Dietz, J. M. & Baker, A. J. 1993. Polygyny and female reproductive success in golden lion tamarins, *Leontopithecus rosalia*. *Animal Behaviour*, **46,** 1067–1078.

Digby, L. 1995a. Infant care, infanticide, and female reproductive strategies in polygynous groups of common marmosets (*Callithrix jacchus*). *Behavioral Ecology and Sociobiology*, **37,** 51–61.

Digby, L. J. 1995b. Social organization in a wild population of *Callithrix jacchus*: II. Intragroup social behavior. *Primates*, **36,** 361–375.

Digby, L. J. 1999. Sexual behavior and extragroup copulations in a wild population of common marmosets (*Callithrix jacchus*). *Folia Primatologica*, **70,** 136–145.

Digby, L. J. & Ferrari, S. F. 1994. Multiple breeding females in free-ranging groups of *Callithrix jacchus*. *International Journal of Primatology*, **15,** 389–397.

Dixson, A. F. 2001. The evolution of neuroendocrine mechanisms regulating sexual behaviour in female primates. *Reproduction, Fertility and Development*, **13,** 599–607.

Doolan, S. P. & Macdonald, D. W. 1997. Band structure and failures of reproductive suppression in a cooperatively breeding carnivore, the slender-tailed meerkat (*Suricata suricatta*). *Behaviour*, **134,** 827–848.

Eastman, S.-A. K., Makawiti, D. W., Collins, W. P. & Hodges, J. K. 1984. Pattern of excretion of urinary steroid metabolites during the ovarian cycle and pregnancy in the marmoset monkey. *Journal of Endocrinology*, **102,** 19–26.

Epple, G. 1970. Maintenance, breeding, and development of marmoset monkeys (Callithricidae) in captivity. *Folia Primatologica*, **12,** 56–76.

Evans, S. 1983. The pair-bond of the common marmoset, *Callithrix jacchus jacchus*: an experimental investigation. *Animal Behaviour*, **31,** 651–658.

Faulkes, C. G., Arruda, M. F. & Monteiro da Cruz, M. A. O. 2003. Matrilineal genetic structure within and among populations of the cooperatively breeding common marmoset, *Callithrix jacchus*. *Molecular Ecology*, **12,** 1101–1108.

Ferrari, S. F. & Diego, V. H. 1992. Long-term changes in a wild marmoset group. *Folia Primatologica*, **58,** 215–218.

French, J. A. 1997. Proximate regulation of singular breeding in callitrichid primates. In: *Cooperative Breeding in Mammals* (Ed. by N. G. Solomon & J. A. French), pp. 34–75. Cambridge: Cambridge University Press.

Fuller, T. K., Kat, P. W., Bulger, J. B., Maddock, A. H., Ginsberg, J. R., Burrows, R., McNutt, J. W. & Mills, M. G. L. 1992. Population dynamics of African wild dogs. In: *Wildlife 2001: Populations* (Ed. by D. R. McCullough & R. H. Barrett), pp. 1125–1139. London: Elsevier.

Harlow, C. R., Gems, S., Hodges, J. K. & Hearn, J. P. 1983. The relationship between plasma progesterone and the timing of ovulation and early embryonic development in the marmoset monkey (*Callithrix jacchus*). *Journal of Zoology*, **201,** 273–282.

Hubrecht, R. C. 1989. The fertility of daughters in common marmoset (*Callithrix jacchus jacchus*) family groups. *Primates*, **30,** 423–432.

Jarvis, J. U. M. 1991. Reproduction of naked mole-rats. In: *The Biology of the Naked Mole-rat* (Ed. by P. W. Sherman, J. U. M. Jarvis & R. D. Alexander), pp. 384–425. Princeton, New Jersey: Princeton University Press.

Johnstone, R. A. 2000. Models of reproductive skew: a review and synthesis. *Ethology*, **106,** 5–26.

Keane, B., Waser, P. M., Creel, S. R., Creel, N. M., Elliott, L. F. & Minchella, D. J. 1994. Subordinate reproduction in dwarf mongooses. *Animal Behaviour*, **47**, 65–75.

Kirkpatrick-Tanner, M., Aeschlimann, C. & Anzenberger, G. 1996. Occurrence of an infanticide within a captive polygynous group of common marmosets, *Callithrix jacchus*. *Folia Primatologica*, **67**, 52–58.

Koenig, W. D., Haydock, J. & Stanback, M. T. 1998. Reproductive roles in the cooperatively breeding acorn woodpecker: incest avoidance versus reproductive competition. *American Naturalist*, **151**, 243–255.

König, A., Rothe, H., Siess, M., Darms, K., Gröger, D., Radespiel, U. & Rock, J. 1988. Reproductive reorganization in incomplete groups of the common marmoset (*Callithrix jacchus*) under laboratory conditions. *Zeitschrift für Säugetierkunde*, **53**, 1–6.

Lazaro-Perea, C. 2001. Intergroup interactions in wild common marmosets, *Callithrix jacchus*: territorial defence and assessment of neighbours. *Animal Behaviour*, **62**, 11–21.

Livesey, J. H., Roud, H. K., Metcalf, M. G. & Donald, R. A. 1983. Glycerol prevents loss of immunoreactive follicle-stimulating hormone and luteinizing hormone from frozen urine. *Journal of Endocrinology*, **98**, 381–384.

Nievergelt, C. M., Digby, L. J., Ramakrishnan, U. & Woodruff, D. S. 2000. Genetic analysis of group composition and breeding system in a wild common marmoset (*Callithrix jacchus*) population. *International Journal of Primatology*, **21**, 1–20.

Oerke, A.-K., Einspanier, A. & Hodges, J. K. 1995. Detection of pregnancy and monitoring patterns of uterine and fetal growth in the marmoset monkey (*Callithrix jacchus*) by real-time ultrasonography. *American Journal of Primatology*, **36**, 1–13.

O'Riain, M. J., Bennett, N. C., Brotherton, P. N. M., Mellrath, G. & Clutton-Brock, T. H. 2000. Reproductive suppression and inbreeding avoidance in wild populations of cooperatively breeding meerkats (*Suricata suricatta*). *Behavioral Ecology and Sociobiology*, **48**, 471–477.

Price, E. C. & McGrew, W. C. 1991. Departures from monogamy in colonies of captive cotton-top tamarins. *Folia Primatologica*, **57**, 16–27.

Ralls, K. & Ballou, J. 1982. Effects of inbreeding on infant mortality in captive primates. *International Journal of Primatology*, **3**, 491–505.

Reeve, H. K., Emlen, S. T. & Keller, L. 1998. Reproductive sharing in animal societies: reproductive incentives or incomplete control by dominant breeders? *Behavioral Ecology*, **9**, 267–278.

Rothe, H. & Koenig, A. 1991. Variability of social organization in captive common marmosets (*Callithrix jacchus*). *Folia Primatologica*, **57**, 28–33.

Saltzman, W. 2003. Reproductive competition among female marmosets (*Callithrix jacchus*): proximate and ultimate causes. In: *Sexual Selection and Reproductive Competition in Primates: New Perspectives and Directions* (Ed. by C. B. Jones), pp. 197–229. Norman, Oklahoma: American Society of Primatologists.

Saltzman, W., Schultz-Darken, N. J., Scheffler, G., Wegner, F. H. & Abbott, D. H. 1994. Social and reproductive influences on plasma cortisol in female marmosets monkeys. *Physiology & Behavior*, **56**, 801–810.

Saltzman, W., Schultz-Darken, N. J. & Abbott, D. H. 1996. Behavioural and endocrine predictors of dominance and tolerance in female common marmosets, *Callithrix jacchus*. *Animal Behaviour*, **51**, 657–674.

Saltzman, W., Schultz-Darken, N. J. & Abbott, D. H. 1997a. Familial influences on ovulatory function in common marmosets (*Callithrix jacchus*). *American Journal of Primatology*, **41**, 159–177.

Saltzman, W., Schultz-Darken, N. J., Severin, J. M. & Abbott, D. H. 1997b. Escape from social suppression of sexual behavior and of ovulation in female common marmosets. *Annals of the New York Academy of Sciences*, **807**, 567–570.

Saltzman, W., Severin, J. M., Schultz-Darken, N. J. & Abbott, D. H. 1997c. Behavioral and social correlates of escape from suppression of ovulation in female common marmosets housed with the natal family. *American Journal of Primatology*, **41**, 1–21.

Saltzman, W., Schultz-Darken, N. J., Wegner, F. H., Wittwer, D. J. & Abbott, D. H. 1998. Suppression of cortisol levels in subordinate female marmosets: reproductive and social contributions. *Hormones and Behavior*, **33**, 58–74.

Savage, A., Giraldo, L. H., Soto, L. H. & Snowdon, C. T. 1996. Demography, group composition and dispersal in wild cotton-top tamarin (*Saguinus oedipus*) groups. *American Journal of Primatology*, **38**, 85–100.

Smith, A. C., Tirado Herrera, E. R., Buchanan-Smith, H. M. & Heymann, E. W. 2001. Multiple breeding females and allonursing in a wild group of moustached tamarins (*Saguinus mystax*). *Neotropical Primates*, **9**, 67–69.

Sokal, R. R. & Rohlf, F. J. 1995. *Biometry*. 3rd edn. New York: W.H. Freeman.

Solomon, N. G. & French, J. A. 1997. The study of mammalian cooperative breeding. In: *Cooperative Breeding in Mammals* (Ed. by N. G. Solomon & J. A. French), pp. 1–10. Cambridge: Cambridge University Press.

Stevenson, M. F. & Rylands, A. B. 1988. The marmosets, genus *Callithrix*. In: *Ecology and Behavior of Neotropical Primates. Vol. 2* (Ed. by R. A. Mittermeier, A. B. Rylands, A. F. Coimbra-Filho & G. A. B. da Fonseca), pp. 131–222. Washington, D.C.: World Wildlife Fund.

Summers, P. M., Wennink, C. J. & Hodges, J. K. 1985. Cloprostenol-induced luteolysis in the marmoset monkey (*Callithrix jacchus*). *Journal of Reproduction and Fertility*, **73**, 133–138.

Sutcliffe, A. G. & Poole, T. B. 1984a. An experimental analysis of social interaction in the common marmoset (*Callithrix jacchus jacchus*). *International Journal of Primatology*, **3**, 591–607.

Sutcliffe, A. G. & Poole, T. B. 1984b. Intragroup agonistic behavior in captive groups of the common marmoset *Callithrix jacchus jacchus*. *International Journal of Primatology*, **5**, 473–489.

Vehrencamp, S. L. 1983. A model for the evolution of despotic versus egalitarian societies. *Animal Behaviour*, **31**, 667–682.

Wallen, K. 2001. Sex and context: hormones and primate sexual motivation. *Hormones and Behavior*, **40**, 339–357.

Webley, G. E. & Hearn, J. P. 1994. Embryo–maternal interactions during the establishment of pregnancy in primates. *Oxford Reviews of Reproductive Biology*, **16**, 1–32.

Widowski, T. M., Ziegler, T. E., Elowson, A. M. & Snowdon, C. T. 1990. The role of males in the stimulation of reproductive function in female cotton-top tamarins, *Saguinus o. oedipus*. *Animal Behaviour*, **40**, 731–741.

Widowski, T. M., Porter, T. A., Ziegler, T. E. & Snowdon, C. T. 1992. The stimulatory effect of males on the initiation but not the maintenance of ovarian cycling in cotton-top tamarins (*Saguinus oedipus*). *American Journal of Primatology*, **26**, 97–108.

Ziegler, T. E., Matteri, R. L. & Wegner, F. H. 1993. Detection of urinary gonadotropins in callitrichid monkeys with a sensitive immunoassay based upon a unique monoclonal antibody. *American Journal of Primatology*, **31**, 181–188.

Ziegler, T. E., Scheffler, G. & Snowdon, C. T. 1995. The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, *Saguinus oedipus*. *Hormones and Behavior*, **29**, 407–424.