



# Reproductive biology of captive male cottontop tamarin monkeys as a function of social environment

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The cottontop tamarin, *Saguinus oedipus oedipus*, is a cooperatively breeding monkey in which mature male and female offspring serve as helpers to assist in rearing younger siblings. Generally, only one female per social group reproduces; breeding restriction is mediated in postpubertal female offspring through low and acyclic levels of reproductive hormones. We investigated (1) reproductive activity of postpubertal male offspring, and (2) whether aggression towards male offspring and a cortisol-mediated stress response might restrict breeding of male offspring in the natal group. We examined sexual behaviour, olfactory communication and urinary hormone levels (testosterone, dihydrotestosterone, luteinizing hormone, cortisol) of the subject males while we manipulated their social environment from housing in natal groups to pairing with a novel female, and after the production of their own offspring. Mounting and erection rates of the male subjects were as high in the natal group as when paired with a novel female. However, most mounts in the natal group were directed towards other males, and complete copulation sequences did not occur with natal-group females. Social environment had no significant effect on olfactory investigation of breeding females. Although hormone levels increased significantly after the subjects were removed from the natal group, the elevation was transient; the hormone levels of subjects in their natal groups did not differ from the levels shown by the same males when successfully producing their own offspring. Male offspring received more contact aggression in the natal group than when paired with the novel female. However, most of the aggression was received from siblings rather than the breeding pair, and levels of cortisol did not correspond with levels of aggression. Thus, at both a behavioural and endocrine level, mature male offspring in captive natal groups were potentially fertile, but sexual activity with natal-group females appeared to be behaviourally restricted and directed instead towards group males. In wild cottontop tamarin groups, this reproductive potential may allow male helpers flexibility to respond to breeding opportunities.

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A cooperatively breeding species is one in which individuals other than the biological parents provide care for offspring. Among vertebrates, cooperative breeding has been documented in birds, fish and mammals, including members of the primate family Callitrichidae (marmoset and tamarin monkeys) (Stacey & Koenig 1990; Emlen 1991; Solomon & French 1997). When alloparents (also called helpers; Skutch 1935) are offspring born to a group member, they typically exhibit delayed dispersal from the group and do not reproduce successfully until after they leave the natal group even though they may be physiologically mature (Stacey & Koenig 1990; Solomon & French 1997). The behaviour and physiology of breeding

restriction in mature group members varies between cooperatively breeding species. Breeding restriction may be manifest at a behavioural level (e.g. male Harris' hawk, *Parabuteo unicinctus*: Mays et al. 1991; male dwarf mongooses, *Helogale parvula*: Creel et al. 1992, 1993; grey wolves, *Canis lupus*: Asa 1997), or endocrine and physiological effects may be linked with restricted reproduction (e.g. male pied kingfishers, *Ceryle rudis*: Reyer et al. 1986; white-browed sparrow weavers, *Plocepasser mahali*: Wingfield et al. 1991; female Harris' hawk: Mays et al. 1991; female dwarf mongooses: Creel et al. 1992; wild dogs, *Lycaon pictus*: Creel et al. 1997; microtine rodents: Carter & Roberts 1997).

The cottontop tamarin, *Saguinus oedipus oedipus*, is a small-bodied, arboreal callitrichid primate endemic to Colombia. Breeding females typically give birth to twins

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once per year in groups of related and unrelated individuals, ranging in size from two to 10 members (Neyman 1978; Savage et al. 1996). Cottontop tamarins breed cooperatively both in the wild (Snowdon & Soini 1988) and in captivity (Snowdon et al. 1985). In captive groups of cottontop tamarins, both male and female offspring can remain in a family group for several years and assist extensively in the rearing of younger siblings. Males apparently mature physically by 19 months of age (A. J. Ginther, A. A. Carlson, T. E. Ziegler, C. T. Snowdon, unpublished data). Female offspring in their natal groups reach puberty by 19 months of age (Tardif 1984; Ziegler et al. 1987b); but most evidence indicates that typically only the dam is fertile, and that reproductive suppression in captive female cottontop offspring occurs in the form of a complete lack of ovarian cyclicity as long as the female remains in her original natal group (French et al. 1984; Ziegler et al. 1987b; Widowski et al. 1990, 1992; Price & McGrew 1991; but see Heistermann et al. 1989). Savage et al. (1996; wild cottontop tamarins) and Price & McGrew (1991; captive cottontops) have documented social groups containing two pregnant females. In all instances, however, infants from only one of the females survived.

The role of males within the singular breeding system of cottontop tamarins is less clear. Polyandrous matings have been documented in both wild and captive groups of callitrichid species (*Callithrix* spp.: Rylands 1985; *Leontopithecus*: Baker et al. 1993; Dietz & Baker 1993; Dietz & Kleiman 1986; *Saguinus* spp.: Terborgh & Goldizen 1985; Goldizen 1987; Garber et al., 1991; Price & McGrew 1991). However, other studies of cottontops have reported only one reproductively active male per captive group (French et al. 1984) or did not observe copulations in wild multimale groups (Savage et al. 1996). Studies on captive males of other callitrichid species provide mixed evidence for constraint of male reproductive function. Some studies indicate at least some effect on sexual behaviour or physiology of male helpers (e.g. common marmosets, *C. jacchus*: Abbott 1984; Anzenberger 1985; black tufted-ear marmosets, *C. kuhli*: French & Schaffner 1995), whereas one study reports no suppression of reproductive hormones in male lion tamarins, *L. rosalia* (French et al. 1989).

The primary aims of this study were to (1) describe the degree of sexual activity and reproductive function in adult male cottontop tamarin offspring living as alloparents in their natal group and (2) compare these natal levels to those of the same males after removal from the natal group. We examined the sexual behaviour and concurrent urinary levels of reproductive hormones (luteinizing hormone, LH; testosterone, T; and a primary testosterone metabolite, dihydrotestosterone, DHT) in adult male offspring while the males were housed in their natal group and after we altered their social environment by removing them from the natal group and pairing them with a novel female. We then measured reproductive hormones in the same males after the successful production of their own offspring in their own family group. We expected to see lower levels of either sexual behaviour or reproductive hormones, or both, in the natal group, with

levels of reproductive hormones in males as fathers higher than levels in the natal group.

Investigation of female genitalia is a male precopulatory behaviour found in callitrichid monkeys (Dixson 1998 for review); and in cottontop tamarins, female scent secretions convey information about identity, ovulatory state and reproductive status (Epple 1988; Ziegler et al. 1993a; Washabaugh & Snowdon 1998). We measured olfactory investigation of the scent marks and genitalia of female group members by the male subjects in the natal group and after pairing to provide an indication of the males' interest in cycling females even in the event that other reproductive behaviours were suppressed in the natal group. Scent-marking rates of male subjects were also measured.

The secondary aim of this study was to examine a mechanism that might be involved in restricting breeding activity or reproductive function of adult male offspring in the natal group. We measured rates of contact aggression received by subject males in the natal group and, for comparison, after pairing with a novel female. We expected subjects to receive higher levels of aggression in the natal group than when paired, with either member of the breeding pair contributing more aggression than other natal group members and more than the male's mate in the paired phase. To determine whether a cortisol-mediated stress response might serve as a physiological intermediate between aggression and any decreased reproductive function in the natal group (Sapolsky 1990, 1992), we measured urinary cortisol during natal housing and through successful production of the subjects' own offspring.

## METHODS

### Animal Care

The subjects belonged to a colony of *S. o. oedipus* at the University of Wisconsin, Madison, Psychology Department (Snowdon et al. 1985). All colony animals were socially housed typically as either a mated pair or in family groups. Cottontop tamarins born in the colony were reared by natal group members. When multiple social groups were in one room, cages were spaced to prevent physical contact between members of different groups; and visual contact was prevented by opaque fabric sheets. Restraint and handling of animals rarely occurred. Most medical and health care procedures were administered without handling or disruption to the animals' environment. Transfer of animals between cages or rooms was usually accomplished with the use of removable runways constructed from ventilation ducting (15.2-cm diameter thermoplastic urethane reinforced with an internal wire helix, Hi-tech Hose Inc., Chicago, Illinois, U.S.A.) and wheeled cages (66 × 188 × 64 cm) or portable carriers.

Cages were constructed of black polyurethane-coated steel mesh on anodized aluminium framing. Cage size for family groups ranged from 236 × 220 × 186 cm (L × H × W) to 285 × 234 × 186 cm; cages for two paired animals were 160 × 236 × 93 cm. The upper half of the

**Table 1.** Natal social environment and subject history

Subject	Natal phase start age (years)	Natal group structure	Natal	Adjacent	Paired	Father*	Father phase start age (years)	Parity sampled
Hu	4.9	D, S, M <sub>7</sub> , F <sub>5</sub> , F <sub>6</sub>	BH	H	BH			
Ni†	2.0	D, S, M <sub>2</sub> , M <sub>2</sub> , m <sub>3</sub> , m <sub>3</sub> , F <sub>1</sub>	BH	H	BH	H	4.4	Third
Ti	2.3	D, S, M <sub>2</sub> , m <sub>3</sub> , m <sub>3</sub> , F <sub>2</sub> , f <sub>4</sub> , f <sub>4</sub>	BH	H	BH	H	3.8	Second
Sq	2.0	D, S, M <sub>2</sub> , m <sub>3</sub> , F <sub>1</sub> , F <sub>2</sub> , f <sub>3</sub>	BH	H	BH	H	4.8	Third
Xy	2.0	D, S, m <sub>3</sub> , F <sub>1</sub> , F <sub>-3</sub> , f <sub>2</sub> , f <sub>2</sub> , f <sub>3</sub>	BH	H	BH	H	5.0	Second
Ng‡	2.4	S, M		H	H	H	6.6	Fifth
Ra§	1.7	S, M		H	H			

Natal group structure (members present in addition to the subject male): Dam (D); Sire (S); male siblings (M) and female siblings (F) older than 19 months of age were designated postpubertal; males (m) and females (f) younger than 19 months of age. The subject male was designated as parity<sub>1</sub>, and siblings were ranked by parity with respect to each subject (subscript numbers). Sampling of behaviour (B) or urine (H) is indicated for each male in each of the phases.

\*Two of the original seven subject males were transferred to other colonies after the paired phase and so were not included in the father phase.

†For a portion of the natal phase the sire of Ni was housed adjacent to the natal cage with full visual and auditory exposure, no physical contact; he was released into the natal group cage and socialized each day. There were no concurrent experimental manipulations or deviations in standard animal care.

‡During the natal phase Ng was housed in a cage adjacent to sire and male sibling (Ra) with full visual and auditory exposure, no physical contact.

§During the natal phase Ra was housed with sire and adjacent to Ng.

cage was equipped with a system of pine planks, natural tree branches, sisal rope, stainless steel and aluminium platforms, and a nestbox of clear acrylic or polycarbonate sheeting. Density of the cage equipment was consistent among cages. Cage floors were covered with pine shavings. Platforms and nestboxes were sanitized and shavings were changed weekly. New branches were fitted approximately every 4 months during sanitization of the entire cage. Animals received full-spectrum overhead fluorescent light (GE Chroma 75) from 0800/0900 to 2000 hours daily with no external source of sunlight. Ambient temperature was maintained within the range of 25.6–27.8°C. Relative humidity, measured over a 12-month period, varied from 11 to 66%.

Animals had access to water ad libitum and were fed three times daily from heavy duty plastic and aluminium bowls on stainless steel and aluminium platforms at least 1 m above the floor. Yogurt, applesauce and supplemental vitamins were provided between 0800 and 0930 hours, shortly after the animals awoke. The main feed occurred between 1145 and 1300 hours and consisted of Zupreem Marmoset Diet and Purina New World Monkey Chow coated with powdered L-ascorbic acid (ICN Biomedicals, Inc., Aurora, Ohio, U.S.A.) dissolved with water, and topped with supplemental fruits, vegetables, potatoes or bread. Between 1430 and 1700 hours, animals received supplemental protein such as peanuts, hard-boiled egg, cottage cheese, canned tuna, or mealworms.

## Subjects and Study Design

All study subjects ( $N=7$ ) were healthy, postpubertal (Gintner et al. 2000, unpublished data), eldest-male offspring born in our colony and were of colony breeding age. Each male subject had been housed with only his natal family group since birth. At the start of the study, five males were each housed with dam, sire, and male and

female siblings; one male (Ra) was housed with his sire only; and one male (Ng) was housed singly in a cage adjacent to his natal group. Details of natal social environment and subject history are summarized in Table 1. Five different family groups were represented. No brothers were sampled simultaneously in a natal group; at least one year passed after sampling and removal of one subject before the next brother was sampled. Subjects were not captured or handled during the experimental periods. Subjects were tested in four experimental phases, which paralleled previous studies of female cottontop tamarins (Widowski et al. 1992) and represented sequential social and reproductive changes experienced by the animals in our breeding colony.

### Natal phase

We tested each subject for 6–15 consecutive weeks while he was housed in his natal group with sire, dam, at least one male sibling, and at least one female sibling. We sampled urine and behaviour of five subjects during this phase; two subjects (Ra, Ng) were excluded from analyses because they were not housed in complete natal groups, and one focal male (Ni) was not included in the analysis of behavioural interactions with sires (Table 1). All subjects, however, were scored for ejaculatory behaviour.

### Adjacent phase

Immediately following the last week of natal phase testing, we removed each subject from his natal group, placed him in a wheeled single-animal cage (66 × 188 × 64 cm) without handling, and transferred him to a different room where we positioned his wheeled cage adjacent to the cage (160 × 236 × 93 cm) of a singly housed postpubertal novel female. The novel female had been housed only in her natal group until that day; subject males and novel females had not been housed in the same colony

room prior to this transition. In this phase, the subject male and novel female were allowed full visual and auditory contact. For each pair, the cages were positioned 15.5 cm apart to prevent physical contact through the cage mesh. Each subject remained in this phase for 8 successive weeks. We collected urine samples from all seven subjects (Table 1); five subjects (Hu, Ng, Ra, Ti, Xy) were scored for ejaculatory behaviour. No other behaviour was sampled.

#### Paired phase

Immediately following the adjacent phase, we released each subject male into his respective female's cage without capture and tested him for 6 more successive weeks. We sampled the behaviour of five subjects during this phase; the same two subjects (Ra, Ng) were excluded as in the natal phase (Table 1). We collected urine samples and scored ejaculatory behaviour for all seven subjects. Data were not gathered during the first 24 h after pairing. Thus, urine samples from all seven subjects were used during the first three study phases; and social behaviour samples were gathered from five subjects during the natal and paired phases, when subjects had physical contact with other animals.

The reproductive state of the breeding female in each of the social phases was consistent among subject males. All dams were pregnant during the entire course of the natal phase, as determined by analysis of urinary LH/chorionic gonadotropin (CG) levels or by backdating from subsequent parturition (Ziegler et al. 1987a, b). During the adjacent and paired phases, all females monitored by urine collection (5 of 7) showed the species-typical hormonal pattern indicating commencement of ovulatory function (Ziegler et al. 1987b, 1995; Widowski et al. 1992).

#### Father phase

Beginning at least 1.5 years after pairing, we sampled urine from five of the original seven subject males (Table 1) while each male was housed with his mate and their offspring. For each male, urine was collected during one of his mate's pregnancies and throughout rearing of the resultant offspring to weaning age (Cleveland & Snowdon 1984). We sampled urine from each of 4 months immediately prior to parturition, and from each of the 2 months immediately following parturition. Gestation in this species lasts approximately 6 months (Ziegler et al. 1987a, b). Thus, the sampling period comprised a typical reproductive cycle for this species in which females conceive prior to weaning of the previous litter. At least one of the delivered offspring survived throughout the sampling period for each subject, and all offspring from the sampled reproductive cycle survived through to adulthood. Urine samples from one male (Xy) were available for 1 month pre- and 1 month postparturition.

#### Collection and Preparation of Urine Samples

We collected urine from male subjects ( $N=7$ , Table 1) two to three times per week over the course of the three

successive phases: natal, adjacent, paired. At least 2 days separated successive collection dates. Urine from subjects in the father phase ( $N=5$ , Table 1) was collected on 3 randomly chosen days from the last 10 days of each sampled month.

Urine has been collected routinely in our colony; all subjects were well habituated to the procedure prior to the start of the experiment. We collected urine from the first void of the day between 0800 and 1000 hours from inside the subject's cage using a hand-held container. A swinging door at the bottom of wheeled cages was opened to insert the collection container. Samples were prepared as described in Ziegler et al. (1987a, b).

#### Urinary Hormone Assays

We assayed urine samples for T and DHT concentration (ng/ml) according to a new assay procedure. In male cottontop tamarins, 94% of urinary T is excreted in the form of complex conjugates, and only 1% is excreted as a free steroid (Ziegler et al. 2000). Thus, prior to assay, all urine samples used for T and DHT analysis were subjected to acid solvolysis and steroid extraction by a technique reported in Ziegler et al. (1996), except that urine was sampled in 500- $\mu$ l aliquots. We made the following improvements to the solvolysis and extraction technique: (1) all ethyl acetate (EA) was added during solvolysis as 5 ml, (2) during extraction, the aqueous phase was quick-frozen in an alcohol-dry ice bath, and the extracted steroids in the EA supernatant were poured off. External recoveries of tritiated T and DHT stock (2500 counts/min  $^3$ HT) were run in duplicate or triplicate to estimate procedural loss.

The T antibody (R156, Munro, University of California, Davis) cross-reacted (50% binding) with the following substances: 92.4% with DHT, 11.2% with 4-androsten 3 $\beta$ , 17 $\beta$ -diol, 5.4% dehydroandrosterone, 3.4% androstane-diol, 2.1% androstenedione, 0.5% androsterone, 0.4% epiandrosterone, 0.2% dehydroepiandrosterone, and less than 0.07% with hydrocortisone, cortisone, corticosterone, desoxycorticosterone, oestrone, oestradiol, progesterone, 17-alpha-hydroxy-progesterone, cholesterol and pregnenolone. We separated T and DHT in the urine samples by celite column chromatography using the System I technique described in Abraham et al. (1972) with the modifications made by Ziegler et al. (1996). Sonification in an ultrasonic cleaner was used to keep extracted steroids suspended in the 96:4 EA/isooctane (EA/ISO) for application to columns. The DHT was eluted with 4 ml 10% EA/ISO, and T was then eluted with 4 ml 20% EA/ISO. External recoveries were  $85.2 \pm 1.1\%$  (mean  $\pm$  SE) for T and  $74.9 \pm 1.4\%$  for DHT. We developed an enzyme immunosorbant assay (EIA) for T and DHT using a procedure modified from Munro & Stabenfeldt (1984): microtitre plates (Nunc-Immuno Plate Maxisorb F96 certified, VWR Scientific, Chicago, Illinois, U.S.A.) were coated with 100  $\mu$ l T antibody diluted 1:35 000 with coating buffer. Column-separated DHT or T fractions (400  $\mu$ l DHT or 50  $\mu$ l T suspended in ethanol) and standards (1–250 pg,  $N=8$ ;  $\Delta$ 4-androsten-17 $\beta$ -ol-3-one for T, 5-alpha-androstan-17 $\beta$ -ol-3-one for DHT,

Sigma Diagnostics, Inc., St Louis, Missouri, U.S.A.) were assayed on microtitre plates. Absorbance was read at 420 nm on a Dynatec MR5000 (Chantilly, Virginia, U.S.A.) or 415 nm (background absorbance of 570 nm subtracted) on a Spectramax 340 (Molecular Devices Corporation, Sunnyvale, California, U.S.A.). Data reductions (log-logit transformation) were analysed by weighted least-squares regression analysis (Rodbard & Lewald 1970) and reported as ng/ml urine.

When assay concentrations for a serial dilution of the T-spiked male tamarin urine pool (100–0.78  $\mu$ l;  $N=8$ ) were compared to T standards, the computed linear regression lines (Brownlee 1960) did not differ in slope ( $t_{60} = -0.74$ , NS). When DHT-spiked male tamarin urine pool concentrations (800–6.25  $\mu$ l;  $N=8$ ) were compared with DHT standards, regression line slopes did not differ ( $t_{28} = -1.495$ , NS). Significance was predefined as  $P < 0.05$ . The sensitivity of the T and DHT EIA at 90% binding was 0.6 pg. Accuracy measured at each standard curve point (1–250  $\mu$ l; 200 ml urine,  $N=8$ ) was  $102.58 \pm 2.36\%$  and  $98.65 \pm 0.42\%$  for the T and DHT EIA, respectively. We processed a male tamarin urine pool along with the urine samples for each set of columns and assayed the pool in duplicate on each microtitre plate; the intra- and interassay coefficients of variation (Rodbard & Lewald 1970) were 1.3 and 11.5% for T ( $N=29$  plates) and 1.2 and 11.4% for DHT ( $N=31$  plates).

We assayed urine samples for LH/CG concentration (ng/ml) according to the radioimmunoassay technique developed by Ziegler et al. (1993b), except that the rhesus reference standard was replaced with purified human chorionic gonadotropin CR-127 (National Hormone and Pituitary Program) diluted serially (0.1–5.0 ng/tube) for the standard curve. A tamarin urine pool was assayed in quadruplicate for each LH assay; the intra- and interassay coefficients of variation were 4.75 and 9.56%, respectively ( $N=34$  assays).

We assayed urine samples for cortisol concentration ( $\mu$ g/ml urine) using the EIA technique cited in Ziegler et al. (1995). Two separate tamarin urine pools were assayed in duplicate on each microtitre plate; the intra- and interassay coefficients of variation were 3.20 and 12.46%, respectively ( $N=79$  plates), and 3.27 and 13.58%, respectively ( $N=74$  plates).

To normalize for variable water content among urine samples, we assayed each urine sample for creatinine content (mg/ml urine) using a modified Jaffe reaction developed by Tietz (1976) and modified by Ziegler et al. (1995). A tamarin urine pool was assayed in duplicate at two different dilutions on each microtitre plate; the intra- and interassay coefficients of variation for the 1:80 dilution were 1.42 and 9.34 %, respectively ( $N=112$  plates), and for the 1:160 dilution they were 1.67 and 11.19%, respectively ( $N=107$  plates).

### Collection of Behaviour Samples

Each subject was observed in 30-min continuous focal-animal sampling sessions (Altmann 1974) two to four times per week, randomly rotated across days and between 0930 and 1130 hours or between 1330 and 1530

hours. These hours corresponded with periods of social group activity undisturbed by animal care routines and excluded primary feeding and nesting times in our colony. The observer sat in front of the cage (eye level at 1 m) in full view of the animals, but out of range of physical contact ( $\geq 15.5$  cm). All colony animals were thoroughly habituated to the presence of the observer. All behavioural observations were made by the first author. Data were entered on a laptop computer using custom-engineered software for the collection of behavioural data.

We scored sexual, aggressive, scent-marking and olfactory investigative behaviour (Table 2) during each session using continuous-frequency sampling. Neither the behavioural process of ejaculation, the composition of the ejaculate, nor the presence of viable spermatozoa could be measured systematically in this study. Furthermore, the large cage size, dark pigmentation of the external genitalia in both males and females, and the copulatory position make intromission difficult to discern reliably. Behavioural indicators of ejaculation have been described (pulsing erect penis following mount: Kendrick & Dixson 1984; female terminate mount: Kuederling et al. 1996) for established pairs of adult common marmosets. However, no validated behavioural indicator of ejaculation for tamarins housed in complex groups is known to us. Previous tamarin studies have presumed ejaculation by such indicators as post-copulatory genital grooming (Savage et al. 1988). Thus, we used two techniques to monitor for ejaculations during focal sampling: (1) 'ejaculation' was scored if fluid discharge was observed directly; (2) 'putative ejaculation' was scored if genital grooming indicated that intromission or ejaculation was likely to have occurred.

### Statistical Analyses

We assessed the effect of social phase on average hormone concentration using the available data from all seven subjects. DHT, LH, T and cortisol concentrations for each sample were divided by the concentration of creatinine (mg/ml) for the same sample to yield hormone concentration per mg creatinine. For each hormone, these corrected concentration values were averaged across each social phase for each subject. Statistical tests were modelled on a randomized complete blocks design, one-factor mixed-model analysis of variance (ANOVA) for unbalanced data, blocked on random subjects. We used the 'proc mixed' command in SAS for analysis as described in Littell et al. (1996; SAS System software, SAS Institute, Inc.). Post-ANOVA paired comparisons of means between individual phases were made using two-tailed protected  $t$  tests as described in Littell et al. (1996). Significance was predefined as  $P < 0.05$ .

For each social phase, we calculated a per-session behaviour rate for each subject; the number of sessions was divided into the total number of behavioural acts for that animal. In addition, mounts or agonistic mounts were grouped and analysed as 'total' (attempted+full) for each subject. For some behaviours, a 'per group member'

**Table 2.** Behaviours sampled during 30-min focal observations of subject males\*

<b>Sexual behaviour</b>	
Attempted mount	One animal (the mounting animal/actor) clasped another (the mounted animal/recipient) about the abdomen with one or both arms. No pelvic thrusting was done by the mounting animal. The mounting and mounted animal were typically positioned ventro-dorsal or ventro-lateral to one another, respectively.
Full mount	Same as attempted mount except that the mounting animal performed one or more pelvic thrusts.
Agonistic mount	An attempted or full mount whereby the mounting animal leapt onto, pounced on, or performed a 'body press' (defined below) to make body contact with the mounted animal. These acts (e.g. 'body press') were not scored as separate instances of contact aggression when they occurred in an agonistic mount. Agonistic mounts typically occurred during rough play or agonistic interactions.
Ejaculation	Direct observation of fluid being discharged from the glans penis, or direct observation of fluid as it was being groomed off the penis or the abdominal fur adjacent to the erect penis with the hands or mouth. The male typically consumed the ejaculate during grooming.
Putative ejaculation	Ejaculatory fluid was not observed directly. The focal male groomed his penis or abdominal fur proximal to the erect penis within 15 s following an interval of pelvic thrusting, which may or may not have occurred during a full mount.
Erection	The penis was judged to be fully extended from the prepuce, and the corpus of the penis was rigid and straight. Because of the unrestricted movement of the animals, the penis could not be viewed continuously. Thus, independent erections were scored after the penis was subsequently observed as completely flaccid or fully retracted into the prepuce.
<b>Aggression received</b>	
Contact aggression	Individual occurrences of the following contact behaviour towards the recipient, including: manual grab, push, swat; bite; wrestling bout (on a surface or while hanging); chase; face press (Savage et al. 1988); or body press (actor quickly and forcefully pushed the ventral surface of its torso against the back or hindquarters of recipient. Neither of the actor's arms were clasped about the recipient's abdomen).
<b>Scent marking and olfactory investigation</b>	
Anogenital scent marking	Animal rubbed anogenital region or underside of scrotum on a substrate or an animal.
Suprapubic scent marking	Animal dragged suprapubic or front/upper side of scrotum on substrate or an animal.
Sternal scent marking	Subject dragged sternal region on substrate.
Sniff scent mark	Subject sniffed or licked the substrate immediately after or while a scent mark was deposited there by another animal.
Anogenital sniff	Animal sniffed or licked the suprapubic or anogenital region of another animal.

\*For behaviours involving two animals, the identity of both the actor and recipient was recorded.

rate was constructed by dividing each subject's per-session behaviour rate by the number of other group members in that social phase. We then calculated a mean behaviour rate across all subjects for each phase. The effect of social phase on per-session behaviour rates was assessed using two-tailed *t* tests for paired data (Ott 1993). Significance was predefined as  $P < 0.05$ . Analyses were made using Minitab statistical software (Minitab Release 7.2 HP-UX version).

## RESULTS

### Hormones: Natal, Adjacent, Paired and Father Phases

Urinary concentrations of reproductive hormones (Fig. 1) varied significantly according to social phase (ANOVA: LH:  $F_{3,14} = 4.40$ ,  $P = 0.02$ ; T:  $F_{3,14} = 3.37$ ,  $P < 0.05$ ; DHT:  $F_{3,14} = 4.38$ ,  $P = 0.02$ ). Average levels of LH and T increased from the natal to the adjacent phase but returned to natal levels during the paired phase. Average levels of DHT increased from the natal to the adjacent phase, remained elevated in the paired phase, and had returned to natal levels by the father phase. For LH and T, all significant differences among post-ANOVA paired

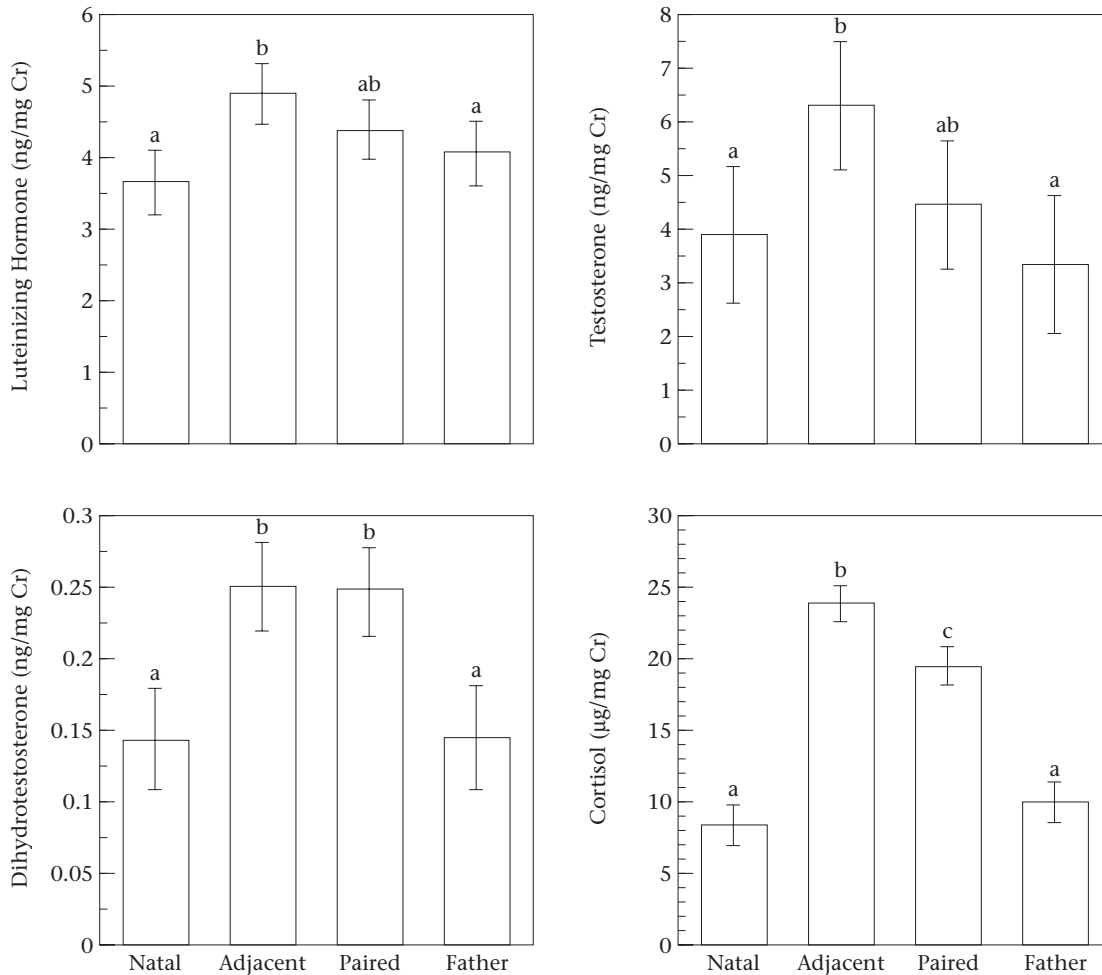
comparisons of means were  $P < 0.04$ . All significant differences among post-ANOVA paired comparisons of means for DHT were  $P < 0.025$ .

Urinary cortisol levels demonstrated a significant and dramatic effect of social phase (ANOVA:  $F_{3,14} = 44.20$ ,  $P = 0.0001$ ). Average cortisol levels were highest in the adjacent phase and had returned to natal levels by the father phase. All significant differences among paired comparisons of means for cortisol were  $P < 0.008$ . For all hormones measured, there were no significant differences found between the natal and father phases.

### Behaviour: Natal and Paired Phases

#### Sexual behaviour

Male siblings rather than females were most often the recipients of full mounts (59% of 32 full mounts; by 3 of 5 subjects) given by focal males in the natal phase. Female siblings received 25% and dams 9% of the full mounts given by subject males. The rate of full mounts was similar in the natal and paired phases (paired *t* test:  $t_4 = -0.66$ ,  $P = 0.54$ ; Table 3). However, the rate of full mounts per sister in the natal phase was lower (paired *t* test:  $t_4 = 2.89$ ,  $P = 0.044$ ) than the rate of full mounts given



**Figure 1.** Mean  $\pm$  SE hormone levels per experimental phase. Within each panel, different letters (a, b, c) represent significant differences ( $P < 0.05$ ) by post-ANOVA paired comparisons of means.

to the mate in the paired phase; the rate of full mounts given to sisters overall in the natal phase tended to be lower, but not significantly (paired  $t$  test:  $t_4 = 2.53$ ,  $P = 0.065$ ).

The rate of full mounts to the dam tended to be lower in the natal than in the paired phase (paired  $t$  test:  $t_4 = 2.55$ ,  $P = 0.063$ ; Table 3); but all full and attempted mounts to the dam were by only one subject (Ni). This male had erections during some of each type of mount. One of the three full mounts was in a position where intromission would have been possible, but the dam responded immediately by pulling away quickly and rotating her body to face the male; no response by the dam to the other full mounts was observed.

The rates of total mounts (attempted+full) in the natal and paired phases were nearly identical ( $1.5 \pm 0.57$  total mounts/30 min; mean  $\pm$  SE; paired  $t$  test:  $t_4 = -0.01$ ,  $P = 0.99$ ; Table 3). The rate of total mounts per group member did not differ (paired  $t$  test:  $t_4 = -2.25$ ,  $P = 0.09$ ; Table 3) between phases. Male siblings received most mounts (74% of 130 total mounts) by focal males in the natal phase (Fig. 2). All subjects (with one exception, Xy) made attempted mounts to male siblings; Xy was the

only male that had no male siblings older than the weaning age of 8 weeks. Mounts to both the dam (by one subject only; 6% of total mounts) and female siblings (12% of total mounts) were observed in the natal phase. The rate of total mounts to the dam and the rate of total mounts per sister in the natal phase each tended to be lower than the rate of total mounts to the mate in the paired phase, but these tendencies were not significant (paired  $t$  tests:  $t_4 = -2.26$ ,  $P = 0.086$ ,  $t_4 = -2.46$ ,  $P = 0.069$ , respectively).

Agonistic mounts occurred more frequently in the natal phase than in the paired phase. The rate of total (attempted+full) agonistic mounts was higher (paired  $t$  test:  $t_4 = -5.05$ ,  $P = 0.007$ ) in the natal than in the paired phase; and the rate of total agonistic mounts per group member was higher in the natal phase (paired  $t$  test:  $t_4 = -5.06$ ,  $P = 0.007$ ; Table 3). The majority (97%) of total agonistic mounts in the natal phase were given to siblings; subjects directed 74% of total agonistic mounts to brothers and 23% to sisters (Fig. 2). No subject directed an agonistic attempted mount to his dam; and in the paired phase, only one agonistic attempted mount to a mate occurred. No agonistic full mounts (i.e. with pelvic

**Table 3.** Mean±SE behaviour rates (frequency/30-min session) per social phase

Behaviour	Natal	Adjacent	Paired
Erections	1.49±0.24		1.23±0.13
Putative ejaculations*: total number (actors)	2 (Hu)	0	15 (Ra, Ng, Xy, Hu)
Ejaculations*	2 (Xy, Hu)	2 (Ti, Ng)	1 (Ra)
Full mounts given	0.36±0.17		0.52±0.16
Full mounts given to breeding female	0.04±0.04		0.52±0.16
Full mounts given per nonbreeding female	0.04±0.03		—
Total mounts given	1.52±0.57		1.53±0.57
Total mounts given per group member	0.30±0.13		1.53±0.57
Total mounts given to breeding female	0.11±0.11		1.53±0.57
Total mounts given per nonbreeding female	0.06±0.04		—
Total agonistic mounts given	0.58±0.12		0.01±0.01
Total agonistic mounts given per group member	0.09±0.02		0.01±0.01
Aggression received	7.67±2.33		0.11±0.05
Aggression received per group member	1.18±0.38		0.11±0.05
Aggression received from breeding female	0.14±0.09		0.11±0.05
Aggression received from sire	0.15±0.05		—
Anogenital sniffing	1.40±0.46		0.96±0.38
Anogenital sniffing to breeding female	0.39±0.14		0.96±0.38
Total scent marking	0.17±0.07		0.93±0.50

\*For ejaculatory behaviour, the total number of occurrences across all subjects and the actors are listed.

thrusting) were directed to any female in either the natal or the paired phases. Only two agonistic full mounts were observed in the natal phase; both mounts were given by the same subject (Sq), one to a brother and one to the sire.

Erections occurred in both natal ( $1.49 \pm 0.24$  erections/30 min) and paired ( $1.23 \pm 0.13$  erections/30 min) phases in all focal males. Even after the males were paired with a novel female, erection rate did not change from the natal phase (paired  $t$  test:  $t_4 = -0.97$ ,  $P=0.39$ ).

Ejaculation by one or more subjects was observed in each social phase (total=5 ejaculations by 5 subjects; Table 3). Putative ejaculations occurred in the natal and paired phases; the largest number were observed in the paired phase (Table 3). In the natal phase, all ejaculations or putative ejaculations by subject males occurred during full mounts given to or received from other males ( $N=3$ ) or following headshaking ( $N=1$ ) (a noncontact visual aggressive or sexual behaviour: Snowdon & Soini 1988). Neither ejaculations nor putative ejaculations were observed during any mounts with females in the natal phase. In the adjacent phase, ejaculations appeared to be spontaneous and occurred while the subject male was staring at the novel female, which was headshaking and tongue flicking at the subject, or during a bout of pelvic thrusting with no genital-substrate contact. No manual or oral masturbation was observed. In the paired phase, the ejaculation was observed as the female pulled away from the subject during a full mount. All putative ejaculations in the paired phase occurred during a full mount to the female. In addition, in the natal phase, one postpubertal male sibling ejaculated during a full mount given by one of the subject males; and in a different natal group, a putative ejaculation by the sire was observed during a mount performed on a subject male.

The novel females that were monitored by urinary hormone assay (5 of 7 females) ovulated twice during the adjacent phase, and ovulated or conceived during the

paired phase. One of the two unmonitored novel females conceived during the paired phase as determined by backdating. All study subjects sired offspring after the paired phase. The two subjects that were not included in the father phase of this study sired offspring after their transfer from the study colony.

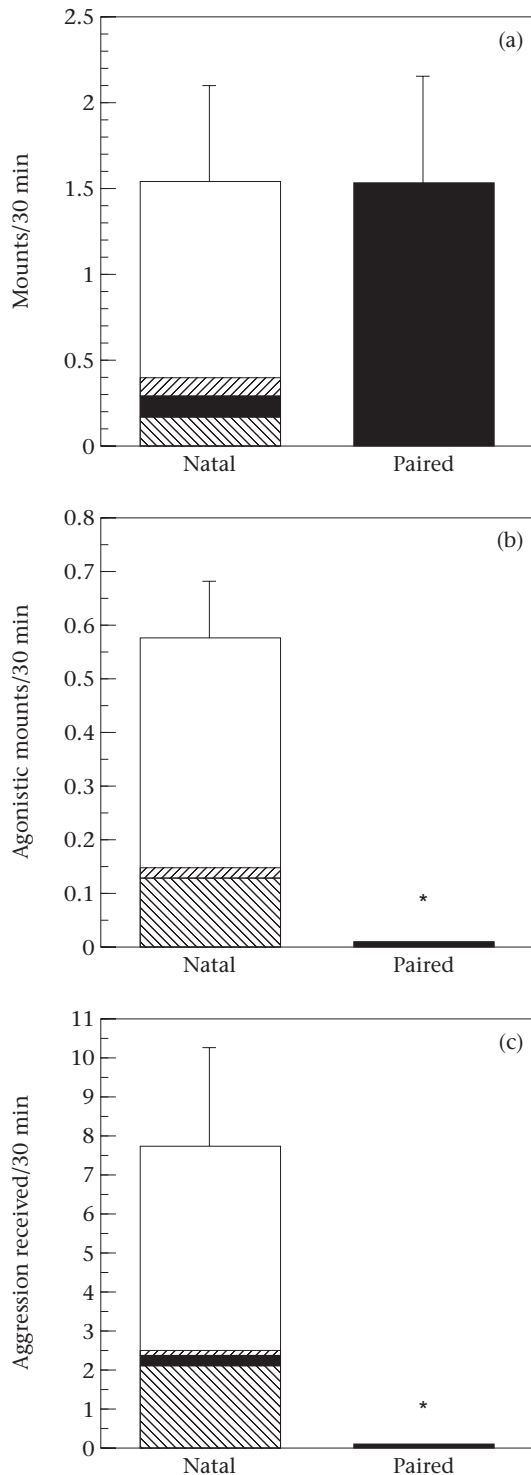
#### Contact aggression

Average rates of aggression per social phase are given in Table 3. Focal males received more contact aggression (paired  $t$  test:  $t_4=3.31$ ,  $P=0.03$ ) in the natal phase than in the paired phase (Table 3). Average rate of aggression received per group member also was higher (paired  $t$  test:  $t_4=3.25$ ,  $P=0.03$ ) in the natal phase than in the paired phase. However, more contact aggression in the natal group was directed at the focal males by male and female siblings (97%) than by the breeding pair; subjects received 68% of contact aggression from brothers and 29% from sisters (Fig. 2). The average rate of aggression received from the dam or from the sire did not differ from that received from the mate in the paired phase (paired  $t$  tests:  $t_4=0.75$ ,  $P=0.49$ ,  $t_3=1.64$ ,  $P=0.20$ , respectively).

#### Olfactory investigation and scent marking

Average anogenital sniffing and scent-marking rates per social phase are given in Table 3. The rates of sniffing the breeding female in the natal and in the paired phase did not differ (paired  $t$  test:  $t_4 = -1.70$ ,  $P=0.16$ ). Average rate of sniffing the mate did not differ from the rate of sniffing for all male and female natal-group members combined (paired  $t$  test:  $t_4=1.12$ ,  $P=0.33$ ) or from sniff rate per natal-group member (paired  $t$  test:  $t_4 = -2.09$ ,  $P=0.10$ ). No instances of sniffing freshly deposited scent marks (Table 2) were observed.

Average rate of scent marking by focal males did not differ between the natal and the paired phases for anogenital scent marking (paired  $t$  tests:  $t_4 = -0.83$ ,  $P=0.45$ ),



**Figure 2.** Mean  $\pm$  SE rates of behaviour per 30-min observation session in the natal and the paired phases. The rate of each behaviour in the natal phase is partitioned into the relative proportion directed towards brother(s) ( $\square$ ), sire ( $\text{diagonal lines}$ ), dam ( $\blacksquare$ ), or sister(s) ( $\text{diagonal lines}$ ). (a) Total mounts (attempted+full) given by subject males; (b) Total agonistic mounts (attempted+full) given by subject males; (c) Contact aggression received by subject males. \*Significant differences ( $P < 0.05$ ) between overall rates of behaviour in the natal and the paired phases.

sternal marking ( $t_4 = -1.49$ ,  $P = 0.21$ ), suprapubic marking ( $t_4 = 0.23$ ,  $P = 0.23$ ), or for all types of marking combined for each phase (total marking:  $t_4 = -1.48$ ,  $P = 0.21$ ).

## DISCUSSION

We found no evidence for hormonal suppression of male offspring housed with their natal group; natal hormone levels for each male were comparable to his average levels as a reproductively successful animal. Although sexual behaviour occurred during the natal phase, reproduction by the subjects may have been prevented by direction of critical sexual behaviour away from the dam.

### Transitional Effect on Reproductive Hormones: LH, T, DHT

Although urinary levels of each hormone increased significantly after subjects were removed from their natal groups, the changes were transient. By the time each subject had become a proven breeding male, the average level of each reproductive hormone had returned to his natal level. The mean increase in the adjacent and paired phases might have been due to a number of acute stimuli including territorial behaviour and establishment of a new territory or stimulation by the novel female. Mating behaviour and introduction to a mate correspond with increased androgen production in some male rodents (e.g. Macrides et al. 1975; Schoech & Matt 1989).

In two other callitrichid species, comparisons of plasma T or LH concentrations between breeding males and adult sons in natal groups have yielded results comparable to this study (*L. rosalia*: French et al. 1989; *C. jacchus*: Abbott & Hearn 1978; Abbott 1984; Baker et al. 1999). However, French & Schaffner (1995) reported that urinary T levels of black tufted-ear marmosets were significantly lower in adult-aged males living in the natal family group than in breeding males, and that T levels in the adult sons rose significantly after they were removed from the natal group and housed in isosexual groups or paired with unrelated females. Low-ranking subordinate male common marmosets in groups of unrelated adults have reduced plasma T or LH compared with the behaviourally dominant male of the group (Abbott 1984; Abbott et al. 1985, 1992); and Sheffield et al. (1989) found different in vitro T steroidogenic pathways for testicular tissue taken from dominant versus subordinate males, suggestive of lower intratesticular androgen concentrations in subordinates.

### Redirection of Sexual Behaviour

Sexual behaviour directed towards all group members combined (full mounts, total mounts) occurred at nearly identical rates in the natal and paired phases. No significant differences were found for rates of erections and olfactory investigation of breeding females across the social phases. Scent-marking rates by subject males did not change, corresponding with previous findings (French et al. 1984). The rates of these behaviours were

not affected by social phase even after the males were exposed to the physical stimulus of a novel, ovulating female. In addition, this study provides evidence that the physiological sequences involved in the process of ejaculation are not prevented in adult male helpers in the natal group.

The relatively higher frequency of ejaculations, putative ejaculations and full mounts to the female in the paired phase compared with females in the natal phase is explained perhaps by acute stimulation from the novel female, ovulatory cues in the adjacent and paired phases, or the process of pair bonding. [Savage et al. \(1988\)](#) reported that newly paired cottontop tamarin females were mounted by males at a significantly higher rate than established breeding females. Parallel findings have also been reported for new and established breeding pairs of other callitrichid species (*C. jacchus*: [Evans 1983](#); [Evans & Poole 1983, 1984](#); *L. rosalia*: [Kleiman 1977, 1978](#)). The similarity of erection and overall mounting rates between natal and paired phases is thus all the more striking.

An unexpected result of this study was the level of sexual behaviour displayed by subjects in the natal environment and the preponderance of these behaviours with other males. The definitions of sexual behaviour in the cottontop ethogram were detailed to monitor species-specific reproductive activity among males and females. As defined, mounting behaviour between males was indistinguishable from those between males and females; and full sequences included accompanying visual and vocal signals typical of male–female copulation in this species (A. J. Ginther, personal observation). The majority of full and attempted mounts by male subjects in the natal phase were given to male siblings. Some mounting behaviour was directed towards both nonbreeding and breeding female natal-group members; yet we did not observe any ejaculations, putative ejaculations, or even suspected intromissions during mounts by male offspring to natal-group females. Ejaculations and putative ejaculations occurred only with natal-group males rather than females. Thus, breeding restriction may not be maintained by preventing sexual behaviour entirely, but by directing full copulatory behaviour away from females. Sexual behaviour directed at other males may help facilitate a singular breeding paradigm, and a ‘functional suppression’ may exist wherein sons are reproductively capable, but only limited sexual behaviour with the fertile female occurs. In this manner, young males may engage in sexual behaviour within the confines of a breeding system that otherwise suppresses their reproduction.

Isosexual behaviour is sometimes incorporated into episodes of play in primates, but its expression in this context generally diminishes with age and is more often displayed in other contexts by adults ([Vasey 1995](#)). In cottontop tamarin family groups, preference for play partners is not dictated by gender. [Cleveland & Snowdon \(1984\)](#) found the most likely play partner to be the twin (regardless of gender) or the nearest age cohort; breeding and nonbreeding adults were involved in play infrequently. As such, we would not expect the adult males of this study to preferentially engage in play with only

brothers in the natal phase. Isosexual behaviour has been documented in males of a wide range of primate species, including free-ranging populations where isosexual behaviours are included in the normal behavioural repertoire (for reviews, see [Vasey 1995](#); [Dixson 1998](#)). A number of hypotheses have been generated to explain the presence of sexual behaviour between adult male primates, including practice for heterosexual copulation, control of dominance and rank, formation of alliances and regulation of tension and reconciliation. In cottontop tamarin males, isosexual behaviour also may be a facet of sexuality that allows a flexible breeding strategy for males; male offspring remain reproductively capable (see also [Baker et al. 1999](#)) within the limits of a cooperative breeding system.

### Aggressive Behaviour and Cortisol Levels

The change in rates of agonistic mounts across social phases resembled that of contact aggression rather than that of mounts. As with contact aggression, the agonistic component of mounting behaviour therefore seemed to disappear when the animals were newly paired. This suggests that in addition to reproductive function, mounting behaviour may be used in the form of agonistic mounts for sociosexual communication of dominance or rank ([Dixson 1998](#) for review) especially among male siblings within the natal group, and that the two types of mounts defined in this study can be reliably discerned. Mounting behaviour may therefore be used in agonistic encounters as well as sociosexual ([Dixson 1998](#)) and reproductive contexts.

The majority of aggressive behaviour towards subjects was given by siblings. Neither the sire nor the dam differed from the novel female in the amount of contact aggression directed towards the subject males. This suggests that sustained, heightened aggression in social groups is not used by parents to restrict breeding by males. It was not always possible to objectively differentiate between aggressive acts that occurred in a context of play versus one of purely agonistic interaction. Although some of the contact aggression scored likely occurred during rough play, parents of this species are less likely to be involved in play with offspring ([Cleveland & Snowdon 1984](#)). Hence, the aggression received from sire or dam by natal-phase subjects is unlikely to represent play. Rate of aggression between the subject and the breeding female did not differ between the natal and paired phases. Although aggression received by males might have been largely attributed to rough play or formation of social hierarchy among siblings, our study provides evidence that continued aggression from the dam was not used to restrict sexual behaviour by sons. Future work should evaluate possible changes in aggression from the dam across the ovulatory cycle or acute responses to specific acts of sexual behaviour by sons. In other primate species, successful mating behaviour is under control of the female or is mediated by complex behavioural sequences and social interactions between male, female, or other social group members ([Goldfoot 1982](#); [Kendrick & Dixson 1983, 1984](#); [Wallen 1990](#)). Consistent with this

idea is the dam's response in this study to subject Ni, wherein the subject's mount was rejected by the dam.

The pattern of change in cortisol levels as subject males changed social phase did not reflect the decrease in levels of aggression received; although aggression received was higher in the family than when males were newly paired, cortisol levels were lower. Cortisol levels in the natal group were low and basal as judged by levels of successfully breeding males, and parallels findings for common marmoset males (Baker et al. 1999). In free-ranging olive baboons, *Papio anubis*, subordinate males have chronically elevated plasma cortisol levels due to a disruption in the hypothalamic feedback mechanism, as well as decreases in testosterone attributable to acute stress (Sapolsky 1990). Our finding fits the pattern described for males of other cooperatively breeding species (Baker et al. 1999) where nonbreeding males have similar or lower levels of stress hormones than breeding, dominant males (Creel et al. 1996). Changes in urinary cortisol levels in our subjects paralleled the pattern of change shown by female tamarins that undergo an equivalent change in social and physical environment (Ziegler et al. 1995). Cortisol levels were highest in the adjacent phase, when males were removed from their natal social group, transferred to a new cage and colony room, and exposed to a novel female. Therefore, cortisol is unlikely to be involved in mediating restricted reproduction in sons.

### Restriction of Reproduction in Male versus Female Offspring

The divergence between male and female cottontop tamarin offspring in hormonal response to removal from the natal environment is clear and striking. The male tamarins in this study excreted nearly equivalent average levels of reproductive hormones as either natal offspring or established breeders, whereas female tamarins in previous studies underwent fundamental changes in endocrine function and potential fertility upon removal from the natal group. Urinary LH and oestrogen levels of captive female offspring are low and acyclic as long as they remain in the natal group with the breeding pair and are organized into functional cycles only after the females are exposed to or paired with a novel male (French et al. 1984; Ziegler et al. 1987b; Widowski et al. 1990, 1992), or in the absence of the dam or other family members, as is the case for eldest female offspring (French et al. 1984; Tardif 1984; Heistermann et al. 1989; Widowski et al. 1990; Price & McGrew 1991). Despite the presence of some sexual behaviour with natal-group males, fertility in female cottontop tamarin offspring is thus prevented in natal groups through the absence of ovulatory cycles, as in females of several callitrichid species (reviewed in French 1997). In cooperatively breeding social carnivores, subordinate males experience weaker reproductive suppression than females as predicted by lower costs of reproduction (Creel & Creel 1991; Creel et al. 1997). Similar gender differences in reproductive strategy have been demonstrated in other cooperatively breeding

species (e.g. common marmosets: Abbott 1984; Harris' hawk: Mays et al. 1991; dwarf mongooses: Creel et al. 1992; African wild dogs: Creel et al. 1997).

Despite extensive controls on the reproduction of female cottontop tamarins and callitrichid females in general (reviewed in French 1997), this study provides evidence for flexibility in reproductive strategies of male cottontop tamarin group members. Multiple males copulate in wild groups of saddle-back tamarins, *S. fuscicollis*, and it has been proposed that facultative polyandry accounts for apparent flexibility in wild groups of tamarins (Terborgh & Goldizen 1985; Goldizen 1987; Sussman & Garber 1987); males mate polyandrously if helpers are needed to successfully raise the offspring of one female, and monogamously only if other helpers are already available. Our results provide a behavioural and physiological basis for polyandry in cottontop tamarin groups. Taken together, our behavioural and hormonal findings for cottontop tamarins, and those of Price & McGrew (1991), parallel those of Baker et al. (1999) for common marmosets. For both species, no differences in levels of cortisol and reproductive hormones were found between fathers and sons, little or no sexual behaviour occurred between sons and mothers, but sons may be sexually active with a novel female in the presence of the father. Thus, although sexual behaviour of male offspring appears to be restricted in the natal group, male offspring may be sexually active under alternative social circumstances, and a basis for father-son polyandry exists. In the natal phase of this study, high overall levels of sexual behaviour were independent of any potential ovulatory stimulus by the breeding female and suggest a sustained state of reproductive readiness. An ability to mate opportunistically outside the group is another possible benefit of continual reproductive readiness in males. During contact with other groups, extragroup copulations have been observed by subordinate male common marmosets (Digby 1999; Lazaro-Perea, in press). Similarly, increased flexibility in the reproductive controls on only one gender may have important implications for patterns of dispersal from the natal group and immigration or formation of new social groups. Callitrichid males, including cottontop tamarins, may have the ability to respond reversibly to subtle changes in the social environment to meet breeding opportunities and increase reproduction. Thus, at a behavioural and hormonal level, male offspring in captive groups have the potential to be reproductive; and this reproductive potential has important implications for understanding the form and flexibility of callitrichid breeding strategies.

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