



## Circadian Variation with a Diurnal Bimodal Profile on Scent-Marking Behavior in Captive Common Marmosets (*Callithrix jacchus*)

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*Scent-marking behavior is associated with different behavioral contexts in callitrichids, including signaling a territory, location of feeding resources, and social rank. In marmosets and tamarins it is also associated with intersexual communication. Though it appears very important for the daily routine of the individuals, very few researchers have investigated distribution through the 24-h cycle. In a preliminary report, we described a preferential incidence of this behavior 2 h before nocturnal rest in families of common marmosets. We expand the data using 8 family groups (28 subjects), 8 fathers, 6 mothers, 8 nonreproductive adults (4 sons and 4 daughters), and 6 juvenile (3 sons and 3 daughters) offspring that we kept in outdoor cages under natural environmental conditions. We recorded the frequency of anogenital scent marking for each group during the light phase, twice a wk, for 4 consecutive wks, from March 1998 to September 1999. Cosinor test detected 24- and 8-h variations in 89.3% and 85.7% of the subjects, respectively, regardless of sex or reproductive status. The 8-h component is a consequence of the 2 peaks for the behavior, at the beginning and end of the light phase. Daily distribution*

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*of scent marking is similar to that others described previously for motor activity in marmosets. The coincident rhythmical patterns for both behaviors seem to be associated with feeding behavior, as described for callitrichids in free-ranging conditions, involving an increase in foraging activities early in the morning and shortly before nocturnal rest.*

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**KEY WORDS:** age; common marmoset; scent-marking behavior; sex; temporal variation.

## INTRODUCTION

Various studies with callitrichids demonstrated that chemical communication via pheromones seems critical to survival of the New World primates. *Callithrix* can present 3 main types of scent-marking patterns: suprapubic, circumgenital or anogenital, and sternal, which differ among species (Stevenson and Rylands, 1988). Anogenital scent-marking is the most usual for common marmosets (*Callithrix jacchus*), and researchers have associated it with aggression (Epple, 1970) and territoriality (Araújo, 1996; Epple, 1970; Lazaro-Perea *et al.*, 1999; Stevenson and Rylands, 1988). However, studies of tamarins (*Saguinus mystax*: Heymann, 2000; *Leontopithecus rosalia*: Miller *et al.*, 2003) showed no evidence for territorial function in either species.

In common marmosets scent marking seems to be involved also with reproductive inhibition between females (Barrett *et al.*, 1990; Smith and Abbott, 1998), but according to Miller *et al.* (2003), only males use scent marks to communicate intrasexual dominance in groups of *Saguinus mystax*. Researchers have also demonstrated the role of scent marking in intersexual communication in common marmosets (Smith and Abbott, 1998) and golden lion tamarins (Miller *et al.*, 2003). In moustached tamarins, Heymann (1998) found that males sniffed more female than male scent marks. In relation to the temporal organization of the behavior, only a previous report from our laboratory showed that scent marking exhibits diurnal variation, with the highest values occurring at the end of the light phase (Nogueira *et al.*, 2001). The profile seems to be associated with either feeding behavior or reinforcement of scents in alimentary sources at the end of the day, thus facilitating feeding localization the next day. We evaluated whether scent-marking behavior in common marmosets fluctuates in a regular way, as others demonstrated for drinking behavior (Saito *et al.*, 1983), locomotor activity (Erkert, 1989; Menezes *et al.*, 1993), and allogrooming in both isolated heterosexual pairs (Mota *et al.*, 1993) and individuals in family groups (Azevedo *et al.*, 1996; Menezes *et al.*, 1994).

## METHODS

### Subjects

We used 28 individuals from 8 families of common marmosets, *Callithrix jacchus*. All of the parents were born in captivity, except 1 pair of adults, which arrived at the colony as juveniles. We observed 3 or 4 focal individuals (mother, father, son, and daughter) within each family. Each group lived in an outdoor cage ( $2 \times 2 \times 1$  m) at the Nucleo de Primatologia of the Universidade Federal do Rio Grande do Norte ( $5^{\circ}50'S$   $35^{\circ}12'W$ ). The focal sample consisted of 8 fathers, 6 mothers, 7 sons, and 7 daughters. Climatic conditions were natural, and we offered food twice a day around 0800 and 1400 h, with water available *ad libitum*.

### Procedure

We observed scent-marking behavior of the focal individuals over a 4-wk interval. We concentrated data recording in 2 d randomly chosen for each wk, when we recorded scent-marking behavior continuously from 0500 to 1700 h, which corresponds to the photo phase.

We previously trained 2 persons to observe scent-marking behavior in the species, and the accuracy test was 92%. Each observer collected data from 1 or 2 individuals in a family depending on the number—3 or 4—of focal individuals we monitored. We recorded each episode with a manual counter and quantified only anogenital scent-marking behavior, consisting of rubbing the anogenital region on a substrate. To allow recognition, we marked each individual with a yellow spot on a specific body region such as ear tufts, forelimbs, hind limbs, or head or dorsal area of the trunk.

### Statistical Analysis

We performed the Cosinor test (Nelson *et al.*, 1979) via El Temps software (A. Diez-Noguera, Spain), which evaluates if a set of data has a regular fluctuation with a defined period by fitting a cosine curve with the period to the data. Accordingly, we show the results as achrophase, time of the day when the variable reaches its highest level; mesor, mean values of the estimated cosine curve; and amplitude, the maximum displacement from the mesor value. We considered the results significant when  $p < 0.05$  and the rhythm percentage (%R) was at least 15%.

We made comparisons considering sex and age via 2-way ANOVA with Tukey's post hoc test. We made hourly profile comparisons via ANOVA for repeated measures.

## RESULTS

Data analysis showed that age ( $p < 0.001$ ), sex ( $p < 0.01$ ), and time of day ( $p < 0.001$ ) influenced scent-marking behavior. The behavior was more frequent in adult females ( $3.3 \pm 6.5$  episodes/h; mean  $\pm$  SD) ( $p < 0.05$ ) than in other age and sex classes, with no difference between adult males ( $1.9 \pm 3.9$  episodes/h) and juvenile males ( $1.0 \pm 2.1$  episodes/h) or females ( $1.4 \pm 3.1$  episodes/h), though even in juveniles, females tended to scent mark more than males did.

Daily distribution of scent-marking behavior of adults and juveniles is in Fig. 1. Adults show a bimodal pattern of fluctuation with higher scent-marking values occurring at the beginning and end of the light phase, but the profile is not so evident for juveniles. The number of scent-marking episodes increased from 1300 h in both adult males and females, reaching the highest values around 1600 h. The values in this interval are statistically greater than others, as indicated by ANOVA for repeated measures ( $p < 0.001$ ). Adult females also showed higher values in the 0600–0700 h interval.

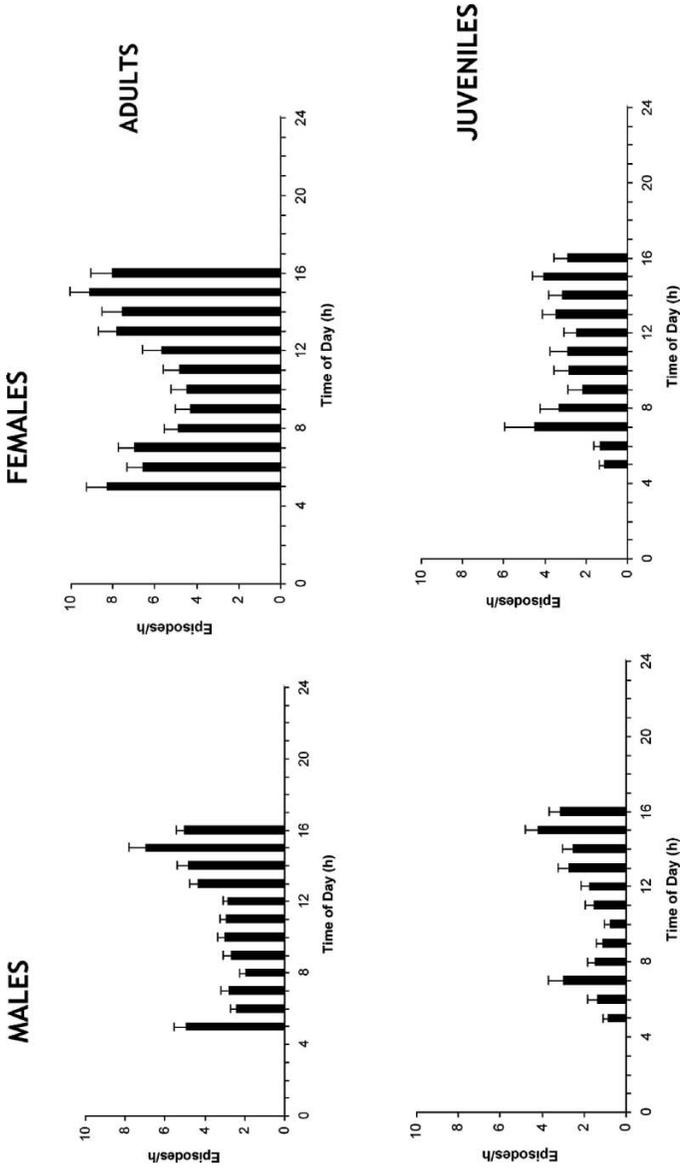
The Cosinor test for both 24- and 8-h components indicated statistical significance for 89.3% and 85.7% of the subjects, respectively. The 8-h component is associated with regularity of a bimodal profile for daily behavior distribution (Menezes *et al.*, 1996, 1998). Tables I and II show the values for mean, mesor, amplitude, acrophase, and rhythm percentage for each individual. The individual acrophases for 24-h periodicity were concentrated near midday for all individuals, regardless of sex or age.

## DISCUSSION

Our results show for the first time that scent-marking behavior in common marmosets has a bimodal profile that we may describe as an 8-h period component. We also confirm data previously reported using 4 families (Nogueira *et al.*, 2001) that demonstrated a diurnal variation pattern for scent-marking behavior in adult common marmoset males and females.

Cosinor test for 24-h period showed significant results for 25 of 28 subjects, including all juveniles. In addition, we detected an 8-h component for 24 of 28 individuals, except 1 adult male, 2 adult females, and 1 juvenile female.

Via the Cosinor test, we demonstrate that scent-marking behavior presents a circadian fluctuation with a diurnal bimodal profile. The consistency of our finding with those previously reported for the locomotor



**Fig. 1** Mean ( $\pm$ SEM) daily profiles of scent-marking behavior observed for adult and juvenile common marmosets.

**Table I.** Individual Cosinor results for frequency of scent-marking behavior in the 24-h period for all studied animals

Animal	Mesor	Amplitude	Acrophase	%R
<b>Adult males</b>				
285	1.5 (1.1–1.9)	12.0 (1.3–2.6)	1225(1105–1344)	21.8
311	1.5 (1.1–1.8)	1.6 (1.0–2.3)	1024 (08.47–1201)	16.0
375	NS	NS	NS	NS
395	0.8 (0.6–1.0)	1.0 (0.7–1.4)	1242 (1120–1404)	20.9
445	2.3 (1.9–2.8)	2.8 (2.1–3.6)	1052 (0953–1152)	33.1
483	1.0 (0.8–1.2)	1.3 (0.9–1.6)	1152 (1045–1259)	28.3
485	1.6 (1.3–1.8)	1.9 (1.4–2.4)	1113 (1011–1214)	31.5
497	1.4 (1.1–1.7)	1.8 (1.3–2.3)	1207 (1059–1314)	28.1
523	1.7 (1.3–2.2)	2.1 (1.4–2.8)	1306 (1144–1427)	21.1
527	5.6 (4.6–6.6)	6.2 (4.5–8.0)	1040 (0934–1146)	29.0
529	2.1 (1.6–2.6)	2.6 (1.8–3.4)	1211 (1056–1326)	23.9
561	2.3 (1.7–2.9)	3.1 (2.1–4.1)	1217 (1059–1334)	23.0
<b>Adult females</b>				
314	NS	NS	NS	NS
408	NS	NS	NS	NS
446	3.2 (2.7–3.8)	3.9 (2.8–4.7)	1104 (1005–1204)	33.1
460	3.8 (3.2–4.4)	4.3 (3.3–5.4)	0940 (0843–1037)	34.8
468	1.2 (0.9–1.4)	1.4 (1.0–1.7)	1032 (0928–1136)	29.9
506	10.4 (9.0–11.8)	12.7 (10.3–15.2)	1111 (1027–1155)	47.1
510	4.3 (3.6–5.0)	4.9 (3.6–6.2)	1105 (1005–1206)	32.1
512	4.5 (3.8–5.2)	5.4 (4.0–6.7)	1050 (0952–1147)	34.1
532	2.6 (2.0–3.1)	2.9 (2.0–3.9)	0922 (0804–1040)	22.4
546	1.1 (0.8–1.4)	1.6 (1.1–2.1)	1258 (1139–1416)	22.3
<b>Juvenile males</b>				
629	0.8 (0.6–1.1)	1.0 (0.7–1.4)	1120 (0952–1249)	18.7
707	0.9 (0.7–1.1)	1.0 (0.6–1.5)	1241 (1110–1412)	17.7
731	1.3 (1.0–1.6)	1.7 (1.2–2.3)	1151 (1034–1307)	23.2
<b>Juvenile females</b>				
600	0.6 (0.5–0.8)	0.9 (0.6–1.3)	1223 (1104–1343)	21.9
676	0.8 (0.6–1.0)	0.9 (0.6–1.3)	1041 (0913–1208)	18.8
686	2.8 (2.3–3.4)	3.9 (2.9–4.8)	1052 (0956–1148)	35.9

*Note.* The results for all animals were significant ( $p < 0.05$ ) except where indicated. Values in parentheses correspond to 95% confidence interval. Acrophase values are in the format hhmm.

activity rhythm of captive (Menezes *et al.*, 1996, 1998) and free-ranging common marmosets (Castro *et al.*, 2003) reinforces the association between scent-marking and feeding behavior, the distribution of which follows locomotor activity.

Our evidence suggests that the diurnal profile of scent-marking behavior in captive marmosets does not follow the artificial feeding schedule of captive conditions but remains associated to the time of foraging activities for wild animals. As Alonso and Langguth (1989) demonstrated, foraging behavior, *i.e.*, ingestion of animal, fruit, and exudate items, has a

**Table II.** Individual Cosinor results for the frequency of scent-marking behavior in the 8-h period for all studied animals

Animal	Mesor	Amplitude	Acrophase	%R
<b>Adult males</b>				
285	1.5 (1.1–1.8)	1.2 (0.5–1.8)	1120 (0911–1329)	22.0
311	1.5 (1.1–1.8)	0.7 (0.0–1.3)	0908 (0411–1404)	16.0
375	NS	NS	NS	NS
395	0.8 (0.6–1.0)	0.6 (0.3–1.0)	1031 (0829–1232)	21.0
445	2.3 (1.9–2.8)	0.7 (0.0–1.4)	1311 (0755–1828)	33.2
483	1.0 (0.8–1.2)	0.6 (0.3–0.9)	0920(0705–1136)	28.4
485	1.6 (1.3–1.8)	0.9 (0.4–1.3)	1138 (0926–1350)	31.6
497	1.4 (1.1–1.6)	0.8 (0.4–1.3)	0853 (0637–1110)	28.2
523	1.7 (1.4–2.1)	1.7 (1.1–2.3)	1122 (0959–1244)	21.2
527	5.6 (4.7–6.4)	4.3 (2.8–5.8)	1104 (0941–1228)	29.0
529	2.1 (1.7–2.5)	1.9 (1.2–2.6)	1102 (0932–1232)	24.0
561	2.3 (1.8–2.9)	1.4 (0.4–2.4)	1305 (1006–1605)	23.2
<b>Adult females</b>				
314	NS	NS	NS	NS
408	NS	NS	NS	NS
446	3.2 (2.8–3.7)	2.9 (2.1–3.6)	1001 (0857–1104)	33.1
460	3.8 (3.3–4.3)	2.4 (1.5–3.4)	1108 (0937–1240)	34.7
468	1.2 (1.0–1.3)	0.7 (0.4–1.1)	1152 (0956–1347)	29.9
506	10.4 (9.1–11.7)	4.8 (2.6–7.1)	1043 (0853–1233)	47.2
510	4.4 (3.6–4.9)	3.3 (2.2–4.5)	1125 (1007–1243)	32.2
512	4.5 (3.8–5.1)	3.4 (2.2–4.5)	1104 (0941–1227)	34.1
532	2.6 (2.1–3.1)	2.5 (1.6–3.3)	1057 (0937–1217)	22.3
546	1.1 (0.8–1.3)	1.2 (0.7–1.6)	1019 (0846–1150)	22.4
<b>Juvenile males</b>				
629	0.8 (0.6–1.0)	0.8 (0.4–1.1)	1059 (0908–1250)	18.7
707	0.9 (0.7–1.1)	1.0 (0.6–1.3)	1210 (1047–1332)	18.0
731	1.3 (1.0–1.6)	0.9 (0.3–1.4)	1239 (1002–1516)	23.4
<b>Juvenile females</b>				
600	NS	NS	NS	NS
676	0.8 (0.6–1.0)	0.7 (0.3–1.0)	1055 (0855–1254)	18.8
686	2.8 (2.3–3.3)	1.6 (0.7–2.5)	1340 (1121–1559)	36.1

*Note.* The results for all animals were significant ( $p < 0.05$ ) except where indicated. Values in parentheses correspond to 95% confidence interval. Acrophase values are in the format hhmm.

bimodal pattern of diurnal distribution very similar to that we found for scent-marking behavior.

Though juveniles also showed both 24- and 8-h components, amplitude fluctuation is lower than for adults, suggesting that the frequency of this behavior increases during growth, as Menezes *et al.* (1996) also observed for locomotor activity. Few researchers have addressed the ontogeny of scent-marking behavior in callitrichids. French and Cleveland (1984) observed that both sexes of immature cotton-top tamarins (*Saguinus oedipus*) living in family groups started scent marking at 2 mo. Ximenes (1991) reported that common marmoset infants started to scent mark during the 4th week

of life. They initially marked near the feeding plate but not on the food, where adults mostly do, increasing levels of food marking until weaning at 3 mo.

Lower frequency of scent-marking behavior in juveniles may result from their social role within the group. Juveniles increase their interactions more with siblings than with parents and are mainly involved with socialization activities such as playing, fighting-like behavior, and eventually mounting same-age or younger siblings (Yamamoto, 1993). In captivity, they may also take care of the infants, which one does not see in free-ranging groups (Yamamoto *et al.*, 1996). Juveniles also did not participate in intergroup encounters in the wild, where scent-marking behavior is important for adult males in defending territorial and alimentary resources (Araújo, 1996).

Scent-marking behavior in callitrichids is associated with multiple social and individual contexts, such as territorial defense, communication of reproductive functioning and feeding sources, among others (Washbaugh and Snowdon, 1998). Smith *et al.* (2001) has showed via linear discriminant analysis of scent marking pools that common marmoset females have a chemical compound that may allow individual identification. Indeed, Ferris *et al.* (2001, 2004), using magnetic nuclear resonance in males, demonstrated that neuroanatomical areas involved in odor discrimination, collected from ovulating and ovariectomized females, are part of a general appetitive circuit associated with both sexual activity and emotion. These findings suggest that olfactory cues provide information on the reproductive functioning of females and that odors modulate sexual behavior in the species.

We found that females scent mark more frequently than males do. Miller *et al.* (2003) found similar results for *Leontopithecus rosalia* and Smith and Gordon (2002) for *Saguinus labiatus*. For *Saguinus mystax*, Heymann (1998) reported that males and females did not differ in the use of anogenital marking, though females showed significantly higher suprapubic marking activity than that of males. Researchers have recorded use of scent marking within a reproductive context by common marmoset in 2 ways: from the dominant to the subordinate female, as part of the mechanisms involved in reproduction inhibition (Abbott, 1984; Smith and Abbott, 1998) and also by wild subordinate females toward males, to deceive them about their reproductive status (Lazaro-Perea *et al.*, 1999). The evidence seems to be consistent with the prediction of the sexual selection theory for species in which males give substantial care to offspring, as Heymann (1998) pointed out for *Saguinus mystax*. In such cases, female competition is high and scent-marking behavior is a component of the expression of the dispute.

In summary, our results indicate that besides being part of reproductive signaling in common marmosets, scent-marking behavior is also temporally associated with natural patterns of locomotor and feeding behavior and its expression depends on sex and age.

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