

**CHRISTINI BARBOSA CASELLI**

**“COMPORTAMENTO TERRITORIAL DE *Callicebus nigrifrons* Spix, 1823  
(PITHECIIDAE): INFLUÊNCIA DA DISPONIBILIDADE DE FRUTOS E  
POSSÍVEIS FUNÇÕES DAS VOCALIZAÇÕES DE LONGO ALCANCE.”**

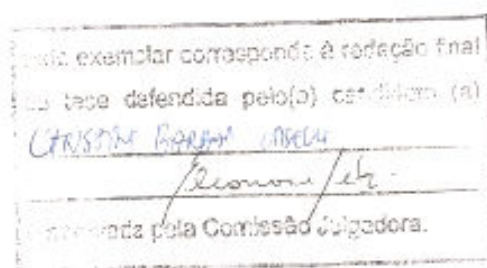
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**UNIVERSIDADE ESTADUAL DE CAMPINAS**  
**INSTITUTO DE BIOLOGIA**

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Biologia para obtenção do Título de  
Doutor em Ecologia.

Orientadora: Profa. Dra. Eleonore Zulnara Freire Setz  
Coorientador: Prof. Dr. Júlio César Bicca-Marques

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## **Resumo**

A territorialidade corresponde a uma forma de competição na qual competidores expulsam uns aos outros de áreas contendo recursos, os territórios. A defesa de territórios pode ser flexível, sendo manifestada apenas quando existirem recursos críticos que limitem o crescimento da população (frequentemente representados pela disponibilidade de alimento) e quando estes forem economicamente defensáveis. Os primatas do gênero *Callicebus* são geralmente descritos como territoriais, embora esta caracterização baseie-se em estudos focados em apenas três das 30 espécies conhecidas desse gênero diverso de primatas Neotropicais. Ainda, a manifestação de comportamentos relacionados à territorialidade se mostrou bastante variável em estudos anteriores, podendo estar relacionada à curta duração desses trabalhos, os quais não contemplaram os efeitos da variação sazonal de recursos alimentares na manifestação desses comportamentos. Nesse contexto, investigamos o comportamento territorial de *Callicebus nigrifrons*, analisando a influência da variação na disponibilidade de frutos sobre este comportamento. Buscamos também analisar o papel das vocalizações de longo alcance, geralmente associadas a defesa conjunta de território, mas que também pode estar relacionada à defesa de parceiros reprodutivos. Para isso monitoramos o comportamento de um grupo habitado de *C. nigrifrons* ao longo de 20 meses (Novembro/2009 - Junho/2011; 730 horas de observação) paralelamente ao monitoramento da disponibilidade de frutos de 22 espécies zoocóricas consumidas por estes primatas. Adicionalmente, analisamos a estrutura dos cantos de longo alcance de nove grupos de *C. nigrifrons* e relacionamos a estrutura desses cantos aos contextos nos quais estes são utilizados. Também realizamos experimentos com “playbacks” para investigar a importância das vocalizações de longo alcance na defesa conjunta de territórios e de parceiros. As vocalizações de *C. nigrifrons* apresentaram estrutura hierárquica, cuja combinação de unidades menores, as sílabas, dão origem as frases, que são então combinadas em sequências mais longas. Estas vocalizações de longo alcance são utilizadas tanto para comunicação entre membros do mesmo grupo, como para comunicação entre grupos vizinhos e, embora sejam compostas por unidades vocais semelhantes (mesmas sílabas e frases), apresentam estruturas diferentes associadas a estes diferentes contextos comportamentais. Os cantos utilizados para comunicação entre grupos vizinhos foram utilizados principalmente para defesa de recursos alimentares importantes na dieta desses primatas. Diferente de outros *Callicebus*, *C. nigrifrons*



não exibiu comportamento de defesa e patrulhamento das bordas de sua área de vida, mas focaram o comportamento de defesa em áreas com recursos alimentares importantes e economicamente defensáveis, como árvores frutíferas. Esse comportamento de defesa, tanto pela emissão de vocalizações de longo alcance, quanto pelo uso de outros comportamentos agressivos, resultaram na expulsão de grupos vizinhos que se aproximassem de sua área de uso, e variou em intensidade de acordo com a disponibilidade de frutos no ambiente. A baixa frequência de encontros entre nosso grupo focal e grupos vizinhos sugere que os comportamentos adotados por *C. nigrifrons* são efetivos para garantir o acesso prioritário a recursos alimentares importantes em sua dieta.

## **Abstract**

Territoriality is a form of competition in which competitors exclude each other from areas containing resources, the territories. Territorial behaviors are flexible and should be adopted only when there is critical resource shortage (which limits population growth) and when these are economically defensible. *Callicebus* monkeys are usually described as territorial, although this characterization is based on studies focused on three of the 30 known species of this diverse genus of Neotropical primates. Still, the expression of behaviors related to territoriality showed a variable pattern in previous studies. Part of these variation within *Callicebus* genus could be a result of the short duration of these studies, which did not contemplate the effects of seasonal variation of food resources on territorial behavior. Here we investigated the territorial behavior of *Callicebus nigrifrons*, evaluating the influence of fruit availability over it. We also evaluated the role of *C. nigrifrons* loud calls in intra and extra group communication, as well as on food and mate defense. We thus monitored the behavior of a habituated group of *C. nigrifrons* over a period of 20 months (November/2009 - June/2011; 730 observation hours in total). In parallel to behavioral data collection we monitored the availability of fruits from 22 species eaten by these primates. We also described the structure of loud calls of nine groups of *C. nigrifrons* and related the structure of these calls to the contexts in which they were used, and performed playback experiments to test the hypotheses of mate and joint territorial defense. *Callicebus nigrifrons* loud calls are higher hierarchical structures formed by different small units, the syllables, which are assembled to form phrases, which are then assembled to form long sequences, the loud calls. These calls are used in different contexts, involving the communication within and between groups. Although these calls are composed by basically the same syllable and phrase types, the proportional contribution of the different syllable and phrases and the way they are assembled differ between these different contexts. The loud calls used for extra group communication was more clearly associated to joint resource defense of important food recourses in *C. nigrifrons* diet, the fruits. Unlike previews studies of *Callicebus* spp., *C. nigrifrons* did not show the behavior of patrolling and marking range boundaries, but advertised the occupancy of its range via loud call emissions, especially from places close to important and economically defensible food sources, such as fruits. The intensity of the defensive behavior, via loud call emissions and aggressive approach and repulsion of neighboring groups, increased with fruit availability. The

low frequency of inter-group agonistic encounter suggest that territories ownership advertisement by *C. nigrifrons* are effective in maintaining the priority access to important food resources.

## **1. Introdução geral**

Territórios são áreas defendidas contra incursões de co-específicos e nas quais o indivíduo (ou grupo de indivíduos) tem acesso exclusivo, ou prioritário, aos recursos ali presentes, como alimento, parceiros reprodutivos ou locais para reprodução (Brown, 1964; Powell, 2000; Wilson, 2000). O território pode corresponder a toda a área de vida utilizada por um indivíduo (ou grupo), ou apenas a uma parte desta, como áreas centrais mais utilizadas (Person & Hirth, 1991; Powell, 2000).

Modelos teóricos que buscam explicar a evolução do comportamento territorial sugerem que a expressão desse comportamento deve variar entre diferentes populações de uma mesma espécie ou mesmo dentro de uma população ao longo do tempo (Carpenter & Macmillen, 1976; Maher & Lott, 2000). Em geral, a defesa de territórios deveria ser adotada quando o ganho com a defesa (seja um ganho alimentar ou de parceiros sexuais) fosse superior ao gasto energético despendido com esse comportamento. Assim, em condições de baixa e alta disponibilidade de recursos a defesa não seria esperada. No primeiro caso porque o gasto energético com a expulsão de co-específicos seria provavelmente superior ao ganho adquirido pela exclusividade ao recurso defendido, enquanto que no segundo caso a competição pelos recursos seria muito baixa. Nesse sentido, a defesa territorial deveria ocorrer quando houver uma quantidade intermediária de recursos, na qual a competição entre os indivíduos pelo acesso não seja muito elevada (Brown, 1964; Carpenter & Macmillen, 1976; Maher & Lott, 2000).

O alimento é considerado o recurso limitante mais comum e, para várias espécies, o comportamento territorial tende a variar de acordo com a qualidade e a distribuição espaço-temporal deste recurso (Carpenter & Macmillen, 1976; Kinnaird, 1992; Maher & Lott, 2000; Strier, 2007).

Alimentos de baixa qualidade, como folhas maduras, podem não compensar a energia, o tempo e nem os riscos envolvidos na defesa dos mesmos. Ainda, devido a sua maior abundância e distribuição mais uniforme, estes não são recursos facilmente esgotáveis. Por outro lado, alimentos de alta qualidade, como frutos, apresentam distribuição mais agregada e produção mais variável ao longo do ano (Clutton-Brock & Harvey, 1977; Grant et al. 1992). Nesse sentido, a defesa de sítios com alta disponibilidade de frutos durante determinados períodos do ano pode resultar em aumento da energia adquirida e compensar os gastos despendidos com a sua defesa (Kinnaird, 1992; Strier, 2007).

Em mamíferos, os territórios podem ser defendidos por meio de vários mecanismos, como interações agressivas, marcações odoríferas, vocalizações ou comportamentos estereotipados sem contato físico (“displays”), sendo estes três últimos mais comumente empregados (Owen-Smith, 1977; Powell, 2000). Várias espécies de primatas produzem vocalizações de longo alcance (“loud calls”), que, devido às suas propriedades acústicas (alta amplitude e baixa frequência), podem ser transmitidas por longas distâncias (Mitani & Stuht, 1998; Waser & Waser, 1977). Estas características permitem que as vocalizações de longo alcance possam ser utilizadas na comunicação entre grupos vizinhos, apresentando importante função na regulação de espaço entre estes (Mitani & Stuht, 1998; Oliveira & Ades, 2004; Wich & Nunn, 2002).

As vocalizações de longo alcance podem ser emitidas individualmente por machos e fêmeas ou em parceria, na qual ambos os sexos, geralmente um casal reprodutor, vocalizam simultaneamente ou alternadamente de forma coordenada (Grafe & Bitz, 2004; Hall, 2004). Estas parcerias, denominadas duetos, foram registradas para várias espécies de aves e de primatas e, em geral, estão associadas à defesa conjunta de território (Farabaugh, 1982; Hall, 2004; Oliveira & Ades, 2004; Serpell, 1981). Os cantos de longo alcance podem ainda ser emitidos em coro,

quando mais de dois indivíduos participam (Bradley & Mennill, 2009; Kitchen, 2004). Embora os coros recebam menos atenção que os duetos, é provável que tenham funções semelhantes na manutenção colaborativa de territórios (Bradley & Mennill, 2009; Fan et al., 2009; Geissmann & Mutschler, 2006), podendo ainda atuar no aumento do alcance de comunicação, uma vez que, ao sobrepor chamados, aumentam a amplitude sonora, como observado nos papagaios galego (*Alipiopsitta xanthops*; de Araújo et al., 2011).

A hipótese de defesa conjunta de territórios está baseada na premissa de que, ao coordenar suas vocalizações em duetos, os parceiros produzem demonstrações mais efetivas da capacidade e disponibilidade de defesa de territórios do que aquelas que seriam produzidas individualmente (Hall, 2004). Por outro lado, emissões coordenadas de vocalizações em duetos podem ser resultado do conflito de interesse entre parceiros, como no caso da hipótese de guarda de parceiro (Levin, 1996; Morton & Derrickson, 1996). De acordo com a mesma, os animais vocalizam para demonstrar o estado de comprometimento de seu parceiro, evitando que este copule com possíveis invasores ou sejam perdidos para estes (Grafe & Bitz, 2004; Hall, 2004).

O uso de vocalizações de longo alcance para comunicação entre grupos vizinhos, principalmente em duetos, corresponde a uma característica marcante do gênero *Callicebus*, popularmente conhecidos como sauás (Kinzey & Robinson, 1983; Mason, 1968; Moynihan, 1966; Robinson, 1979a). Os primatas desse gênero são animais monogâmicos de pequeno porte (pesando até 2Kg), predominantemente frugívoros, os quais vivem em grupos familiares compostos pelo casal reprodutor e até quatro filhotes (Bordignon, 2008; Kinzey, 1997). Em geral, os sauás são descritos como territoriais (Kinzey, 1997; Robinson et al., 1986), embora poucos estudos tenham procurado investigar esta questão. Ainda, nestes poucos estudos, o comportamento territorial se apresentou de forma variável entre as espécies estudadas (Kinzey &

Robinson, 1983; Price & Piedade, 2001), possivelmente devido à curta duração dos trabalhos, os quais não contemplaram a variação sazonal na disponibilidade de recursos alimentares na presença do comportamento.

Em *C. ornatus* observou-se que o uso frequente de vocalizações de longo alcance promove encontros de grupos vizinhos nas bordas dos territórios, resultando na demarcação de áreas de uso exclusivo (Mason, 1968; Robinson, 1979a). Em *C. torquatus*, grupos vizinhos vocalizam em reação a vocalizações de outros grupos, anunciando a localização dos grupos em determinadas áreas e raramente resultam em encontros (Kinzey & Robinson, 1983). Em *C. personatus* (Price & Piedade, 2001) a emissão de vocalizações de longo alcance é infrequente e ocorre principalmente durante encontros entre grupos, não resultando em áreas de uso exclusivo.

Com relação à estrutura das vocalizações de longo alcance, as espécies de *Callicebus* mais bem estudadas são *C. cupreus* (Müller & Anzenberger, 2002) e *C. ornatus* (Robinson, 1979b). Nestas espécies, machos e fêmeas vocalizam sequências praticamente idênticas de forma simultânea, alternando as diferentes partes de uma sequência repetidas vezes. Os duetos geralmente são iniciados por vocalizações denominadas gemidos (“moaning”) de ambos os sexos, os quais levam à primeira frase da sequência de um dueto. Em geral, os animais iniciam os “moanings” simultaneamente, porém, algumas vezes o macho pode iniciar as vocalizações e, em outras, a fêmea. Essa diferença pode ser um indicativo da motivação de cada sexo em iniciar os duetos (Müller & Anzenberger, 2002; Robinson, 1979b). Robinson (1979b) observou que em testes com “playbacks” de vocalizações individuais de machos e fêmeas, os indivíduos geralmente iniciavam os duetos em resposta a vocalizações de outros de mesmo sexo.

A estrutura e função das vocalizações de longo alcance nas demais espécies do gênero *Callicebus* foi pouco explorada, sendo estes cantos basicamente associados a defesa conjunta de

territórios (Kinzey & Robinson, 1983; Mason, 1968; Price & Piedade, 2001; Robinson, 1979a, 1979b). Consequentemente, mais estudos são necessários para uma maior compreensão sobre o comportamento de vocalização desses primatas e como as vocalizações de longo alcance podem atuar na comunicação interna entre membros do mesmo grupo e entre grupos vizinhos, especialmente na defesa de recursos, sejam estes alimentares ou parceiros reprodutivos.

Aqui neste trabalho nos focamos no comportamento dos sauás-da-cara-preta (*Callicebus nigrifrons*; figura 1) com o intuito de trazer mais informações para a discussão a respeito da territorialidade em *Callicebus* e o papel das vocalizações de longo alcance nesse contexto, diferenciando essas vocalizações daquelas utilizadas para outras possíveis finalidades, como comunicação interna entre membros de um mesmo grupo e na defesa de parceiros reprodutivos. Para isso, no capítulo um, nós analisamos primeiramente a estrutura das vocalizações de longo alcance de *C. nigrifrons* e relacionamos vocalizações com diferentes estruturas aos seus respectivos contextos comportamentais, discutindo o papel destas vocalizações na comunicação intra e inter grupos. Posteriormente, no segundo capítulo, nos focamos nas vocalizações de longo alcance utilizadas para a comunicação entre grupos e testamos a função destas vocalizações na defesa de parceiros e de territórios. Por fim, no terceiro capítulo, investigamos o comportamento territorial em *C. nigrifrons*, avaliando a influência da disponibilidade de recursos sobre esses comportamentos e como as vocalizações de longo alcance inter grupo são utilizadas nesse contexto.

## **2. Objetivos**

Propomos aqui:

1) descrever a estrutura dos cantos de longo alcance de *C. nigrifrons* e analisar os contextos nos quais estes são utilizados;



- 2) testar as hipóteses de defesa conjunta de territórios e de parceiros para os cantos de longo alcance utilizados na comunicação entre grupos;
- 3) investigar o comportamento territorial de *C. nigrifrons*, analisando a influência da variação na disponibilidade de frutos sobre a manifestação desse comportamento.



**Figura 1.** Indivíduo adulto de *Callicebus nigrifrons* (Foto: Caselli, C.B)

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## Capítulo 1

"Comportamento de vocalização de sauás-da-cara-preta (*Callicebus nigrifrons*):  
propriedades acústicas e contextos comportamentais das vocalização de longo  
alcance."

1 Title: **Black-fronted titi monkeys (*Callicebus nigrifrons*) vocal behavior:**  
2 **acoustic properties and behavioral contexts of loud calls**

3  
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12  
13 Short title: **Vocal behavior of *Callicebus nigrifrons***

14  
15 Word count: 7.059 words plus 4 figures and 2 tables



## **Abstract**

The use of loud calls is wide spread through many bird and primate species and due to their high amplitude and low frequency, can be transmitted over long distances, being commonly used by for both intra and extra group communication. In the Neotropics, the titi monkeys are known by the emission of conspicuous coordinated loud calls, which have been related to territorial defense, yet this understanding is based on studies of only three of the 30 known species of the genus. Here we describe the acoustic properties of *Callicebus nigrifrons* loud calls and compare the behavioral and ecological contexts associated with these vocalizations to investigate their role in intra and extra group communication, as well as on food and mate defense. *Callicebus nigrifrons* uses different small units, the syllables, to form higher hierarchical structures, the phrases, which are then assembled to compose the loud calls. Structural different calls are used in different contexts, involving the communication within and between groups. Our results also provide support for the hypothesis that calls used for communication between neighboring groups are a cooperative display, used by the mated pair and other group members, to regulate the access to important food resources, such as fruits. Our observations of monkeys behavior and structural analysis of loud calls, on the other hand, lend only partial support to the mate defense hypothesis.

**Key words:** loud calls; territorial defense; mate defense; animal communication.

## 1. Introduction

Due to their high amplitude and low frequency, loud calls are transmitted over long distances (Wich & Nunn, 2002), and are thus commonly used by many birds and primates for both intra and extra group communication (Hall, 2004; Mitani & Stuht, 1998; Serpell, 1981). These calls can be emitted by males and females as solos or as jointly produced signal by two or more individuals, such as duets or choruses, respectively (Geissmann, 2002; Heimoff, 1986; Kitchen, 2004; Pollock, 1986, Wilson et al., 2001).

Coordinated emissions of loud calls, especially as duets by mated pairs, are commonly observed in monogamous primates and their functions are usually associated with joint territorial defense or mate guarding (Cowlshaw, 1992; Mitani & Stuht, 1998; Oliveira & Ades, 2004, Serpell, 1981). In joint territorial defense the individuals signal to outsiders their resource holding potential and willingness to defend territories (Hall, 2004), whereas in mate guarding contexts animals join its partners' vocalization to signal their mated status and thus repel same sex rivals (Grafe & Bitz, 2003; Levin, 1996).

In the Neotropics, the titi monkeys (genus *Callicebus*) are known by the emission of conspicuous coordinated loud calls, which have been related to territorial defense, yet this understanding is based on studies of only three of the 30 known species of the genus (Kinzey & Robinson, 1983; Mason, 1968; Price & Piedade, 2001; Robinson, 1979a, 1981). In *Callicebus ornatus* the frequent emission of loud calls is associated to the defense of territory boundaries and may also play a role in mate defense, since individuals of the mated pair usually initiate the duets in response to same-sex vocalizations (Mason, 1968; Robinson, 1979a). In *C. torquatus* (Kinzey & Robinson, 1983) and *C. personatus* (Kinzey & Becker, 1983; Price & Piedade, 2001) groups engage in counter-calling events in

reaction to duets or presence of other groups and seem to be used to alert the group's location to neighbors, avoiding encounters between them.

The structure of these loud calls were comprehensively described only for *C. ornatus* (Robinson, 1979b) and *C. cupreus* (Müller & Anzenberger, 2001), and in both species these vocalizations are formed by small units (calls) which are assembled to form higher hierarchal structures, the phrases, that are then cycled to compose sequences of variable duration. The form and function of the loud calls in the remaining species of *Callicebus* have not been explored. Besides, poor attention was given to the function of these calls in intra group communication or their possible function in mate defense (Kinzey & Robinson, 1983; Mason, 1968; Price & Piedade, 2001; Robinson, 1979a, 1979b).

Although mate defense may not seem a very compelling hypotheses to duets function in monogamous mammals, *Callicebus* monkeys, for example, can occasionally engage in extra-pair copulations (Defler, 2004; Mason, 1966) and partner change has been observed in the field (pers. obs.). The same was observed for Asian arboreal rainforest hylobatids (Palombit, 1994a, b; Reichard & Sommer, 1997), which are a good comparative to *Callicebus* in the sense that they are frugivorous territorial primates that forms long-term pair-bonds (Bartlett, 2007). Given the potential for extra-pair copulation and turnover in pair bonds, the mate guarding can be an important strategy to guarantee male reproductive success (Palombit, 1999) and gibbon songs are likely to serve in mate defense (Cowlshaw, 1992). In fact, mammalian social monogamy have been suggested to evolve in conditions in which not gregarious females live apart of each other in small territories. In this scenario males can effectively guard its mate and thus mate defense may play an important role in the evolution of mammalian monogamy (Komers & Brotherton, 1997; Palombit, 1999).

91 In this study, we focus on the loud calling behavior of black-fronted titi monkeys  
92 (*Callicebus nigrifrons*) that, like other *Callicebus*, are predominantly frugivorous primates  
93 that live in family groups composed of an adult breeding pair and one to three offsprings  
94 (Kinzey, 1997; Robinson et al., 1986). During preliminary field observations on this  
95 species we came to recognize two types of loud calls that are apparently used in different  
96 contexts: for intra and extra group communication. We thus temporary named these calls as  
97 type 1 and 2, respectively. These calls can be distinguished by hearing and also by the  
98 monkeys' behavior in the field. The type 1 sounds more cyclic (because of the repetitive  
99 use of chirrups, as described by Robinson, 1979b) and is usually spontaneous, triggered by  
100 one or two individuals of the same group, resulting in visible changes in group members  
101 movements. The type 2 calls is usually followed by an increasing arousal state, with no  
102 obvious cyclical sound, and is usually associated to the vocalizations or the presence of  
103 neighboring groups.

104 Here we describe the acoustic properties of *C. nigrifrons* type 1 and 2 loud calls to  
105 evaluate if they have structural differences and characterize and compare the social and  
106 ecological contexts associated with these vocalizations to investigate if calls with different  
107 structures are associated with different roles, such as in intra and extra group  
108 communication.

109 If *C. nigrifrons* loud calls used for extra group communication are important in food  
110 resource defense we expect that (1) animals should call more often as choruses or as duets  
111 when facing outsiders, because all members of the group would have a common interest in  
112 defending their shared resources from other groups or individuals; (2) animals should  
113 vocalize more frequently in periods when defensible and worth-contesting critical

resources, such as fruits, are available. On the other hand, if these calls are important in mate defense we expect that (3) they should be emitted more often as solos or as duets with perceptive differences in sex contributions, since males and females would have individual interests in repelling same-sex rivals; (4) *C. nigrifrons* should also vocalize more frequently in periods when the female is fertile, the time in which the risk of extra pair copulation is higher.

## **2. Methods**

### **Subjects and study site**

We recorded the vocalizations of six groups of *C. nigrifrons* at three Atlantic Forest sites in the state of São Paulo in southeastern Brazil: one habituated and three non-habituated groups at Serra do Japi Municipal Ecological Reserve (23°11'S, 46°52'W), municipality of Jundiaí; one habituated group at Cantareira State Park (23°27'S, 46°37'W), municipality of São Paulo; and one habituated group at Ribeirão Cachoeira (23°27'S, 46°37'W), municipality of Campinas. We complemented our recordings of wild animals with recordings of three captive groups at the Bauru Zoo (Bauru, state of São Paulo), Guarulhos Zoo (Guarulhos, state of São Paulo) and Center of Environmental Development of Companhia Brasileira de Metalurgia e Mineração (Araxá, state of Minas Gerais). Captive animals were born in the wild and were capable of performing the species typical song when stimulated by playbacks. Subsets of recordings were used for different analysis depending on the quality of the recording and the specific purposes, as described below.

To investigate the natural context of loud calls we focused our observations on a habituated *C. nigrifrons* group (hereafter group 1) composed of five to six individuals at Serra do Japi, a 350-km<sup>2</sup> remnant of Atlantic Forest. Group 1 was followed from dawn to

dusk (50 complete days) or from the moment it was found until it was lost (29 incomplete days) for 3 to 5 days each month over a period of 20 months (November 2009 - June 2011). Since loud calls can be heard up to 1 km away (Robinson et al., 1986), we also registered the loud calls uttering frequency of at least four neighboring groups to assess the daily and seasonal variation based on the vocal behavior of these five groups. Between March and November of 2007, we also observed a mated pair of *C. nigrifrons* (hereafter group 2) in an adjacent area to group 1 and registered the frequency of loud calls emitted for this couple and their neighboring groups. To estimate the period that females were fertile, we used the number of observed copulations and birth events for groups 1 and 2 (Caselli, unpublished data); copulations are frequent during the breeding season and gestation length is known to range from 122 to 167 days (Defler, 2004; Valeggia, 1999).

#### **Fruit availability**

To evaluate the relationship between vocal behavior of group 1 and its neighbors and fruit availability, we monitored the fruit production of 211 plants of 22 zoochoric species eaten by *C. nigrifrons* in the territory of group 1. To access the monthly fruit production for each plant we assigned scores from 0 to 4 for the percentage of the crown bearing mature fruits (0: no fruit; 1: 1 to 25% of the crown bearing fruit; 2: 26 to 50%; 3: 51 to 75%; 4: 76 to 100%; following Sun et al., 1996). We used the monthly fruit scores attributed to each individual plant to calculate an index of relative fruiting (following Kinnaird, 1992). This index sums the monthly scores attributed to all individuals and divides this value by the number of individuals of each species and then by the number of species.

#### **Loud call recordings and acoustical analysis**

We recorded vocalizations in WAV format, using a Sennheiser ME-67 directional microphone and a Marantz PMD-671 digital recorder at 48 kHz sampling rate and 16-bit resolution. We conducted the recordings between June 2010 and June 2011 at distances of up to 20 m (mean = 5 m) from the tree where the monkeys were calling. Whenever possible, we recorded all occurrences of loud calls of group 1, both spontaneous and in response to neighboring groups or to playback. For other groups we recorded calls only in response to loud calls of neighboring groups or to playback. For playbacks to group 1 we used recordings of the Ribeirão Cachoeira group; for playbacks to all other groups we used recordings of group 1. When recording the vocalizations of non-habituated groups, the observer remained hidden in understory vegetation to minimize influencing the recorded animals' behavior. Non-habituated titi monkeys emit characteristic alarm calls in the presence of observers (Cäsar et al., 2012) and these alarm calls were not observed during the recordings.

We did not take into account the identity, age and sex of individuals recorded, since distinguishing between individuals in these categories is difficult in the wild. For habituated groups however, we were able to distinguish between the male and female of the mated pair and between them and their offspring based on their size when animals were close to each other. Males are slightly larger than females (Robinson et al., 1986) and adults (> 30 months of age) of the mated pair were larger than the subadults (18 - 30 months) and juveniles (6 - 18 months). Infants (< 6 months) were easily recognized due to their small size (de Luna et al., 2010; Valeggia et al., 1999). Hereafter we will refer to both subadults and juveniles as young.

To visualize the structure of loud calls we generate spectrograms of the recordings using Syrinx-PC software (John Burt, Seattle, WA; Blackman window type; 2048 FFT). Based on visual inspection of the spectrograms of recordings of all groups, we identified different types of syllables. We defined syllables as an uninterrupted spectrographic tracing separated by silent intervals (following Robinson, 1979b). These units, rarely produced in isolation, are assembled by the monkeys to form phrases that are then combined to form long vocal sequences.

Since the recordings often consisted of overlapping vocalizations, we selected a subset of 20 recordings (one or more recordings from each of the nine groups) in which we were able to select individual syllables with no overlap. We randomly selected up to 10 examples of each type of syllables for each group (average:  $6.4 \pm 0.6$  (SE) syllables per type per group). We then measured the following fine structural parameters: duration of the entire syllable, number of harmonics under 2 kHz; harmonic-to-noise ratio (a parameter for quantifying the noise within a signal); minimum and maximum frequency; and total number of harmonic peaks above -15 dB at 5 ms of the beginning and the end of the syllable. We conducted these measurements in Avisoft-SASLab Pro 5.2.05 (R. Specht, Berlin, Germany) using the automatic parameter measurements tool (settings: two thresholds of -16 and -20 dB, 23 ms hold time, 1024 FFT size, 98.43% overlap, Blackman window, 1 ms time resolution and 22 Hz frequency resolution). Before conducting these measurements we normalized each song to the same amplitude (-1dB) and filtered all sounds above 20 kHz (High-pass filter) and below 80 Hz (Low-pass filter) to standardize the songs measurements between recordings and eliminate background sounds outside the frequency spectrum of *C. nigrifrons* vocalizations. The use of automated parameter



measurements minimized the influence of subjectivity in measuring the structural features of these calls.

Based on these syllable pre-categories, we identified five qualitatively different phrases, formed by the combination of one or two different syllables. We quantified the proportional contribution of syllable and phrase types for loud calls temporarily named type 1 and 2 to access possible structural differences between these calls. We also measured call mean duration, the mean rate of syllables and phrases and the transition probability between two consecutive syllable and phrase types. To carry out such measurements, we selected a subset of 26 recordings (13 of type 1 and 13 of type 2) that allowed us to analyze the sequence of these vocal units for at least one animal vocalizing in each recording (either because the vocalization of one of the individuals in the recording was louder, consequently more visible in the spectrogram, or because the other animals in the group were silent for a period of the recording). For type 1 loud calls this subset included vocalizations of four groups (three wild and one captive), for type 2 loud calls we used only recordings of two wild groups (group 1 and Ribeirão Cachoeira group). The procedure of analyzing the sequence of vocal units consists of drawing boxes around these units (syllables or phrases) using Syrinx-PC's time cursors. For measuring the duration of each type of loud call, we included 12 additional vocalizations of each type, totaling 25 examples of each call type.

### **Callicebus nigrifrons vocal behavior**

We registered all occurrences of group 1 loud calls, their context and time of day. Context description included: (i) the occurrence of neighboring groups loud calling before and after group 1 vocalizations events; (ii) the number of animals vocalizing; (iii) the

behavior of the animals while vocalizing; (iv) the duration of the call; (v) the reaction time and (vi) the group's spatial reaction (neutral, retreat or approach) based on the estimated group's angle-of-turn after a call given by a neighbor group. We considered a neutral response if group 1 remaining stationary or turned between  $0^{\circ}$  and  $15^{\circ}$ , an approach when it turned between  $15^{\circ}$  and  $180^{\circ}$  and moved toward the calling group, and a retreat when it turned between  $15^{\circ}$  and  $180^{\circ}$  and moved towards the opposite direction of the calling group.

### **Daily distribution of loud calls**

To analyze the temporal distribution of loud calls, we calculated the vocalization uttering frequency for each hour of day for groups 1 and 2 and for their neighboring groups. We transformed the time of calling events into times with respect to sunrise to control for seasonal variation on days' length.

### **Statistical analyses**

We conducted a discriminant function analysis (DFA) of the syllables types that we had categorized visually. We randomly selected one example of each of six syllable types from each of nine groups and constructed a DFA based on these 54 syllables. To test the significance of the discriminant model we performed a MANOVA. We then cross-validated this analysis to determine whether we could correctly predict the type of syllable on the basis of seven measured fine structural parameters (independent variables) using a second example of each syllable type from each group. Since three of the 10 independent variables were highly correlated with at least one of other variables (Spearman's rank correlation coefficient ranging from -0.60 to 0.59,  $P < 0.05$ ), we performed the DFA with seven independent variables. We report the accuracy of the discriminant analysis as the

proportion of these second syllables correctly assigned to the appropriate syllable type. These analyses were performed using the packages MASS version 7.3-18 (Venables & Ripley, 2002) and stats of R software version 2.15.0 (R development core team, 2011).

To determine whether the transitions between any two consecutive syllables or phrases occur at rates significantly different than expected by chance, given the observed frequency of syllable and phrase types, we used the winnowing log-linear-based approach (Bakeman & Quera, 2011). This analysis is based on the chi-square test of independence, where the events in the rows (lag 0) of contingency tables are preceded by the events in the columns (lag 1). The dimension of contingency tables is determined by the number of syllable and phrase types that labels both rows and columns. For phrase transition tables, for example, we included “start” and “end” as new categories, resulting in a 7 x 7 contingency table. These new categories were introduced to investigate which type of phrase is significantly associated with start and end of each type of call. We performed the winnowing log-linear-based approach using ILOG 3 program (Bakeman et al., 2010) to analyze phrase transitions for both type 1 and 2 loud calls and syllable transitions for loud calls type 1. To describe the pattern of syllable transitions in loud calls type 2 we could not use the log-linear approach due to the reduced size of the contingency table, since one of the syllable types were rare in this type of call. In this case we run a lag sequential analysis using the GSEQ program 5.1 (Bakeman & Quera, 2011), producing a 3 x 3 table of p-values for the transition probabilities. To reduce the risk of type 1 error we applied Bonferroni correction, adjusting the alpha level of significance by the number of tests (Sokal & Rohlf, 1995).

To test if frequency of loud calls emission varied along the day we performed a nonparametric Friedman test implemented in R software version 2.15.0 (R Development

Core Team, 2011). For daily variation analysis, we considered only the first 11 hours after sunrise because the monkeys were active after the 11th hour in only seven of the 20 months of the study. To test if the frequency of loud calls used for extra group communication is higher when food resource is abundant we used a Chi-squared test considering the number of call events and the number of observation hours in months with higher and lower fruit availability than the mean fruit availability at the study site. We used the same approach to test whether monkeys vocalize more frequently in periods when females are in estrus.

### 3. Results

#### Loud call acoustic properties and structure

*Syllable structure and transition patterns.* We identified six pre-categories of syllables, named from A to F (figure 1, table I): "A" syllables are composed by high (between 3 to 12 kHz) and low frequency components (near 1 kHz) with some faint or absent middle harmonics (see between the dashed lines in figure 1); "B" syllables are composed of many tight harmonics and sub-harmonics, with little frequency modulation, and most of the energy is concentrated under 2 kHz; "C" syllables are composed by many loose harmonics and with greater frequency modulation than "B" syllable's harmonics; "Ds" and "Fs" are similar to "As", but with middle harmonics between the top and bottom components (see between the dashed lines in figure 1); "F" syllables are longer than "As" and "Ds" (table I), with a notable extension and modulation of higher components. "E" syllables are similar to "As", but the low frequency components are absent.

A discriminant analysis based on seven fine structural features (table I) significantly differentiated between these six pre-categories of syllables (Wilks Lambda = 0.015,  $P < 0.0001$ ,  $N = 54$  syllables). In a cross-validated analysis, syllables were assigned to the

correct type with 67.0% accuracy, significantly higher than the 16.7% accuracy expected by chance (Binomial test:  $P = 0.01$ ). This analysis correctly predicted most "Bs", "Cs" and "Es", but had difficulty in distinguishing "As", "Ds" and "Fs". For this reason, we lumped "Ds", "Fs" and "As" in all subsequent analyses (hereafter "As").

The proportional contribution of each syllable category and their transition patterns differed between type 1 and 2 loud calls (figure 2). While type 1 are composed mainly by "As", "Bs" and "Cs", type 2 are composed mainly by "Bs" and "Cs". Although we detected a few "Es" on type 2 calls during preliminary inspections of sonograms, these syllables were not present at the recordings sampled for this analysis. In type 1 loud calls the transitions between consecutive "As" and "Es" and between "Cs" and "Bs" occurred at higher rates than expected and transitions between "As" and "Bs" and between "As" and "Cs" occurred at lower rates than expected (complete model before the removal of significant transitions between syllables:  $G^2 = 3066.24$ ,  $df = 9$ ,  $N = 2565$ ,  $P < 0.001$ ; resulting model after the removal of the four significant transitions from the 16 possible:  $G^2 = 14.83$ ,  $df = 1$ ,  $N = 297$ ,  $P < 0.001$ ; figure 2). In type 2 loud calls the transitions between syllables also showed a significant pattern ( $G^2 = 2651.07$ ,  $df = 4$ ,  $N = 3767$ ,  $P < 0.0001$ ). The transitions between "As" and "Bs" and between "Cs" and "Bs" (figure 2) occurred at higher rates than expected and the transitions between consecutives "Bs" and "Cs" and from "As" to "Cs", occurred at lower rates than expected (all these transitions are significant at  $\alpha = 0.005$  Bonferroni corrected levels).

*Phrase structure and transition patterns.* We recognized five types of phrases (figure 1): "ab" and "bc" phrases are formed by alternated emissions of each syllable; "aa" and "bb" are formed by consecutive emissions of each syllable and "ae" is formed by combined

emissions of "As" and "Es" with no particular order. Except for "ae", which was registered only for type 1 loud calls, all phrases are used for composition of both type of loud calls, but their proportional contribution and transitional pattern differed between these calls (figure 2). Type 1 calls are composed mainly by "bc", "ab" and "aa" phrases while type 2 calls are composed mainly by "bc" and "ab" phrases. In type 1 calls "aa" and "ae" phrases are longer than other phrases and "ab" phrases are much shorter (table II), possibly because monkeys avoid combining "As" and "Bs". Although the monkeys avoid transitions between "Bs" in type 2 calls, some short "bb" phrases (table II) are used in the beginning and end of these calls. The "aa" phrases are rare and are used only at the beginning of calls. Both "ae" and "aa" phrases were also produced alone, out of loud calls, in alarm contexts or when animals were foraging close to the ground.

The transitions between phrase types for both type 1 and 2 loud calls showed a significant pattern (type 1 calls:  $G^2 = 357.60$ ,  $df = 29$ ,  $N = 265$ ,  $P < 0.001$ ; type 2 calls:  $G^2 = 83.18$ ,  $df = 19$ ,  $N = 215$ ,  $P < 0.0001$ ). Eight of all 49 possible transitions observed for type 1 loud calls occurred at higher and lower rates than expected by chance (resulting model after all eight removals:  $G^2 = 12.73$ ,  $df = 1$ ,  $N = 67$ ,  $P < 0.001$ ). Thus, type 1 calls typically start with "aa" phrases, followed by "abs", "bcs" and "bbs", commonly on this order, going then back to "aas". The call could then end or the individual could go back again to "abs" (figures 2 and 3). No phrase type was significantly related to the end of type 1 calls. For type 2 calls only the presence of "aa" and "bb" phrases at the start of calls and "bb" phrases at the end occurred at rates greater than expected by chance (resulting model after all three removals:  $G^2 = 20.20$ ,  $df = 1$ ,  $N = 203$ ,  $P < 0.001$ ). Thus, after starting, "ab" and "bc" (and eventually some "bb" phrases), are cycled by chance to compose type 2 loud calls with

variable duration (figures 2 and 3, table II). This pattern persists even when we restrict the sequential analysis to calls of group 1 (model before removals:  $G^2 = 60.1$ ,  $df = 19$ ,  $N = 102$ ,  $P < 0.01$ ; resulting model after removals:  $G^2 = 22.1$ ,  $df = 1$ ,  $P < 0.001$ ;  $N = 7$  type 2 calls of group 1). Altogether these results are in agreement with our initial prediction that type 1 and 2 loud calls have different composition and structural organization.

#### **Callicebus nigrifrons vocal behavior**

A total of 290 loud calls (80 of type 1 and 210 of type 2) were detected during 730 observation hours of group 1. Most of type 1 loud calls (74%) were spontaneously emitted when group members were spread out within their range (i.e. not in reaction to neighbors groups), while 23% were emitted after vocalizations of neighboring groups. When type 1 calls were emitted after vocalizations of nearby groups, group 1 usually emitted type 1 followed by type 2 calls (in 67% of these events). The contrary was detected for type 2 loud calls: only 20% were spontaneously emitted (not in reaction to neighbors), while 80% were emitted in reaction to other groups, in encounters (43%) or after other groups' vocalizations (37%). These results are in agreement with our initial prediction that type 1 loud calls are related to communication within group, while type 2 are related to communication between groups.

Most of type 1 loud calls were performed in chorus by all individuals of group (60%) and only 4% were emitted as solo. After type 1 calls, group members usually coordinated their activities: they often joined each other again (in 61% of occasions), changed their travel direction (18% of occasions) and/or emitted type 2 loud calls in sequence (28%). We eventually heard type 1 loud calls of neighboring groups (25 events), but this calls were ignored by group 1 in 40% of these events. However, when these calls were produced by

nearby groups (60% of events), group 1 produced type 2 loud calls, or type 1 followed by type 2 calls.

Most of type 2 loud calls were performed in duets (52%) by the mated pair or in chorus (46%) with the young's participation. Solos (2%) were emitted only by youngsters, that continued calling alone after calling in chorus. While calling the mated pair always stayed together in physical contact, sometimes with entwined tails, or separated by less than 0.5 m. In the field we could not detect differences in which animal started the duets, but from spectrogram inspections of 25 recordings we noted that individuals could start calling together or with a small difference of  $1.13 \pm 1.58$  s (Mean  $\pm$  SD, N = 18).

In 60% of type 2 calls spontaneous emissions, neighbors counter-called. Group 1 usually reacted fast to neighbors' vocalizations, taking the mean time of 3 min (range: 0-29 min) to counter-call and oriented themselves towards the neighbors' direction. Before counter-calling the group frequently moved in the direction of the group that was previously calling (55%), but sometimes they could also move in the opposite direction (9%) or stay in the same place (36%). The choruses were more frequent in intergroup encounters (45%) and in reaction to other groups' vocalizations (38%).

#### **Daily variation in loud calling**

The uttering frequency of type 1 loud calls emissions did not vary significantly along the day (Friedman test:  $X^2 = 14.39$ ; df = 10,  $P = 0.16$ ; figure 4), but the frequency of type 2 loud calls emissions did (Friedman test:  $X^2 = 28.57$ ; df= 10,  $P < 0.01$ ). Although group 1 can emit type 2 calls during all times of its daily active period, these calls are more concentrated in the morning (figure 4). Group 1's neighbors, group 2 and group 2's neighbors also showed variable emission of type 2 loud calls along the day (Friedman test:



1's neighbors:  $X^2 = 93.23$ ;  $df = 10$ ,  $P < 0.001$ ; group 2:  $x^2 = 41.63$ ;  $df = 10$ ,  $P < 0.001$ ; group 2's neighbors:  $X^2 = 53.90$ ;  $df = 10$ ,  $P < 0.001$ ), with a similar pattern to group 1 (figure 4).

#### **Loud calling vs. fruit availability and female estrus**

The output of type 2 loud calls was higher than expected by chance in months with higher fruit availability (Chi-squared test:  $X^2 = 12.82$ ;  $df = 1$ ,  $P < 0.001$ ), but the output of type 1 loud calls was not (Chi-squared test:  $X^2 = 0.18$ ;  $df = 1$ ,  $P > 0.05$ ).

The female of group 1 gave birth between the end of July and beginning of August in three consecutive years (2009 - 2011). Although we have not observed copulations that indicated the period when the female was fertile, we estimated that this period happened between March and April, based on the gestation length period. This estimate is supported by the observation of frequent copulation events in group 2 between March and April of 2007. We found that group 1 and 2 vocalized less than expected by chance in periods when females were supposed to be fertile (group 1:  $X^2 = 11.97$ ;  $df = 1$ ,  $P < 0.01$ ; group 2:  $X^2 = 6.16$ ;  $df = 1$ ,  $P < 0.05$ ). Altogether, these results are in agreement with the prediction that loud calls involved in extra group communication are used for food resource defense.

#### **4. Discussion**

Here we show that *C. nigrifrons*, like *C. ornatus* and *C. cupreus* (Robinson, 1979b; Müller & Anzenberger, 2002), uses different small units, the syllables, to form higher hierarchical structures, the phrases, which are then assembled to compose the loud calls. Confirming our initial prediction, structural different loud calls, type 1 and 2, are used in different contexts, involving the communication within and between groups. These calls are basically formed by the same syllable and phrase types, but the proportional contribution of the different syllable and phrases and the way they are assembled differ.

Differently from previous studies on *Callicebus* loud calls (Müller & Anzenberger, 2001; Robinson, 1979b) that based their analysis in phrase types, here we based our analysis on lower hierarchical structures, the syllables. Thus, we adopted a different terminology. However, following the descriptions and sonograms presented for *C. ornatus* vocalizations by Robinson (1979b), we could still identify similarities between our phrase types: “aa” are similar to chirrups, “ab” to bellows, “bc” to pants and pumps, and “bb” to bellows and honks. For “ae” phrases we could not find correlates on *C. ornatus* repertoire. This phrase type was rarely used even in *C. nigrifrons* loud calls, although it could be produced alone in alarm contexts or when animals are foraging close to the ground. Thus, it is possible that “ae” phrases are more commonly used as simple call in other circumstances, as the “cheep” alarm calls described by Cäsar et al. (2012) for *C. nigrifrons*.

Note that some of our phrase types could be assigned to more than one type described by Robinson (1979b). From our perspective, pants and pumps, for example, are alternations of Bs and Cs, and the difference between them seems to be the interval between syllables and their duration, which can also be a result of animal motivation while singing (Bradbury & Vehrencamp 1998). Thus, although our classification may seem an oversimplification, our approach reduces the subjectivism in vocal units’ classification.

Type 1 loud calls used by *C. nigrifrons* in intra group communication shows a cyclical and stereotyped pattern of emission of phrases. This structure resembles the one of the “short sequences” described for *C. ornatus* (Robinson, 1979b). The short sequences are produced by *C. ornatus* while approaching neighboring groups, during and after boundary interactions and short sequences emitted by one group can elicit response from nearby groups (Robinson, 1979b, 1981). Although Robinson (1979b, 1981) have related these calls

to events of interaction between groups, their usage while approaching and withdrawing from territory borders could also be related to coordination of group members' movements as we observed at Serra do Japi. *Callicebus nigrifrons* uses type 1 calls in contexts of coordination and cohesion of group members, and usually just react to type 1 loud calls of neighboring groups when emitted from nearby places.

The structure of type 2 loud calls resembled that of “long sequences” described for *C. ornatus* and *C. cupreus* (Müller & Anzenberger, 2001; Robinson, 1979b). However, different from *C. ornatus* long sequences, and from type 1 loud calls, type 2 calls did not show a stereotyped pattern of phrases emission. After type 2 calls start, a variable number of phrase types are delivered by chance. This less stereotyped pattern could be related to three possible explanations: 1) the fact that we analyzed calls recorded from four groups from different areas, 2) the fact that we did not consider possible sex-related structures of calls, and 3) the more complex social contexts to which these calls are associated.

The first explanation seems less likely because when we restricted the analysis to calls of group 1, the pattern of phrases delivery by chance persisted. Besides, the analysis of type 1 calls included vocalizations of two groups from different areas and revealed a very stereotyped sequence of phrases.

In other duetting primates, such as gibbons, indris and tarsiers (Geissmann, 2002; Haimoff, 1986; Nietsch, 1999), sex differences in mate contributions are usually associated with more conspicuous differences in the calls of males and females, such as the types of notes (Geissmann, 2002; Giacoma et al. 2010) and/or sequences (Geissmann, 2002) that are produced by only one of the sexes. Inspections of duet's spectrograms revealed that both individuals use the same syllable and phrase types, resulting in similar contributions by

male and female, as observed for *C. ornatus* and *C. cupreus* (Müller & Anzenberger, 2001; Robinson, 1979b). Besides, while analyzing vocal units sequences, we always chose to analyze the highest energy vocalization in the recording. Since mates always duet side-by-side, the darker sequence in the spectrogram cannot be attributed to an individual that is closer to the microphone. Robinson (1979b) and Müller & Anzenberger (2001) observed that male calls were louder (more marked in spectrogram) than the female calls. So, we may have analyzed mainly the calls of males. Thus, the more complex structure of type 2 calls are less likely related to possibly ignored sex-specific contributions.

Type 2 loud calls were emitted in basically three different circumstances: spontaneously, in response to other groups or during group encounters. When in response to other groups, the animals could also show different reactions: stay at the same place, approach or move away. These different circumstances and reactions could reflect different levels of motivation and could be linked to different signals with particular structures (Bradbury & Vehrencamp 1998). Thus, type 2 loud calls could be actually a set of different signals linked with different contexts. Alternatively, these calls may also correspond to an arbitrary signal, whose meaning will depend on what other nearby animals are singing (Vehrencamp, 2001). The behavior of singing a similar signal type in response to a rival's signal is termed 'matching' (Krebs et al., 1981). This is a commonly used mechanism for directing signals to a particular rival in birds and the extent of vocal matching can reveal the signaler's intentions or motivational level (Beecher et al., 2000; Krebs et al., 1981; Vehrencamp, 2001). Countersign matching can happen through matching of entire song types (Beecher et al., 2000) or through partial matching, such as using songs that contains phrases in common with the rival's song (Marshall-Ball & Slater, 2004) and have been shown to function as

territorial signals in solos and duets (Beecher et al., 2000; Vehrencamp, 2001). Thus, the fact that loud calls type 2 are used for communicating in more complex behavioral contexts can possibly explain the less stereotyped organization pattern found.

Loud calls type 1 and 2, are composed by loud, modulated and broadband elements (figures 1 and 3) that besides allowing long range transmission, also provide information about the location and distance of the signaler (McComb & Reby, 2005). Although the use of long-range vocalizations for intra group communication may seem unnecessary, group members can spread over a large area while foraging, being out of each other's sight (Müller, 1996; *pers. obs.*). Golden lion tamarins (*Leontopithecus rosalia*), for example, use louder and longer calls when separated from the group (Boinski et al., 1994). Thus, the acoustic property of loud calls encode information that are important not only when groups are monitoring their neighbors, but also when group members are dispersed throughout their range (Boinski, 2000).

Because the coordination of group members would be necessary in any time of the animals' activity period and at all times of the year, the animals need to emit calls for intra group communication occasionally (Geissmann & Mütschler, 2006). Thus, the proposed function of type 1 loud calls for coordinating group members' activities and keeping group cohesion is reinforced by its regular pattern of emission, with no preference for a particular period along the day or periods with higher fruit availability.

Differently from type 1 loud calls, type 2 calls emissions were more concentrated in the morning, in a similar pattern to that one observed for Indris (Geissmann & Mütschler, 2006) and gibbons (Mitani, 1985). These primates concentrate the emission of loud calls in the first hours of the morning, when sound transmission is supposed to be more effective

(because of the higher humidity and lower temperatures; Mitani, 1985; Wiley & Richards, 1978). Thus, like indris and gibbons, *C. nigrifrons* calling behavior may also favor the transmission of type 2 loud calls over greater distances. This pattern is in agreement with the extra group communication role of type 2 loud calls, since the signal needs to reach neighbors outside its range, not only the individuals inside the group's ranges, as in the case of type 1 loud calls.

The loud calls used for extra group communication in other *Callicebus* are usually associated to joint territorial advertisement, specially the duets (Kinzey & Robinson, 1983; Mason, 1968; Robinson, 1979b, 1981). In *C. nigrifrons* it also seems to fill this role, corroborating our initial prediction. These calls were commonly emitted during intergroup conflicts, and were emitted mainly as duets or choruses, suggesting a common interest of group members in defending their shared resources. In indris, as in *C. nigrifrons*, the chorus produced by male, female and juveniles has also been suggested to serve as a territorial advertisement and are commonly used when a group approaches or enters another group's territory (Pollock, 1986).

Another function attributed to loud calls used for extra group communication is mate defense (Hall, 2004; Levin, 1996; Robinson, 1981). Here, we found only a small support for this function, as we observed that duets can be started by only one of the participants on some occasions, suggesting that one of the individuals duetting could be more motivated to start calling than the other. In disagreement with this hypothesis, we found that the uttering frequency of type 2 loud calls were lower in months when the females were supposed to be fertile. Besides, type 2 loud calls were more frequent in months when defensible critical resources, such as fruits, were available, what is accordance with territorial defense

hypothesis (Maher & Lott, 2000). The fact that the animals called less when females were fertile could be a strategy to avoid signaling the female's position, or can just be a reflex of decreased fruit availability at the same period (mean = 1.71, inferior then the monthly mean value = 2.33). In this second scenario, the effect of resource availability is possibly overcoming any eventual effect of mate defense on the vocalization behavior, and future studies should try to disentangle both effects in places where fruit availability and breeding show a different phase relation. In territorial gibbons the highest calling rate also coincided with periods of food resource abundance (Bartlett, 2007).

This is the first description of *C. nigrifrons* loud calls and the first study addressing the function of the loud calls in this species. We confirmed our initial prediction that *C. nigrifrons* uses structurally distinct loud calls for communicating between individuals of the same group and between neighboring groups. Our results also provide support for the hypothesis that calls used for communication between neighboring groups are a cooperative display, used by the mated pair and other group members, to regulate the access to important food resources, such as fruits. Our observations of monkeys behavior and structural analysis of loud calls, on the other hand, lend only partial support to the mate defense hypothesis. During the study period, however, we did not witness interactions of our group with solitary individuals. Thus, we do not know how the individuals would behave in these circumstances. Therefore, more studies are necessary to understand the possible role of *C. nigrifrons* loud calls on mate defense. Controlled playback experiments simulating invasions of solitary individuals and mated pairs would be helpful to test this hypothesis. Detailed analysis focusing in each sex contributions to loud calls can also bring new and robust evidence in support to the role of these vocalizations on the mate defense.

However, because of the great overlap in the individual contributions to the duets, this is another challenge in studying these primates' vocalizations using traditional acoustic analysis. Another further interesting investigation would be to explore the countersign matching behavior, which have not been explored yet in primates. For *C. nigrifrons*, this investigation would bring new insights about the complex pattern of phrases transition in calls used for communication between groups. Thus, besides the new information about *Callicebus* vocal behavior and loud calls structure, the present study also brought many important and interesting questions that could be explored in further investigations in order to complement the present knowledge about the inter and intra group communication in this highly diverse and poorly studied genus of Neotropical primates.

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685 **Tables**

686 **Table I.** Mean values of fine structural parameters measured for all pre-category of syllables for nine groups of *Callicebus*  
687 *nigrifrons*. The values in brackets indicate the standard deviation, except for number of harmonics ( $\leq 2$  kHz) and number of  
688 peaks, for which the numbers in brackets corresponds to maximum and minimal values. Abbreviations: HNR, harmonic-to-noise  
689 ratio; N peaks, number of harmonic peaks above -15 dB. The asterisk indicates the parameters excluded from the DFA.

Syllable	N	N harmonics ( $\leq 2$ kHz)	Duration (s)*	Start			N peaks	End			N peaks
				Min freq. (Hz)*	Max freq. (Hz)	HNR (dB)		Min freq. (Hz)*	Max freq. (Hz)	HNR (dB)	
A	38	2 (1-3)	0.08 ( $\pm 0.03$ )	3312.89 ( $\pm 949.01$ )	6426.84 ( $\pm 1873.82$ )	39.29 ( $\pm 9.41$ )	2 (1 - 3)	344.21 ( $\pm 661.07$ )	1117.37 ( $\pm 1440.31$ )	50.84 ( $\pm 8.56$ )	1 (1 - 2)
B	75	6 (3-11)	0.14 ( $\pm 0.05$ )	221.33 ( $\pm 109.45$ )	1811.60 ( $\pm 985.41$ )	41.29 ( $\pm 12.80$ )	2 (1 - 6)	170.00 ( $\pm 82.72$ )	1552.80 ( $\pm 754.79$ )	42.75 ( $\pm 12.95$ )	2 (1 - 4)
C	73	3 (1-4)	0.10 ( $\pm 0.04$ )	463.43 ( $\pm 207.75$ )	3107.12 ( $\pm 1502.24$ )	40.09 ( $\pm 13.62$ )	3 (1 - 6)	252.74 ( $\pm 109.59$ )	2007.53 ( $\pm 1364.36$ )	46.65 ( $\pm 11.45$ )	2 (1 - 8)
D	42	2 (1-5)	0.10 ( $\pm 0.04$ )	3882.62 ( $\pm 1325.38$ )	6195.95 ( $\pm 1765.28$ )	39.98 ( $\pm 12.31$ )	1 (1 - 5)	272.14 ( $\pm 95.09$ )	1614.29 ( $\pm 857.93$ )	46.06 ( $\pm 12.01$ )	2 (1 - 3)
E	31	0 -	0.05 ( $\pm 0.02$ )	3829.68 ( $\pm 916.88$ )	6160.97 ( $\pm 1953.49$ )	39.59 ( $\pm 6.44$ )	1 (1 - 3)	3917.42 ( $\pm 866.13$ )	6817.42 ( $\pm 2139.50$ )	45.70 ( $\pm 5.72$ )	2 (1 - 5)
F	67	2 (1-5)	0.18 ( $\pm 0.07$ )	2851.05 ( $\pm 1234.98$ )	5695.22 ( $\pm 1850.54$ )	40.80 ( $\pm 9.97$ )	2 (1 - 5)	270.75 ( $\pm 149.02$ )	1473.43 ( $\pm 841.11$ )	48.30 ( $\pm 13.07$ )	2 (1 - 5)

**Table II.** Mean values ( $\pm$  SD) of duration of loud calls and phrases used to compose loud calls and mean rate values ( $\pm$  SD) of syllables and phrases for each type of loud calls. The values in brackets indicate number of samples).

Loud calls	Mean duration (s)	Mean rate of syllables (n/s)	Mean rate of phrase (n/min)	Phrases mean duration (s)				
				aa	ab	Ae	bb	bc
1	62.2	3.6	23.34	4.8	0.4	9.52	1.7	1.8
	$\pm 64.0$	$\pm 1.2$	$\pm 8.5$	$\pm 3.2$	$\pm 0.3$	$\pm 6.8$	$\pm 0.9$	$\pm 0.6$
	(25)	(13)	(13)	(67)	(72)	(13)	(29)	(71)
2	63.2	5.1	17.6	1.1	2.1	-	2.6	5.1
	$\pm 34.6$	$\pm 0.9$	$\pm 3.1$	$\pm 0.5$	$\pm 1.7$		$\pm 2.2$	$\pm 3.9$
	(25)	(13)	(13)	(4)	(68)		(41)	(89)

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### **Figure legends**

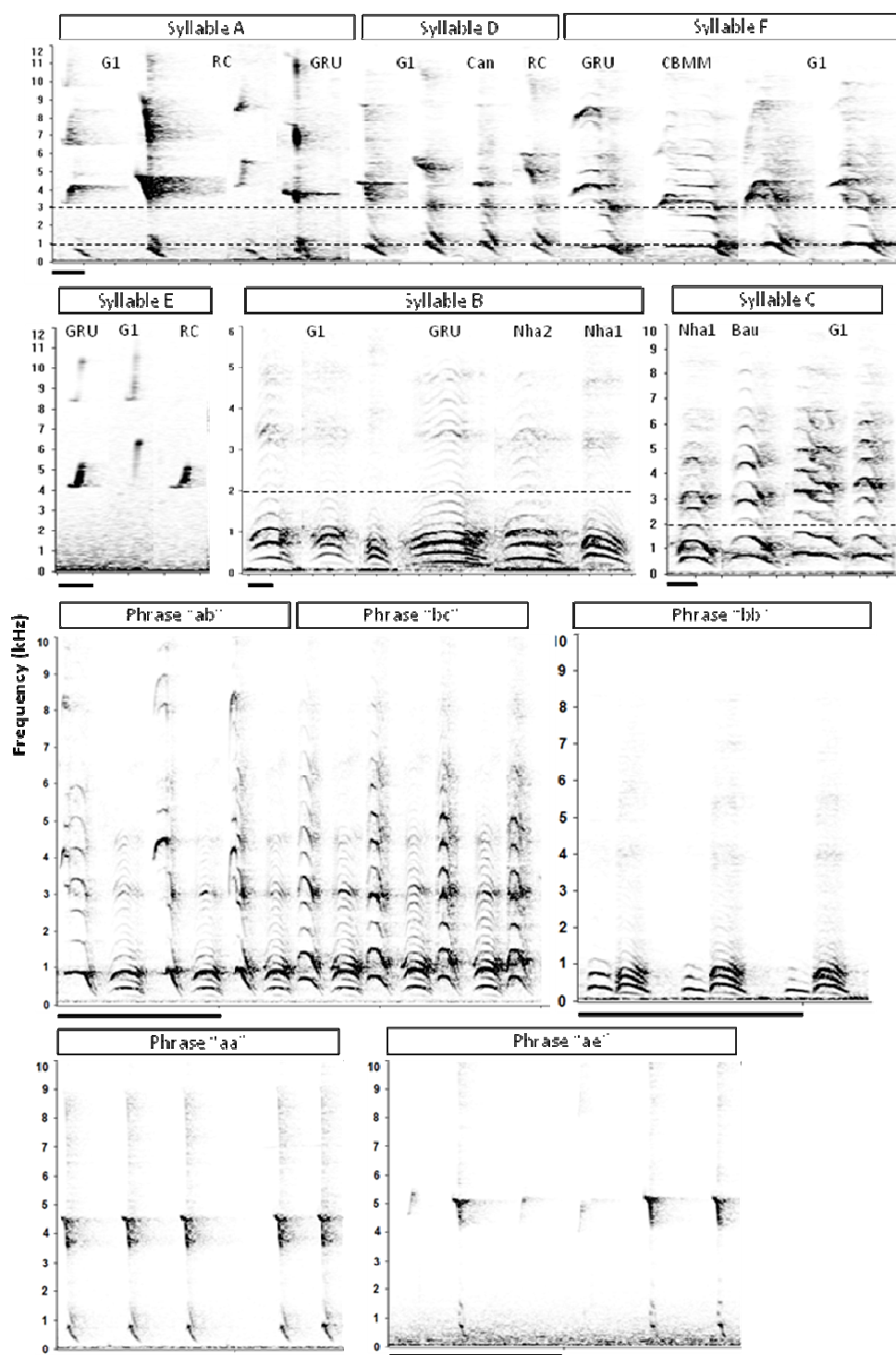
**Figure 1.** Sound spectrograms of syllables and phrases used by *Callicebus nigrifrons* to compose loud calls. For syllable spectrograms, the scale at horizontal axes corresponds to 0.1 ms, for phrases spectrograms the scale corresponds to 1 s. Syllable samples of different groups are shown (Wild groups: group 1(G1), Ribeirão Cachoeira (RC), non-habituated groups from Serra do Japi (Nha1 and Nha2), Cantareira (Can); Captive groups: Guarulhos (GRU), Araxá (CBMM) and Bauru (Bau)). Phrase samples from group 1. The dashed lines at syllables A, D and F panels draw attention to the middle harmonics and the dashed lines at syllables B and C panels draw attention to the number of harmonics under 2 kHz (see text for further detail).

**Figure 2.** Flow diagrams showing the probabilities at which syllable (left) and phrase (center) types follow from one type to another for each type of loud calls (group 1). The arrows represent the path way between consecutive vocal units and the different arrows type indicates the probability of each transition as shown in the scale in the right. We only represented the transitions that happened more than expected by chance. The different color in the boxes shows the proportional contribution of each type of vocal unit to each type of loud call as shown in the scale in the right. The boxes with the dashed lines indicates that a particular vocal unit do not occur in loud calls composition or at a particular position of loud calls. In the center panel the first column shows the phrases that can be used in the beginning of each type of loud vocalization; the second column shows the ones used in the middle, which are cycled during de song emission; and the third column shows the phrases that can be used in the end of the songs. The phrases showed in the third column can finalize the song or can lead to another phrase (as shown in the middle column). Intra-group loud calls: N of syllables = 2578, N of phrases = 252,

725 N of calls = 13; Extra-group loud calls: N of syllables = 3767, N of phrases = 228, N of calls =  
726 13.

727 **Figure 3.** Sound spectrograms of intra (top) and extra-group (bottom) loud calls showing the  
728 phrase sequence pattern for each call.

729 **Figure 4.** Daily variation of type 1 loud calls of group 1 and type 2 loud calls of group 1 and 2  
730 and their neighboring groups. For all graphics the bars represent the standard error. The time is  
731 represented as number of hours after sunrise.



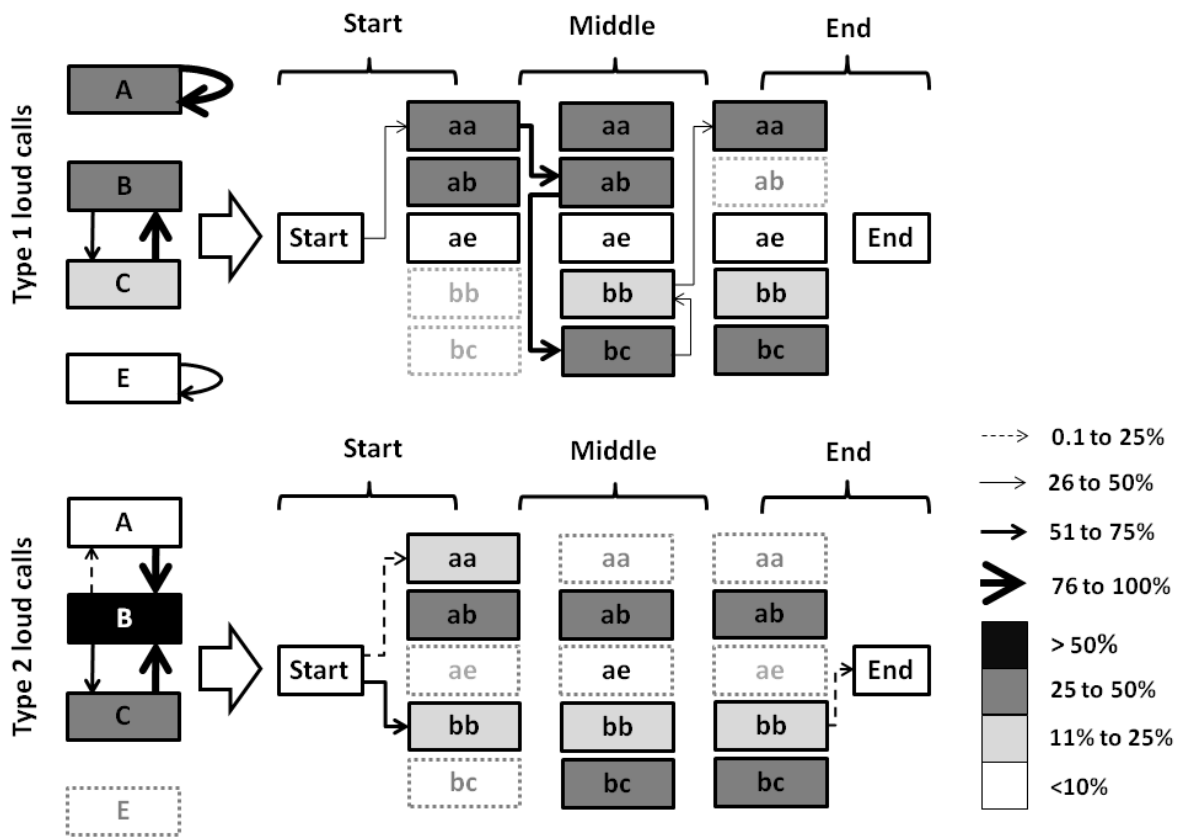


Figure 2.

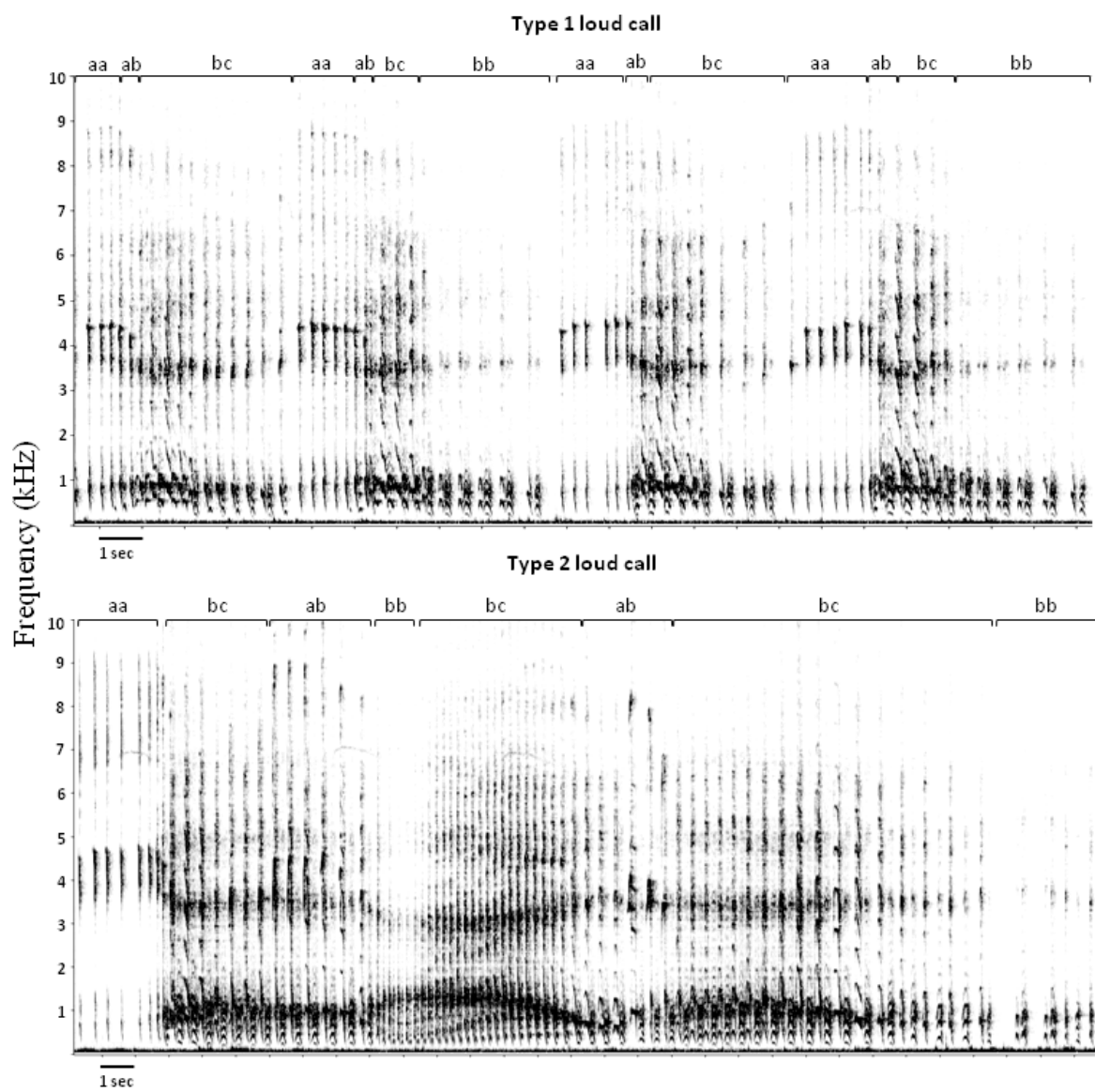


Figure 3.

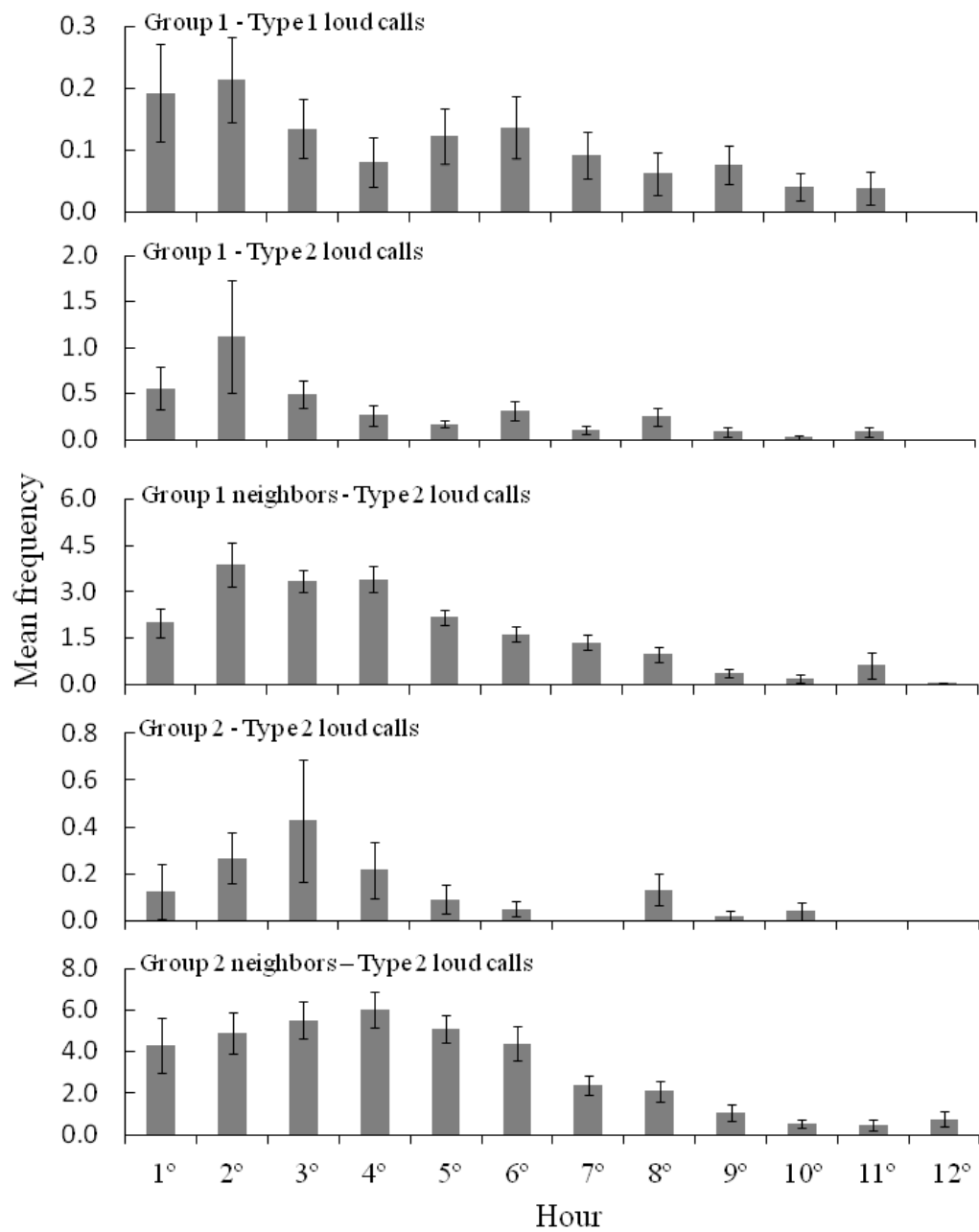


Figure 4.

## Capítulo 2

"Resposta de sauás-da-cara-preta de vida livre (*Callicebus nigrifrons*) a playbacks de solos e duetos."

1 Title: **Response of free-ranging black-fronted titi monkeys**  
2 **(*Callicebus nigrifrons*) to playbacks of solos and duets**  
3  
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12 Sul, Brazil.  
13  
14 Short title: **Response of *Callicebus nigrifrons* to solos and duets**  
15  
16 Word count: 4.353 words plus 4 figures  
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26    **Abstract**

27    Many birds and primates use loud calls to mediate agonistic contests with conspecifics  
28    either as solos by males or females, or as coordinated emissions, like duets. The high  
29    variation in complexity and contribution of each sex to coordinated vocal emissions  
30    suggests that they may have several functions, including territory defense and mate  
31    guarding. *Callicebus* monkeys are proposed to defend their range via coordinated  
32    emission of loud calls, especially duets. However, due to the difficulties in obtaining  
33    data about titi monkeys ' behavior in the field, there are few studies about the function  
34    of these calls and, although some evidences point that these coordinated loud calls may  
35    serve to mate defense, this hypothesis has not been tested yet. Observing interactions  
36    between established groups and solitary individuals is rare in the field. Therefore,  
37    controlled experimental design is required to simulate such situations and evaluate the  
38    mate and joint territorial defense hypotheses. Here we conducted playback experiments  
39    with three habituated groups of *Callicebus nigrifrons* to test these hypotheses. Except  
40    for the control stimuli, the titi monkeys reacted to all playback treatments (duet, female  
41    solo and male solo) and did not show sex-specific qualitative responses to solos. In most  
42    of trials males and females counter-called in duets, and started to call at the same time.  
43    Although the male started moving first, both sexes, and their young, approached the  
44    speaker together rather than alone. The groups response strength was also similar to all  
45    three conspecifics treatments. The behavior of groups is in accordance with the joint  
46    territory defense hypothesis rather than with the mate defense hypothesis.

47           **Key words:** conflict, cooperation, duet, playback, territory defense, mate  
48    defense.

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## 51 **1. Introduction**

52 Many birds and primates use loud calls to mediate agonistic contests with  
53 conspecifics (Hall, 2004; Serpell, 1981; Wich & Nunn, 2002), either as solos by males  
54 or females, or as coordinated emissions, like duets or chorus, by two or more  
55 individuals, respectively (Bradley & Mennill, 2009; Geissmann, 2002; Grafe & Bitz,  
56 2003; Heimoff, 1986; Langmore, 1998; Wich & Nunn, 2002; Wilson et al., 2001). The  
57 high variation in complexity and contribution of each sex to coordinated vocal  
58 emissions suggests that they may have several functions, including territory defense and  
59 mate guarding (Bradbury & Vehrencamp, 1998; Hall, 2004; Wich & Nunn, 2002).

60 The joint territorial defense hypothesis is based on the assumption that when  
61 coordinating their vocalization emissions, animals produce more effective  
62 demonstrations of resource holding potential and willingness to defend territories than  
63 when calling alone (Hall, 2004; Robinson, 1981). In this cooperative scenario, the  
64 signal is directed to outsiders and partners are more likely to coordinate their songs than  
65 to sing alone when facing intruders of any sex, paired or not (Hall, 2004; Hall & Peters,  
66 2008). Nonetheless, stronger responses should be given to calls of paired individuals  
67 than to solos, because two individuals are more threatening than one (Hall, 2004).

68 From the mate guarding perspective, animals participate in coordinated vocalization  
69 emissions to advertise their partner's mated status in an attempt to prevent same-sex  
70 rivals from copulating with its mate or even takeovers (Hall, 2004; Langmore, 1998). In  
71 this scenario the individuals' response can be directed to same-sex outsiders to avoid  
72 their partner usurpation (reflecting conflicting interests; Grafe & Bitz, 2003; Seddon et  
73 al., 2002), or to opposite-sex outsiders to avoid their own usurpation (reflecting  
74 individuals' cooperation; Hall, 2000, 2004). Evidence from monogamous gibbons  
75 (*Hylobates lar*) and titi monkeys (*Callicebus ornatus*) are in accordance with this

76 cooperative mate guarding behavior: playback of female loud calls elicit duets started  
77 by females and joined by males (Raemaekers & Raemaekers, 1985; Robinson, 1981).

78 Titi monkeys (genus *Callicebus*) are predominantly frugivorous small Neotropical  
79 primates that live in family groups composed of an adult breeding pair and one to three  
80 offsprings (Kinzey, 1997). Two of the currently 30 recognized species (Defler et al.,  
81 2010; van Roosmalen et al., 2002; Wallace et al., 2006) are proposed to defend their  
82 range via coordinated emission of loud calls, especially duets (*C. torquatus*: Kinzey &  
83 Robinson, 1983; *C. ornatus*, formerly *C. moloch*: Mason, 1968 and Robinson, 1979a,  
84 1981). However, due to the difficulties in obtaining data about titi monkeys' behavior in  
85 the field (Pinto et al., 2013), there are few studies about the function of these calls  
86 (Kinzey & Robinson, 1983; Mason, 1968; Robinson, 1979a, 1981). Although Robinson  
87 (1979b, 1981) provided evidence that *C. ornatus* vocalizations may serve to mate  
88 defense, this hypothesis has not been tested yet.

89 Black-fronted titi monkeys (*Callicebus nigrifrons*) use conspicuous loud calls for  
90 both intra and extra group communication. These calls are emitted as solos, duets and  
91 choruses, although the first are rare. The contribution of each mate to duets does not  
92 seem to be sex-specific and either sex can initiate these coordinated emissions (chapter  
93 1). Although we have found that extra group loud calls are more frequent in periods  
94 with higher fruit availability and less frequent when females are supposedly fertile  
95 (chapter 1), lending strong support to the joint territorial defense, observing interactions  
96 between established groups and solitary individuals is rare in the field. Therefore,  
97 controlled experimental design is required to simulate such situations and evaluate the  
98 mate and joint territorial defense hypotheses.

99 In this study we tested these hypotheses by presenting three types of stimuli (duets,  
100 male solos and female solos) to paired mates. If duets are used for mate guarding, we

101 predicted that (1) individuals should vocalize more as solos or start duets more often in  
102 response to same-sex solo stimuli; (2) males and females should start to move first or  
103 lead the movement towards the playback more often in response to same-sex solo  
104 stimuli; (3) solo stimuli should evoke more intense responses than duet stimuli because  
105 a paired individual is less sexually threatening or attractive to mated individuals than an  
106 unpaired one. On the other hand, if duets are used as a cooperative mechanism of  
107 territorial defense, we predicted that (1) individuals in the breeding pair should vocalize  
108 more often as duets than as solos or start the duets together regardless of the stimulus  
109 type (solos or duets); (2) mates should start to move or lead group movement together  
110 regardless of the stimulus type; (3) duet stimuli should evoke more intense responses  
111 than solos or equivalent strong responses (because all intruders are seen as potential  
112 competitors for the food supply; Benedict, 2010).

## 113 **2. Methods**

### 114 **Study site and study subjects**

115 Playback experiments were conducted between August and September 2012, with  
116 three habituated groups of *Callicebus nigrifrons* at three Atlantic forest remnants  
117 (approximately 54 km apart from each other) in the state of São Paulo, Southeastern  
118 Brazil. Group 1 (a mated pair and three young) was studied at Serra do Japi Municipal  
119 Ecological Reserve (23°14'3.38"S, 46°56'8.81"W), municipality of Jundiaí; group 2 (a  
120 mated pair and one young) was studied at Ribeirão Cachoeira (22°49'48.43"S,  
121 46°55'26.06"W), municipality of Campinas; and group 3 (a mated pair and two young)  
122 was studied at municipality of Nazaré Paulista (23°11'30.95"S, 46°21'34.42"W).

123 The three study sites are characterized by semideciduous secondary forest and  
124 temperate humid climate. Serra do Japi is a 350-km<sup>2</sup> remnant and at this site the focal  
125 group has at least four neighboring groups. At Ribeirão Cachoeira, a 2.45-km<sup>2</sup> remnant,

126 and Nazaré Paulista, a 0.5-km<sup>2</sup> remnant, approximately, the focal groups has at least  
127 two neighboring groups. All groups are known to show site fidelity and to interact with  
128 neighbouring groups by vocal means and agonistic behaviors (chapter 1; Knogge C.,  
129 *pers. comm.*; Nagy-Reis, 2012).

130 Although *Callicebus* monkeys are sexually monomorphic (Robinson et al., 1986), we  
131 were able to distinguish between male and female of the mated pair and between them  
132 and their offspring based on body size (when animals were close to each other). Males  
133 are slightly larger than females (Robinson et al., 1986) and adults (> 30 months) are  
134 larger than subadults (18–30 months) and juveniles (6–18 months; de Luna et al., 2010;  
135 Valeggia et al., 1999). Marks on the face and tail also helped in the identification of  
136 some individuals. No study subject was captured or handled and all research reported in  
137 this manuscript adhered to Brazil's legal requirements.

#### 138 **Stimulus recording and preparation**

139 The vocalizations used to produce the stimuli were recorded in WAV format, using a  
140 Sennheiser ME-67 directional microphone and a Marantz PMD-671 digital recorder at  
141 48 kHz sampling rate and 16-bit resolution. We recorded duets and solos of free-  
142 ranging habituated groups (group 1 and 2) and from three captive groups at the Bauru  
143 Zoo (Bauru, São Paulo state), Guarulhos Zoo (Guarulhos, São Paulo state) and Center  
144 of Environmental Development of Companhia Brasileira de Metalurgia e Mineração  
145 (Araxá, Minas Gerais state). Recordings from captive monkeys were necessary to obtain  
146 solos, which are rare in the wild. However, the captive study subjects were born in the  
147 wild and knew how to perform the species typical song when stimulated via playbacks.

148 We recorded duets and solos in response to playback of duets from groups 1 and 2.  
149 Then we selected 30 seconds of recordings with a high signal-to-noise ratio, filtered  
150 them to remove background noise below 100 Hz and normalized all stimuli to a

151 standard amplitude of -1 dB (in order to standardize the amplitude of calls with respect  
152 to their peak amplitudes because recording distances were not always the same across  
153 subjects). For the duet recordings, we removed the beginning of the vocalizations (solo  
154 part) to simulate duets in which mates started calling together.

155 To evaluate whether study subjects were responding to the playback of conspecifics  
156 rather than to any vocalization broadcasted by the loudspeaker, we selected recordings  
157 of dusky-legged guan (*Penelope obscura*) as a control stimulus. We obtained recordings  
158 from the "Fonoteca Neotropical Jacques Vielliard" collection  
159 (<http://proj.lis.ic.unicamp.br/fnjv/>). We also selected 30 seconds of recordings with a  
160 high signal-to-noise ratio, filtered and normalized them as we did for the *C. nigrifrons*'  
161 recordings. Since this bird is commonly found at our three study sites and also produces  
162 loud calls, we considered this an appropriate control stimulus. Besides, titi monkeys  
163 usually pay little attention to these calls when they occur naturally (*pers. obs.*).

#### 164 **Playback experimental design**

165 Playbacks consisted of four treatments (stimuli): (1) duets, (2) male solos, (3) female  
166 solos, and (4) dusky-legged guan vocalizations (control). Each group was submitted to  
167 all treatments, resulting in four trials with each group and a total of 12 trials. To avoid  
168 pseudo replication, each study group received a single presentation of each stimulus and  
169 each stimulus was used only once (Wiley, 2003), except for the female solos, because  
170 we had recordings of only two individuals. To avoid habituation or interference between  
171 trials, we conducted only one trial per day, randomizing the order in which each  
172 treatment was assigned to each group. We also randomized the stimulus exemplar  
173 assigned to each pair, taking care to avoid assigning to a pair its own recording.

#### 174 **Playback presentation**

175 All playbacks were conducted from 7:00 to 11:00 am, when monkeys are normally  
176 vocally active (chapter 1). We began each playback trial once when we had the  
177 individuals of the mated pair in sight, preferably while resting, and if no calls from  
178 neighboring groups were heard for at least 30 minutes. We broadcasted the stimulus  
179 from random places inside the groups' home range from a distance of 40 to 60 m to the  
180 tree in which the monkeys were resting using a T-120B CSR loudspeaker (audio output:  
181 40W; frequency response: 25 Hz - 15 kHz) connected to a Marantz PMD-671 portable  
182 digital recorder. We set the loudspeaker volume to levels closer to what we hear in the  
183 field and held this volume constant across all trials. During these trials while one  
184 observer played the stimulus, another observed the groups and recorded their reactions.  
185 We recorded the reaction of the groups in the next 30 minutes after the start of the  
186 playback.

### 187 **Response Measures**

188 To assess the level of motivation in the group members reaction to the stimuli, we  
189 measured the following vocal and physical responses: latency to start to move, latency  
190 to first song, number of songs in response, mean duration of songs, speed to arrive  
191 within 10 m of the loudspeaker (distance of groups to 10 m from the loudspeaker /  
192 latency to arrive within 10 m of the loudspeaker), who starts to moving towards the  
193 loudspeaker and the type of song emitted (solo of male or female, duet started by male  
194 or female, or duets with no apparent difference in who starts singing).

195 If groups did not react to the stimuli, we assumed that the latency to start to move  
196 and the latency to first song were 30 min (the total duration of the trial) and the number  
197 of songs in response, mean duration of songs and speed to arrive within 10 m from the  
198 loudspeaker were zero. We adopted this criterion to avoid empty cells in the analyses,  
199 especially in the case of control stimulus (see results). We opted to record the animals'

200 reaction for 30 minutes after the start of playbacks because in natural conditions group 1  
201 could take up to 29 minutes to counter-call to a neighbors' vocalization (chapter 1).

## 202 **Data Analysis**

203 To test whether female, male or pair motivation to start calling or moving was  
204 dependent on the playback condition, we used an extended form of the Fisher's exact  
205 test (from 2 x 2 tables to general  $n \times m$  tables), also known as Freeman-Halton test  
206 (Freeman & Halton, 1951).

207 Since the five quantitative response variables (latency to start to move, latency to  
208 first song, number of songs in response, mean duration of songs, speed to arrive within  
209 10 m from the loudspeaker) were significantly correlated with each other (Spearman's  
210 rank correlation coefficient ranging from 0.61 to 0.89,  $P < 0.05$ ), we followed McGregor  
211 (1992)'s recommendation to use Principal Component Analysis (PCA) to quantify  
212 responses to playback based on the measurement of many reaction types. Another  
213 reason for combining several measures of response into one is to include different types  
214 of groups' reaction and make the interpretation of the results easier. For example, while  
215 one group can approach the playback, another can sing, but both have responded to the  
216 playback (McGregor, 2000). Therefore, to test whether the response of the groups  
217 differed among the four stimuli, we first combined all five quantitative response  
218 variables into a single composite response variable using a PCA based on correlation  
219 matrices. After performing the PCA, we chose the first unrotated principal component  
220 factor (PC1), which accounted for 63% of the variance (eigenvalue = 3.14). Following  
221 Burt et al. (2001) and McGregor (1992), we then extracted the PC1's coefficients to  
222 calculate the response score for each trial as follows:  $(0.49 \times \text{number of songs}) + (0.51 \times$   
223  $\text{mean song duration}) + (0.20 \times \text{displacement velocity}) - (0.48 \times \text{latency to move}) - (0.48$   
224  $\times \text{latency to sing})$ . Stronger reactions are indicated by greater scores. We compared the



225 score values (combined response variables) between the stimulus types using a  
226 generalized linear mixed model (GLMM), including the stimuli as experimental factors  
227 and the identity of groups as a random effect.

228 To check whether the distance from where the stimuli were broadcasted to the groups  
229 (which varied from 40 to 60 m) interfered in the animals' response, we used a Spearman  
230 rank correlation between the response index and the distance of playbacks from groups.

231 All the analyses were preformed in R software version 2.15.0 (R development core  
232 team, 2011) using the Stats package, except the GLMM. The GLMM were implemented  
233 in R using the add-on package lme4 version 0.999999-0 (Bates et al., 2012).

### 234 **3. Results**

235 Playbacks of dusky-legged guan (control) failed to elicit any response (figure 1).  
236 Upon hearing control stimulus, the animals just looked towards the playback at the  
237 moment it started and they usually stopped looking before the broadcasted sound  
238 stopped. Upon hearing the playback of conspecific vocalizations, on the other hand, titi  
239 monkeys quickly adopted a watchful stance, looking towards the playback direction.  
240 They usually started to move towards the broadcasted sound in the first minute after the  
241 playback started, sometimes even before it ended (mean latency time to move after the  
242 playback starts =  $87 \pm 121$  seconds; mean  $\pm$  SD) and usually started to counter-call in  
243 the first 10 minutes after the playback started (mean latency time to counter-call after  
244 the playback starts =  $8 \pm 10$  minutes; mean  $\pm$  SD). The lack of response to control  
245 stimuli and the monkeys' behavior during the trials suggest that the playback was  
246 effective in simulating intrusions by conspecifics.

247 The way mated pairs reacted to playbacks was independent of the type of conspecific  
248 stimulus (Fisher's exact test: which sex started to sing:  $P = 1.00$ ; which sex started to  
249 move:  $P = 0.23$ ; figure 2). Mated pairs always counter-called as duets and 86% of these

250 duets were started together by male and female (N of calls in response to playbacks =  
251 14; figure 2). In five of these events, their offspring joined them in chorus. Although  
252 males were the first to start moving in most trials (figure 2), females and other group  
253 members followed males closely, and the animals alternated in the leading positions  
254 along the way towards the playback. These reactions are in accordance with the joint  
255 territorial defense hypotheses.

256 The response scores varied from -28.98 (no reaction) to 42.91 (duet:  $24.50 \pm 10.31$ ;  
257 male solo:  $15.49 \pm 7.79$ ; female solo:  $14.33 \pm 16.61$ ; control:  $-28.98 \pm 00.00$ ; mean  $\pm$   
258 SE; figure 3). The reaction of the groups to conspecific stimulus types was similar  
259 (GLMM:  $x = 0.75$ ;  $P = 0.687$ ; figure 3). In fact, groups appear to have reacted  
260 differently from each other and the order of playback presentation to each group seemed  
261 to have no influence on the level of response (figure 4). The small variation in groups'  
262 distance from the playbacks did not affect their response (Spearman rank correlation:  $R$   
263  $= -0.32$ ,  $N = 12$ ,  $P = 0.40$ ). This response pattern also appears to be in agreement with  
264 joint territory defense hypothesis.

#### 265 4. Discussion

266 Except for the control stimuli, the titi monkeys reacted to all playback treatments.  
267 Differently from *C. ornatus* (Robinson, 1979b, 1981), *C. nigrifrons* individuals did not  
268 show sex-specific qualitative responses to solos. In most vocal responses males and  
269 females counter-called in duets, and started at the same time. In some occasions, the  
270 young also joined them in chorus. Under natural conditions, young participation in loud  
271 calls emissions is common during threatening situations, as simulated here (chapter 1).  
272 Although the male started moving first, both sexes, and their young, approached the  
273 speaker together rather than alone.

274 According to the joint territory defense hypothesis mated pairs would respond to  
275 invaders in a coordinated manner, by approaching speakers together or vocalizing  
276 together (Hall, 2004; Hall & Peters, 2008). Thus, in this aspect, the behavior of groups  
277 is in accordance with the joint territory defense hypothesis rather than with the mate  
278 defense hypothesis, which in turn predicts uncoordinated responses by individuals of  
279 the mated pair (Hall, 2004).

280 The groups response strength was similar to all three conspecifics treatments, what is  
281 also in agreement with the joint territory hypothesis, since all intruders are seen as  
282 potential competitors for the food supply (Benedict, 2010; Dahlin & Wright, 2012). The  
283 support for the joint territory defense hypothesis, in this case, however, cannot rule out  
284 the mate defense function of loud calls. On playback experiments with *C. ornatus*, for  
285 example, the groups response strength did not differ between solos and duets as well  
286 and this lack of difference was inferred to be a consequence of the fact that the stimuli  
287 were played from inside the groups' range, that would have triggered an over-response  
288 in face of increased threat of an invasion (Robinson, 1981). Since the cost of responding  
289 to a threat would be inferior to the costs of losing the territory to an invader, the animals  
290 should strongly repel all potential invaders (Dahlin & Wright, 2012). Thus, any vocal  
291 stimulus representing the presence of a rival within an animals' territory may elicit a  
292 similar, high level territory defense response, what would possibly override subtle  
293 differences in responses to solos and duets (McGregor, 2000).

294 Moreover, we cannot eliminate the possibility that our small sample size have  
295 influenced this result. Reduced sample size only allows the detection of relatively large  
296 differences. In order to detect more subtle differences, we would probably need an  
297 increased sample size (see Wiley, 2003). Large sample sizes are not a trivial when  
298 dealing with habituated groups of primates. Indeed, previous investigations of monkey

299 loud call functions that used a playback approach faced the same experimental  
300 compromise and had to perform experiments with just a few groups or use non  
301 habituated groups or, still, repeat trials with the same groups, resulting in increased  
302 pseudoreplication (Bezerra et al. 2010; Cunha & Byrne, 2006; Cunha & Jalles-Filho,  
303 2007; Kinzey & Robinson, 1983; Mitani, 1985; Raemaekers & Raemaekers, 1985;  
304 Robinson, 1981). Titi monkeys are not easy to habituate (Pinto et al. 2013) and  
305 performing these tests with non-habituated monkeys would interfere in the animals'  
306 response since it is difficult to conceal the observer presence. Besides, it would have  
307 been more difficult to control the distance from groups to speaker, find the groups in the  
308 field and be sure about the groups' identity. Thus, it would be interesting to conduct this  
309 kind of experiments in research areas established for long periods, with an increased  
310 number of habituated groups.

311 Another possible explanation for the observed lack of difference in *C. nigrifrons*  
312 response intensity is that our response variables may not have been sensitive enough to  
313 detect the difference shown by the animals (McGregor, 2000). In some bird species, for  
314 example, the selection of song types that most resembles the song type of rivals (Burt et  
315 al. 2001; Vehrencamp, 2001) or the behavior of matching the frequency of the song  
316 with the frequency of rival's song (Mennill & Ratcliffe, 2004), can also be used to  
317 encode sender level of motivation or likelihood to attack (Bradbury & Vehrencamp,  
318 1998). In the plain wren, *Thryothorus modestus zeledoni* (Marshall-Ball & Slater,  
319 2004), within the apparent joint performance of duets, males and females target their  
320 aggression at same-sex competitors, using their duet contributions to match the song  
321 types of same-sex rivals. Frequency song matching has been described in call exchanges  
322 of captive Japanese macaques (*Macaca fuscata*), demonstrating that these monkeys are  
323 able to modify the acoustic parameters of their calls according to the features of prior

324 calls of another group members (Sugiura, 1998). Although this match was observed in  
325 contexts not involving agonistic interactions, it shows that in monkeys, as in birds  
326 (Mennill & Ratcliffe, 2004), acoustic changes of song features can occur in the short  
327 term as a response to calls of other individuals.

328     One more reasonable alternative explanation for the lack of difference in monkeys  
329 response strength to playback stimuli is that the subjects possibly did not perceive the  
330 difference between the sounds broadcasted (McGregor, 2000) and thus, their variation  
331 in response would be related to other factors not measured here. In some previous  
332 studies a single-speaker playback was successfully used for simulating solos and duets  
333 (Douglas & Mennill, 2010; Mitani, 1985, 1987). However, in species where individuals  
334 can perform both parts of a duet, as seems to be the case in *Callicebus* monkeys  
335 (chapter 1; Robinson, 1979b; Müller & Anzenberger, 2001), duet stimuli broadcasted  
336 through a single speaker may simulate one individual producing both parts of a duet.  
337 Nonetheless, we do not believe that this problem occurred in our experiment. In  
338 *Callicebus* monkeys, mates usually do not emit the same song part at the same time.  
339 Even being able to perform both parts of a duet, the mates alternate phrases types while  
340 singing (Robinson, 1979b; Müller & Anzenberger, 2001). From sonogram inspections  
341 of *C. nigrifrons* recordings, it seems that these monkeys behave in the same way. Thus,  
342 different phrases can be (and usually are) heard at the same time in a duet, but not in a  
343 solo, and this can possibly indicate to listeners the number of individuals calling.

344     In spite of the experimental limitations, this study brought important and  
345 complementary information to previous field observation of *C. nigrifrons* vocal  
346 behavior (chapter 1). While in the field we had no opportunity to observe how animals  
347 would react to the presence of solitary individuals, here we were able to simulate these  
348 contexts and observed that animals of the mated pairs and their offspring responded to

349 invaders in an collaborative manner, by approaching speakers or vocalizing together,  
350 regardless of the kind of intruder, what is in agreement with the join territorial defense  
351 hypothesis. This study also revealed that *C. nigrifrons* does not behave like *C. ornatus*,  
352 in which same sex individuals start the duets more often in response to same-sex stimuli  
353 (Robinson, 1979a; 1981). The participation of other group members was not mentioned  
354 on these playback experiments. In fact, chorusing have not received much attention in  
355 previous studies about loud calls function in *Callicebus* or in other monogamous  
356 primates (Cowlshaw, 1992; Heimoff, 1986; Minati, 1985, 1987; Müller &  
357 Anzenberger, 2001; Robinson, 1979b, 1981; Wich & Nunn, 2002). These vocalizations,  
358 however, is likely to have similar functions to duets in the collaborative maintenance of  
359 territories and group cohesion (Bradley & Mennill, 2009; Fan et al., 2009; Geissmann &  
360 Mütschler, 2006), and thus should receive more attention in further studies.

361 It's not easy to interpret the differences between these closely related species of  
362 *Callicebus* based on the few available studies. Further studies within and across species  
363 of this diverse and widespread genus (van Roosmalen et al. 2002) are necessary in order  
364 to bring new insights on the use and functions of loud calls in these primates.  
365 Experimental investigation exploring finer responses of animals, such as structural  
366 differences in songs performed in response to playbacks, checking if the pattern of vocal  
367 response is somewhat similar to the vocalization in the stimuli broadcasted, may reveal  
368 possible hidden conflicting interest in vocal contributions of members of the mated  
369 pairs (Marshall-Ball & Slater, 2004).

370 Although here and in previous field observation we have found more support for  
371 joint territorial defense hypothesis in detriment of the mate defense one, they are not  
372 exclusive. Hall (2004), for example, has argued that the benefit gained through the  
373 alliance formed to cooperatively defend access to resources would increase the value of

the partnership and, thus, mate defense would follow from joint resource defense. By performing a mutually cooperative, coordinate display, such as duetting and chorusing, the social interactions themselves may also have a positive effect on the reinforcement, maintenance and cohesion of pair and group bond (Fan et al. 2009). In studies of primates loud calls, some mutually non exclusive functions are proposed to explain the function of these vocal displays (Cowlshaw, 1992; Fan et al. 2009; Geissmann, 1999; Heimoff, 1986; Méndez-Cárdenas & Zimmermann, 2009). In gibbons, besides the function in resource defense, the coordinated emission of loud calls in duets was proposed to have a hole in the mated status and pair bond announcement, reducing the attractiveness of both adults to the opposite sex and, consequently, the risk of invasion by an unpaired individual, extra-pair copulation and maybe mate desertion (Cowlshaw, 1992; Reichard, 1995). It is likely that pair bond advertisement is also involved in *C. nigrifrons* loud calls displays. The fact that the mated pair, but not necessarily the other individuals of the group, sing in very close proximity, sometimes with intertwined tails, and that individuals call much more often in duets than in solos or choruses (chapter 1), corroborates this idea. Besides, defending one's territory against all kinds of intruders will also result in the exclusion of same-sex rivals to both individuals in the mated pair (Robinson, 1981). Thus, through the announcement of territory ownership and defense, titi monkeys can also regulate the access of invaders to its mates, and thus, mate defense can be a side effect of territory defense.

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521        **Figures legends**

522        **Figure 1.** Number of trials in which groups reacted (moved towards the playback or  
523 counter-called) to the different stimuli broadcasted (control, duet, male solo, female  
524 solo). N of trials per stimulus type = 3; N of total trials= 12.

525        **Figure 2.** Frequency that each sex or the mated pair started to move towards the  
526 playback or counter-call in response to conspecific stimuli. The animals could start to  
527 move only once at each trial, but they could start calling many times. N of trials with  
528 conspecifics stimuli = 9; N of calling events in response to playbacks = 14.

529        **Figure 3.** Mean response scores for the four experimental conditions (control, duet, solo  
530 male and solo female). The vertical bars show the standard error.

531        **Figure 4.** Response scores for the four treatments (left) and in order of playback stimuli  
532 presentation (right) by groups.

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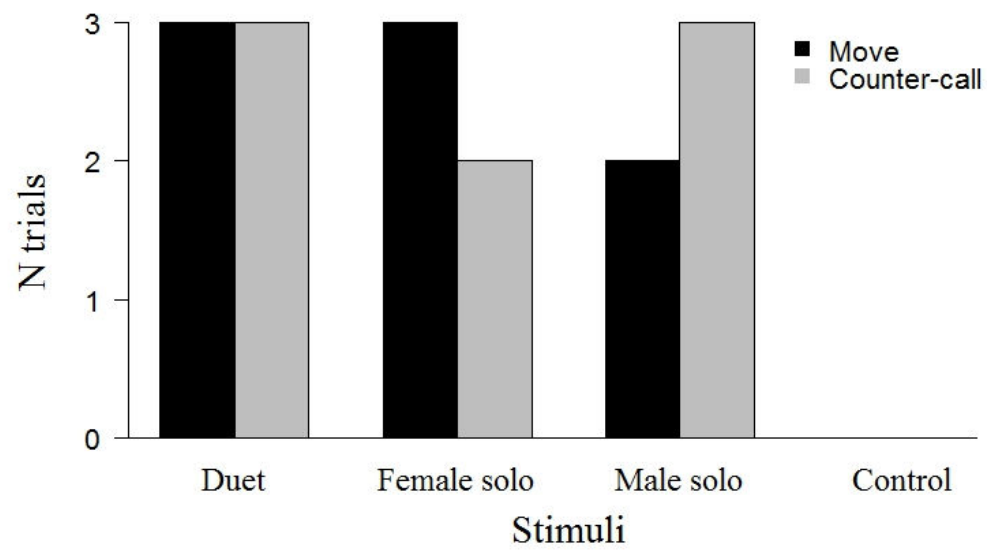
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544 **Figures**



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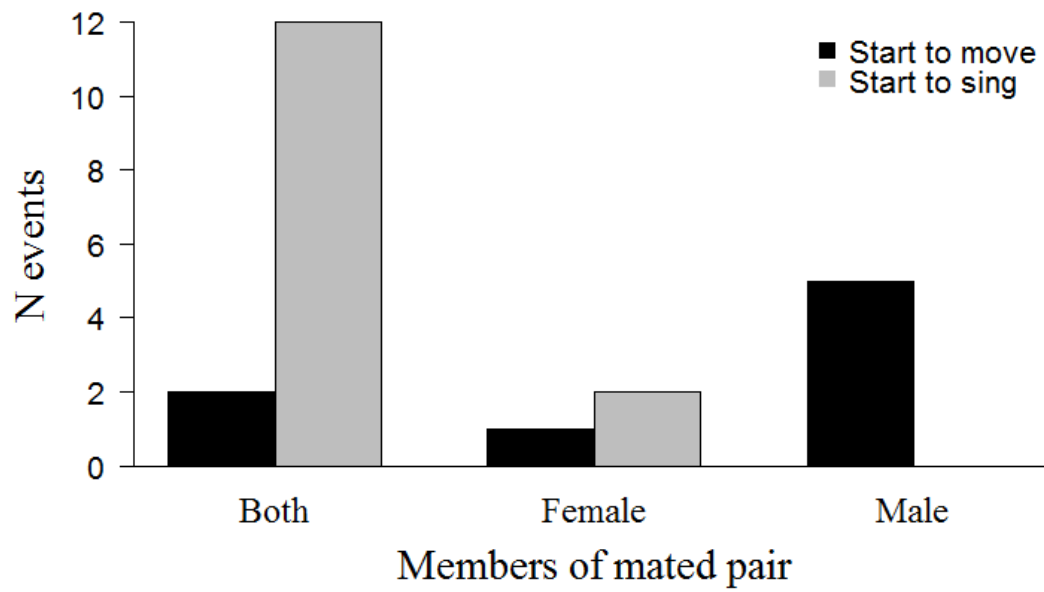
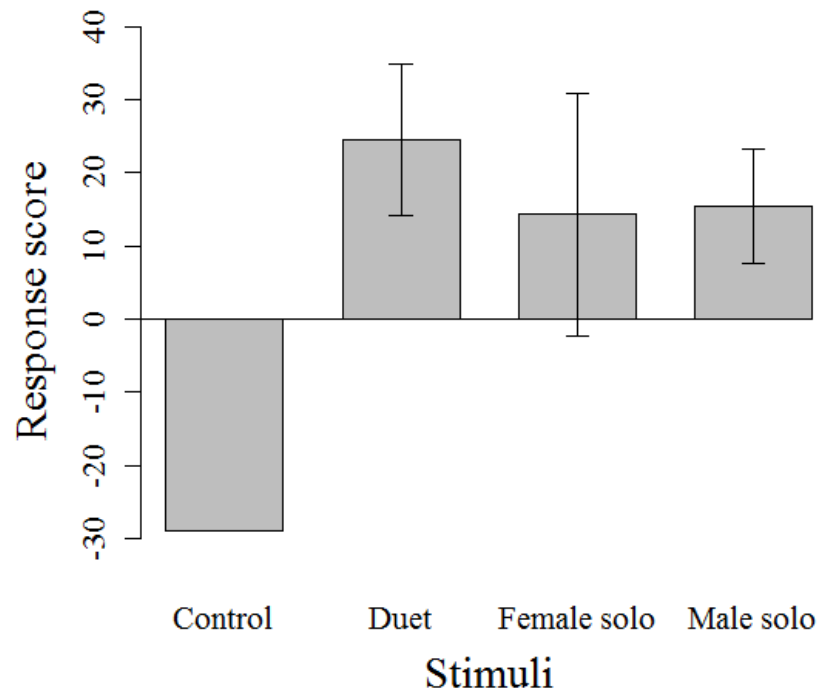


Figure 2.



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578 Figure 3.

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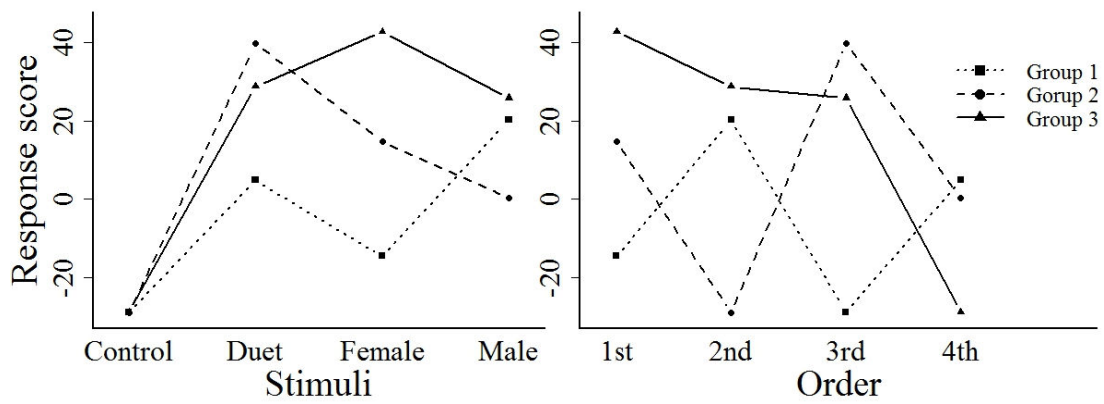


Figure 4.

**Capítulo 3**

"Variação na disponibilidade de frutos e no comportamento de defesa de recursos de sauás-da-cara-preta (*Callicebus nigrifrons*)."

1 Title: **Variation in fruit availability and resource defense by black-**  
2 **fronted titi monkeys (*Callicebus nigrifrons*)**  
3  
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24    **Abstract**

25       *Callicebus* primates are known to behave territorially yet this understanding is based  
26    on studies focused on just three of the 30 known species of this genus. Still, some of  
27    these few studies could not find support for territoriality. Although territoriality have  
28    been related to the defense of scarce critical resources, the effect of seasonal food  
29    availability on *Callicebus* territorial behavior was not evaluated. Here we investigated  
30    the influence of fruit availability on the frequency of behaviors associated with resource  
31    defense in a free range group of *C. nigrifrons* and evaluated the possible mechanisms  
32    used to guarantee priority access to food resource. Over a period of 20 months we  
33    monitored the fruit availability and recorded all agonistic behaviors during encounters  
34    with at least four neighboring groups and all loud calls emissions. Fruit production at  
35    the study site varied in a predictable way along the months and the monkeys strongly  
36    relied on this food resource. The monkeys advertised their presence in the territory  
37    through loud call emissions along all months and their vocal behavior increased with  
38    fruit production. Intergroup encounters were rare and most of these events occurred at  
39    places close to important feeding sites. The degree of aggression on encounters  
40    followed the variation in fruit availability. We concluded that intergroup encounters are  
41    probably result of disputes over important food resources, such as fruits, and the  
42    availability of these resources is an important determinant of *C. nigrifrons*' territorial  
43    behavior. The low frequency of inter-group agonistic encounter suggest that territories  
44    ownership advertisement by *C. nigrifrons* are effective in maintaining the priority  
45    access to important food resources.

46       **Key words:** territoriality, competition, resource defense, loud calls, primates.

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## 49    **1. Introduction**

50        Territoriality is a form of competition in which competitors exclude each other from  
51    areas containing resources, the territories (Maher & Lott, 1995). At one extreme, the  
52    territories may encompass the animal's entire home range and have sharp delimited  
53    limits (Hamilton III et al., 1976; Mason, 1968; Robinson, 1979a). At the other extreme,  
54    animals can use overlapped home ranges, defending only portions of this area, such as  
55    core areas, over which the animal has priority access to resources, such as food, mates  
56    or breeding sites (Burt, 1943; Powell, 2000).

57        Territoriality is not a fixed property of a species and can vary between populations of  
58    the same species or even within the same population over time (Carpenter &  
59    Macmillen, 1976; Kinnaird, 1992, Maher & Lott, 2000). According to economic models  
60    focused on resident behavior, territory defense should be adopted only when there is a  
61    shortage of critical resources (which limits population growth) and when these are  
62    economically defensible (Carpenter & Macmillen, 1976; Maher & Lott, 2000). In  
63    periods of low resource availability, for example, territory defense would not be  
64    expected, since the costs of defending a territory are likely higher than the gain acquired  
65    through exclusive access to the limiting resource. On the other hand, when resource  
66    productivity is high, competition for resource is low and territory defense would not be  
67    necessary. In this scenario, the territorial behavior would be expected in periods of  
68    intermediate resource availability, when competition for access to resources is moderate  
69    and the gain acquired through exclusive access to the limiting resource exceeds the  
70    costs of its defense (Brown, 1964; Carpenter & Macmillen, 1976).

71        Food is one of the most common limiting resources and territorial behavior of several  
72    species can be predicted from variation on food productivity (Carpenter & Macmillen,  
73    1976; Maher & Lott, 2000; Powell, 2000). Terborgh and Diamond (1970) argue that

74 animal reaction to food resource abundance, however, is not necessarily immediate. For  
75 example, some feeding trees with big crops at the ripening time can offer an ephemeral  
76 superabundance of resource that would decrease as its usage accumulates. Hence the  
77 competition must come as a result of the depletion of the standing crop and will be felt  
78 at a subsequent time, perhaps weeks after its heaviest use.

79 The quality of the food is another important determinant factor on defense decision.  
80 Low quality food, such as mature leaves (Clutton-Brock & Harvey, 1977; Grant *et al.*  
81 1992), may not compensate for the energy, time and even the risks involved on its  
82 defense. Besides, due to its greater abundance and more uniform distribution, these are  
83 not an easily monopolized resource. On the other hand, high quality food, such as fruits,  
84 has a more clumped distribution over space and variable, but more predictable,  
85 production throughout the year (Clutton-Brock & Harvey, 1977; Grant *et al.* 1992;  
86 Maher & Lott, 2000). Spatially and temporally predictable resources are more easily  
87 monopolized and defended (Grand & Grant, 1994; Maher & Lott, 2000). Accordingly,  
88 the defense of sites with high fruit availability during certain periods of the year may  
89 result in large increases in acquired energy and may also compensate for the energy  
90 spent on their defense (Kinnaird, 1992).

91 Territories with resources may be defended by an individual, a mated pair, or a  
92 group, and different behaviors may be employed on this task (Carpenter & Macmillen,  
93 1976; Gaston, 1978; Powell, 2000). Brown (1969) described three basic categories of  
94 territorial defense behavior: attack, threat and advertisement. In mammals, territories  
95 can be defended through attack (physical agonistic interactions), announcement (scent  
96 marking and vocalizations) and threats (stereotyped physical displays, vocalizations;  
97 Powell, 2000). Many birds and primates produce far-carrying loud calls on territorial  
98 contests or announcements (Cowlshaw, 1992; Hall, 2004; Oliveira & Ades, 2004),

99 which allow the communication between conspecific competitors over long distances  
100 (Mitani & Stuht, 1998; Waser & Waser, 1977).

101 The vocal interaction between neighboring groups through loud calls is a  
102 conspicuous behavior of Neotropical titi monkeys (genus *Callicebus*) (Mason, 1968;  
103 Kinzey, 1997; Robinson, 1979a). These predominantly frugivorous and monogamous  
104 primates live in familiar groups composed of a mated pair and two to four offspring  
105 (Kinzey, 1997). They are usually described as territorial, although these blanket  
106 characterization are based on studies focused on three of the 30 known species (*C.*  
107 *torquatus*: Kinzey & Robinson, 1983; *C. ornatus*: Mason, 1968 and Robinson, 1979a;  
108 *C. personatus*: Price & Piedade, 2001). Still, some of the few conducted studies could  
109 not find support for territoriality (Kinzey & Robinson, 1983; Price & Piedade, 2001).  
110 While in *C. ornatus*, the emission of loud calls is very frequent, leading to encounters at  
111 well demarked territory boundaries (Mason, 1968; Robinson, 1979a), the emission of  
112 loud calls and intergroup encounters are less frequent in *C. personatus* (Price &  
113 Piedade, 2001) and *C. torquatus* (Kinzey & Robinson, 1983). In *C. personatus* (Price &  
114 Piedade, 2001) loud calls emissions at intergroup encounters do not lead to exclusive  
115 use of home ranges and in *C. torquatus* (Kinzey & Robinson, 1983) groups seem to use  
116 counter-calling events to advertise their location.

117 Part of these behavioral differences within *Callicebus* genus could be a result of the  
118 short duration of these studies, which did not contemplate the effects of seasonal  
119 variation of food resources on territorial behavior. In order to fully understand the  
120 expression of territorial behaviors on this diverse genus of Neotropical primates, long  
121 term research across and within species, taking into account food resource availability,  
122 are necessary.

123 Here we investigated the influence of fruit availability on the frequency of behaviors  
124 related to resource defense in *Callicebus nigrifrons* living on an environment with  
125 pronounced seasonality at the southern limit of the tropical zone. Like other titi  
126 monkeys, *C. nigrifrons* use loud calls to communicate with neighboring groups and  
127 these calls seem to have a role in resource defense (chapters 1 and 2). To investigate  
128 how loud calls are used on resource defense, we also analyzed the spatial and temporal  
129 emission pattern of these vocalizations.

130 To guide our discussion about the influence of fruit availability on *C. nigrifrons*  
131 territorial behavior, we elaborated two main predictions: (1) if fruits are indeed an  
132 important food resource for these monkeys, it should comprise a great proportion of the  
133 group diet, and fruit consumption should increase with its availability; (2) if fruit  
134 availability is an important determinant of territorial behavior of *C. nigrifrons*, the  
135 expression of behaviors related to territoriality should vary in response to variation on  
136 fruit availability.

137 In order to understand the mechanisms used by *C. nigrifrons* for resource defense we  
138 predicted that: (1) if *C. nigrifrons* defends its entire range, delimiting and reinforcing its  
139 territory limits, the monkeys should vocalize more often from territory boundaries and  
140 intergroup encounters should occur more often at these areas; (2) if they only defend  
141 particularly valuable regions of their territory, such as important feeding sites, fruit  
142 production should be predictable, the monkeys should vocalize more from areas close to  
143 feeding sites and encounters should occur more often at these areas; (3) if they just  
144 advertise territory ownership, they should vocalize throughout their range, with no  
145 preference for particular areas.

## 146 2. **Methods**



147        **Subjects and study site**

148        We focused our observations on a habituated group of *Callicebus nigrifrons*,  
149        composed of 5 to 6 individuals, at Serra do Japi (23°14'3.38"S, 46°56'8.81"W), a 350-  
150        km<sup>2</sup> remnant of Atlantic Forest at the southern limit of the tropical zone in southeastern  
151        Brazil. Serra do Japi is characterized by a secondary semideciduous forest and  
152        temperate humid climate (Morellato, 1992).

153        Although *Callicebus* monkeys are sexually monomorphic (Norconk, 2007), we were  
154        able to distinguish between male and female of the mated pair and between them and  
155        their offspring based on size (when animals were close to each other). Males are slightly  
156        larger than females (*pers. obs.*) and adults (> 30 months) of the mated pair were larger  
157        than the sub-adults (18–30 months) and juveniles (6–18 months; de Luna et al., 2010;  
158        Valeggia et al., 1999). Hereafter we will refer to both sub-adults and juveniles as young.

159        **Fruit availability**

160        In parallel to behavioral data collection, we monitored the fruit production of 211  
161        plants of 22 zoochoric species eaten by *C. nigrifrons* (Caselli & Setz, 2011). To access  
162        the monthly fruit production for each plant we assigned scores from 0 to 4 for the  
163        percentage of the crown bearing mature fruits (0: no fruit; 1: 1 to 25% of the crown  
164        bearing fruit; 2: 26 to 50%; 3: 51 to 75%; 4: 76 to 100%; following Sun et al., 1996).  
165        We used the monthly fruit scores attributed to each individual plant to calculate an  
166        index of relative fruiting (following Kinnaird, 1992). This index sums the monthly  
167        scores attributed to all individuals and divides this value by the number of individuals of  
168        each species and then by the number of species.

169        **Data collection**

170        We followed monkeys from dawn to dusk (50 complete days) or from the moment  
171        they were found until they were lost (29 incomplete days) for three to five days each

172 month over a period of 20 months (from November 2009 to June 2011). During daily  
173 follows, we conducted activity scan samples of 1-min duration every 5 min (Altmann  
174 1974) and categorized the behaviors of any visible monkey as feeding, traveling,  
175 resting, and others (which includes all behaviors not included in any of the previous  
176 categories). When animals were feeding we recorded the type of item consumed (flesh  
177 fruit parts, seeds, flowers, vegetative plant parts, insects and others). Using these data,  
178 we calculated the proportion of feeding records spent on fruit consumption.

179 To quantify the focal group territorial behavior we recorded all occurrence of  
180 agonistic behaviors during group encounters, such as displays, chases, physical  
181 aggressions and counter-calling, as well as all extra group loud call emissions out of  
182 encounters. We define an encounter as all the events in which we were sure that our  
183 focal group was in visual contact with another group. We define as displays the  
184 behavior of assuming a semi-erect position (arched position), that could be accompanied  
185 by piloerection and tail rising, and the behavior of jumping in the canopy, resulting in  
186 noise of shaking branches and leaves. Chases consisted in following individuals of the  
187 other groups very close and fast and physical aggression the acts of grabbing and biting.

188 Later, we assigned scores to all agonistic behaviors recorded based on their presumed  
189 costs and risks and used these scores to calculate an index representing the degree of  
190 aggressivity in encounters for each month. For example, we assigned four points to the  
191 occurrence of physical aggressions, three points to chases, and one to three to loud call  
192 emissions, depending on the amount of calls emitted at each encounter. We gave three,  
193 two or one point if the monkeys emitted more, the same number or less calls than the  
194 mean number (mean = 4) of calls at encounters, respectively. For displays, we assigned  
195 one point. Thus, if in a particular month we recorded chases, displays and calls (in an  
196 amount inferior to the mean number of calls at encounters), the month score in this case

197 would be four: 3 (chases) + 1 (displays) + 1 (calls in an amount inferior to the mean  
198 number of calls at encounters). This way, monthly scores (hereafter aggressivity index)  
199 can vary from zero to eleven. We also recorded the location of all encounters and the  
200 location from where the monkeys emitted loud calls out of encounters.

#### 201 **Daily path length and home range size**

202 In order to calculate group's daily path length, home range size and intensity of use  
203 (utility distribution), we recorded the group's location every ten minutes with a GPS.  
204 We then plotted these points on a map with a scale of 1:25 m and superimposed a  
205 virtual grid with 25 x 25 m<sup>2</sup> cells over it. To calculate the total home range size we  
206 summed all the 625 m<sup>2</sup> cells entered by the group. We considered as “boundaries” all  
207 cells with at least one side in contact with the perimeter of group’s range plus all cells  
208 with a shared side with these edge cells. Thus, the boundary includes all the area within  
209 a minimum distance of 25 m from the edge (modified from Price & Piedade, 2001).

210 To explore the use of home range area on a monthly basis, we also calculated and  
211 plotted the monthly home range estimates from minimum convex polygons (MCP)  
212 using 95% of the locations recordings. We generated the MPCs in R software version  
213 2.15.0 (<http://www.r-project.org/>) and the add-on package adehabitatHR version 0.4.2  
214 (Calenge, 2006).

#### 215 **Home range intensity of use and loud calls distribution**

216 In order to evaluate the use of space by *C. nigrifrons* and the spatial distribution of  
217 call emissions we counted all the records in which monkeys entered each cell  
218 representing the group's home range and the number of loud calls emitted from each of  
219 these cells. We thus calculated the “variance to mean ratio index” (Payandeh, 1970),  
220 that is suitable to describe distributions on different scales (Payandeh, 1970, Neumann  
221 & Starlinger 2001). This index should assume values equal to one for random

distributions, values smaller than one for uniform distributions, and values greater than one for clumped distributions (Neumann & Starlinger, 2001).

Finally, we plotted all important feeding sites exploited by titi monkeys and all sleeping trees on the grid and polygons representing group's home range. We considered as important feeding sites those trees or vines in which at least half of the group foraged for fruits during at least four scans.

### **Statistical Analysis**

To evaluate the predictability of fruit production at the study site we used a Linear Regression with the index of relative fruiting as the dependent variable and the index of relative fruiting of the previous month (one month time lag) as the predictor variable. To evaluate the importance of fruit resources on titi monkeys' diet we used a Linear Regression to model the relationship between fruits' consumption (the monthly mean proportion of fruit on titi monkeys' diet) with the index of relative fruiting. To evaluate the influence of fruit availability over the frequency of loud calls we used a Spearman rank correlation between the monthly frequency of loud calls and monthly index of relative fruiting. For this analyses we used Spearman rank correlation because our data did not fit the requirements of normality and homogeneity of variance.

By means of Generalized Linear Models (using quasi-Poisson distribution to control for overdispersion of the data; Crawley, 2007) we related the monthly aggressivity index with the monthly index of relative fruiting to test whether fruit availability affects the frequency of behaviors related to territoriality. We performed this analysis with no time lag and with one month time lag, to detect possible delays in the animals' response to fruit depletion, as argued by Terborgh and Diamond (1970). To evaluate if the number of intergroup encounters increases with the distances travelled by the monkeys, we also used Generalized Linear Models (using Poisson distribution), relating the

247 number of encounters on each month with the monthly mean values of daily path  
248 length.

249 Finally, we used a Chi-square to test whether the number of loud calls emitted from  
250 places close to important feeding sites (cells with important feeding trees plus cells with  
251 a shared side with these cells) was greater than that expected by chance. We also used a  
252 Chi-square to test whether the monkeys emitted loud calls more often from its range  
253 boundaries than from its inner area, taking into account the proportional contribution of  
254 these areas.

255 All the analyses were implemented in R version 2.15.0 (<http://www.r-project.org/>)  
256 using significance level of 0.05.

### 257 **3. Results**

#### 258 **Fruit availability**

259 Fruits from two to 12 species were available per month and the fruit availability  
260 index ranged from 0.17 to 5.58 ( $2.33 \pm 1.53$ ; Mean  $\pm$  SD), confirming the fluctuation on  
261 fruit production at Serra do Japi (Morellato, 1992). Fruit production in a given month  
262 could be reliably predicted by its availability in the previous month (Linear Regression:  
263  $R^2 = 0.30$ ;  $F_{(1,17)} = 8.54$ ;  $P < 0.01$ ; model equation: fruit availability in a given month =  
264  $0.96 + 0.62$  fruit availability in the previous month)).

#### 265 **Fruit consumption vs. fruit availability**

266 Flesh fruit parts were the most consumed items (accounting for 56% of the feeding  
267 records;  $N = 6121$ ) and their consumption increased with fruit availability (Linear  
268 regression:  $R^2 = 0.28$ ;  $F_{(1,18)} = 7.18$ ;  $p < 0.01$ ; figures 1), confirming the importance of  
269 these resource on titi monkeys' diet (Caselli & Setz, 2011).

#### 270 **Loud calls vs. fruit availability**

271 The monkeys consistently emitted loud calls along the months (outside encounters),  
272 except for July of 2010, when animals only called during intergroup encounters. The  
273 mean monthly frequency of loud calls per hour ( $0.2 \pm 0.1$ ; Mean  $\pm$  SD) was positively  
274 correlated with fruit availability (Spearman rank correlation:  $r_s = 0.45$ ,  $N = 20$ ,  $P < 0.05$ ;  
275 figure 2).

#### 276 **Intergroup encounters vs. fruit availability**

277 We observed a total of 108 agonistic behaviors (73% of which were loud calls, 18%  
278 chases, 5% fights and 5% displays) in 19 intergroup encounters (range: 0 to 3  
279 encounters per month) with, at least, four neighboring groups during 730 observation  
280 hours (mean =  $39 \pm 6$  hours per month). Intergroup interactions ranged from 8 min to 1  
281 h 18 min (mean = 27 min). None of these encounters involved affiliative or sexual  
282 interactions between animals of different groups, and involved at least counter-calling  
283 between groups facing each other. Both male and female of the mated pair and their  
284 offspring participated in counter-calls, chases, displays and physical aggressions.  
285 Counter-calling was observed in all encounters, chases were observed in 84% of the  
286 encounters and displays in 26%. Physical aggressions were observed in 26% of the  
287 encounters and only after all other agonistic behaviors have been performed. When  
288 infants were present, one of the individuals (not necessarily the male or female of the  
289 mated pair) stayed with the infant on its back away from the immediate confrontation  
290 area. However, this individual could participate in loud calls emissions. In two  
291 occasions, when the infant was already able to move independently, it was attacked by  
292 individuals of the other group, felt on the ground and ran away from the confrontation  
293 site on the forest floor. After 53% of the encounters the animals of the focal group  
294 participated in grooming sections.

295 The monthly aggressivity index ranged from 0 to 10 and was positively related to  
296 fruit availability of the previous month (GLM, quasi-Poisson:  $t = 2.73$ ,  $df = 17$ ,  $P <$   
297  $0.05$ ; figure 3), but showed no relationship with fruit availability in the same month  
298 (GLM, quasi-Poisson:  $t = 0.71$ ,  $df = 18$ ,  $P = 0.49$ ; figure 3).

#### 299 **Daily path length**

300 The monthly mean daily path length was  $1.01 \pm 0.23$  Km (Mean  $\pm$  SD) and the mean  
301 distance travelled per day each month had no influence in the number of encounters  
302 with neighbors (GLM, Poisson:  $z = 1.59$ ,  $df = 17$ ,  $P = 0.11$ ). Thus, the number of  
303 encounters did not increase in months in which the group travelled more.

#### 304 **Home range use and spatial distribution of loud calls, intergroup encounters** 305 **and important feeding sites**

306 The monkeys used an area of 28 ha (figure 4) and the use of space by the group was  
307 clumped (variance to mean ratio index = 10.14), with the inner area of its home range  
308 being used more than expected by chance (Chi-square test:  $X^2 = 568.53$ ,  $df = 1$ ,  $P <$   
309  $0.0001$ ). This area concentrates 83% of the important feeding sites ( $N = 155$  ; figure 5)  
310 and 89% of the sleeping trees ( $N = 28$ ). The group did not use all parts of their home  
311 range along months (figure 6). They used a monthly mean area of  $14 \pm 4$  ha and only a  
312 small central area of 3 ha was consistently used in all 20 months (figure 6). This area  
313 does not exhibit a pronounced concentration of important feeding sites or sleeping trees,  
314 but it connects all parts of the group home range, which are used and reused at different  
315 times along months (figure 6).

316 Only 28% of the extra-group loud calls ( $N = 205$ ) was emitted from the group's  
317 home range boundaries (figure 5). Consequently, the monkeys vocalized less often from  
318 its range boundaries than expected by chance (Chi-squared test:  $X^2 = 12.62$ ;  $df = 1$ ;  $P <$   
319  $0.001$ ). On the other hand, the monkeys emitted more extra-group loud calls from places

close to important feeding sites than expected by chance (Chi-squared test:  $\chi^2 = 10.28$ ;  $df = 1$ ;  $P < 0.01$ ). The encounters with other groups were observed not only at the boundaries of group's home range (figure 5). Indeed, 58% of the encounters occurred at the group's inner area and 84% at places close to important feeding sites (figures 5).

#### 4. Discussion

Fruit production at the study site varied in a predictable way along the months and the monkeys strongly relied on this food resource, reinforcing the importance of fruits in *C. nigrifrons*' diet (Caselli & Setz, 2011). The monkeys advertised their presence in the territory through loud call emissions along all months (except July 2010) and their vocal behavior increased with fruit production. Intergroup encounters were rare and their occurrence did not increase in months in which animals traveled more, indicating that they do not occur by chance, as a mere consequence of how much the group moves each month (Harris, 2007). Most encounters occurred at places close to important feeding sites and the degree of aggression on encounters followed the variation in fruit availability, increasing after months of great fruit production. Thus, intergroup encounters are probably result of disputes over important food resources, such as fruits, and the availability of these resources is an important determinant of *C. nigrifrons*' territorial behavior, corroborating our initial prediction.

The time lag of one month between fruit production and the degree of aggressivity on intergroup encounters can be consequence of the group's response to the competition that follows fruit crop depletion after periods of superabundance at times of fruiting (Terborgh & Diamond, 1970). Another possible scenario is that the intensification of group's aggressive behavior is a consequence of the increase of intruders attracted by the enlarged food production (Myers et al., 1979). As it is probably more difficult to locate resources on an unfamiliar area, like the neighbors' range, the intruders may take



345 some time to locate fruiting trees inside other groups' territories. Thus, the intruder  
346 pressure is not immediate to the increase in fruit production, delaying the detection of  
347 changes in monkeys defensive behavior measured during encounters. Because the vocal  
348 behavior of titi monkeys accompanies the increase in fruit availability with no time lag,  
349 this second scenario is more likely to explain the observed delayed response of monkeys  
350 to the increase in fruit availability.

351 Here, we used the degree of aggression between neighboring groups during  
352 encounters as an indicative that the animals are defending their food resource. However,  
353 other factors, such as mate defense, can also result in intergroup antagonism whenever  
354 two groups meet (Harris, 2007). Nonetheless, if agonistic encounters were result from  
355 males attempting to defend its mate against males from other groups, these agonistic  
356 encounters should occur when females are fertile (Sicotte & Macintosh, 2004), which  
357 was not the case. Only one of the nineteen encounters occurred between March and  
358 April, the months in which females are supposed to be fertile at Serra do Japi (chapter  
359 1). Besides, confronts do not involve only males, the female and young actively  
360 participated in these agonistic encounters, demonstrating a common interest. Indeed, if  
361 food resource defense plays a role in agonistic encounters, these events should take  
362 place more often in food sites and the rate of encounters should be positively related to  
363 fruit availability (Garber et al., 1993; Steenbeek et al., 1999), which is exactly what we  
364 found.

365 Variable resource defense in response to fruit production was also observed in  
366 crested mangabey groups (*Cercocebus galeritus galeritus*) at eastern Kenya: as fruit  
367 availability increases, aggressive interactions occur between groups when they feed on  
368 species with patchy distributions (Kinnaird, 1992). However, differently from  
369 mangabeys, we never observed neutral encounters between titi monkey's groups. It is

370 possible that periods of food resource superabundance do not occur at Serra do Japi, in  
371 which competition between groups should not be necessary (Carpenter & Macmillen,  
372 1976; Maher & Lott, 2000).

373 Black-fronted titi monkeys did not show the behavior of patrolling and marking  
374 range boundaries, but they advertised the occupancy of its range via loud call emissions,  
375 especially from places close to important food sources, and reacted aggressively to all  
376 groups during encounters within its range. The constant advertisement of occupancy of  
377 an area by neighboring territory owners can possibly result in familiarity between each  
378 other and, thus, reduce unnecessary chases and fights, that will be necessary only when  
379 one come closer or trespasses each other ranges (Bradbury & Vehrencamp, 1998).  
380 Black-crested gibbons (*Nomascus concolor jingdongensis*), as black-fronted titi  
381 monkeys, called more often close to important food patches and inter-group conflicts  
382 were not frequent (Fan et al., 2009). The rarity of inter-group conflicts suggest that  
383 territories ownership advertisement by these two primates, especially from places close  
384 to important feeding sites, are effective in maintaining the priority access to important  
385 food resources. At the same time, this behavior do not restrict the access to less favored  
386 areas of the home range, resulting in a flexible system with spatial overlap while  
387 ensuring protection of important resource.

388 The behavior of *C. nigrifrons* differed from that of *C. ornatus* (formerly *C. moloch*),  
389 which reinforces and defends well defined territory boundaries through frequent  
390 agonistic encounters at these areas. *Callicebus ornatus* encounters are usually provoked  
391 by emission of male solo loud calls early in the morning and involve approaching the  
392 border, counter-singing between mated pairs and chases of same sex individuals  
393 (Mason, 1966, 1968; Robinson, 1979a).

394 It is not easy to infer the factors responsible for these interspecific variations on titi  
395 monkeys' behavior based on the few conducted studies, but it is possible that the  
396 differences in population density at study sites may play an important role (Table I).  
397 *Callicebus ornatus* were studied in more dense population, and groups occupied  
398 contiguous small areas, four to five times smaller than the ranges reported for *C.*  
399 *torquatus*, *C. personatus* and *C. nigrifrons* (Table I). In conditions of higher density,  
400 besides the intensified competition for resources (Maher & Lott, 2000), attempts of  
401 territorial expansion can also occur (Tilson, 1981), and, thus, the very marked and  
402 defended boundaries would be efficient in repelling potential intruders and would also  
403 make territorial expansion by neighbors difficult. Yet, in this scenario of small  
404 contiguous areas, there is also an increased chance of encounters between members of  
405 different groups, what increases the chances of extra pair copulation (Mason, 1966).  
406 Thus, the increased male participation on territory demarcation would be an attempt to  
407 decrease the chances of their mates to meet stranger males in other moments when  
408 female and male are not in close proximity, as they are during these provoked  
409 encounters at the range borders (Mason, 1966; Robinson, 1979a). At Serra do Japi, due  
410 to the greater size of their home range, maybe the risk of having its food usurped by  
411 unnoticed intruder incursions is greater than undesired encounters of mated females  
412 with strange males. Thus, not only male, but also female, as well as their offspring,  
413 would be more motivated to defend their shared resource from common rivals.

414 As observed for *C. personatus* and *C. torquatus*, *C. nigrifrons* did not call more often  
415 from their home range boundaries and encounters were not frequent (Kinzey et al. 1977;  
416 Price & Piedade, 2001). Large ranges demand more time and energy to patrol  
417 (Schoener, 1987). Thus, calling regularly from parts of its range or from more valuable  
418 sites (as important feeding trees), can be a more economical strategy to defend

419 important resources in this circumstance. For *C. nigrifrons*, which uses around 50% of  
420 its home range per month, the defense of its entire range is likely impracticable.

421 Price and Piedade (2001) concluded that *C. personatus* is not markedly territorial  
422 because home ranges overlapped partially and the monkeys did not called very often.  
423 However, 93% of their observations were concentrated in three months of atypical low  
424 precipitation, what possible affected food availability during the study. Here we  
425 observed that, in some months, loud call emissions were infrequent or even inexistent,  
426 and this variation was related to fluctuations on fruit production. Consequently, this  
427 could be the reason for the lack of observations of behaviors related to territoriality on  
428 *C. personatus*.

429 Most of previous studies addressing resource defense in monogamous primates,  
430 focus on the mated pairs behavior and the importance of maintaining and reinforcing  
431 pair bonds, not mentioning or informing about the participation of offspring in resource  
432 defense (Cowlishaw, 1992; Kinzey & Robinson, 1983; Mitani, 1985, 1987; Raemaekers  
433 & Raemaekers, 1985; Robinson, 1979a, 1979b, 1981, Robinson et al., 1986). In *C.*  
434 *nigrifrons*, the young not only help in infant care, eventually carrying it and keeping it  
435 away from the confrontation area during encounters, but also actively participate in joint  
436 loud call emissions and agonistic behaviors during and outside intergroup encounters  
437 (chapter 1).

438 The mere participation on intergroup displays and aggressions implies commitment  
439 of the individuals involved, since it requires partners-directed effort in coordinating  
440 behaviors, are energetically costly and risky (Fan et al., 2007; Kitchen & Beehner,  
441 2007; Smith, 1994). Extended family cooperative territory defense was observed in bird  
442 and primate species in which non-breeder offspring remain longer in their natal group  
443 (Gaston 1978; Walters et al. 1992; Lazaro-Perea, 2001; Garber et al. 1993). Although

444 we cannot reject the possibility that offspring involvement in intergroup interactions can  
445 also be related to breeding opportunities assessment in neighboring groups, as observed  
446 in common marmosets (*Callithrix jacchus*; Lazaro-Perea, 2001), it does not seem to be  
447 the main reason for young participation on these events. The young participated in 50%  
448 of the loud calls in choruses during encounters. If they were just interested in accessing  
449 mating opportunities, they would not need to join loud call emissions in these events,  
450 since individuals can visually evaluate each other. Besides, no affiliative or sexual  
451 interactions between animals of different groups were observed.

452 In sum, established groups of *Callicebus* monkeys seem to occupy stable ranges and  
453 defend their resource from neighboring conspecific groups through different  
454 mechanisms, that goes from defending and marking ranges boundaries (*C. ornatus*;  
455 Mason, 1968; Robinson, 1979a), passing through the advertisement of the ownership of  
456 areas with important food sources (*C. nigrifrons*; this study), to the simple  
457 advertisement of groups' location (*C. torquatus*; Kinzey & Robinson, 1983). The  
458 participation of group members also differed between species and the importance of  
459 offspring on resource defense has possibly been overlooked in previous studies,  
460 ignoring important information about social interactions of these species. Variations on  
461 population density, home range size, as well on fruit availability, neglected on previous  
462 studies, are likely to have an important contribution to the differences on territorial  
463 behavior. Fluctuation in fruit availability has proven to be an important determinant of  
464 territorial behaviors expression in *C. nigrifrons* and, thus, further long-term field studies  
465 should take these fluctuations into account.

466 Because we focused on the behavior of one group, we could not address other  
467 important issues involved in intergroup interactions, such as the degree of home range  
468 overlap between neighbors' ranges and the influence of previous interactions on

consecutive encounters, which can also influence the groups' motivations and, consequently, the degree of aggression between competitors on further contests (Harris, 2007). Since in *Callicebus* individual and sex identification is not trivial (and may need capturing and marking group members), we also could not evaluate details about sex-specific interactions during intergroup encounters. More detailed information on sex-specific interactions could have revealed behaviors associated to mate defense, like same-sex chases, as observed in *C. ornatus* and other primates (*Callithrix jacchus*: Lazaro-Perea, 2001). The history of group formation is another valuable information. In areas where neighboring individuals may be closely related, kinship might have complex effects on the degree of aggression and spatial exclusion (Bartlett, 2003). Therefore, many factors not yet explored, or poorly explored, can be acting on intergroup interactions and further long term field studies addressing these points are fundamental to corroborate and complement the conclusions drawn from this study and improve our understanding about the strategies used by *Callicebus* monkeys for resource defense and whether, how and why this behavior varies across species and habitats.

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641      **Tables**

642      **Table I.** Comparison of home range size and population density across previous studies  
643      on different *Callicebus* species.

Species	Home range size (ha)	Population density (n/km <sup>2</sup> )	Reference
<i>C. ornatus</i>	0.3 - 0.5	400	Mason, 1968
	3.3 - 4.2	57	Robinson, 1979a
<i>C. torquatus</i>	20	20	Easley & Kinzey, 1986; Kinzey et al., 1977
<i>C. personatus</i>	22	12.3 - 12.6	Price & Piedade, 2001; Price et al. 2002
<i>C. nigrifrons</i>	28	3.5 - 14.86*	Trevelin et al., 2007

644      \* Since we have no information about population density at Serra do Japi, we used data of *C.*  
645      *nigrifrons*' populations at other localities reviewed in Trevelin et al., 2007  
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660 **Figure legends**

661 **Figure 1.** Variation in fruit consumption (% of feeding samples) with in response to  
662 fruit availability (index of relative fruiting). N of feeding records = 6121.

663 **Figure 2.** Variation in loud call emission frequency (number of loud calls per  
664 observation hour) and in fruit availability (index of relative fruiting) along the months.

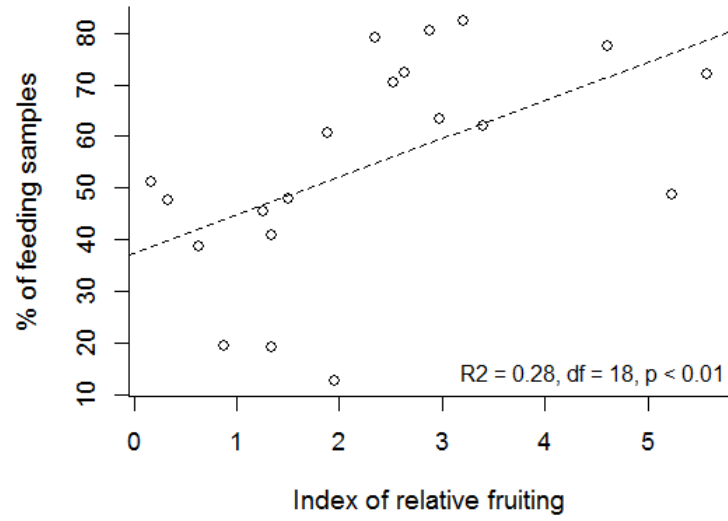
665 **Figure 3.** Variation of aggressivity index (combined agonistic responses during  
666 encounters) in response to fruit availability (index of relative fruiting) of the previous  
667 (A) and the current month (B).

668 **Figure 4.** Intensity of use (utility distribution) of different areas of the home range. The  
669 quadrants with bold lines delimitate the home range boundary and the ones with dashed  
670 lines represent the inner area. The intensity of colors indicates the number of location  
671 records in which the group was observed in each cell (as shown in the caption at the  
672 bottom left). N of location records = 4610.

673 **Figure 5.** Home range representation indicating the distribution of extra group loud  
674 calls (left) and important feeding sites (right). The quadrants with bold lines delimitate  
675 the home range boundaries and the ones with dashed lines represent the inner area. The  
676 intensity of colors indicates the number of important feeding sites and the number of  
677 calls events registered in each quadrant (as shown in the caption at the bottom left). The  
678 numbers plotted at the home range representation on the right indicate the number of  
679 intergroup encounters in each cell. N of loud calls = 205; N of feeding sites = 155; N of  
680 encounters = 19.

681 **Figure 6.** Representation of total home range used in 20 months (bold line) showing the  
682 overlaid ranges used in each month (dashed lines; A), the central area consistently used  
683 in all months (in grey; A) and the distribution of encounters (B), important feeding sites  
684 (C) and sleeping trees (D).

685 **Figures**



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687 Figure 1.

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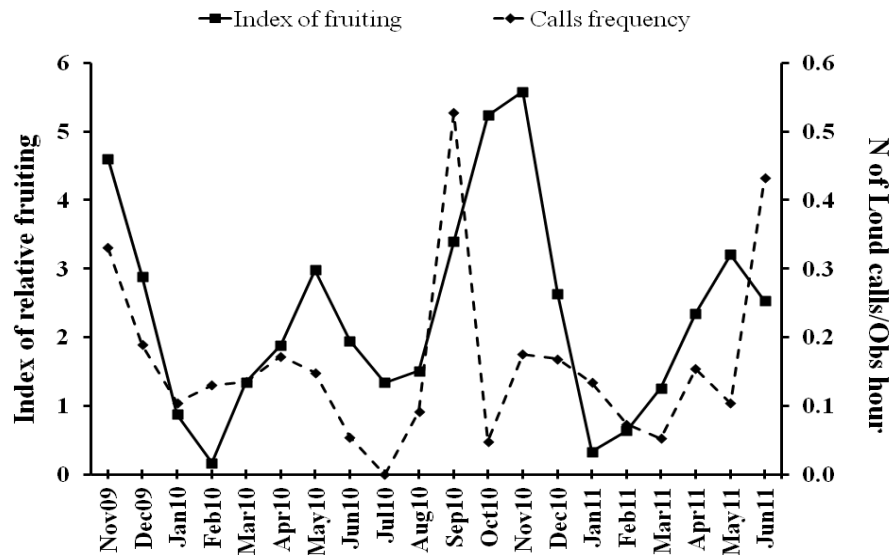


Figure 2.



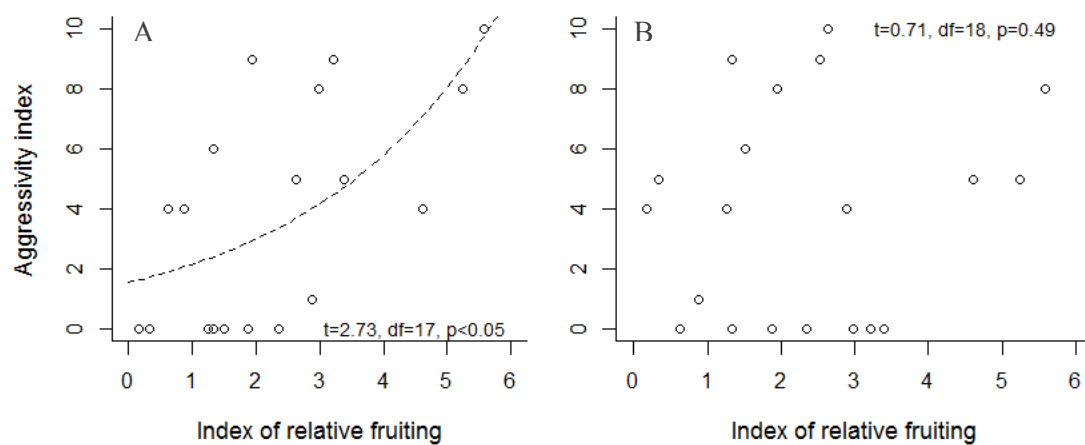
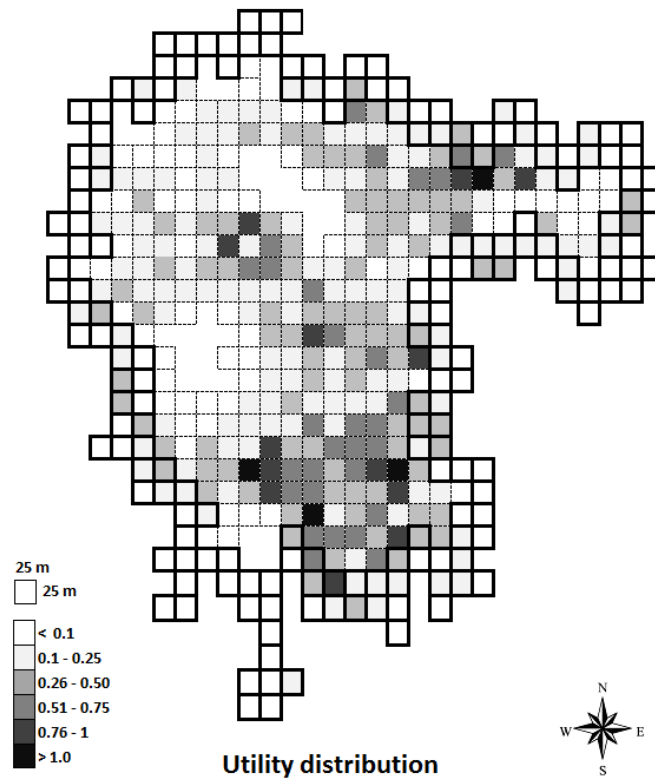


Figure 3.



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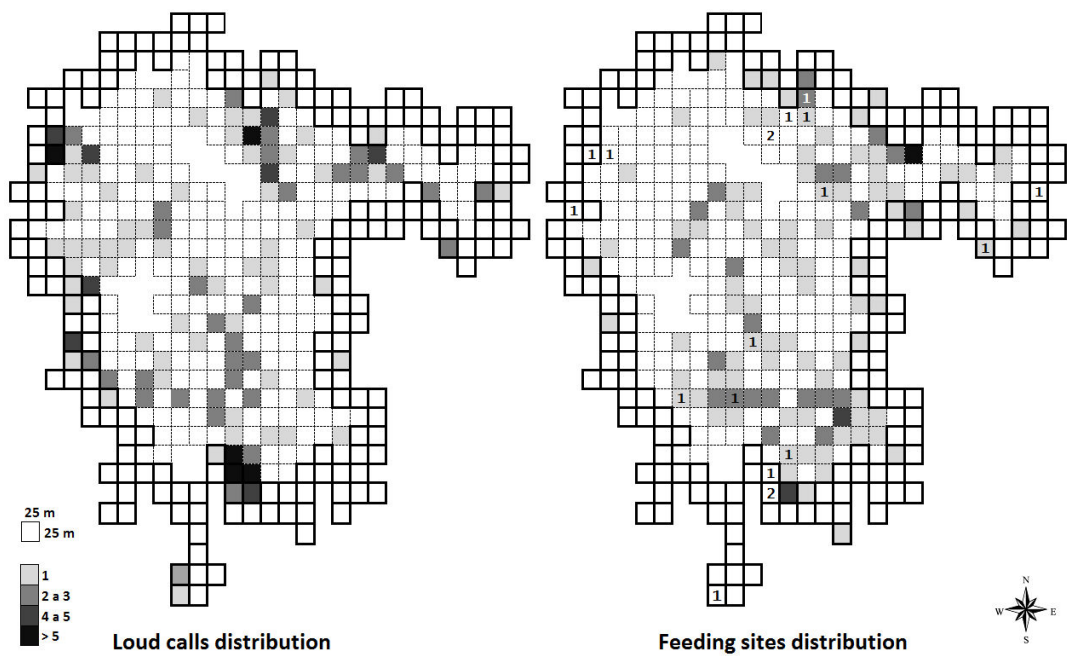
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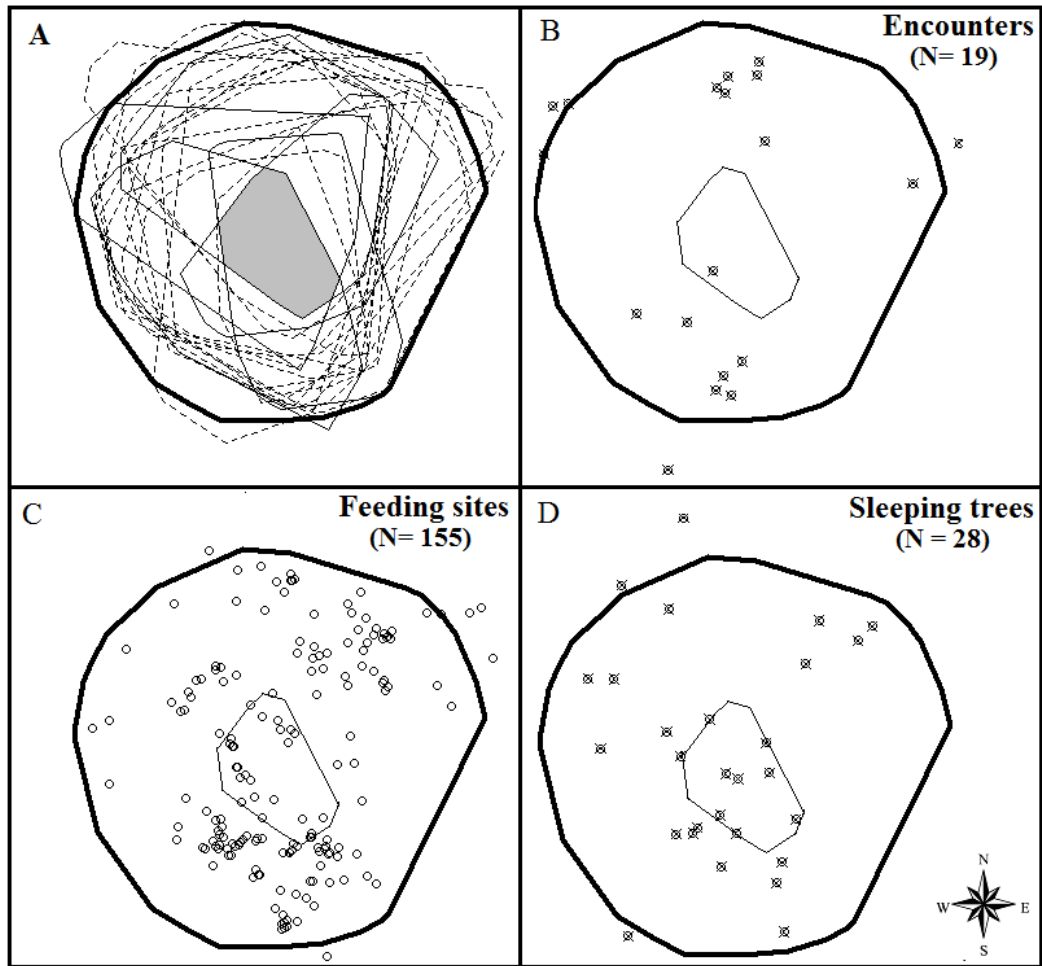
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**Figure 6.**

### **Conclusões gerais**

Como descrito para *C. ornatus* (Robinson, 1979b) e *C. cupreus* (Müller & Anzenberger, 2001), os cantos de *C. nigrifrons* são estruturados de forma hierárquica, partindo da combinação de unidades menores, as sílabas, para formação de frases, que então são combinadas para dar origem a sequências mais longas, os cantos propriamente ditos. Confirmando observações preliminares, *C. nigrifrons* utilizam vocalizações de longo alcance tanto para comunicação entre membros do mesmo grupo como para comunicação entre membros de grupos vizinhos e estes cantos, embora sejam compostos de unidades vocais semelhantes (mesmas sílabas e frases), apresentam estruturas diferentes (quanto a proporções e padrões de transições de sílabas e frases).

Os cantos utilizados para comunicação intra-grupo apresentam uma estrutura mais estereotipada que os cantos utilizados para comunicação externa e, possivelmente, esta diferença esteja relacionada com a natureza mais complexa dos contextos envolvidos na comunicação entre grupos vizinhos. Aqui encontramos suporte para a hipótese de que os cantos empregados para comunicação entre grupos vizinhos possui função de defesa de recursos alimentares importantes na dieta de *C. nigrifrons*, embora a hipótese de defesa de parceiro não possa ser descartada. Observamos também que esse comportamento de defesa envolve a atuação conjunta não apenas do casal reprodutor dos grupos, mas também dos jovens, que participam ativamente do coros de cantos de longo alcance e também de comportamentos agonísticos durante encontro entre grupos, sugerindo um interesse comum dos membros dos grupos.

Diferente de outros *Callicebus*, *C. nigrifrons* não exibiu comportamento de defesa de e patrulhamento das bordas de sua área de vida, mas focaram o comportamento de defesa a áreas com recursos alimentares importantes e economicamente defensáveis, como árvores frutíferas mais utilizadas em sua alimentação. Esse comportamento de

defesa, que se dá tanto pela emissão de cantos de longo alcance, quanto pela expulsão de grupos vizinhos, variou em intensidade de acordo com a disponibilidade de frutos no ambiente. A baixa frequência de encontros entre nosso grupo focal e grupos vizinhos sugere que os comportamentos adotados por *C. nigrifrons* são efetivos para garantir o acesso prioritário a recursos alimentares importantes em sua dieta. O uso exclusivo de áreas ou recursos protegidos dentro dessas áreas parece algo irreal na natureza, pois como comentado por Burt (1943, pg. 350), "*It is to be expected that the territory of each and every individual will be trespassed sooner or later regardless of how vigilant the occupant of that territory might be.*".

Em resumo, grupos estabelecidos de sauás parecem ocupar áreas de vida estáveis e defender o seu recurso de grupos coespecíficos por meio de mecanismos diferentes, que vão desde a defesa e demarcação de bordas bem definidas, como em *C. ornatus*, ao anúncio da posse e defesa de áreas com importantes fontes de alimento, através de emissão de vocalizações de longo alcance e outros comportamentos agressivos a grupos que se aproximem, como em *C. nigrifrons*. Desta forma, embora *C. nigrifrons* não defendam toda sua área de vida, estes podem ser ditos territoriais, visto que defendem áreas importantes de sua área de vida quando essa defesa é economicamente viável. Novamente, como colocado por Burt (1943, pg. 351), "*Only those that protect some part of the home range, by fighting or aggressive gestures, from others of their kind, during some phase of their lives, may be said to have territories.*".

A participação de todos os membros do grupo, não apenas do casal reprodutor, também diferiu do relatado em estudos anteriores com *Callicebus*, sendo possível que a importância dos jovens na defesa dos recursos tenha sido negligenciada nestes estudos anteriores, ignorando informações importantes sobre as interações sociais dessas espécies. Variações na densidade populacional, no tamanho das áreas de vida utilizadas,

bem como na disponibilidade de frutos (também negligenciada em estudos anteriores), podem ajudar a explicar as diferenças no comportamento entre as espécies de sauás estudadas. Flutuação na disponibilidade de frutos demonstrou ser um determinante importante da expressão do comportamento de defesa de recursos em *C. nigrifrons* e, portanto, mais estudos de longo prazo devem levar estas flutuações em conta.

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