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Responses of wild titi monkeys, *Callicebus coimbrai* (Primates: Platyrrhini: Pitheciidae), to the habituation process

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ABSTRACT. Adequate habituation of free-ranging subjects is essential for any field study, but is generally unsystematic. Here, attempts to habituate three titi monkey (*Callicebus coimbrai* Kobayashi & Langguth, 1999) groups are described, and factors determining the effectiveness of the process are discussed. The "relentless pursuit" approach was aided by playback recordings of vocalizations but only one group was habituated adequately. Average contact in 13 encounters with group 1 was just over one minute, whereas in 32 encounters with group 2, it averaged 3,5 minutes (maximum = 22 minutes). Group 3 was more tolerant of observers, and was considered fully habituated by the seventh encounter. The factors determining this disparity remain unclear, although vegetation density seems important. Whereas group 3 occupied an area of relatively undisturbed forest, with a sparse understory, the other groups occupied a habitat with dense undergrowth and an irregular canopy. The subjects' tolerance may have been affected by reduced visibility and less discreet behavior of the observers. On 10 occasions, the members of group 2 leapt to the ground and fled through the undergrowth. The results indicate the need for a careful evaluation of habitat characteristics prior to the selection of groups for habituation.

KEY WORDS. Behavior; habitat; habituation; observation.

The behavioral monitoring of free-ranging primates can provide a wide range of data on ecological parameters such as diet, home range size and habitat preferences, although the reliability of observations depends on the degree of habituation of the subjects (Williamson & Feistner 2003). In the behavioral sciences, the concept of habituation has been adopted from the field of physiology, where it refers to a reduction in response time as a consequence of repetitive stimulation (Thorpe 1963, Williamson & Feistner 2003). The habituation of a subject to the presence of human observers involves primarily the suppression of its instinctive response to the approach of potential predators (Williamson & Feistner 2003). Primates are especially appropriate subjects for this type of process, given their behavioral flexibility and ability to adapt to novel conditions (Blom *et al*. 2001, 2004, Bertolani & Boesch 2008, Jack *et al*. 2008).

The observation of unhabituated primates is vulnerable to a wide range of bias, varying from the distortion of natural behavior patterns to the inadequate identification of subjects (Williamson & Feistner 2003). By contrast, fully habituated subjects will often allow observers to approach to within a very short distance, and collect detailed data on specific behavior patterns, including such subtle traits as facial expressions. However, while an extensive literature is available on behavioral sampling procedures (e.g. Altmann 1974, Fragaszy *et al*. 1992, Martin & Bateson 1993, Altmann & Altmann 2003), few studies have described or analyzed specific techniques for the habituation of free-ranging primate subjects. In fact, few field study (Blom *et al*. 2001, 2004, Boere *et al*. 2006, Bertolani & Boesch 2008, Jack *et al*. 2008) reports provide any details of this process.

The titis (*Callicebus* spp.) are among the shyest and most discreet of all monkeys (Mason 1968, Kinzey 1981, Kinzey & Wright 1982), due to a combination of factors, including their relatively small size and cryptic behavior. They are notoriously difficult to observe under most conditions (Kinzey 1982), although they will normally respond decisively to playback broadcasts of recordings of conspecific duet vocalizations (Robinson 1979, Melo & Mendes 2000). While playbacks provide a useful aid for locating titi groups, they are of only limited potential for the habituation of subjects. Once they recognize the source of the playback, however, titis will typically engage in cryptic behavior and, with experience, they may even avoid them altogether.

In the present study, the response of titi monkeys, *Callicebus coimbrai* Kobayashi & Langguth, 1999, to the habituation process in the context of fragments of Brazilian Atlantic Forest is described and evaluated in relation to differences among groups, and the effects of habitat structure on this process. A number of tentative guidelines for the development of a more systematic approach to the habituation process are provided.

MATERIAL AND METHODS

The study took place at the Fazenda Trapsa (11°12'S, 37°14'W), an abandoned shrimp farm in the municipality of Itaporanga d'Ajuda in the northeastern Brazilian state of Sergipe. The property encompasses eight Atlantic Forest fragments, with a total area of approximately 500 ha. The fragments vary in size, habitat quality and connectivity but even the smallest are occupied by the three local primate species – *C. coimbrai*, *Callithrix jacchus* Linnaeus, 1758, and *Cebus xanthosternos* Wied-Neuwied, 1826. The original vegetation has been classified as arboreal restinga (Scarano 2002), which is similar in composition to the typical Atlantic Forest, but generally lower in stature (canopy 15 m or lower) and located on very sandy soils in areas close to the coast. The fragments are located in the centre of the farm, at least 3 km away from the nearest human habitations, and there is no human transit in either of the two fragments included in the present study.

Two of the fragments were selected for behavioral monitoring of resident titis. One fragment, denominated Viveiro, covers an area of 62 ha, approximately half of which was devastated by a fire approximately 10 years ago. This left the fragment with a highly heterogeneous structure, varying from secondary forest to early successional habitat, characterized by a highly irregular canopy, with frequent gaps and very dense undergrowth. The terrain is hilly and irregular, with a number of steep-sided gulleys.

The second fragment, known as Camboinha, is much smaller than Viveiro (14.4 ha), but is relatively well-preserved, with sparse undergrowth, except for a small area that was affected by fire in March, 2009. The terrain is mostly flat. These two fragments were selected for a number of practical reasons, including their relative accessibility, lack of areas prone to flooding, and their density of titis.

Initial preparation of each site involved the installation of access trails, cut along the main axes of the fragment. Additional trails were added as information was accumulated on the distribution of the titis within the fragment, as proposed by Williamson & Feistner (2003), with the ultimate objective of establishing a 50 x 50 m grid within each group's home range, the standard layout for ecological studies of titis (Müller 1996, Palacios *et al*. 1997, Heiduck 2002).

Habituation of the subjects was based on the standard "relentless pursuit" procedure used in the majority of primate field studies (Setz 1991), in which the animals are followed continuously until their behavior remains unaltered, or virtually so, in the presence of the observer. Initially, contact with the subjects was obtained primarily through the use of playback of the vocalizations of the closely-related *Callicebus nigrifrons* Spix, 1823, to which the local titis were known to respond systematically (R.R.D. Chagas, Universidade Federal de Sergipe, unpubl. data). Use of the recording of this species was necessary because of the lack of good recordings of *C. coimbrai* vocalizations, at the time.

When the observer first entered the forest, the trail system was searched systematically for signs of the titis, concentrating on areas in which they had been observed on previous occasions. If the animals were not encountered, the trails were walked again, this time with the playback being broadcast at 30 minute intervals. The playbacks were presented at strategic locations, where the vegetation was relatively dense, in order to minimize the visualization of the observer by the subjects.

Once one or more titis responded vocally to the playback, the observer would take a bearing in relation to the trail system, and move in the direction of the animals. If the subjects stopped vocalizing before the observer was able to locate the group, the playback would be activated again until a response was obtained. When at least one subject was visualized by the observer, a set of data recorded, time and location of the encounter; the number of animals observed; the behavioral response of the first animal observed (Tab. I); and the height above the ground of the first animal sighted. Additional information was then collected on the subsequent behavior of the subjects, and the time at which contact was lost. This data was converted to relative frequencies for comparisons among groups.

Habitat quality was evaluated within each study area based on the approach of Chagas & Ferrari (2010), which was applied to the assessment of habitat at the same study site. The procedure is based on the comparative assessment of a series of variables: mean canopy height; percentage canopy cover; understory density; and the density of lianas and pioneer trees (e.g. *Cecropia* spp.). This information was then used to define three basic habitat types: mature, secondary and disturbed (Tab. II). Once defined, each 50 x 50 m quadrant formed by the trail system was assigned to a category, based on the predominant habitat observed within the quadrant. The proportion of each category in each fragment was used to determine the expected values in a contingency table of habitat use, analyzed with the *G* test. Statistical analyses were performed in BioEstat, version 5.0 (Ayres *et al*. 2007).

Data were collected on up to three days per week between July, 2008, and June, 2009. Observations were conducted between 06:00 and 17:00 h by an observer and a field assistant, who helped with the monitoring of subjects. The "relentless pursuit" of the groups consisted of following the animals cautiously and continuously without attempting to approach them to within a distance that would cause them to take evasive ac-

Table I. Categories of the behavioral response of unhabituated titis to the presence of human observers (adapted from Blom *et al.* 2004).

Category	Description
Ignore	Animal(s) completely still (may be defecating or urinating) with no reaction to the presence of the observer
Curious	Animal(s) watching the observer, or moving to a position in order to obtain a better view of the observer
Avoid	On sighting the observer, the animal(s) move(s) away rapidly and silently while emitting alarm vocalizations
Vocalize	Animal(s) emitting alarm vocalization toward the observer without moving away
Feed	Animal(s) continue(s) feeding normally even after noticing the presence of the observer
Move	Having detected the presence of the observer, the animal(s) move(s) away at a normal pace without vocalizing or exhibiting any marked behavioral modification

Table II. Habitat categories used to classify the vegetation within the trail system in each forest fragment at the Fazenda Trapsa, Sergipe, Brazil. Based on Chagas & Ferrari (2010).

Category	Diagnosis
Mature	Continuous canopy 12-15 m tall (occasionally ⩾ 15 m) with few gaps (<25% of total area), relatively sparse understory, low density of lianas and few pioneer trees (e.g. *Cecropia* spp.)
Secondary	Relatively continuous canopy 8-10 m tall (25-75% open), with well-developed understory, medium density of lianas and pioneer trees
Disturbed	Discontinuous canopy 5-10 m tall with frequent gaps (>75%), very dense understory, high density of lianas and pioneer trees

tion (judged qualitatively by the animals' reaction). In the case of the Viveiro fragment, as there were two resident groups, the first group encountered was monitored until contact was lost.

Observations were initially conducted in the Viveiro fragment, between July, 2008, and March, 2009. The fragment was home to two resident groups, one with an adult pair, a subadult and an infant (group 1), and one with an adult pair, two subadults, a juvenile, and an infant (group 2). Following the failure to adequately habituate either of these groups (see below), attention shifted to a third group (group 3), resident in the Camboinha fragment. This group contained an adult pair, a subadult, and an infant, and was monitored in June, 2009. Subsequent observations (J.P. Souza-Alves, unpubl. data) are not included here, as the group was considered to be fully habituated by the end of June.

RESULTS

General patterns

The analysis of habitat quality revealed that Camboinha was overall better preserved than Viveiro (Tab. III), and in particular, that it contained a small proportion of mature forest, which was absent from the Viveiro fragment. The three groups were encountered in these different habitats (Fig. 1) more or less as expected according to their availability (G test: $p > 0.48$ for all three groups). This corresponded to a very significant difference among groups ($G = 27.643$, d.f. = 4, $p < 0.0001$), with most (84.8%) sightings of groups 1 and 2 in disturbed forest, in contrast with group 3, which was invariably observed in the better-preserved habitat.

Table III. Availability of the three habitat categories in the Viveiro and Camboinha fragments at the Fazenda Trapsa, Sergipe.

Habitat category	Number (%) of records	
	Viveiro	Camboinha
Mature	0 (0.0)	5 (17.9)
Secondary	4 (20.0)	23 (82.1)
Disturbed	16 (80.0)	0 (0.0)

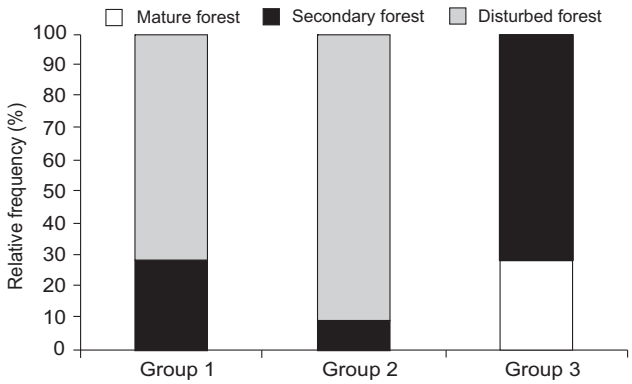

Figure 1. Relative frequency of encounters with the three *C. coimbrai* study groups at the Fazenda Trapsa by habitat category.

In general, the titis reacted in three distinct manners to the presence of human observers: ignoring them, approaching them with apparent curiosity, and taking flight, although not

all animals reacted in all three ways. Typically, the initial encounters with an unhabituated group were characterized by "chirrup" (cf. Robinson 1979) alarm vocalizations, followed by curiosity and territorial calls, and finally a rapid retreat.

While the animals normally moved away through the forest canopy, the members of group 2 exhibited a unique avoidance pattern (never seen in any of the other 14 groups observed at the site), where they would jump down to the ground and either flee through the dense undergrowth or remain motionless until the observer moved away. This pattern was observed on 10 occasions (almost a third of the encounters), and only in the disturbed habitat, and appears to be a response to the characteristics of this type of vegetation, in which this group was encountered most frequently (Fig. 1). It is unclear, however, why these same factors should not have affected the behavior of group 1 in the same way, although this group was observed only a third as frequently as group 1 (see below), and proportionately much less often in disturbed habitat.

While the members of groups 1 and 2 would often show curiosity towards the observers, their basic reaction remained unchanged throughout the study period, and they would almost invariably take flight after only a few minutes of contact. By contrast, the reaction of the members of group 3 was far less intense, even during the initial encounters. Rather than taking flight, in fact, these animals would often hide in the crown of a tree.

The members of group 1 never allowed observers to approach them in any way, whereas group 2 would occasionally stay put as the observers came closer, although on at least three of these occasions, some of the animals urinated and defecated when the observers closed in to within 8 m. Once again, group 3 contrasted considerably in its reactions to the approach of the observers, and would allow them to come within 5 m as early as the second encounter.

Specific patterns

The two Viveiro groups were encountered on 45 occasions in 381 hours of fieldwork. Total contact with group 1 in 13 encounters was only 14 minutes, that is, a mean duration of just over one minute per encounter. Many encounters (n = 5) lasted only a few seconds, and the longest, five minutes. On three occasions, however, the animals reacted to the observers with curiosity (Fig. 2), although such behavior was not reflected in any major increase in contact.

The average duration of contact was slightly longer (3:30 min) in group 2, although encounters the 32 encounters were highly variable. While almost half (n = 15) lasted only a few seconds, contact was maintained for as long as 22 minutes in one case. Subsequent encounters would nevertheless follow the normal pattern, and little progress had been made by the 32nd and final encounter. These differences, in relation to group 1, are reflected in the respective behavior patterns of the two groups (Fig. 2). The members of group 2 not only avoided the observers much less frequently than those of group 1, they were also much less curious, although in almost a third of encounters, their initial reaction was to ignore the observers. One possible factor in these differences is the greater frequency of contact with group 2, which was contacted almost three times more often than group 1 (36 encounters versus 13).

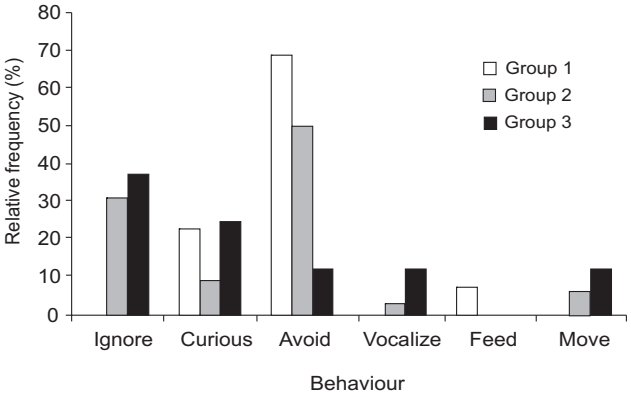

Figure 2. Relative frequency of different responses to the presence of observers in the three *C. coimbrai* groups at the Fazenda Trapsa, Sergipe, Brazil.

In contrast, whereas the first encounter with group 3 lasted only a few minutes, the second one was longer than any recorded with either of the other two groups, and most subsequent encounters lasted even longer. By the seventh day of monitoring (only one individual was observed in encounter 6), when the animals could be approached without provoking any noticeable reaction, thus the group was considered to be fully habituated. From this moment onwards, it was possible to monitor the group continuously and systematically throughout the daily activity period, permitting the collection of quantitative behavioral data between July and December, 2009 (J.P. Souza-Alves, unpubl. data), and regular monitoring, which has continued to the present day.

An additional pattern among groups was the number of individuals observed per encounter. Whereas contact with a single individual was relatively common in groups 1 (35.7% of sightings) and 2 (46.6%), it occurred on only one occasion in group 3, in which almost half the encounters (44.4%) involved all four group members. Obviously, this is at least partly a result of the much longer encounters recorded in this group. In groups 1 and 2, the pattern of variation was exactly the opposite.

The reduced visibility of the members of first two groups appears to be related primarily to differences in habitat (Fig. 1), and may not only reflect their more cryptic behavior in the much denser vegetation of the Viveiro fragment, but also implies in reduced contact with observers per individual. In other words, each group member was much less likely to have direct contact with an observer during an encounter than the mem-

bers of group 3. It seems reasonable to assume that this further reinforced the relative difficulty of habituating the members of the Viveiro groups.

One other difference among groups was their vertical distribution within the forest. Group 1 was invariably observed in the upper levels of the canopy (> 9 m), whereas group 2 was encountered at heights below 9 m in approximately a third (31.3%) of sighting, and occasionally (n = 2), very close to the ground. It was on these occasions that the members of this group fled along the ground. However, there was no obvious difference between these two groups and group 3 (which was encountered on all but one occasion at 9-14 m) that might account for the contrasts in the results of the habituation process.

DISCUSSION

While considerable differences were found in many of the variables analyzed, the exact factors determining the success or difficulty of habituating one or another of the groups remains unclear. A cautious examination of the results nevertheless permits some speculation.

Prior experience of contact with humans, in particular hunters, may affect the habituation process considerably (Williamson & Feistner 2003, Bertolani & Boesch 2008), although Atlantic Forest titis are almost never hunted (Chiarello 2003), and there is no evidence of any such activity at Fazenda Trapsa (R.R.D. Chagas, unpubl. data; pers. obs.). However, a primate predator, the capuchin (*C. xanthosternos*), is present at the site and has been observed in both fragments. Capuchins are known to prey on titis (Sampaio & Ferrari 2005), and the members of group 3 were observed avoiding a group of capuchins systematically on two occasions (pers. obs.). The capuchins at the Fazenda Trapsa range widely among the different forest fragments, and as Viveiro is much larger than Camboinha, it seems likely that the capuchins visit this fragment more frequently. In this case, groups 1 and 2 would not only have more frequent contact with the capuchins, but this contact may also be more threatening due to the structure of the highly disturbed habitat in this fragment. Such a conclusion is nevertheless highly speculative.

The differences in habitat structure between the two fragments may also have played a role in the variation in the success of the habituation process. Possibly the most important difference between fragments is the much denser understory found at Viveiro, in comparison with Camboinha, where relatively undisturbed forest with little undergrowth predominates. Additionally, while the canopy is continuous at Camboinha, the tree crowns at Viveiro are irregular and often widely-spaced, hindering the displacement of arboreal quadrupeds such as the titis. Boere *et al*. (2006) also reported that the dense vegetation of the central Brazilian Cerradão hampered the habituation of pencil-tufted marmosets, *Callithrix penicillata* É. Geoffroy, 1812, at a site near Brasília.

In contrast, Tutin & Fernandez (1996) and Blom *et al.* (2004) have argued that dense undergrowth was an advantage for the habituation of gorillas, because it offered them more cover and made contact with observers less stressful. However, unlike titis or marmosets, gorillas are large terrestrial primates with few predators, so it seems unlikely that the same considerations apply here.

Two factors may be relevant here. The denser vegetation at Viveiro offers more cover in which the animals are able to hide, and thus avoid contact with observers. This may have contributed to the smaller numbers of animals observed in this fragment, and the reduced contact time, which may have been crucial to the difficulty of the habituation process. A second consideration may have been the noise factor – in denser undergrowth, the observers are less able to move and follow the subjects without making a disturbance.

The habit of fleeing across the ground observed in members of group 2 is an atypical pattern in the platyrrhines, which are highly adapted for an arboreal lifestyle. Similar behavior has been observed in the Amazonian *Callicebus brunneus* Wagner, 1842 (S.F. Ferrari unpubl. data), and has been reported by local residents at a number of other sites, indicating that it may be a typical predator-avoidance strategy in this genus, although one which may vary considerably among individuals or groups, depending on either intrinsic (individual variation or experience) or environmental factors. Titis generally prefer the lower forest strata (Kinzey 1981, Palacios *et al.* 1997, Ferrari *et al*. 2000), a pattern that may be reinforced at the present study site, due to the low stature of the forest (Chagas & Ferrari 2010).

In the absence of any systematic variation in other features, habitat structure appears to be the primary factor determining the differences in the effectiveness of the habituation process among groups. This factor has received little attention in other studies (e.g. Jack *et al*. 2008), but may be especially relevant in the case of small-bodied species such as titis, and at sites where there is significant variation in habitat structure. Hopefully, such considerations will contribute to the much-needed development of more effective habituation strategies (Forthman *et al*. 1996, Butynski & Kalina 1998a, Williamson & Feistner 2003).

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