Functionally Referential Alarm Calls in Tamarins (*Saguinus fuscicollis* and *Saguinus mystax*) – Evidence from Playback Experiments

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**Introduction**

Although animals do not intend to provide information for their conspecifics, there are many examples demonstrating that listeners are able to acquire information from vocal signals (Seyfarth & Cheney 2003). Vervet monkey alarm calls are the classical example for a functionally referential alarm call system (Struhsaker 1967; Seyfarth et al. 1980; Evans 1997). Vervet monkeys give acoustically distinct alarm calls in response to different types of predators such as aerial and terrestrial predators or snakes. When conspecific listeners hear these alarm calls they respond with the adequate escape strategy. In
the case of an aerial alarm call vervet monkeys climb down the tree and scan the sky. In the case of a terrestrial alarm call they climb up into trees, and in the case of a snake alarm call they often stand bipedal and scan the ground. Regardless of whether or not the caller has an intention to communicate the presence of a certain predator, evidently these calls serve a referential function for the listeners.

Further studies on nonhuman primate vocalisations have revealed other types of alarm call systems, such as a continuum of graded signals with the usage based on response urgency (chacma baboons: Fischer et al. 2001) or mixed systems (redfronted lemur and white sifakas: Fichtel & Kappeler 2002) with one functionally referential call and other unpecific calls. Scientists have identified two factors that appear to explain the evolution of such different alarm call systems: the presence or absence of different types of predators and hence the presence or absence of different escape strategies in a certain species (Macedonia 1990; Pereira & Macedonia 1991). Following Macedonia & Evans (1993) animals with different escape strategies, like ring-tailed lemurs (*Lemur catta*), tend to have a functionally referential alarm call system, while animals with similar or only one escape strategy, like ruffed lemurs (*Varecia variegata*), tend to have a gradual or urgency-based system. Subsequent studies on several Old World monkeys confirmed this hypothesis. Diana monkeys (*Cercopithecus diana*: Zuberbühler et al. 1997) and Campbell’s monkeys (*Cercopithecus campbelli*: Zuberbühler 2001) show different locomotive patterns towards eagles and leopards and have functionally referential call types for these predators. On the contrary, larger and predominantly terrestrial primates like chacma baboons seem to fulfil the prediction that primates without specific escape strategies encode arousal or escape urgency in their alarm calls. Field studies on chacma baboons in the Okavango delta in Botswana have shown that these primates have no distinct alarm call types in their repertoire (Fischer et al. 2001). Their alarm calls continuously grade into ‘lost calls’, calls that are uttered when an individual has lost contact with other group members. Of course, the same species may have acoustically discrete, predator-specific alarm calls that show urgency-based acoustic gradation within each class. Manser (2001) demonstrated experimentally that suricates give acoustically distinct alarm calls to terrestrial predators, avian predators, and snakes, and within each class have high urgency and low urgency variants that reflect the immediacy of danger and elicit stronger or weaker responses.

As former studies of alarm call systems have all been carried out on lemurs or cercopithecines, some of whom are closely related to each other, it seems important and useful to gain similar knowledge also on New World monkeys. These have undergone an independent radiation within the order primates and differ in essential socio-ecological and life-history characteristics from the other primate taxa (Strier 1994; Kappeler & Heymann 1996). Therefore, acoustic studies on New World monkeys cannot only fill a taxonomic gap, but also lead, in comparison with prosimians and Old World monkeys, to a deeper understanding of alarm call systems in general.

Tamarins of the New World genus *Saguinus*, and most other callitrichids, suffer from a severe predation pressure because of their small body size. Most important among their predators are large raptors, but also felids, mustelids, and snakes have been reported to prey on callitrichids (e.g. Terborgh 1983; Emmons 1987; Heymann 1987). In field studies, it has been observed that saddleback and moustached tamarins show distinct behavioural reactions towards different types of predators (Heymann 1990; Peres 1993). In response to aerial predators, they look upwards and quickly fall or climb downwards, while in response to terrestrial predators, they look downwards and sometimes approach the predator. Tamarins live in dense forest habitats with limited visibility and therefore need to maximize the efficiency of their acoustic communication. This is especially true in the case of anti-predatory behaviour, where quick and appropriate reactions are required. Hence, tamarins are very suitable subjects for the investigation of acoustic warning signals.

In this study, we conducted playback experiments to test if the acoustic information of aerial and terrestrial alarm calls is sufficient to elicit the predator-specific reactions in saddleback and moustached tamarins. In accordance with the reactions observed during natural predator encounters we established the test paradigm: in response to the playback of aerial alarm calls the focal animal should look significantly longer upwards, while in response to the playback of terrestrial alarm calls the focal animal should look significantly longer downwards.

**Methods**

**Study Site and Subjects**

The study was carried out from May to Nov. 2001 and Oct. to Nov. 2002 at the Estación Biológica Quebrada Blanco (EBQB) in northeast Peru.
station is located at 4°21'S 73°09'W, approximately 90 km southeast of Iquitos, near the river (Quebrada) Blanco. The rain forest is of the ‘terra-firme’ type (Encarnación 1985, 1993) and has a canopy height ranging from 25 to 30 m with some outstanding trees rising up to 40 m. The study site is approximately 1.5 km² large and run through by a system of parallel trails in north–south and west–east direction, with a 100 m spacing in between. For details of the study site see also Heymann (1995). There is not much information available on the predator fauna of the area, but several case studies of sightings and attacks report on a wide variety of tamarin predators, such as large raptores (Heymann 1990; Overslujs Vasquez & Heymann 2001) and snakes (Bartecki & Heymann 1987; Shahuano Tello et al. 2002). Attacks of terrestrial predators like cats and mustelids are of course difficult to observe, as these animals are strongly disturbed by the human observer following the monkeys. Nevertheless, during this study, the first author had the opportunity to see two tayras (Eira barbara) and two bush dogs (probably Atelocynus microtis) in immediate proximity of the study groups. An ocelot (Felis pardalis) has been seen at EBQB by a first author’s co-worker in 2001 (P. Löttker, pers. comm.). For more information on predator fauna see also Smith et al. (2004). There is no data available on the predation pressure.

During the study period two fully habituated groups of tamarins inhabited the area. A third group was habituated by the first author and a field assistant starting late Oct. 2001. Each group consisted of the two species Saguinus mystax and Saguinus fuscicolis, which formed stable associations. Group sizes ranged from eight to 14 individuals, including between four and six S. fuscicolis and between three and nine S. mystax. The number of individuals changed in the course of time because of migration, birth or death. Individuals could be identified by a combination of natural features such as body size, body shape, and fur characteristics as well as size, coloration, and pigmentation of genitals or other body parts. At the EBQB the observers can approach any habituated tamarin up to a proximity of approximately 1 m (S. fuscicolis) or 3 m (S. mystax), which facilitates individual recognition. By means of binoculars the identification is also possible from larger distances and at greater heights.

**Experimental Procedure**

For the playback experiments we used a SONY TCD-D100 DAT-Recorder connected to a BOSE ‘Roommate II’ (BOSE Corporation, Framingham, MA, USA) loudspeaker. The loudspeaker was positioned at approximately 8–12 m distance from the focal animal and tied to the trunk of a tree at approximately 1.5 m of height, presumably invisible to the monkey. The subject’s behaviour was recorded with a CANON UC-X50 Hi 8 mm video camcorder (Canon, Tokya, Japan).

The playback was performed only when the focal animal was engaged in a quiet activity with little or no locomotion, i.e. resting, grooming, feeding or relaxed foraging. To avoid interfering stimuli we conducted all playback experiments when no territorial encounters or natural predator alarms took place. The focal animals were chosen opportunistically when all the required conditions were met. Altogether, 10 S. fuscicolis and 11 S. mystax individuals were tested. We aimed to test any one focal individual with all three playback types (aerial, terrestrial, and control). We used an ‘all combination design’, i.e. we played S. mystax calls both to S. mystax and to S. fuscicolis subjects, and S. fuscicolis calls both to S. fuscicolis and S. mystax subjects. All playback experiments were conducted during the two study periods (see study site and subjects). To avoid habituation, we attempted to conduct subsequent experiments in the same focal group with at least 1 d of spacing in between. Only in three cases, a subsequent experiment had to be carried out the following day.

**Playback Stimuli**

All calls used as playback stimuli were recorded in the same study groups at EBQB during 6 mo of preliminary studies in 2000 and during the first playback study period from May to Nov. 2001. We adjusted the volume of the calls to that observed during naturally occurring predator encounters. Figure 1 shows spectrograms of the characteristic aerial and terrestrial alarm calls of S. fuscicolis and S. mystax. The figure represents typical examples of the call series we used as playback stimuli. A call series is a unit of calls in the same sequence as naturally recorded. Note that both species’ aerial and S. mystax terrestrial alarm call series consist exclusively of repetitions of one and the same call type, whereas S. fuscicolis terrestrial alarm call series contain several different call types. A multi-parametric acoustic analysis of the various call types will be given in a separate paper (J. Kirchhof & K. Hammerschmidt, unpubl. data).

We used only alarm calls of which the eliciting predator or model type (aerial or terrestrial) was
known. As it was difficult to record a sufficient number of alarm calls with known stimuli, not every experiment could be conducted with a unique call series. Therefore, some call series were used in more than one experiment. For the 19 aerial playback experiments we used 10 different call series, which were all recorded during natural predator encounters. For the 20 terrestrial playback experiments we used 11 different call series, which were recorded during natural encounters and presentations of a live dog or a stuffed toy serving as an ocelot (*F. pardalis*) model. For the 17 control playback experiments we used 11 different stimuli: nine of these were different song series of common birds in the area which are known to be harmless to the tamarins (n = 5: *Lipaugus vociferans*, screaming piha, n = 4: *Ramphastus spec.*, toucan), one was the observer’s voice and one was ambient noise of the forest. Table 1 gives the distribution of playback experiments regarding playback type (aerial, terrestrial, and control), focal species and call origin (conspecific or heterospecific).

All playbacks were designed as follows: an initial call series with a duration of 8–9 s was followed by a pause of 20–25 s, and then the same call series was repeated. Thus, the entire playback had a total duration of approximately 35–40 s. The recording of the focal animal began several seconds before the first call series and ended between 30 and 50 s after the second call series.

### Behavioural Analysis

All video recordings were digitised and viewed with the program ADOBE PREMIERE 4.2 with a time resolution of 25 frames per second (duration of a single frame = 0.04 s). We measured the latency to the first reaction of the focal animal (mostly a turning of the head) by counting the frames, beginning from the start of the first call series. We then counted the frames the focal animal spent looking up, down, and to remaining directions, ending 25 s after the start of the second call series. The durations of all directions were summed up to build the duration of total looking. By doing so all analysed playback videos had a duration of approximately 60 s (x = 54.70 ± 5.35 s, n = 64). For each playback experiment, we also determined the direction of the focal animal’s first glance following the stimulus. We recorded a change of gaze direction only when the subject had turned its head in response to the call series. If the subject maintained the same gaze direction before as after the stimulus, we scored it as ‘no direction’. Tamarins usually make obvious head movements while scanning their environment. The looking direction was therefore considered as ‘up’ or ‘down’ when the angle of the tamarin’s head reached at least 45° declination from the horizontal plane.

### Table 1: Number of conducted playback experiments

<table>
<thead>
<tr>
<th></th>
<th>S. fuscicollis</th>
<th>S. mystax</th>
<th>S. fuscicollis</th>
<th>S. mystax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Terrestrial</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td></td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Spectrograms of the characteristic (a) aerial and (b) terrestrial alarm calls of *S. fuscicollis* and *S. mystax*. The call series represent typical examples of the ones we used as playback stimuli.
line. All smaller angles were considered as ‘straight’.
It was also sampled when the subject looked to the
loudspeaker. Glances of the animals to their own
body, the body of grooming partners or glances at
items close by, such as hand-held food, were not
sampled as any looking direction.

Statistical Analysis
Although we tried to test each focal animal within
all three playback types and both with conspecific
and heterospecific calls, some subjects were not
available frequently enough in an appropriate test
situation, and others died or disappeared before the
test series was completed. Thus, the test tables includ-
ed some missing values. Therefore, we were not
able to use a test design, which includes the different
playback types at the same time. We used a general-
ized linear model analysis (GLM, SPSS 12) to iden-
tify differences in latency related to playback type,
species and call origin. To test our paradigm that
subjects look significantly longer upwards in
response to the playback of aerial alarm calls than in
response to the playback of terrestrial alarm calls we
used the GLM repeated measurement procedure
(SPSS 12). We did separate tests for the three play-
back types (aerial, terrestrial and control). To test
the direction of the first glance, we grouped the
possible directions in three categories (up, down,
and other directions) and used Pearson chi-square
test (SPSS 12).

Results

Latency
For *S. fuscicollis*, we found similar short latencies in
all three playback types (Fig. 2). For *S. mystax* we
found short latencies only in aerial playbacks, in ter-
restrial playbacks *S. mystax* showed intermediate
latencies and the longest in control playbacks
(Fig. 2). Therefore, we found significant differences
in latency between the two species only after play-
back a control call (GLM: F_{1,11} = 12.88, p = 0.004).
We found no significant differences in latency after
playback of an aerial alarm call and a terrestrial
alarm call.

Looking Durations
For *S. fuscicollis* and *S. mystax*, we found significant
differences in the looking directions between aerial
and terrestrial alarm call playbacks (Fig. 3). After
playback of an aerial alarm call both species spent
significantly more time looking up than looking
down (GLM repeated measurement: F_{1,16} = 51.3,
p = 0.000). After playback of a terrestrial alarm call
both species spent significantly more time looking
down than looking up (GLM repeated measure-
ment: F_{1,15} = 14.5, p = 0.002). After playback of a
control call neither species showed stimulus signifi-
cant differences in the time looking up or down
(GLM repeated measurement: F_{1,16} = 0.221,
p = 0.645).

![Fig. 2: Mean and SEM of latencies in response to the different play-
back types](image)

![Fig. 3: Durations (mean and SEM) of looking up (a) and looking down
(b) given in percent of the total looking (TL)](image)
After playback of an aerial alarm call we found no significant differences between the two species (GLM repeated measurement: $F_{1,16} = 2.33, p = 0.146$), or origin of the alarm calls (GLM repeated measurement: $F_{1,16} = 1.88, p = 0.19$). We also found no significant interactions between looking direction and species or call origin (looking duration $\times$ species: $F_{1,16} = 2.83, p = 0.111$; looking duration $\times$ call origin: $F_{1,16} = 0.89, p = 0.361$). We also found no significant differences between the two species after playback of a terrestrial alarm call (GLM repeated measurement: $F_{1,15} = 3.63, p = 0.076$), but because of the small sample size this result should be taken with caution. We found no significant difference related to call origin (GLM repeated measurement: $F_{1,16} = 0.198, p = 0.863$) and no significant interactions (looking duration $\times$ species: $F_{1,16} = 0.52, p = 0.481$; looking duration $\times$ call origin: $F_{1,16} = 2.43, p = 0.14$). In a similar way, we found neither significant differences between species after playback of a control stimulus (GLM repeated measurement: $F_{1,16} = 0.01, p = 0.92$), nor a significant interaction between looking duration and species (looking duration $\times$ species: $F_{1,16} = 0.007, p = 0.936$).

Direction of the First Glance

Table 2 shows the directions of the subjects’ first glances after hearing the playback stimulus. Saguinus mystax never looked immediately down after aerial playbacks, and never immediately up after terrestrial playbacks (chi-square two-tailed: $df = 4, p = 0.025, n = 19$). Likewise, S. fuscicollis never looked immediately down after aerial playbacks, but by contrast, showed no clear tendency in the first glances after terrestrial playbacks (Table 2). Corresponding to that, the chi-square test revealed no significant differences comparing the direction of the first glance (chi-square two-tailed: $df = 4, p = 0.181, n = 26$). In control experiments, both species’ first glances were into various directions and in three cases the subjects maintained the same gaze direction as prior to the playback.

### Table 2: Direction of the first glance. The table indicates the frequencies of the different looking directions immediately after hearing the playback stimulus

<table>
<thead>
<tr>
<th>Looking direction</th>
<th>S. fuscicollis Playback type</th>
<th>S. mystax Playback type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerial</td>
<td>Terrestrial</td>
</tr>
<tr>
<td>Up</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Down</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Straight/to loudspeaker</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

**Discussion**

Saddleback and moustached tamarins looked significantly longer upwards in response to aerial alarm calls and significantly longer downwards in response to terrestrial alarm calls. Thus, they reacted as if external referents, i.e. information about the predator type or the appropriate reaction, were encoded in the acoustic features of the calls. This conclusion is supported by the fact that none of the two species showed any preference after playback of control calls. In addition, we found no differences in the responses of S. fuscicollis and S. mystax whether the alarm call stimulus was produced by a conspecific or a heterospecific caller. This finding indicates that the call origin had no influence on the response of either species.

The direction of the first glance corresponded in most cases to the direction from which the simulated predator would have attacked. The only exception was the response of S. fuscicollis to the terrestrial alarm calls. Here, we found no preference of the first glance. This could correspond to an apparently more general usage of their terrestrial alarm calls: while all the other calls, i.e. both species’ aerial alarm calls and S. mystax terrestrial alarm calls, were exclusively used in the predator-specific situation, calls acoustically similar to S. fuscicollis terrestrial alarm calls were also given in other contexts, such as aggressive inter-group encounters. There are other studies in nonhuman primates (Fichtel & Kappeler 2002; Fichtel et al. 2005) and birds (Seyfarth & Cheney 1990) that also suggest a more general usage of terrestrial alarm calls in comparison with aerial alarm calls. This is in line with our observation that aerial and terrestrial alarm vocalisation of S. mystax and aerial alarm vocalisation of S. fuscicollis contained only one call type, whereas the terrestrial alarm vocalisation of S. fuscicollis consisted of several different call types (see Fig. 1).

As far as we know, this study is the first to demonstrate functionally referential alarm calls in New World primates. Functionally referential alarm call systems have been found among primates in three cercopithecine species (Cercopithecus aethiops: Seyfarth et al. 1980; C. diana and C. campbelli: Zuberbühler et al. 1997; Zuberbühler et al. 2001) and in ring-tailed lemurs (L. catta: Macedonia 1990). Furthermore, functionally referential alarm call systems also exist among carnivores (Suricata suricatta: Manser 2001;
Manser et al. 2001), and among birds (Gallus gallus: Gyger et al. 1987; Evans et al. 1993a), and are supposed to exist in some species of rodents (e.g. Tamura & Young 1993; Weary & Kramer 1995; Greene & Meagher 1998). Functionally referential alarm call systems hence have evolved independently in several different vertebrates, and taxonomic affiliation is unlikely to be a major selective factor to develop such systems. Mixed alarm call systems so far have been described by Fichtel & Kappeler (2002) for red-fronted lemurs (Eulemur fulvus rufus) and white silakas (Propithecus verreauxi verreauxi). The assumption of a mixed system in a callitrichid species, a group quite distantly related to prosimians, adds further support that a species’ taxonomic classification is not the only predictor for its alarm call system. This is further enhanced by the fact that saddleback and moustached tamarins are closely related to each other, but still seem to exhibit differences in their alarm call systems.

Although S. fusciollis and S. mystax both show predator-specific reactions which is in line with the hypothesis of Macedonia & Evans (1993) that different escape strategies are the main selective force for the evolution of referential signalling, the difference between the alarm call behaviour of S. fusciollis and S. mystax found in the present study suggests there might be more factors involved. One obvious difference between S. fusciollis and S. mystax is that they use different height levels in the forest. S. fusciollis usually range lower in the forest than S. mystax and even sometimes come down to the ground (Norconk 1990; Smith et al. 2004). Therefore, they generally seem to be more vulnerable to terrestrial predators than S. mystax. In this context, it is interesting to note that S. fusciollis mobbed terrestrial predator models significantly longer than S. mystax did (Kirchhof 2003). On the other hand, S. fusciollis, closer proximity to the ground might also imply that for them terrestrial predators are easier to recognize than aerial predators. Hardie & Buchanan-Smith (2000) studied mixed-species groups of S. fusciollis and S. labiatus, which exhibit a similar height division as associated S. fusciollis and S. mystax (S. fusciollis in lower strata and S. labiatus in higher strata: Buchanan-Smith 1990). They found that S. fusciollis detected novel non-threatening objects faster when those objects were on the ground, while S. labiatus detected objects faster that were at greater heights. In the present study, it could sometimes be seen that in terrestrial playback experiments S. fusciollis only shortly checked the ground visually, whereas S. mystax often exhibited a rather conspicuous searching behaviour (Kirchhof, personal observation). It remains open to which extent an impeded visual contact to the predator can shape the structure of an alarm call system, but these data suggest a certain connection.

Another noteworthy aspect is that functionally referential systems can also develop in species with only small differences in their ways of escaping from aerial and terrestrial predators. Diana and Campbell’s monkeys approach the predator in natural encounters with both eagles and leopards. The subtle difference between the two situations is that in response to aerial predators typically only adult males approach and the rest of the group stays behind, while in response to leopards often the whole group approaches the predator (Zuberbühler et al. 1997; Zuberbühler 2001). Nevertheless, both species have evolved functionally referential alarm calls for the two predator types. The same phenomenon can be seen in the suricate (S. suricatta), a social mongoose, inhabiting the dry plains of southern Africa. Although these animals do not show strongly distinct predator-specific reactions, they give functionally referential alarm calls to aerial and terrestrial predators (Manser et al. 2001). In natural encounters with both aerial and terrestrial predators, they scan the area and move to a shelter, but in response to aerial predators they sometimes additionally scan the sky, while in response to terrestrial predators they often gather and move away after looking around (Manser et al. 2001). This fine-tuned adjustment of reactions hints at the possibility that for them it might be useful enough to know where the predator approaches from, to look into the respective direction, and to decide then what to do. More generally spoken, it may not be necessary to have clearly distinct escape strategies, like vervets, but the mere need of a fast predator recognition and, accordingly, an appropriate scanning response might well be sufficient to develop a referential signalling system.

In this study, we found evidence for a functionally referential alarm call system in S. mystax and a combined system of one functionally referential aerial alarm call and more non-specific terrestrial alarm calls in S. fusciollis. It has been suggested that the type of alarm call system might be shaped on the one hand by certain ecological key factors, such as the body size, a terrestrial or arboreal habitat, and the density of a habitat, and on the other hand by the presence or absence of different escape modes from predators (e.g. Evans et al. 1993b; Macedonia & Evans 1993; Evans 1997). However, saddleback
and moustached tamarins are of more or less the same body size, live both highly arboreal in the same dense forest habitat, and respond both in distinct ways to aerial and terrestrial predators. Nevertheless, they vary considerably in the degree of their call specificity. Thus it seems that, although ecological conditions and predator-specific reactions apparently are important, they may not be sufficient to explain the evolution of a certain type of alarm call system. It would be interesting to investigate more species that are as similar as *S. fuscicollis* and *S. mystax* and exhibit different alarm call systems.

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**Literature Cited**


