1   # Contextual encoding in titi monkey alarm call sequences

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ABSTRACT

11 Many primates produce one type of alarm call to a broad range of events, usually terrestrial

12 predators and non-predatory situations, which raises questions about whether primate alarm

13 calls should be considered "functionally referential". A recent example is black-fronted titi

14 monkeys, *Callicebus nigrifrons*, which emit sequences of B-calls to terrestrial predators or

15 when moving towards or near the ground. In this study, we reassess the context-specificity of

16 these utterances, focussing both on their acoustic and sequential structure. We found that B-

17 calls could be differentiated into context-specific acoustic variants (terrestrial predators vs.

18 ground-related movements) and that call sequences to predators had a more regular sequential

19 structure than ground-related sequences. Overall, these findings suggest that the acoustic and

20 temporal structure of titi monkey call sequences discriminate between predator and non-

21 predatory events, fulfilling the production criterion of functional reference.

SIGNIFICANCE STATEMENT

23 Primate terrestrial alarm calls are at the centre of an ongoing debate about meaning in animal

24 signals. Primates regularly emit one alarm call type to ground predators but often also to

25 various non-predatory events, raising questions about the referential nature of these signals. In

26 this study, we report observational and experimental data from wild titi monkeys and show

27 that terrestrial alarm calls are usually given in sequences of acoustically distinct variants

28 composed in structurally distinct ways depending on the external event. These differences are

29 salient and could help recipients to distinguish the nature of the call eliciting event. Since

30 most previous studies on animal alarm calls have not checked for acoustic variants within

31 different call classes, it may be premature to conclude that primate terrestrial calls do not meet

32 the criteria of functional reference.

KEYWORDS

ACKNOWLEDGMENTS

INTRODUCTION

Animal alarm calls can potentially convey a rich set of information, used by receivers to make adaptive behavioural decisions. Alarm calls have been shown to convey information about predator species (Randall et al. 2005; Suzuki 2014), predator size (Templeton et al. 2005), predator behaviour (Griesser 2008; Cunningham and Magrath 2017) or threat level (Blumstein and Armitage 1997; Manser 2001). Such information is encoded in a wide range of vocal features, including spectral properties (Manser 2001), temporal structure (Templeton et al. 2005), call rate (Warkentin et al. 2001), or call combinations (Ouattara et al. 2009a; Suzuki 2014).

The fact that some animal signals are structurally linked to distinct external events has created a debate about the cognitive nature driving signalling behaviour. Humans use a range of communication strategies, from simple index finger pointing to complex linguistic utterances, to refer an audience to an external event. In animals, signals that provide reliable information to the recipients about external events are often termed "functionally referential" because the underlying mental processes of call production are usually unclear. The criteria for functional reference have been that the signal has to be stimulus-specific (production criterion) and sufficient for receivers to display an appropriate response (perception criterion), even in the absence of the eliciting stimulus or any correlated contextual cues (Macedonia and Evans 1993). Various examples of animal communication qualify as functionally referential (Townsend and Manser 2013) because they are elicited by a feature of the environment (e.g. predator type). Importantly, this chain of events can be the result of different underlying mechanisms. For example, an event-specific alarm call can be "affective" if its production is mediated by a specific arousal level, without impacting the referential properties of the signal. In other words, although signals can be linked to external events, they may be simple reflections of undetermined emotional states without carrying any semantic properties

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72   (Seyfarth and Cheney 2003; Price et al. 2015). The current debate is less about the

73   psychological mechanism driving call production, but about the referential specificity of the

74   calls (Wheeler and Fischer 2012).

75   Many animal species possess two alarm call types; one for aerial and one for terrestrial

76   predators (see Kiriazis and Slobodchikoff 2006). A consistent finding in primates is that aerial

77   alarms are typically highly predator-specific while terrestrial alarms tend to be more general

78   and can be used in many contexts (Fichtel and Kappeler 2002; Fichtel et al. 2005; Kirchhof

79   and Hammerschmidt 2006; Wheeler 2010; Wheeler and Fischer 2012; Zuberbühler and

80   Neumann 2017). For example, red-fronted lemurs (*Eulemur fulvus rufus*) give "woof" calls to

81   fossas and dogs, but also in non-predatory situations of seemingly high arousal, while

82   "chutter" calls are exclusively given to hawks (Fichtel and Kappeler 2002). Similarly, tufted

83   capuchins (*Cebus apella nigritus*) give "bark" calls to aerial threats and "hiccup" calls to

84   terrestrial predators, but also in non-predatory, seemingly stressful situations (Wheeler 2010).

85   Strictly speaking, the terrestrial alarm calls of these species do not fulfil the production

86   criterion by Macedonia and Evans (1993), and hence cannot be classified as functionally

87   referential. Instead, they are more similar to human pointing insofar as they attract the

88   attention of other group members, who then either consider pragmatic cues, such as other

89   recent events (Arnold and Zuberbühler 2013) or simply follow the caller's gaze direction to

90   the cause of his or her calling (Crockford et al. 2015).

91   However, there are additional complexities regarding the hypothesis that primate terrestrial

92   alarms are referentially unspecific. In particular, recent progress in acoustic and statistical

93   analyses continues to highlight the richness of information encoded in animal signals (e.g.

94   Griesser 2008). Moreover, the recent introduction of automated feature extraction technology

95   and unsupervised learning algorithms can highlight fine-grained contextual variation related

96   to external events that may not be readily perceivable by human observers (e.g. Fedurek et al.

2016). Since most of the studies reporting unspecific terrestrial alarm calls lack the necessary detailed acoustic analyses (e.g. Fichtel and Kappeler 2002; Kirchhof and Hammerschmidt 2006; Wheeler 2010; but see Wheeler and Hammerschmidt 2013; Price et al. 2015), a sensible hypothesis is that terrestrial alarm calls in primates differ acoustically depending on whether they are given to predators or in non-predatory situations. Without such detailed acoustic analyses, it may be premature to conclude whether a contextually unspecific terrestrial alarm call is in fact a collection of contextually specific terrestrial call variants (e.g. Fischer et al. 1995).

Another complexity arises from findings that some alarm calls are organised sequentially, often in context-specific ways. An example is the alarm roaring of Guereza colobus monkeys *Colobus guereza*. One finding has been that vocal utterances elicited by leopards contain fewer roars per phrase but a higher number of phrases compared to those elicited by crowned eagles, which show the opposite pattern (Schel et al. 2009). In this case, there is also evidence that receivers respond to these structural differences as if they perceived the corresponding predators themselves (Schel et al. 2010).

In this study, we reassess the context-specificity of alarm utterances of wild black-fronted titi monkeys, *Callicebus nigrifrons,* focussing both on the acoustic and sequential levels. The species has been subject to a series of previous studies that have reported soft, structurally simple B-call sequences to terrestrial predators, such as oncillas *Leopardus tigrinus*, puma *Puma concolor* and tayra *Eira barbara* (Cäsar et al. 2012a, 2013) but also when moving or foraging near the ground (Cäsar 2011; Cäsar et al. 2012b) (Fig. 1). Sequences to predators can last up to two hours, although B-calls are then gradually replaced by other call types (Cäsar 2011). B-call sequences during foraging appear to be much shorter, lasting only a few seconds, with multiple sequences uttered during the same movement events, usually in synchronization with the movements (MB, personal observation).

The small size of these primates (0.8-1.3 kg; Norconk 2011) exposes them to high predation pressure (Ferrari 2009). Since titi monkeys live in dense forests with low visibility, natural selection may have favoured the evolution of context-specific signalling. We were therefore puzzled by the fact that monkeys emitted B-calls to both terrestrial predators and while descending to the ground to forage, despite the two situations carrying different degrees of risk. If calls given in these two situations cannot be discriminated, then receivers have to consider additional information to determine whether a predator is present or not. Establishing visual contact with the caller and determining its gaze direction is one possible strategy, but this can be costly as it requires more time to react adaptively. On the other hand, maintaining visual contact with the caller is generally adaptive for the latter because it facilitates the location of a hidden predator (Wheeler 2010). This strategy only works, however, if alarm signals occur at low rate in the absence of predators.

Pilot observations suggested that titi monkey B-calls are emitted in a more regular fashion in predatory situations than when descending near the ground in non-predatory situations. Moreover, B-call sequences emitted in alarm situations appear to elicit vigilance (Cäsar et al. 2012b) while B-call sequences emitted during foraging do not (MB, personal observations). We therefore hypothesised that B-sequences to predators and during descents are different at two different levels: in the acoustic structure and in the sequential structure.

METHODS

Study Subjects and Site

Our study took place at the Reserva Particular do Patrimônio Natural Santuário do Caraça, a private reserve of 11,000 ha in the Espinhaço Mountain range, Minas Gerais, Brazil (20°05' S, 43°29'W). Our study took part in the central part of the reserve, in the two forests of Tanque Grande and Cascatinha. The two forests are located one kilometre apart from each

other and are composed of transition zones between native Atlantic forest, "cerrado" (savannah), "campo rupestre" (rocky grassland) and "capoeira" (deforested areas), ranging from 1,200 to 1,300 metres of altitude (Brandt and Motta 2002). The climate is characterised by a rainy season (from October to March) and a dry season (from April to September).

We studied six groups of *Callicebus nigrifrons* that have been habituated to human presence since 2003 (Cäsar 2011) (Table 1). Four groups reside in the forest of Tanque Grande and two groups in the forest of Cascatinha. Titi monkeys typically live in family groups comprising an adult heterosexual pair, monogamous for life, and up to four offspring (Bicca-Marques and Heymann 2013). Both sexes disperse after reaching sexual maturity, at around 3-4 years of age (Bossuyt 2002). We considered an individual as adult from the age of 30 months, as sub-adult between 18 and 30 months, as juvenile between 6 and 18 months and as infant if less than 6 months old (Cäsar 2011). Recognition of individuals was based on morphological cues, such as size, fur pattern and facial or corporal characteristics.

The research reported in this article was conducted in compliance with all relevant local and international laws, and has the approval of the ethical committee CEUA/UNIFAL, number 665/2015.

Data Collection

We monitored groups on a daily basis during two field seasons (April to June 2015 and October 2015 to August 2016). We followed each group and collected data on at least four days per month. It was not possible to record data blind because our study involved focal animals in the field. In order to assess acoustic and sequential differences in B-call utterances, we recorded natural B-call sequences and conducted predator presentations. We used two stuffed terrestrial predators as stimuli: one tayra, *Eira barbara,* and one oncilla, *Leopardus tigrinus*. Each model was presented twice to each group, once in the canopy (between 3 and

170 10 metres high, depending on the structure of the arboreal strata) and once on the ground. The

171 context of emission was categorised as (a) "terrestrial predator" (natural or experimental

172 terrestrial predator encounters), (b) "ground" (caller descends or moves horizontally near the

173 ground, at 2-3 m high maximum, usually to forage, no predator presence). Spectrograms of

174 calls and sequences associated with each context are in Fig. 1 and example sound files are

175 presented in the supplementary material. We recorded vocalizations in WAV format with a

176 Marantz solid-state recorder PMD661 (44.1 kHz sampling rate, 16 bits accuracy) and a

177 directional microphone Sennheiser K6/ME66 or K6/ME67 (frequency response: 40-20,000

178 Hz ± 2.5 dB).

179 *Acoustic structure*

180 **Call selection and data sets**

181 We extracted single calls from the original recordings of sequences given in the two contexts

182 using Praat 5.3.84 (Boersma and Weenink 2009). We removed calls from the data set for the

183 following reasons: if recorded from more than about 7 metres away, if given by immature

184 (infant or juvenile) or unidentified individuals, or if the context could not be determined.

185 Alarm calling typically involved all group members joining in a chorus. Therefore, the

186 selected calls generally were taken from the beginning and end of calling sequences to ensure

187 reliable identification of callers. We created two data sets, one for females and one for males

188 to remove the confounding effects of sex in the subsequent statistical analyses. Each

189 individual (seven males and seven females) provided at least six calls in each context (ground:

190 N=14 individuals, N=3 sequences/individual; terrestrial predator: N=14 individuals; N=1

191 sequence/individual). We considered a total of 271 calls from 68 sequences (Table 2).

192 **Acoustic Analysis**

193   We visually inspected spectrograms (FFT size: 512, Hanning window, time resolution: 3.54

194   ms, frequency resolution: 86.1 Hz) to exclude recording sections disturbed by other sounds or

195   with low signal-to-noise ratio. We adapted acoustic parameters used in Podos (2001). For

196   each call, we first measured directly on the spectrogram (1) the duration, and (2) the number

197   of harmonics. We then measured frequency parameters from the power spectra: (3) the peak

198   frequency, (4) the minimum and (5) the maximum frequency at which the amplitude exceeds

199   -20 dB relative to peak frequency, (6) the frequency range (maximum-minimum frequency),

200   the peak frequency at the (7) first 10 ms of the call (referred later as "first peak") and (8) last

201   10 ms of the call (referred later as "last peak") (Fig. 2). The measurement of the minimum

202   and maximum frequency relative to the peak frequency allows to maximize the proportion of

203   signal measured, by not including background noise nor excluding signal energy (Podos

204   2001; Zollinger et al. 2012). All measurements were conducted using Raven Pro 1.5 Beta

205   Version. Raw data are provided in the supplementary materials.

206   Acoustic analyses were done by two raters (MB, GM). To assess between-rater reliability, we

207   used a subset of 51 randomly selected calls (19% of the total dataset). We calculated the

208   interclass correlation coefficient (ICC) for each of the acoustic parameters, and the level of

209   between-rater agreement reached the required reliability level for all acoustic parameters ($r \geq$

210   0.8, Cicchetti 1994).

211   **Statistical Analysis**

212   For each acoustic parameter, we visually inspected histograms and transformed data to

213   approach symmetric distributions (log, square root or fourth root) if necessary. We excluded

214   strongly correlated parameters ($r \geq 0.7$) (Quinn and Keough 2002). Thus, we excluded

215   maximum frequency (both sexes) because it was strongly correlated with the minimum

216   frequency.

217   We used discriminant function analysis (DFA) to test for acoustic differences between

218   contexts. The aim of this analysis is to determine whether certain objects (here the calls) can

219   be discriminated into classes (caller identity, context) by parameters measured from each

220   object (acoustic parameters). However, a DFA requires independence of data (i.e. it only

221   allows the consideration of a single factor at a time, for example "individual" or "context"),

222   and violating this assumption leads to increased probability of type I errors (Mundry and

223   Sommer 2007). We therefore used permuted discriminant function analysis (pDFA; Mundry

224   and Sommer, 2007), which combines a permutation approach with a DFA. We conducted a

225   crossed pDFA for each sex separately to assess whether the B-calls could be differentiated

226   among contexts based on their acoustic structure. We set "context" as the test factor and

227   "individual" as the control factor to test for contextual differences while controlling for

228   multiple calls of each individual (Mundry and Sommer 2007).

229   In order to extract the key variables, i.e. the variables that enable discrimination of context in

230   the pDFA, we re-ran 1000 permuted DFA and recorded those variables that had the highest

231   coefficient of linear discriminant in at least 800 DFAs out of 1,000, i.e. the variables allowing

232   for discrimination in more than 80% of the discrimination tests.

233   The ICC was conducted with the rptR package (Stoffel et al. 2017) in R version 2.14.0 (R

234   Development Core Team 2011). All other tests were conducted in R version 3.4.1 (R

235   Development Core Team 2017). The pDFA was generated using a function kindly provided

236   by R. Mundry, based on the function "lda" of the R package MASS (Venables and Ripley

237   2002). The R script is provided in the supplementary materials.

238   *Sequential structure*

239   **Sequence selection**

240   Responses to predator presence must be rapid, suggesting that alarm signals should convey

241   any potential predator information as early as possible, i.e., once the caller has identified the

242   disturbance. For this reason, we only focused on the first eleven calls of each sequence to

243   measure ten call intervals (mean = 6.69 seconds, SD = 3.38). Hence, what we refer to as

244   "sequence" in the following are the first eleven calls of a sequence.

245   For the predation context, we only considered sequences of pure B-calls, i.e., with no other

246   alarm call type interspersed (e.g., A-call, Cäsar et al. 2012a). Since B-call sequences can be

247   emitted in synchronization with movements during foraging bouts, we only considered as a

248   new sequence an utterance preceded by at least 30 seconds of silence. As for call selection,

249   we did not consider sequences if given by several individuals at the same time, by immature

250   (infant or juvenile) or unidentified individuals, or if the context could not be determined.

251   **Dataset and analysis**

252   A total of 36 sequences from 12 individuals were considered for this analysis (Table 3).

253   For each sequence, we extracted two features. First, we measured the time interval between

254   two subsequent calls for each of the eleven first calls (i.e. a total of ten duration per

255   sequence). Second, we quantified the level of variability of the call interval for each sequence

256   by calculating the coefficient of variation of the call intervals (CV= standard deviation /

257   mean). A low CV indicates that calls are regularly emitted in the sequence, while a high CV

258   indicates that calls intervals are variable in the sequence, with a mix of longer and shorter

259   intervals. Raw data are provided in the supplementary materials.

260   **Statistical analysis**

261   We fitted two generalized linear mixed models (GLMM). The first one was on the

262   relationship between duration of the call interval and the context of emission with a gamma

263   error structure. The second one was on the relationship between the CV of the sequence and

264   the context of emission, again with a gamma error structure (Payton 1996). For both, we

265   entered context (terrestrial predator vs. ground) and sex of the caller as fixed factors. Identity

266   of the caller was controlled for by including it as a random factor nested within the group

267   identity. We obtained P-values with likelihood ratio tests (LRT) of the full models against the

268   null models, i.e. models without the fixed factor context. The fit of the models was evaluated

269   by the proportion of variance explained (the marginal coefficient of determination R^2_m, i.e. the

270   variance accounted for by fixed factors, and the conditional coefficient of determination R^2_c,

271   i.e. the variance accounted for by both fixed and random factors) estimated with the delta

272   method for variance estimation described in Nakagawa et al., (2017).

273   Both GLMM were fitted using the lme4 package (Bates et al. 2015) in R version 3.4.1 (R

274   Development Core Team 2017). The R script is provided in the supplementary materials.

275   **RESULTS**

276   *Acoustic structure*

277   In females, B-calls could be distinguished on the basis of emission context with 82% of calls

278   correctly classified, significantly higher than the 63% expected by chance (p=0.001) (Fig. 3).

279   The key parameter allowing for discrimination was the minimum frequency in 937 DFAs out

280   of the 1,000 permutations: minimum frequency was about 0.5 kHz higher in the terrestrial

281   predator context than in the ground context (Fig. 4).

282   In males, classification of B-calls to the correct emission context was 69%, which was not

283   significantly higher than the 60% expected by chance (p=0.153).

284   *Sequential structure*

285   Context did not affect significantly the duration of inter-call intervals (LRT: $\chi^2(1)$=0.63,

286   p=0.4252; R^2_m=0.019, R^2_c=0.133) (Table 4, Fig. 5), but it affected the coefficient of variation

287   of the inter-call intervals (LRT: $\chi^2(1)=6.57$, p=0.010, $R^2_m =0.303$, $R^2_c =0.334$). Variation of

288   inter-call intervals was greater during descent sequences than in sequences in response to

289   terrestrial predators (Table 4, Fig. 6): in the predator context, calls were given with a more

290   regular rhythm than in the ground context calls.

291   **DISCUSSION**

292   We tested whether B-call sequences to predators and during descent differed in terms of call

293   acoustic structure and/or on the sequential structure level. In female titi monkeys, B-calls

294   could be differentiated probabilistically, mostly based on their minimum frequencies, with the

295   terrestrial predator context being higher-pitched than the ground context (Fig. 3,Fig. 4). B-

296   calls were also typically emitted in more regularly structured sequences during the terrestrial

297   predator compared to the ground context (Fig. 6). These results suggest that B-call sequences

298   can convey information about the emission context on at least two levels: the acoustic

299   structure of individual calls and the structure of the entire call sequences.

300   Context-specific acoustic variants within one alarm-call type have also been reported in other

301   primate species, notably Barbary macaques, *Macaca sylvanus*, that produce acoustically

302   different variants depending on the predator type (Fischer et al. 1995), and these variants are

303   perceived by receivers (Fischer and Hammerschmidt 2001). This is also the case in

304   chimpanzees *Pan troglodytes*, whose barks are emitted in two different contexts (hunt and

305   snake presence) correlated with two acoustic variants (Crockford and Boesch 2003).

306   We found acoustic variants in B-calls, but one might consider the classification results as

307   weak. Indeed, the difference between the number of correctly classified calls and the ones

308   expected by chance was only moderately significant in females and not significant in males.

309   These levels of correct classification to the emission context are low compared to other

310   studies (e.g. Price et al. 2015), and thus raise the question of whether the differences are

311 biologically relevant and sufficient to allow discrimination by receivers. In the end, playback

312 experiments are needed, but in the meantime it is worth pointing out that the sample sizes

313 were small, the statistical tests were performed on only one call type and B-calls are

314 structurally very simple calls (Fig. 1), especially if compared to other primate alarm calls (e.g.

315 Crockford and Boesch 2003; Ouattara et al. 2009b; Price et al. 2015). In this view, it was

316 noteworthy that the classification rate was significant. Moreover, it is possible that sequences

317 emitted in the predator context represent a mix of predatory and ground B-calls because of

318 movements of callers towards the ground to check on the threat. As such, it seems likely that

319 the classification results underestimate the true differences between the two contexts.

320 Therefore, our results suggest the existence of at least two context-specific variants of B-calls,

321 but only future playback experiments will show whether these subtle differences can actually

322 be perceived by receivers.

323 The minimum frequency was the main parameter allowing for discrimination between the B-

324 call acoustic variants, with the B-calls given to terrestrial predators being higher-pitched than

325 those given in the ground context. Similar increases of minimum frequency with higher

326 arousal have been frequently observed in mammals and birds (Perez et al. 2012; Briefer

327 2012), in line with Morton's (1977) motivation-structural rules. The presence of a predator

328 may be a more stressful situation for the caller, and should result in a higher minimum

329 frequency compared to the arguably less stressful situation of moving towards or near the

330 forest floor.

331 We found acoustic differences between the alarm and descending contexts in females but not

332 in males. In general, the hypothesis is that pair-living primates, such as titi monkeys, do not

333 show sex differences in vocal repertoires and use their calls in similar ways (Snowdon 2017)

334 in contrast to species with other breeding systems (e.g. Gautier and Gautier-Hion 1982;

335 Stephan and Zuberbühler 2016). Male titi monkeys may indeed produce two acoustic variants

336 but our study failed to show it. In many animal species, males are more engaged in anti-

337 predator behaviour (e.g. van Schaik and van Noordwijk 1989; Brunton 1990), suggesting that

338 male alarm call sequences to terrestrial predators consisted of a mix of predator and ground

339 B-calls, likely emitted while descending near the predator to check on it, more so than in

340 females. This hypothesis needs to be tested in the future with systematic data.

341 Our study also went beyond more traditional analyses insofar as we also analysed differences

342 at the level of the sequential structure. Here, we found that B-calls were emitted more

343 regularly in the predator than in the ground context. Similar effects have been reported in

344 black-capped chickadees (*Poecile atricapilla*), which produce "chick-a-dee" calls with a

345 shorter time interval between the "chick" and "dee" syllables and more "dee" syllable when

346 encountering small, manoeuvrable raptors than large ones (Templeton et al. 2005).

347 Snowdon et al. (1997) suggested that non-social calls (e.g. alarm calls) show less variability

348 than calls used in intragroup social interactions (e.g. contact calls) because alarm calls require

349 quick responses from recipients. This has been shown at the spectral level for primates and

350 birds (Charrier et al. 2001; Lemasson and Hausberger 2011; Bouchet et al. 2012) but to the

351 best of our knowledge has not been tested on call sequence structure. Our results can be

352 interpreted such that temporal variability in call sequences is also linked to the degree of

353 social significance of the signal. B-sequences emitted in response to predators may be less

354 socially relevant and thus more regular, than B-sequences when the caller is signalling his

355 movement towards the ground to other members of the group.

356 Since the coefficient of variation of the call interval is a sequence feature, it may be too costly

357 for receivers to wait until the emission of (at least) three calls to perceive this feature. Thus,

358 differences in acoustic structure may be more important for early decisions about the call-

359 eliciting event, which does not prevent variation in the call interval to convey further

360 information about the context later on. Moreover, although B-call sequences are redundant,

361   call intervals will reassure recipients and enhance discriminability after a few repetitions.

362   However, whether titi monkeys rely on acoustic and / or sequential parameters to attribute

363   meaning about the eliciting context needs be tested with playback experiments.

364   Alarm calls to predators can have various functions, such as signalling detection to a predator

365   or warning members of the group (see review in Zuberbühler 2009), but the function of the

366   ground B-call sequences are less evident. We can think of several possibilities. First, ground

367   B-calls may signal the caller's own perception of enhanced risk. Foraging in lower strata may

368   be more dangerous, due to higher predation risk (Mourthé et al. 2007). B-calls sequences thus

369   provide relatively specific information about the caller's whereabouts, which may be relevant

370   to other group members, as also documented in pied babblers *Turdoides bicolor* or Diana

371   monkeys *Cercopithecus diana* (Uster and Zuberbühler 2001; Radford and Ridley 2007).

372   Callers, for example, may elicit higher levels of vigilance from other group members, which

373   increases their own safety. Second, ground B-calls sequences could indicate that no predator

374   is around and that it is safe to forage near the ground, like the "guarding" close calls in

375   meerkats *Suricata suricatta* (Townsend et al. 2011). However, we regard this as a less

376   plausible scenario, simply because the two B-call variants are very similar, with a

377   corresponding high risk of misunderstanding, which is also indicated by the less than 100%

378   classification results. Further playbacks are needed to understand the main function of the

379   ground B-call sequences, but it is likely that titi monkeys categorise both event types, going

380   near the ground and terrestrial predator, in similar ways, e.g. as threats (real or feared) related

381   to the ground (Zuberbühler and Neumann 2017). Going down may be perceived as dangerous,

382   simply because terrestrial predators are likely to be encountered (Mourthé et al. 2007).

383   It is a common finding, across many nonhuman primate species, that calls associated with

384   terrestrial disturbances are also given in other contexts (e.g. Fichtel and Kappeler 2002;

385   Wheeler 2010), which has questioned the notion of functionally referential alarm calls

386 (Macedonia and Evans 1993; Fischer and Price 2016). Our current study adds an additional

387 layer of complexity to this debate, because of context-dependent acoustic and sequential

388 structures in titi monkey "terrestrial alarm" calls. Also relevant is that the production criterion

389 of functional reference is generally difficult to operationalize, since context is always defined

390 by the observer, and this may be different from how animals categorise the world

391 (Zuberbühler and Neumann 2017). Moreover, calls can exhibit different degrees of context-

392 specificity, varying from a classification success of 100% to a statistically significant

393 classification success, like the B-calls of titi monkeys. As such, it appears important that

394 future work explores the concept of context-specificity to get a better understanding of what

395 constitutes context-specific and -unspecific, or better even, to develop a continuous measure

396 of *how* context-specific call types are (Zuberbühler and Neumann 2017; see also Scarantino

397 and Clay 2015). Such research seems essential to understand better the "potentially more

398 complex processes underlying responses to more unspecific calls" (Wheeler and Fischer,

399 2012, p. 195).

400 To conclude, titi monkey B-calls seem to have the potential to provide listeners with

401 information about external events, which encourages careful analyses of terrestrial alarm calls

402 and other vocalizations to check for the presence of acoustic and sequential variants. From the

403 recipient's perspective, further experiments are needed to determine whether call variants are

404 discriminated and whether additional contextual cues are taken into account (Scarantino and

405 Clay 2015). Future work on the evolution of referential signalling and its potential roots in

406 primate signalling will need to address these points, notably if callers direct their calls to

407 specific recipients and, in doing so, take their mental states into account.

COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of potential conflict of interest

Ethical approval

The research reported involving animals in this article was conducted in compliance with all relevant local and international laws, and has the approval of the ethical committee CEUA/UNIFAL, number 665/2015.

DATA AVAILABILITY STATEMENT

The datasets generated and the Rscripts used for the current study as well as audio examples of B-sequences are available in the following Figshare repository:

https://figshare.com/projects/Contextual_encoding_in_titi_monkey_alarm_call_sequences/23248

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580

FIGURE CAPTIONS

Fig. 1 Spectrograms of B-calls from (a) the terrestrial predator context and (b) the ground context, and spectrograms of B-call sequences from (c) the terrestrial predator context and (d) the ground context, all from the same individual

Fig. 2 Measure of acoustics parameters on a B-call from the ground context, on the spectrogram (top panel) and the power spectrum (bottom panel) with 1: duration, 2: number of harmonics, 3: peak frequency, 4: minimum frequency, 5: maximum frequency, 6: frequency range, 7: first peak, 8: last peak. Figures were drawn using the "seewave" package (Sueur et al. 2008)

Fig. 3 Distribution of the discriminant scores of female B-calls given to terrestrial predators and in the ground context. Note that the pDFA does not allow for graphic representation. Hence, this figure is drawn from the results of a DFA, and only serves to illustrate discrimination, but does not represents the results of the actual pDFA

Fig. 4 Median and quartiles of the minimum frequencies in ground and predator context, in females (a) and in males (b)

Fig. 5 Median and quartiles of the call interval duration in the ground and predator context

Fig. 6 Median and quartiles of the coefficient of variation of the call intervals in the ground and predator context

TABLES

601 **Table 1** Composition of the six study groups

Forest	Group	# Individuals	Paired ad.	Unpaired ad.	Sub.	Juv.	Inf.
Tanque Grande	A	6	2	1-3	1	0-1	0-1
	D	4-5	2	0-1	1	0-1	0-1
	R	4-6	2	0-2	0-1	0-1	0-2
	S	4-5	2	0-2	0-1	0-1	0-1
Cascatinha	M	5-6	2	1-2	1	1	0-1
	P	4-5	2	1-2	0-1	0-1	0-1

602   Paired ad.: mated pairs; Unpaired ad.: other adults; Sub.: Subadults, Juv.: Juveniles and Inf.:

603   Infants

604

605 **Table 2** Data sets used for call analysis. The first number indicates the number of calls, the

606 second indicates the number of different sequences the calls were extracted from

Ind		Context	
		Terrestrial predator	Ground
Females	AL	7/1	11/3
	AU	7/1	13/5
	DN	9/2	13/4
	DT	8/1	11/4
	ML	6/1	9/3
	PL	7/1	16/4
	SV	6/1	15/4
	Total	50/8	88/27
Males	AP	6/1	11/5
	AR	12/2	11/3
	PC	12/2	13/3
	PT	6/1	15/4
	RK	6/1	11/3
	RT	6/1	9/3
	SG	7/1	8/3
	Total	55/9	78/24

607

608   **Table 3** Data sets used for sequence analysis, the numbers indicate the number of sequences

Ind	Context	
	Terrestrial predator	Ground
AP	2	2
AR	1	1
DN	1	1
DT	2	2
MK	1	1
ML	2	2
MN	1	1
PC	2	2
PP	1	1
RK	2	2
SG	2	2
SV	1	1
Total	18	18

609

610 **Table 4** Estimated coefficients of the duration and coefficient of variation (CV) models

Model	Effect	Estimate	Standard Error	t-value
Full model duration	Intercept	1.334	0.202	6.593
	Context: Predator	- 0.088	0.109	- 0.804
	Sex: Male	0.330	0.241	1.369
Null model duration	Intercept	1.288	0.194	6.650
	Sex: Male	0.330	0.241	1.369
Full model CV	Intercept	1.114	0.185	6.015
	Context: Predator	0.557	0.212	2.621
	Sex: Male	0.138	0.226	0.611
Null model CV	Intercept	1.341	0.181	7.407
	Sex: Male	0.130	0.229	0.569

611

612