

1 **Contextual encoding in titi monkey alarm call sequences**

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9

10 **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.

22 **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.

33 **KEYWORDS**

34 *Callicebus nigrifrons*, titi monkey, alarm call, sequence, acoustic variant, context specificity

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46

47 INTRODUCTION

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

56 The fact that some animal signals are structurally linked to distinct external events has created
57 a debate about the cognitive nature driving signalling behaviour. Humans use a range of
58 communication strategies, from simple index finger pointing to complex linguistic utterances,
59 to refer an audience to an external event. In animals, signals that provide reliable information
60 to the recipients about external events are often termed “functionally referential” because the
61 underlying mental processes of call production are usually unclear. The criteria for functional
62 reference have been that the signal has to be stimulus-specific (production criterion) and
63 sufficient for receivers to display an appropriate response (perception criterion), even in the
64 absence of the eliciting stimulus or any correlated contextual cues (Macedonia and Evans
65 1993). Various examples of animal communication qualify as functionally referential
66 (Townsend and Manser 2013) because they are elicited by a feature of the environment (e.g.
67 predator type). Importantly, this chain of events can be the result of different underlying
68 mechanisms. For example, an event-specific alarm call can be “affective” if its production is
69 mediated by a specific arousal level, without impacting the referential properties of the signal.
70 In other words, although signals can be linked to external events, they may be simple
71 reflections of undetermined emotional states without carrying any semantic properties

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.

97 2016). Since most of the studies reporting unspecific terrestrial alarm calls lack the necessary
98 detailed acoustic analyses (e.g. Fichtel and Kappeler 2002; Kirchhof and Hammerschmidt
99 2006; Wheeler 2010; but see Wheeler and Hammerschmidt 2013; Price et al. 2015), a sensible
100 hypothesis is that terrestrial alarm calls in primates differ acoustically depending on whether
101 they are given to predators or in non-predatory situations. Without such detailed acoustic
102 analyses, it may be premature to conclude whether a contextually unspecific terrestrial alarm
103 call is in fact a collection of contextually specific terrestrial call variants (e.g. Fischer et al.
104 1995).

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

112 In this study, we reassess the context-specificity of alarm utterances of wild black-fronted titi
113 monkeys, *Callicebus nigrifrons*, focussing both on the acoustic and sequential levels. The
114 species has been subject to a series of previous studies that have reported soft, structurally
115 simple B-call sequences to terrestrial predators, such as oncillas *Leopardus tigrinus*, puma
116 *Puma concolor* and tayra *Eira barbara* (Cäsar et al. 2012a, 2013) but also when moving or
117 foraging near the ground (Cäsar 2011; Cäsar et al. 2012b) (Fig. 1). Sequences to predators can
118 last up to two hours, although B-calls are then gradually replaced by other call types (Cäsar
119 2011). B-call sequences during foraging appear to be much shorter, lasting only a few
120 seconds, with multiple sequences uttered during the same movement events, usually in
121 synchronization with the movements (MB, personal observation).

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

134 Pilot observations suggested that titi monkey B-calls are emitted in a more regular fashion in
135 predatory situations than when descending near the ground in non-predatory situations.
136 Moreover, B-call sequences emitted in alarm situations appear to elicit vigilance (Cäsar et al.
137 2012b) while B-call sequences emitted during foraging do not (MB, personal observations).
138 We therefore hypothesised that B-sequences to predators and during descents are different at
139 two different levels: in the acoustic structure and in the sequential structure.

140 **METHODS**

141 *Study Subjects and Site*

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

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

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

159 The research reported in this article was conducted in compliance with all relevant local and
160 international laws, and has the approval of the ethical committee CEUA/UNIFAL, number
161 665/2015.

162 ***Data Collection***

163 We monitored groups on a daily basis during two field seasons (April to June 2015 and
164 October 2015 to August 2016). We followed each group and collected data on at least four
165 days per month. It was not possible to record data blind because our study involved focal
166 animals in the field. In order to assess acoustic and sequential differences in B-call utterances,
167 we recorded natural B-call sequences and conducted predator presentations. We used two
168 stuffed terrestrial predators as stimuli: one tayra, *Eira barbara*, and one oncilla, *Leopardus*
169 *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 \pm 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 ($CV = \text{standard deviation} /$
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.

408 **COMPLIANCE WITH ETHICAL STANDARDS**

409 *Disclosure of potential conflict of interest*

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413 The authors declare that they have no conflict of interest.

414 *Ethical approval*

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

418 **DATA AVAILABILITY STATEMENT**

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

421 https://figshare.com/projects/Contextual_encoding_in_titi_monkey_alarm_call_sequences/23

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- 580

581 **FIGURE CAPTIONS**

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

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

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

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

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

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

599

600 **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