SEMANTIC CONTENT IN TITI MONKEY ALARM CALL SEQUENCES

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By

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ABSTRACT

The comparative approach aims to understand the uniqueness of human language and how it evolved from primitive communication systems. Black-fronted titi monkeys *Callicebus nigrifrons* possess two soft alarm calls: A-calls are specific to threats within the canopy while B-calls are general calls emitted to terrestrial predators but also in non-predatory contexts, while moving near the ground. Titi monkeys combine these two simple calls into rule-governed sequences that can convey information about the predator type and location, suggesting a sophisticated syntax/semantic interface unique in animals. However, studies leading to these conclusions were preliminary, and more research is needed to conclude on vocal capacities of these primates. The first aim of my study is to reassess the context-specificity of sequences of B-calls both on their acoustic and temporal structure. The second is to investigate the encoding mechanisms of predator type and location in alarm sequences. The third is to assess what information titi monkeys extract from the alarm sequences and what sequential feature they attend to. Data were collected on six free-ranging groups of *C. nigrifrons* at the Santuário do Caraça, Brazil. Sequences of B-calls were recorded from individuals exposed to predators or descending near the ground. I found that B-calls could be differentiated into context-specific acoustic variants (terrestrial predators vs. ground-related movements) and that call sequences to predators had a more regular sequential structure than ground-related sequences. I then presented monkeys to two terrestrial predators and one aerial predator, either on the ground or in the canopy, and I modelled what information is encoded by sequences. I found that information encoded in the sequence focused more on the predator type rather than on the predator location. Finally, I carried out playback experiments and found that listeners extracted information about predator type and location by using the proportion of B-call combinations in the sequences, suggesting that meaning was probabilistic rather than categorical. This work shows that call combinations are a key component of titi monkey vocal communication and can convey information about predator type, location and behaviour of the caller. Overall, my results suggest that titi monkeys possess a unique sequential system that may provide new insights in our understanding of the evolution of human language.

KEYWORDS

Syntax, semantics, titi monkeys*, Callicebus nigrifrons*, A-call, B-call

RESUME

L'approche comparative cherche à comprendre ce qui rend le langage humain si unique et comment il a évolué. Les singes titi à front noir *Callicebus nigrifrons* possèdent deux cris d'alarme de courte portée : les cris A, spécifiques aux menaces de la canopée, et les cris B, des cris généraux émis en présence d'un prédateur terrestre ou lorsque les singes descendent près du sol. Les titis combinent ces deux cris en séquences régies par des règles précises qui transmettent des informations sur le type de prédateur et l'endroit où il se trouve, ce qui suggère des capacités de syntaxe et de sémantique uniques dans le règne animal. Cependant, les études menant à ces conclusions sont préliminaires et nécessitent d'être approfondies. Le premier objectif de mon étude est d'évaluer la spécificité contextuelle des séquences de cris B, à l'échelle acoustique et temporelle. Le second objectif est d'étudier les mécanismes par lesquelles les singes encodent le type de prédateur et sa localisation dans les séquences d'alarme. Le dernier objectif est de déterminer quelle information les titis extraient de ces séquences et sur quel mécanisme ils s'appuient. Les données ont été collectées sur six groupes de *C. nigrifrons* sauvages au Santuário do Caraça, au Brésil. Des séquences de cris B émises par des individus exposés à des prédateurs ou descendant au sol ont été collectées, et les résultats ont montré que la structure acoustique des cris B dépendait du contexte d'émission (prédateur terrestre vs descente vers le sol) mais aussi que les séquences avaient une structure temporelle plus régulière lorsqu'il y avait un prédateur terrestre. J'ai ensuite présenté aux singes titis deux prédateurs terrestres et un aérien, au sol ou dans la canopée, et j'ai modélisé quelle information ils encodaient dans leurs séquences vocales. Mes résultats ont montré que l'information encodée était plus liée au type de prédateur qu'à sa localisation. Enfin, j'ai mené des expériences de repasse et j'ai montré que les singes comprenaient le type de prédateur et l'endroit où il se trouvait en se basant sur la proportion de combinaisons de 2 cris B dans la séquence, suggérant que le sens de la séquence est probabiliste et non catégorique. Dans son ensemble, ce travail montre que la combinaison de cris est un élément clé de la communication vocale des singes titis et qu'elle peut transmettre des informations sur le comportement de celui qui émet des vocalisations, sur le type de prédateur et sa localisation. Mes résultats suggèrent donc que les singes titis possèdent un système séquentiel unique, et contribuent de façon significative à notre compréhension de l'évolution du langage humain.

MOTS CLES

Syntaxe, sémantique, singe titi, *Callicebus nigrifrons*, cri A, cri B

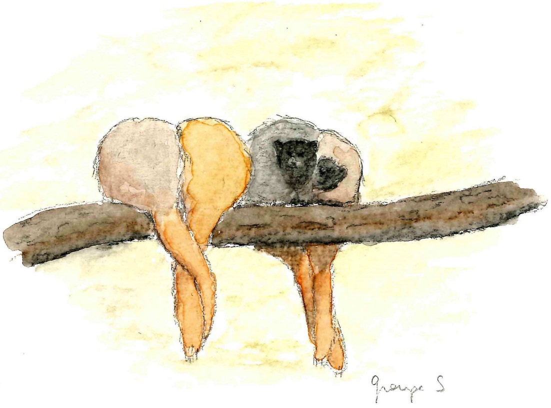
TABLE OF CONTENT

LIST OF FIGURES

Illustrations of chapters by Camille Rostan, used with permission

LIST OF TABLES

CHAPTER 1. INTRODUCTION



HUMAN LANGUAGE AND ANIMAL COMMUNICATION

Language is a uniquely human trait and has been considered as such since the eve of the long quest to identify what make humans different from animals. But what makes language so special compared to animal communication? To answer this question, many linguistics and biologists, starting from Darwin (1872), tried to define language and its characteristics, and compared it to animal communication. Debates were so vigorous that the Société de Linguistique de Paris banned discussions on language evolution in 1866 for over a century (Christiansen and Kirby 2003). Debates are still intense nowadays. For example, Hockett (1960) defined the 13 features of human language but they are regularly contested (e.g. Everett 2005). Chomsky (1965) argued that language is based on a universal grammar that is uniquely human. Hauser *et al.* (2002) claimed later that language can be conceptualised in two ways, a narrow and a broad one, with the narrow sense being unique to humans and the broad one also found in animals. Tomasello (2003) demonstrated that language is usage-based and not innate, that language capacities are connected to cognitive capacities that phylogenetically evolved from animals and that the only uniquely human capacity that differentiates language from animal communication is the comprehension of intentions, which has also been contested by more recent experiments on chimpanzees (Call *et al.* 2004). Nowadays, language uniqueness and evolution are still hard to define: indeed, studies regularly reveal surprisingly high capacities in animal communication, making the comparative approach efficient to understand the uniqueness of human language and how it evolved from primitive communication systems.

Comparative approach

The comparative method uses empirical data from living species to infer hypotheses about extinct ancestors (Hauser *et al.* 2002). In other word, to claim that a trait is unique to humans, one has to search for this trait in a large set of species and demonstrates that no other animal possesses this trait. When comparing data, researcher search for homologies, i.e. traits derived from a common ancestor, to study the evolution of this trait. They can also search for analogies, i.e. traits that are present in different taxa but not in their last common ancestor, suggesting that these traits evolved independently in different taxa. Analogous traits can be very useful to understand the adaptation constraints, the development, or the function of a trait (Hauser *et al.* 2002). Typically, nonhuman primates have a fixed, innate vocal repertoire, while songbirds develop specific songs by learning from conspecifics: thus, to better understand the adaptive function of vocal learning and why it appeared in humans, it is more relevant to focus on songbirds, while it is interesting to study nonhuman primates to understand how human capacities were built on abilities present in the primate lineage (Hauser *et al.* 2002).

To fully understand how human language evolved, one should not restrain research to animal vocal capacities, but also extend this framework to other modalities, especially gestural communication. Indeed, more and more studies reveal than gestural communication in nonhuman primates also reveal signs of continuity with human language, especially because gestures are learnt, flexible, and intentional (see review in Genty *et al.* 2009), characteristics that are less common in nonhuman primate vocal utterances (e.g. Schel et al. 2013). Combining both modalities to understand the origin of human language is therefore crucial (Zuberbühler 2015).

Many other human language characteristics are also seen in animal communication (Fedurek and Slocombe 2011; Zuberbühler 2015), but in this research, I will restrict the rest of my arguments on semantics, the study of meaning, i.e. the relationship between an signal (the signifier) and what it stands for (the signified).

MEANING IN ANIMAL VOCALIZATIONS

What information is conveyed

Shannon (1948) defined information as a statistical measure of uncertainty. The definition was then applied to the field of animal communication as "…a reduction of uncertainty in the recipient" (Seyfarth *et al.* 2010). When an acoustic structure is elicited only by a narrow range of events and when other contextual factors do not influence this structure, then the signal potentially provides listeners with information (Seyfarth and Cheney 2003). To do so, a vocal signal must possess three characteristics (Seyfarth and Cheney 2017). First, it possesses informative value: the signal is associated with a narrow range of context (e.g. an individual, a situation) and is never emitted in the absence of this stimulus. Second, the referential specificity of the acoustic structure indicates the potential to convey reliable information. For example, a signal associated with a large set of stimuli conveys less information than a signal elicited by a very narrow set of stimuli. This is the case in several species of primates, in which one call is typically given to aerial predators, while the call given to terrestrial predators is also given in other non-predatory contexts (Wheeler and Fischer 2012): these general calls possess a similar informative value than the aerial alarm calls because they are associated with a range of stimuli, but are less referentially specific, because the range of stimuli is broader. Finally, the third condition for a signal to convey information is its specificity, i.e. its acoustic uniqueness. In fact, a call can only convey reliable information if it can be distinguished from other calls of the repertoire.

But what is the information that can be conveyed? An abundant literature is available on the subject, in nonhuman primates but also in other vertebrate taxa. Information conveyed in vocal signals can be of three types. First, acoustic signals can convey information about the features of the caller. Indeed, acoustic structure of a call is influenced by the vocal tract of the animals, with larger animals uttering longer calls with lower fundamental frequency, smaller formant dispersion and energy concentrated in lower frequencies (Ey *et al.* 2007; Bowling *et al.* 2017). Since age and sex are mostly correlated with body size, acoustic structure may also convey this information (Blumstein and Munos 2005). Dominance status is also encoded in calls. For example, high-ranking baboon males *Papio cynocephalus ursinus* produce “wahoos” with longer “hoo” syllable and higher fundamental frequency (Fischer *et al.* 2004) and hyenas *Crocuta crocuta* encode information about their dominance status in the spectrum mean frequency of their “giggles” (Mathevon *et al.* 2010). Male quality can also be conveyed in acoustic signals: high-quality warbler males *Setophaga discolor* produce songs with longer elements, lower frequencies and with greater consistency than low-quality males (Byers *et al.* 2016). Individual and group signatures can also be encoded in the signals. For example, great gerbils *Rhombomys opinus* emit rhythmic alarm calls to predators that differ with individual and family group (Randall *et al.* 2005).

Second, vocalizations can encode information about internal states of the caller, since the length and shape of the vocal apparatus can be modified by the emotional state. Indeed, Morton (1977) showed that birds and mammals decrease their fundamental frequency and produce longer calls in hostile contexts but use tonal high-pitched sounds in case of fear or to appease the receiver. It has also been showed in several species of mammals that arousal modifies several acoustic structures like fundamental frequency range, energy distribution and peak frequency (Briefer 2012).

Finally, acoustic signals can convey information about external events. For example, meerkats encode the level of urgency of a predator threat in their alarm calls (Manser 2001), Siberian jays *Perisoreus infaustus* convey information about the behaviour of the predator (Griesser 2008) and prairie dogs *Cynomys gunnisoni* encode the type of predator in their alarm calls (hawks, humans, coyotes and dogs) but also features of the predator, like their colour (Slobodchikoff *et al.* 2009).

However, it is hard to disambiguate whether a call conveys information about the internal state of the caller or an external event. Indeed, the two stimuli can be interdependent, since an external event like a predator presence or an agonistic interaction influences the emotional state of the caller. It has often been considered that animal communication was different from the human language because of their affective component (i.e. call emission is based on emotion), while human language was a referential system based on the relation between words and their referents (e.g. Macedonia and Evans 1993). However, Seyfarth and Cheney (2003) argue that the information content of the call depends on the specificity of its production, regardless of the mechanism underlying this specificity. According to them, knowing that a call is affective, i.e., due to the emotional state of the caller, does not tell anything about its referential properties, i.e., its capacity to convey information. Indeed, affective property of a signal depends on the mechanisms of call production in the caller, while the referential properties of this call depends on the listener's capacity to extract information from the vocalization. This suggests that to conclude on the informational value of a vocal signal, it is crucial to verify that this information is salient to receivers.

How information is extracted

Playback experiments, in which calls are broadcasted to subjects in the absence of eliciting stimulus, are crucial to determine whether listeners can extract information from the vocal signals. When listeners react differently to calls encoding different information, then it can be concluded that they can extract information from the vocal signals, and thus, that the signals are meaningful (Wheeler and Fischer 2015).

Several mechanisms are proposed to explain how listeners adapt their behaviour according to the vocalizations. First, the manipulative hypothesis argues that the acoustic structure of alarm calls induces direct responses in the nervous system of listeners (Owren and Rendall 2001). For example, mammal alarm vocalizations typically exhibit sharp onsets, high frequencies, amplitude fluctuations and high-amplitude noisiness that can impact on receiver attention and emotion and so modify their behaviour. However, this simplistic view is largely debated in the literature (e.g. Seyfarth *et al.* 2010) mainly because receivers can display flexibility in their reaction based on contextual cues, memory and personal experience, and because some calls similar in their acoustic structures do not elicit the same reaction in listeners. Thus, the information theory argues that listeners can learn to associate external events to different call structures and thus adjust their behaviour accordingly when hearing the calls (Seyfarth and Cheney 2003, 2010). For example, after being exposed to the association of a hawk with a neutral tone, Golden-mantled ground squirrels *Spermophilus lateralis* respond similarly to neutral tones than to natural conspecifics hawk alarm calls, suggesting that they learn the association between an acoustic structure and an event (Shriner 1999). Two explanations are proposed. Thompson (1995) suggested that the signal and the event are categorized as similar by the animals because they elicit the same behavioural response. Thus, the signal and the event are functionally equivalent and the association between the two does not require information transfer. A second explanation argues that call structures elicit a mental representation of the referent associated with the call. For example, when primed with snake alarm calls, Japanese tits *Parus minor* become more efficient in finding snake-resembling objects, contrary to when primed with other alarm calls (Suzuki 2018). When primed with eagle alarm or eagle shrieks, Diana monkeys *Cercopithecus diana* do not respond when hearing an eagle shriek, but they do when primed with leopard alarm calls (Zuberbühler et al. 1999a). All these results suggest that listeners modify their behaviour not only because of the acoustic structures of the calls but also because the calls elicit mental representation of their referents. This may also be the case for acoustic signals with little context-specificity: since it is not possible for the listeners to establish a strong association between an event and the call, listeners can rely on pragmatics to infer the eliciting context. This is the case for example for putty-nosed monkeys *Cercopithecus nictitans*: playbacks of general calls associated with contextual noise, like a tree falling, make listeners spend less time looking for the cause of the call than playbacks of general calls alone (Arnold and Zuberbühler 2013), suggesting that they can infer the referent by integrating contextual cues. Similarly, listeners adapt their reaction to their own situation. For example, in vervet monkeys *Cercopithecus aethiops*, individuals on the ground do not react the same way as those in the trees when hearing a leopard alarm call (Scarantino and Clay 2015).

In some cases, information conveyed in signals is ignored by animals. This is the case of example for meerkats *Suricata suricatta*. Close calls of meerkats encode a group signature, and their alarm calls encode information about the caller, but listeners do not seem to discriminate calls from different groups or callers (Schibler and Manser 2007; Townsend *et al.* 2010). This could be because acoustic differences highlighted by statistical analyses are not perceived by animals, or because they are not relevant.

This shows that a diverse range of information can be encoded in single call utterances, and that they can be understood by listeners. However, another layer of complexity arises when it comes to call combinations.

Information at the sequence level

Duality of patterning is a property of human language to generate combination at two levels (Zuberbühler in press; Collier *et al.* 2014). Combination, or phonology, is the capacity of combining meaningless sounds (phonemes) into meaningful elements (morphemes). For example, the morpheme “break” is composed of the phonemes \b\, \ʁ\, \ɛ\ and \k\. Composition, or syntax, is the capacity of combining morphemes into larger structures, like words or phrases, of which meaning depends on the meaning of its parts and of the rules that combine them. For example, the word “unbreakable” is composed of the three morphemes “un” (meaning “not”), “break” (the root) and “able” (meaning “can be done”).

To extend the size of a fixed vocal repertoire, like the one of nonhuman primates, animals can combine vocalizations into sequences (Zuberbühler and Lemasson 2014). Syntax is widespread in animals and takes several forms. Meaning of the syntactic structure can be the linear addition of the meaning of its components. This is the case of females Diana monkeys that combine two morphological units, one conveying information about the external event and one about the identity of the caller, and receiver can extract this two information types from the call combinations (Coye *et al.* 2016). On the other hand, meaning of the call combination can be derived from the meaning of its components. For example, Campbell’s monkeys produce six different alarm call types and combine them into nine context-specific sequences (Ouattara *et al.* 2009a). Among them, leopard alarms calls “Krak” can be combined with a suffix “oo”, a vocalization that is never given alone. “Krak-oo” are given to a wide range of events, from predator presence to inter-group encounters and falling branches (Ouattara *et al.* 2009a, b). Playback experiments showed that the suffixation of the predatory call is salient to receivers, suggesting that suffixation alters the meaning of the predatory call (Coye *et al.* 2015). On the opposite of syntax, examples of animal phonology are still scarce. One clear example is the case of the Chestnut-crowned babbler *Pomatostomus ruficeps* that combines A and B, meaningless vocalizations that are never produced alone, into AB combinations that refer to flight, and BAB combinations that refer to nestling provisioning (Engesser *et al.* 2015).

Two theories are proposed to explain the emergence of syntax. The exaptive perspective states that it emerged as a functional change of pre-existing systems during a sudden evolutionary event in early hominids (Fitch 2011). The continuist perspective states that human syntactic capacities emerged from simpler combinatorial systems in animals (Engesser *et al.* 2016). Based on this idea, Collier *et al.* (2014) suggested that the fact that so few animal species exhibit phonology compared to the numerous example of syntax shows that syntax developed before phonology in human language. Moreover, they suggested that phonology is not genetically-based, since some human language do not share this characteristic, and is probably culture-based. Its emergence would be favoured by a highly social system, for situations that do not require urgent reaction from the listeners, and in an environment in which the number of events to be communicated is large.

Another view criticizes the comparative approach, especially driven by linguists. For example, Bolhuis *et al.* (2014) state that syntax capacity is the main difference between language and nonhuman communication, and believe that the capacity of *merge* (i.e., the capacity to put together two syntactic elements to form a new set) is the key evolutionary innovation that allowed for the emergence of language. They consider that *merge* was a minor change built upon primitive communication faculties but did not derive from them, making the language acquisition not a slow and gradual event as traditionally thought, but rather a single and rapid emergent event in the human lineage, between 70,000 and 100,000 years ago. For all these reasons, Bolhuis *et al.* (2014) conclude that comparative approach make no sense to study the evolution of syntax and language. However, this view is contested. For example, Townsend *et al.* (in prep) argue that human syntactic capacity cannot be restricted to one operation, mainly because syntax can be decomposed in different layers: it can be simple or complex, and can be productive (infinite possible combinations) or non-productive (usage of fixed combinations, like in prefabricated expressions). Since animals use simple and non-productive structures, authors maintain that comparative approach is still relevant to understand the phylogeny of syntax.

All together, these results show that it is crucial to extend the research framework from single call utterances to call combination, since rich information can be conveyed by those arrangements, reflecting greater flexibility in usage than in production (Zuberbühler and Lemasson 2014), higher cognitive capacities than expected before, and providing helpful insight in the evolution of human language.

Limitations in the study of animal semantics

The pioneering work by Seyfarth and Cheney (1980a, b; 1988) originally searched for noun-like signals to denote objects and events (Macedonia and Evans 1993). However, since the cognitive mechanisms of signal production remained unknown, it was suggested to switch focus from noun-like signals to functionally referential signals to remain agnostic about the underlying mental processes (Wheeler and Fischer 2012; Scarantino and Clay 2015). 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). Functionally referential calls or sequences are found in a diverse set of taxa, from birds to rodents and non-human primates, and are used in food-related contexts, alarm contexts, and even in social contexts (Townsend and Manser 2013).

Functionally referential alarm signals convey information about, among other, predator type (Suzuki 2014), predator location (Macedonia and Evans 1993), predator size (Templeton *et al.* 2005), urgency of the situation (Manser *et al.* 2002) and even predator colour (Slobodchikoff *et al.* 2009). However, growing evidences suggest that alarm calls, especially those given to terrestrial predators, are also given to non-predatory events and may function as general alarm calls (e.g. Fichtel and Kappeler 2002; Wheeler 2010; Cäsar *et al.* 2013). This statement raises concerns about the relevance of production specificity criteria to study animal symbolic capacities, especially because listeners must use contextual cues to infer the eliciting context of a signal (Seyfarth and Cheney 2006, 2017; Wheeler and Fischer 2012; Scarantino and Clay

2015). Hence, Wheeler and Fisher (2012), suggested to switch focus from context-specific calls to so called “general calls”, because processing of the latter suggests a higher cognitive capacity than reacting to a referential stimulus.

One problem raised during this debate is that it is nearly impossible to assign context objectively (Zuberbühler and Neumann 2017). First, context specificity is a vague concept, since a context can be highly specific (e.g. prairie dogs alarm calls are specific of the type and the colour of the predator, Kiriazis and Slobodchikoff 2006; Slobodchikoff *et al.* 2009) to sparsely specific (e.g. Japanese great tits’ “chicka” are emitted to aerial and terrestrial predators, Suzuki 2014). Second, context is always determined as categorical concepts according to human’s own perception of the world. Indeed, in the very definition of functionally referential signals, Macedonia and Evans stated that “…all eliciting stimuli must belong to a common category” (p. 179) and stated later that “…ground squirrel alarm calls thus provide only probabilistic information about predator identity and cannot be considered functionally referential” (p. 184). However, human perception of the world may be different from the natural categorisation taking place by an animal mind.

We fail to highlight high symbolic capacities in animals: even Seyfarth and Cheney (1997) are puzzled by the fact that animals have so few semantic labels. Further research on animal communication are needed to understand why.

TITI MONKEYS

This work aims to provide new insights in our understanding of the evolution of human-like semantic capacities. As mentioned earlier, the target species should be phylogenetically close to humans to allow for the search for homologies. However, most vocal studies have been conducted on Old World monkeys (especially Cercopithecines) and Great Apes (Seyfarth and Cheney 2003), which share a most recent common ancestor some 32 and 6-17 million years ago, respectively (Perelman *et al.* 2011). New World monkeys are an independent radiation within the primate order, sharing a common ancestor with humans some 43 million years ago (Perelman *et al.* 2011). Focussing research on a New World monkey species allows for an investigation of potentially older roots of human semantic capacities, to get a clearer picture of its emergence from nonhuman primate-like communication. Moreover, research on New World monkey vocal communication has led to crucial discoveries. For example, pygmy marmosets *Cebuella pygmaea* have been the focus of one of the first studies challenging the supposed vocal fixity of nonhuman primates: Snowdon and Elowson (1999) paired individuals with a new mates and observed that the acoustic structure of their trills converged until being similar. In the same fashion, babbling was considered as being a trait found in human and some songbirds only, until Elowson *et al.* (1998b, a) found that it also occurs in infant pygmy marmosets as a developmental stage. They showed that infant produce long sequences of calls that are mostly similar in structure to those of adults but that are used in different pattern and contexts, suggesting that they have to learn the association between context and call type. All these results suggest that New World monkeys are promising to increase the power of the comparative perspective and better track the phylogenetic history of the many components of human language.

Titi monkeys are New World primates that produce long sequences of calls in several different contexts, such as to predators (Cäsar *et al.* 2012a) or during territorial defence (Caselli *et al.* 2014). Focussing on a species that produces sequences is promising: nonhuman primates have a very limited flexibility in call production (Snowdon 2009), making it interesting to study call combination to assess how they can create meaning from fixed call units (Zuberbühler and Lemasson 2014).

This work focuses on alarm sequences in titi monkeys. Alarm signals are vocalizations emitted in response to the presence of a predator or other dangers. They are interesting to study because they are easy to identify, and they are useful tools to investigate the cognitive mechanisms of a species, including the messages conveyed and the mental representations generated by the receivers (Zuberbühler, 2009). Moreover, as the eliciting stimulus is easy to identify and because the behavioural response of the receivers is usually obvious and dependent on the predator hunting strategy, alarm calls represent an ideal subject for experimental playbacks (Macedonia and Evans, 1993). Moreover, preliminary studies on titi monkey alarm system revealed promising results about their semantic capacities (Cäsar *et al.* 2012b, 2013).

For all these reasons, titi monkey alarm sequences make an ideal candidate to study semantics capacities of human relatives.

Taxonomy

Titi monkeys were long considered as being part of one unique genus, the genus *Callicebus* (family Pitheciidae, subfamily Callicebinae). This genus was divided into four species-groups: *torquatus, personatus, donacophilus* and *moloch* groups, and was considered the most species rich of primate genus with 34 species discovered so far (Carneiro *et al.* 2016), the latest being described in 2015 (Vermeer and Tello-Alvarado 2015). However, recent molecular analysis showed that this genus is divided into three genera: *Cheracebus* (corresponding to the former *torquatus* group*), Callicebus* (former *personatus* group*)* and *Plecturocebus* (former *donacophilus* and *moloch* groups) (Byrne *et al.* 2016).

Ecology

Titi monkeys are small (weight: 0.8-1.4 kg), diurnal neo-tropical primates (Bicca-Marques and Heymann 2013) that live up to 12 years in captivity (Rowe 1996). They are found in South America, in the forests of the Amazon and Orinoco basins, the Chaco and dry forests of Paraguay and Bolivia, and in Brazilian Atlantic rainforests (van Roosmalen *et al.* 2002) (Figure

1.1). Titi monkeys of the new genus *Callicebus* are endemic to Brazil, and are mainly found in tropical rainforest (annual rainfall between 500 to 3800 mm), like in Atlantic forests and forest patches of Caatinga in the South Eastern part of Brazil, in the states of Paraná, São Paulo, Rio de Janeiro, Espirito Santo, Minas Gerais, Goias and Bahia (Byrne *et al.* 2016) (Figure 1.1). They live at an altitude comprised of 0 and at least 1500m (review in Bicca-Marques and Heymann 2013). They are mainly arboreal, and anecdotally go to the ground, mostly to forage (Souza-Alvez *et al.*, in prep). Their diet is composed of fruits, small animal preys (mostly insects) and leaves (Caselli and Setz 2011; Bicca-Marques and Heymann 2013).

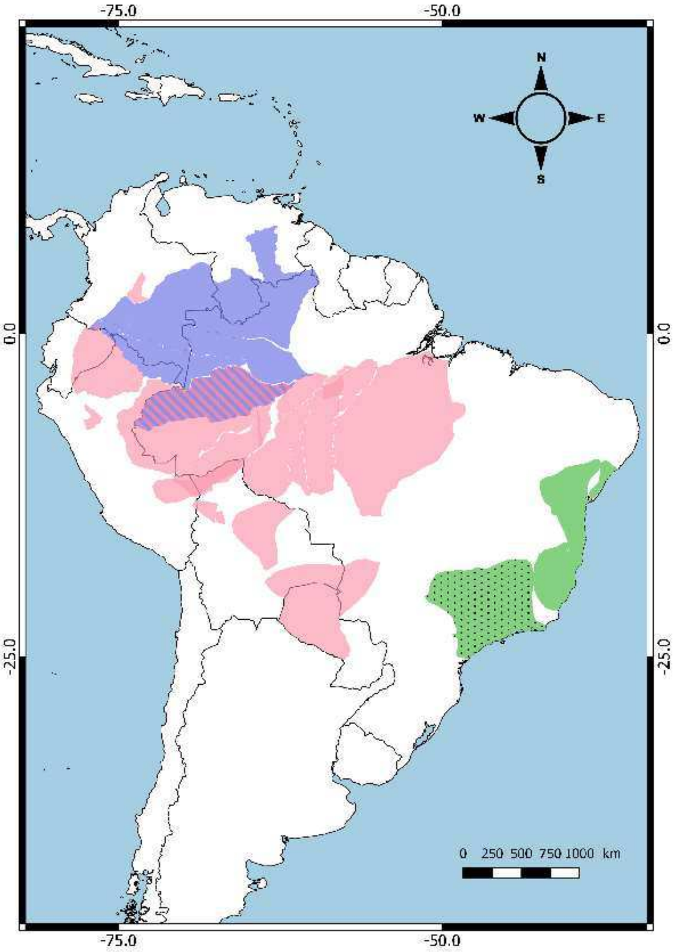


Figure 1.1 Distribution of titi monkeys of the new genera *Cheracebus* (purple), *Plecturocebus* (pink) and *Callicebus* (green). The green dotted area is the distribution range of *C. nigrifrons*. Map adapted from Byrne *et al.* (2016) by Geoffrey Mesbahi. Coordinate system: WGS84, base map from thematicmapping.org (Sandvik 2018), used with permission

Social system

Titi monkey exhibit no sexual dimorphism (Rowe 1996). They live in family groups composed of two mated adults, monogamous for life, and up to four offspring (Bicca-Marques and Heymann 2013). They live in a territory of 1 to 50 ha, but rarely above 25 ha, depending on the species and the site (Bicca-Marques and Heymann 2013). Studies have shown a high bond between the two mates: they are often in close proximity (Mason 1966), many studies report that removal of one of the partner elicits high distress in the remaining one (e.g. Fernandez-Duque *et al.* 1997), and they exhibit aggressive behaviour when exposed to a same-sex intruder (Cubicciotti III and Mason 1978). All these behaviours probably occur to prevent the mate from extra-pair copulations, even if it has already been witnessed (Cubicciotti III and Mason 1978).

The mating pair gives birth to one infant per year, rarely twins (Knogge and Heymann 1995; Valeggia *et al.* 1999), mostly at the end of the period of food scarcity or at the beginning of the period of food availability (Di Bitetti and Janson 2000), between July and January (Bicca-Marques and Heymann 2013). Most of the parental cares are provided by the father, who carries the infant up to 98% of the time and actively transports the infant to the mother to breastfeed (Jantschke *et al.* 1995; Spence-Aizenberg *et al.* 2016). This high disequilibrium in parental care investment between the mother and the father results in a high attachment of the infant to the father but not the mother (Mendoza and Mason 1986; Hoffman *et al.* 1995). However, this attachment does not seem reciprocal: neither parent show distress sign when separated from the young (Mendoza and Mason 1986) and the mother appears to avoid contact with the infant and is agitated in its presence if alone with him (Hoffman *et al.* 1995). A case of maternal infanticide has even been reported (Cäsar *et al.* 2008). Overall, these studies show that the attachment between mates is stronger than between parents and infants, highlighting an interesting social system in which the relationship between mates is similar to filial bond observed in other species (Mendoza and Mason 1986).

Both sex disperse when they reach three years old (Bossuyt 2002), but it remains unknown how new groups are formed. However, splitting of the parents' territory has been observed, probably to allow the mature offspring to move to a different part of the territory, away from the parents (Easley and Kinzey 1986). Replacement of one of the mate of the group by a new individual has also been observed, suggesting another mechanism of group formation (Cäsar 2011).

Predator pressure

Titi monkeys are small primates, which make them subject to high predation pressure (Ferrari 2009). Predators include birds of prey, mammalian carnivores (felid and tayra), snakes and capuchin monkeys (Ferrari 2009; Cäsar 2011; Bicca-Marques and Heymann 2013).

Titi monkeys use sleeping sites randomly distributed among the territory and avoid using the same two nights on a row, probably to make their location less predictable for predators (Caselli *et al.* 2017). They choose high and large trees to avoid detection and access by terrestrial predators, and trees with dense vegetation to prevent aerial predators to detect and attack them (Caselli *et al.* 2017).

Vocal communication

Vocal repertoire of titi monkeys has first been described in *Callicebus cupreus* by Moynihan (1966), who classified 10 vocalization types and suggested that titi monkey vocal communication "…may illustrate the maximum elaboration which can be attained by species-specific language" (p. 125). The vocal repertoire was then described by Robinson (1979a) with 13 call types. Vocalizations of titi monkeys can be classified into high-pitched and soft vocalizations, medium pitched calls and low-pitched, loud calls (Moynihan 1966; Robinson 1979a).

Loud calls are the most conspicuous vocalizations of titi monkeys, audible up to 500m away (Robinson 1981). Therefore, studies on titi monkey vocal communication mainly focused on this behaviour. Loud calls types were described by Moynihan (1966), Robinson (1979a, 1981) and Müller and Anzenberger (2002) in *Callicebus cupreus*, and by Caselli *et al.* (2014) in *Callicebus nigrifrons*. Loud calls are combined into sequences whose order and composition is dependent on the context (Robinson 1979a; Caselli *et al.* 2014). These sequences are emitted in solo (one individual calling), in duet (the two mates calling) or in chorus (more than two individuals calling) (Caselli *et al.* 2014) and mates synchronize their vocal production when duetting (Robinson 1979a; Müller and Anzenberger 2002). The function of these loud calls is puzzling. In *Callicebus lucifer* and *Callicebus personatus*, duets may serve to indicate the location of the calling group to neighbours, to avoid inter-group encounters (Kinzey and Robinson 1983). In *Callicebus ornatus*, however, duets may serve to define territory boundaries and promote neighbour encounters, but they are also used in mate defence, since playbacks simulating a same-sex intruder elicit vocal reactions (Robinson 1979b, 1981). Finally, in *Callicebus nigrifrons*, duets are used as a joint defensive mechanism, to protect food resources against intruders (Caselli *et al.* 2014, 2015). The adult pair reacts strongly to simulated intruders so that playbacks of loud calls are often used in population monitoring, to assess the density of a population in a forest (e.g. Dacier *et al.* 2011; Gestich *et al.* 2016). Individual separated from the rest of the group also emit loud call sequences whose organisation and composition are different from sequences elicited in territorial defence (Caselli *et al.* 2014). Finally, loud calls are also emitted in sequences during predatory situations, but little work has been done on this context (Cäsar 2011). Overall, these studies show that loud calls are used in within- and between-group communication.

The only other vocal behaviour of titi monkey that has been studied is alarm vocalisations. In *Callicebus nigrifrons* the first part of an alarm sequence is composed of two soft call types, the A-calls that are arch-shaped with a down-sweep modulation and the B-calls, S-shaped with an upsweep modulation (Cäsar *et al.* 2012a) (Figure 1.2). Cäsar (2011) reported that some C-calls could also be present in the sequences but these calls seem to function as travelling calls rather than alarm calls. Soft calls are gradually replaced in the sequence by medium and loud calls (Cäsar 2011).

A-call sequences are produced in response to non-terrestrial predators, like raptors and capuchin monkeys (Cäsar *et al.* 2012a), and playbacks of A-calls make the listeners look upwards (Cäsar *et al.* 2012b), suggesting that A-calls refer to threats within or above the canopy. B-call sequences are given in response to terrestrial predators, such as oncillas, pumas and tayras (Cäsar *et al.* 2012a), but also in non-predatory contexts, such as when monkeys approach the ground (Cäsar 2011; Cäsar *et al.* 2012b). These results, added to playbacks experiments showing that B-calls make the listeners look towards the speaker (Cäsar *et al.* 2012b), suggest that B-calls function as general alarm calls. Interestingly, predator presentations showed that when aerial predators are on the ground, titi monkeys intersperse B-calls within the A-call sequence, while when terrestrial predators are found in the canopy, titi monkeys emit a single A-call at the beginning of the B-call sequence (Cäsar *et al.* 2013).

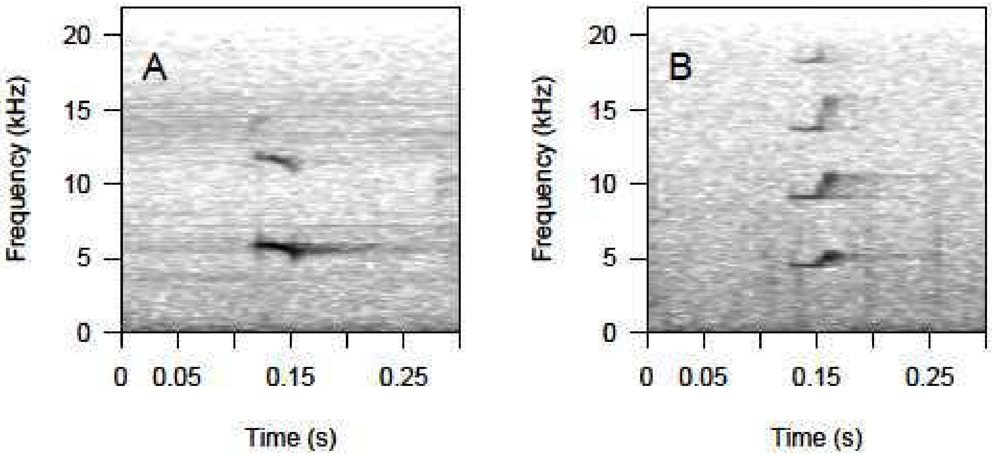


Figure 1.2 Spectrograms of adult titi monkey alarm calls (A) A-call, (B) B-call

From these results, Cäsar *et al.* (2013) concluded that titi monkeys combine two simple calls into rule-governed sequences that can convey information about the predator type and location, with variation in the predator-specific sequences indicating an uncharacteristic location. Overall, this suggests a sophisticated syntax/semantic interface in titi monkeys, possibly the most sophisticated meaningful nonhuman primate sequences that have been studied so far (Cäsar and Zuberbühler 2012).

When re-analysing Cäsar's data with linguistic tools, Schlenker *et al.* (2016a) concluded that the titi monkey alarm sequences should not be considered as a whole, but that each call provides relatively simple information about the situation at the time it is uttered. In their analysis, the A-call would refer to a serious non-ground threat, while the B-call would refer to a noteworthy event, including the non-serious non-ground threats. Thus, in the raptor on the ground condition, the A-calls would refer to a serious non-ground threat (a raptor) but the monkeys would then switch to B-calls (non-serious non-ground threat) because the raptor on the ground is not as dangerous as a flying one. In the terrestrial predator in the canopy condition, the first call would refer to a serious non-ground threat, because the predator is dangerous and in the canopy, but since a deterred predator stops hunting (Zuberbühler et al. 1999b), the predator would stop being dangerous after emission of the first call, so the caller would switch to B-calls (non-serious non-ground predator).

These conclusions led to two hypotheses: Either titi monkeys use non-trivial combinatorial rules to encode for predator location and type, or they do not, and each call provides information about the situation at the time it is uttered.

Black-fronted titi monkeys

Not much research has been conducted on titi monkeys (but see Cäsar 2011), mainly because they live in an environment that is challenging for human observers and because they are very cryptic and discrete animals (Pinto *et al.* 2013).

My research focuses on the black-fronted titi monkey *Callicebus nigrifrons* (von Spix 1823) (Figure 1.3). This species is one of the largest titi monkeys (Rowe 1996), found in the Atlantic forests and Caatinga patches of South-East of Brazil, in the States of São Paulo, Minas Gerais, Goiás and Rio de Janeiro (van Roosmalen *et al.* 2002) (Figure 1.1). This species was the focus of Cäsar's study on alarm sequences (Cäsar 2011; Cäsar *et al.* 2012a, b, 2013).

Figure 1.3 Black-fronted titi monkey *Callicebus nigrifrons*

AIMS OF THE RESEARCH

Titi monkeys may possess a unique system in which the semantics of A and B-calls and the syntax of the sequences are tangled to convey information about predator type and location (Cäsar *et al.* 2012b, 2013). However, studies leading to these conclusions were preliminary, and more research is needed to be able to conclude on these statements.

First, the B-calls are given to terrestrial predators or when the caller is moving towards the ground. This raises questions about the meaning of B-calls, considered as general calls. However, no acoustic investigation was carried out to conclude on the context-specificity of these calls. I investigated both the acoustic and sequential structure of the B-sequences, to define their semantics. Second, the study suggesting that alarm sequences composed of A and B-calls convey information about predator type and location (Cäsar *et al.* 2013) was preliminary. Thus, other sequence features that convey information may have been overlooked. I investigated the information conveyed in the sequences by exposing titi monkeys to predator models on the ground and in the canopy, to refine our understanding of the encoding in titi monkey alarm sequences. Third, it was not investigated how listeners can perceive and extract information from the alarm sequences, making it impossible to conclude on the semantic capacities of the titi monkeys. I carried out playback experiments to assess what information titi monkeys extract from the alarm sequences, and what encoding mechanism they rely on.

Overall, this work aims to bridge the existing gaps to conclude on the semantic and syntactic capacities of titi monkeys, providing new insights in the evolution of vocal capacities in the primate lineage.

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CHAPTER 2. GENERAL METHODS

STUDY SITE

My study took place at the Reserva Particular do Patrimônio Natural Santuário do Caraça (Figure 2.1), a private reserve of 11,000 ha in the Espinhaço Mountain range, Minas Gerais, Brazil (20°05’ S, 43°29’W).

Figure 2.1 Location of the field site in Brazil by Geoffrey Mesbahi. Coordinate system: WGS84, base map from thematicmapping.org (Sandvik 2018), used with permission

History

In 1770, Brother Lourenço de Nossa Senhora built a chapel and founded the Irmandade e Romaria de Nossa Senhora Mãe dos Homens, a brotherhood and pilgrim dedicated to Marie. In 1820, Dom João VI, king of Portugal and Emperor of Brazil, gave the responsibility of the property to the Provincia Brasileira da Congregação da Missão (Brazilian Congregation of the Mission), according to Lourenço's will. The place was turned into a college and a seminar of great reputation that welcomed more than 11,000 students. After a fire destroyed a building in 1968, the Congregação da Missão decided to turn the complex into a spiritual, touristic and cultural centre. 11,000 ha out of the 12,403 of the Santuário were recognized as Reserva Particular do Patrimônio Natural (Particular Reserve of Natural Heritage, RPPN) in 1994 by the IBAMA (Brazilian Institute of the Environment and Renewable Natural Resources).

Environmental impact of economic activities

Since 1770, when the property was purchased by Brother Lourenço, activities outside of educational and spiritual ones have been restricted to farming, cattle raising and forest industry. Logging was common for coal extraction (on Eucalyptus *Eucalyptus sp*), especially in the two forests of interest of this study, but also to clean space for cattle and in 1930, to build a road leading to the complex. This road represents the limit of one of the forest of interest, Tanque Grande. Mining is a very common activity in the region since the end of the XVII century, and some mines were present in the reserve.

Since the creation of the RPPN in 1994, no farming, cattle raising, or forestry is allowed in the protected area. Outside of this zone, in the 1,000-remaining ha, logging (to build fences and for property usage) and burn farming (to clean pastures) are still performed at small scale by the farms belonging to the Sanctuary.

All mine activities were stopped in 2011 in the reserve but are still very intense on the border, threatening to turn it into an "island" isolated from other natural forests: this region is indeed one of the most fragmented Atlantic forest of Brazil (Machado and Bouchardet Da Fonseca 2000). Coal extraction and culture of *Eremanthus erythropappus* (a Brazilian plant that produce popular oil) are very important in the region and are found at the limits of the park. Accidental fires are very common, especially in the borders due to coal exploitations and burning farming, and difficult to manage since most of the region is difficult to access for fire-fighters. Illegal charcoal kilns are present in the region and are targeted by the local environmental police but are difficult to control since the headcounts are very low and the area to manage is wide.

Tourism impact

The place is very popular with tourists and attracts between 60,000 and 70,000 visitors (Brazilians and foreigners, as well as local schools) per year. Although it represents a large amount of people walking in the forest, they follow specific path leading to natural curiosities and are required to stay on these designed trails. Thus, when crossing the forest that monkeys inhabit, visitors can only see the animals along the trails, which only represents a small portion of their life ranges. Moreover, it is explicitly forbidden to interact with animals from the reserve in any way (e.g. feeding, touching, hunting) and we could see that monkeys were habituated to see people on the trails but never approached them to beg for food, which confirms that tourists do not interact with monkeys. Moreover, illegal hunting in the reserve has never been registered. Then, impact of tourists on titi monkeys behaviour -and this study- was limited to noise since they often speak loudly when walking in the forest. We used signs delimiting the study area to quieten tourists but because it had limited efficiency in case of big affluence, we adjusted our schedule to avoid running crucial experiments on these periods (e.g. weekends, public holidays)

Ecology

This 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, hot season (from October to March) and a dry, colder season (from April to September).

The reserve presents a rich biodiversity. Four species of primates inhabit it: the black capuchin *Cebus nigritus*, the white-headed marmoset *Callithrix geoffroyi*, the black-tufted marmoset *Callithrix penicillata* and the back-fronted titi monkey *Callicebus nigrifrons*. Brown howler monkeys *Alouatta guariba* have not been seen since the 2000s but one group has been observed in the reserve in 2016 (Cäsar 2011; Província Brasileira da Congregação da Missão 2013; Douglas Henrique da Silva, personal communication).

Many titi monkey predators - confirmed or suspected – are also present: among mustelids, the tayra *Eira barbara* is common. Among felids, the reserve is home of the ocelot *Leopardus pardalis*, the margay *Leopardus wiedii*, the puma *Puma concolor*, the jaguarundi *Puma yagouaroundi* and the jaguar *Panthera onca* (Província Brasileira da Congregação da Missão 2013). Many raptors are found in Caraça, among them the caracara *Caracara plancus*, the crowned eagle *Harpyhaliaetus coronatus*, the black-chested buzzard-eagle *Geronoaetus melanoleucus* and the black hawk eagle *Spizaetus tyrannus* (Cäsar 2011).

STUDY SUBJECTS

Groups and individuals

I studied five groups of *Callicebus nigrifrons* that have been habituated to human presence since 2003 (Cäsar 2011) but it is important to note that they were not systemically monitored between the end of Cäsar's study in 2011 and my study. To increase the dataset, I habituated a sixth group, the S group, from December 2014 to January 2015 (Table 2.1). Four groups reside in the forest of Tanque Grande and two groups in the forest of Cascatinha, located one kilometre apart from each other (Figure 2.2). As mentioned earlier, both sexes disperse after reaching sexual maturity, at around 3-4 years of age (Bossuyt 2002), so group composition was different between Cäsar's and my study, with only some paired adults being present in both studies (Table 2.1).

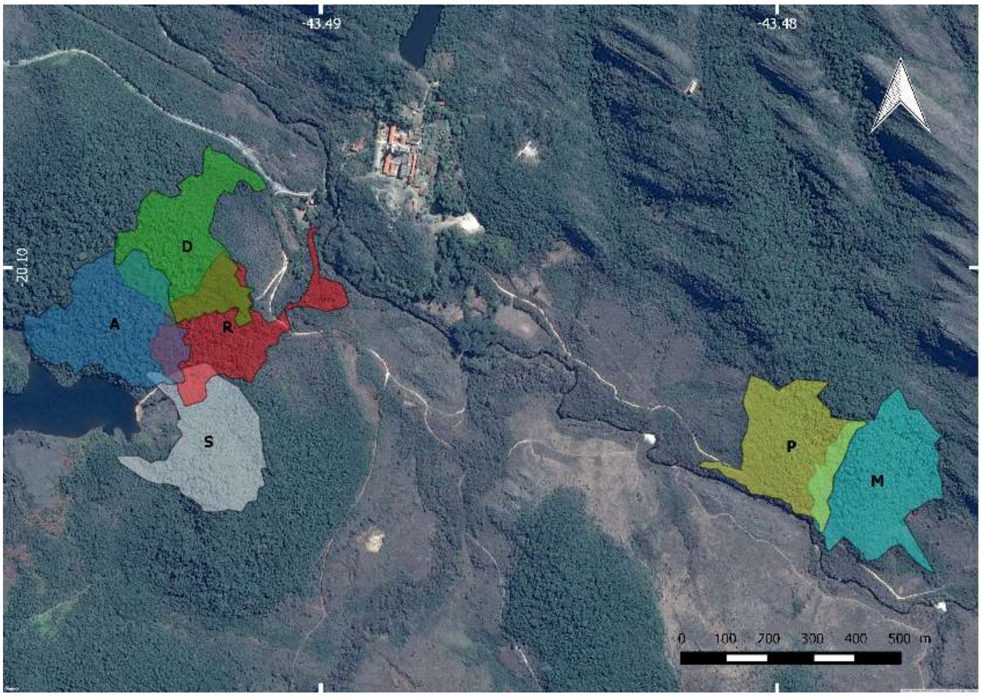

Figure 2.2 Map of the territories of the six habituated groups, by Geoffrey Mesbahi. Coordinate system: WGS84, base map from Google Maps (2018), used with permission

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. Each individual was given a name whose first letter corresponded to the name of the group (ex: Savi from the S group, Muffin from the M group).

Table 2.1 Characteristics of the six habituated groups during my study (2015-2016)

Forest	Group	# Individuals	Paired ad.	Unpaired ad.	Sub.	Juv.	Inf.	Habituation	Territory size (ha)
Tanque Grande	A	6 (1)	2 (1)	1-3	1	0-1	0-1	2008	8.2
	D	4-5 (2)	2 (2)	0-1	1	0-1	0-1	2004	8.0
	R	4-6 (1)	2 (1)	0-2	0-1	0-1	0-2	2004	7.1
	S	4-5	2	0-2	0-1	0-1	0-1	2015	7.0
Cascatinha	M	5-6 (2)	2 (2)	1-2	1	1	0-1	2008	8.5
	P	4-5 (2)	2 (2)	1-2	0-1	0-1	0-1	2008	6.8

Paired ad: mated pairs; Unpaired ad: young adults prior to dispersal; Sub: Subadults, Juv: Juveniles and Inf: Infants. Number between brackets: individuals already present in Cäsar (2011).

DATA COLLECTION

Study period and monitoring teams

Data were collected over a 17-month study period divided into three field seasons: a pilot study (October-December 2014), and two main study periods (April-June 2015 and October 2015-August 2016) by several teams.

Teams were composed of either a master student and a field assistant (Nina Buffenoir and Guilhem Duvot, April-June 2015; Clément Ludcher and Anaïs Pessato, January-March 2016) or two field assistants (Camille Rostan and Arthur Colliot, October-December 2015; Aude Pajot and Franziska Müschenich, April-June 2016) or me and a field assistant (Breno Henrique, December 2015; Geoffrey Mesbahi, April-June 2015 and October 2015-August 2016). After completing the training (about a month), each team was assigned a research topic and collected data autonomously for the rest of their stay. Some of the data collected by these teams were used in this thesis if comparison of data collection at the end of the training phase showed that inter-observer reliability was correct.

Daily monitoring

Each group was followed at least four days per month. Groups were monitored for 1,715 hours during this study: 746 hours by the teams of one master and one assistant or of two assistants, and 969 hours by me and my assistant (Table 2.2).

Table 2.2 Monitoring effort of the six groups by my team (me and one field assistant) and the other teams (one master student + one field assistant or two field assistants)

	Monitoring (in hours) by	
Group	My team	Other teams
A	201.3	24.5
D	163.3	34.2
M	138.5	131.0
P	145.3	189.7
R	193.8	67.2
S	126.5	298.8
Total	968.8	745.3

Teams used to arrive in the territory of the target group at dawn and walked along the trails until finding the monkeys, mainly by acoustic cues (movements in the trees, vocalizations like duets or feeding calls). On experimental days, i.e. days with planned experiment(s), groups were followed until they were lost, stopped at a sleeping site, or after completion of the experiment(s). If conditions allowed it, we sometimes ran experiments on several groups within a day: in this case, we either joined another team that was working with another group, or we searched another group following the same procedure as above.

To avoid habituation, we interspersed “monitoring days” in between experiment days, i.e. days when we only collected opportunistic data and did not run any experiments. On these days, groups were followed until they stopped at the sleeping tree, or after at least 6 hours of monitoring.

Regardless of the aim of the day, scans were collected every 10 min and included the activity, height and strata of every individual of the group, as well as the maximum inter-individual distance. The GPS position of the centre of the group was recorded with a GARMIN GPSMAP60CSx GPS.

Opportunistic data collection

It was not possible to blindly record data because this study involved focal animals in the field.

We recorded natural alarm sequences (i.e. given during natural predator encounters, even if it was not possible to identify the predator) and descending sequences (i.e. when the caller descends or moves horizontally near the ground, at 2-3 m high maximum, usually to forage, no predator presence). Each sequence was labelled with the context of emission and the identity of the caller(s), if identified.

We recorded vocalizations in WAV format with a Marantz solid-state recorder PMD661 (44.1 kHz sampling rate, 16 bits accuracy) and a directional microphone Sennheiser K6/ME66 or K6/ME67 (frequency response: 40-20,000 Hz ± 2.5 dB).

Other data were collected opportunistically along the day, like social events (neighbouring encounters, duets and choruses, copulations), but were not systematically used in this study.

Experimental data collection: Predator presentations

We used four stuffed predators as stimuli (Figure 2.3): one aerial predator (two models of caracaras *Caracara plancus*) and two terrestrial predators (one model of tayra *Eira Barbara* and one of oncilla *Leopardus tigrinus*). The models were all borrowed from the Natural History Museum of the Pontifícia Universidade Católica de Minas Gerais.

Figure 2.3 Predator models (A) Tayra *Eira barabara*; (B) Oncilla *Leopardus tigrinus*; (C) and (D) Caracara *Caracara plancus*

Each predator species was presented twice to a group, once in the canopy and once on the ground, i.e. 36 expected trials in total. It was plausible to present these terrestrial predators in the canopy, since tayras and oncillas climb with ease (Asensio and Gómez-Marín 2002; Sunquist and Sunquist 2002). The order of presentation was randomized among the groups. Model presentations to a group were separated by at least 10 days and the groups were monitored between trials. We monitored the group for at least 30 minutes before the beginning of an experiment (i.e. detection of the model by an individual) and if possible, 30 minutes after the end of the experiment (i.e. after the entire group stopped calling or left the area). We made sure that no duet, group encounter, loud calls from a lost individual, or predator encounter occurred in the 30 minutes preceding the experiment, otherwise the setup was dismantled, and we waited for another 30 minutes to set it up again.

When presented within the canopy, we placed the model at 3-10 meters high (mean ± s.e. = 6.3 ± 1.6 m), depending on the structure of the arboreal strata. When presented on the ground, we placed the model at 0 m high. We considered a trial as failed if the recording quality was bad (n=1), if the model was detected before the setup was completed (n=5), if the model was detected by an individual less than two years old (n=2), if another species gave alarm calls to an observer or to the predator model and elicited a vocal reaction from the monkeys before visual detection (n=2), if an individual fell on the model before detection (n=1), and if another predator was detected just before detection of the model (n=1). If a trial was failed, we waited at least two months to re-run it, except in one case where we only waited 35 days: the monkeys spotted leaf movement in the canopy due to the installation but probably did not see clearly the model (tayra in the canopy, M group). One experiment (Caracara in the canopy, D group) failed three times, and we did not run the experiment a fourth time to avoid a potential bias in the vocal response due to habituation. In total, we run 35 successful trials.

Vocal reactions were recorded in WAV format with a Marantz solid-state recorder PMD661 (44.1 kHz sampling rate, 16 bits accuracy) and a directional microphone Sennheiser K6/ME66 or K6/ME67 (frequency response: 40-20000 Hz ± 2.5 dB). Distance of detection (i.e. distance between the first individual to call and the model at the time of detection, in meters) as well as identity of the caller(s) was noted for each experiment.

VOCAL REPERTOIRE

I used the vocal repertoire established by Cäsar et al, (2012a). To estimate the accuracy of the call recognition, a between-rater reliability test was carried out between me and Cristiane Cäsar. I used a subset of 200 randomly selected calls that each of the two observers labelled (A-call, B-call, C-call or NA). I calculated the Cohen’s kappa and the level of between-rater agreement reached the required reliability level ($k \geq 0.8$).

STATISTICAL ANALYSES

Tests were conducted in R version 2.14.0 (R Development Core Team 2011) and R version 3.4.1 (R Development Core Team 2017). To increase open science and allow replication of the results, all datasets and R scripts are provided on the data repository Figshare (https://figshare.com/), whose exact link can be found in each chapter.

ETHIC STATEMENT

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

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CHAPTER 3. HOW CONTEXT-GENERAL ARE TITI MONKEY ALARM SEQUENCES?

Results presented in this chapter have been published in:

Berthet M, Neumann C, Mesbahi G, Cäsar C, Zuberbühler K (2018)

Contextual encoding in titi monkey alarm call sequences.

Behavioral Ecology and Sociobiology 72:8

ABSTRACT

Many primates produce one type of alarm call to a broad range of events, usually terrestrial predators and non-predatory situations, which raises questions about whether primate alarm calls should be considered "functionally referential". A recent example is black-fronted titi monkeys, *Callicebus nigrifrons*, which emit sequences of B-calls to terrestrial predators or when moving towards or near the ground. In this study, I reassessed the context-specificity of these utterances, focussing both on their acoustic and sequential structure. I found that B-calls could be differentiated into context-specific acoustic variants (terrestrial predators vs. ground-related movements) and that call sequences to predators had a more regular sequential structure than ground-related sequences. Overall, these findings suggest that the acoustic and temporal structure of titi monkey call sequences discriminate between predator and non-predatory events, fulfilling the production criterion of functional reference.

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 (Seyfarth and Cheney 2003; Price *et al.* 2015). The current debate is less about the psychological mechanism driving call production, but about the referential specificity of the calls (Wheeler and Fischer 2012).

Many animal species possess two alarm call types; one for aerial and one for terrestrial predators (see Kiriazis and Slobodchikoff 2006). A consistent finding in primates is that aerial alarms are typically highly predator-specific while terrestrial alarms tend to be more general and can be used in many contexts (Fichtel and Kappeler 2002; Fichtel *et al.* 2005; Kirchhof and Hammerschmidt 2006; Wheeler 2010; Wheeler and Fischer 2012; Zuberbühler and Neumann 2017). For example, red-fronted lemurs (*Eulemur fulvus rufus*) give "woof" calls to fossas and dogs, but also in non-predatory situations of seemingly high arousal, while "chutter" calls are exclusively given to hawks (Fichtel and Kappeler 2002). Similarly, tufted capuchins (*Cebus apella nigritus*) give "bark" calls to aerial threats and "hiccup" calls to terrestrial predators, but also in non-predatory, seemingly stressful situations (Wheeler 2010).

Strictly speaking, the terrestrial alarm calls of these species do not fulfil the production criterion by Macedonia and Evans (1993), and hence cannot be classified as functionally referential. Instead, they are more similar to human pointing insofar as they attract the attention of other group members, who then either consider pragmatic cues, such as other recent events (Arnold and Zuberbühler 2013) or simply follow the caller gaze direction to the cause of his or her calling (Crockford *et al.* 2015).

However, there are additional complexities regarding the hypothesis that primate terrestrial alarms are referentially unspecific. In particular, recent progress in acoustic and statistical analyses continues to highlight the richness of information encoded in animal signals (e.g. Griesser 2008). Moreover, the recent introduction of automated feature extraction technology and unsupervised learning algorithms can highlight fine-grained contextual variation related 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, I 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) (Figure 3.1). 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). I 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

This study took place at the Reserva Particular do Patrimônio Natural Santuário do Caraça, Minas Gerais, Brazil, where I studied the six groups of *Callicebus nigrifrons* described earlier (see Chapter 2 for details).

Data Collection

We monitored groups daily during two field seasons (April to June 2015 and October 2015 to August 2016). To assess acoustic and sequential differences in B-call utterances, we recorded B-call sequences categorised as (a) "terrestrial predator" (natural or experimental terrestrial predator encounters), (b) "ground" (caller descends or moves horizontally near the ground, at 2-3 m high maximum, usually to forage, no predator presence) from natural events and predator presentations. For 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 10 metres high, depending on the structure of the arboreal strata) and once on the ground. More details on these predator presentations and data collection can be found in Chapter 2. Spectrograms of calls and sequences associated with each context are in Figure 3.1.

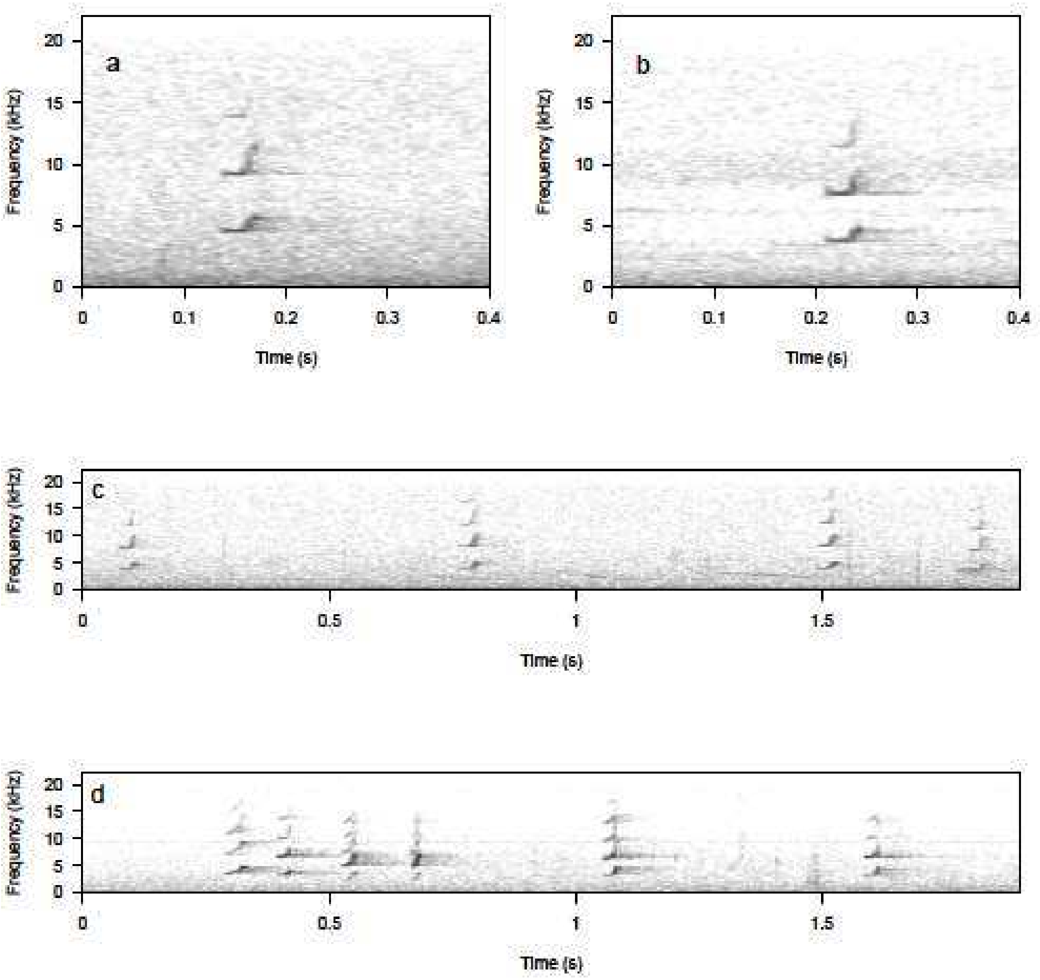

Figure 3.1 Spectrograms of B-calls from (a) the terrestrial predator context and (b) the ground context, and spectrograms of B-call sequences from (c) the terrestrial predator context and (d) the ground context, all from the same individual

Acoustic structure

Call selection and data sets

I extracted single calls from the original recordings of sequences given in the two contexts using Praat 5.3.84 (Boersma and Weenink 2009). I removed calls from the data set for the following reasons: if recorded from more than about 7 metres away, if given by immature (infant or juvenile) or unidentified individuals, or if the context could not be determined. Alarm calling typically involved all group members joining in a chorus. Therefore, the selected calls generally were taken from the beginning and end of calling sequences to ensure reliable identification of callers. I created two data sets, one for females and one for males to remove the confounding effects of sex in the subsequent statistical analyses. Each individual (seven males and seven females) provided at least six calls in each context (ground: N=14 individuals, N=3 sequences/individual; terrestrial predator: N=14 individuals; N=1 sequence/individual). I considered a total of 271 calls from 68 sequences (Table 3.1).

Table 3.1 Data sets used for call analysis The first number indicates the number of calls provided by each individual, the second indicates the number of different sequences the calls were extracted from

		Context	
Ind		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

Acoustic Analysis

I visually inspected spectrograms (FFT size: 512, Hanning window, time resolution: 3.54 ms, frequency resolution: 86.1 Hz) to exclude recording sections disturbed by other sounds or with low signal-to-noise ratio. I adapted acoustic parameters used in Podos (2001). For each call, we first measured directly on the spectrogram (1) the duration, and (2) the number of harmonics. We then measured frequency parameters from the power spectra: (3) the peak frequency, (4) the minimum and (5) the maximum frequency at which the amplitude exceeds -20 dB relative to peak frequency, (6) the frequency range (maximum-minimum frequency), the peak frequency at the (7) first 10 ms of the call (referred later as “first peak”) and (8) last 10 ms of the call (referred later as “last peak”) (Figure 3.2). The measurement of the minimum and maximum frequency relative to the peak frequency allows to maximize the proportion of signal measured, by not including background noise nor excluding signal energy (Podos 2001; Zollinger *et al.* 2012). All measurements were conducted using Raven Pro 1.5 Beta Version. Raw data are provided in the data repository.

Acoustic analyses were done by two raters (MB, GM). To assess between-rater reliability, we used a subset of 51 randomly selected calls (19% of the total dataset). We calculated the interclass correlation coefficient (ICC) for each of the acoustic parameters, and the level of between-rater agreement reached the required reliability level for all acoustic parameters ($r \geq 0.8$, Cicchetti 1994).

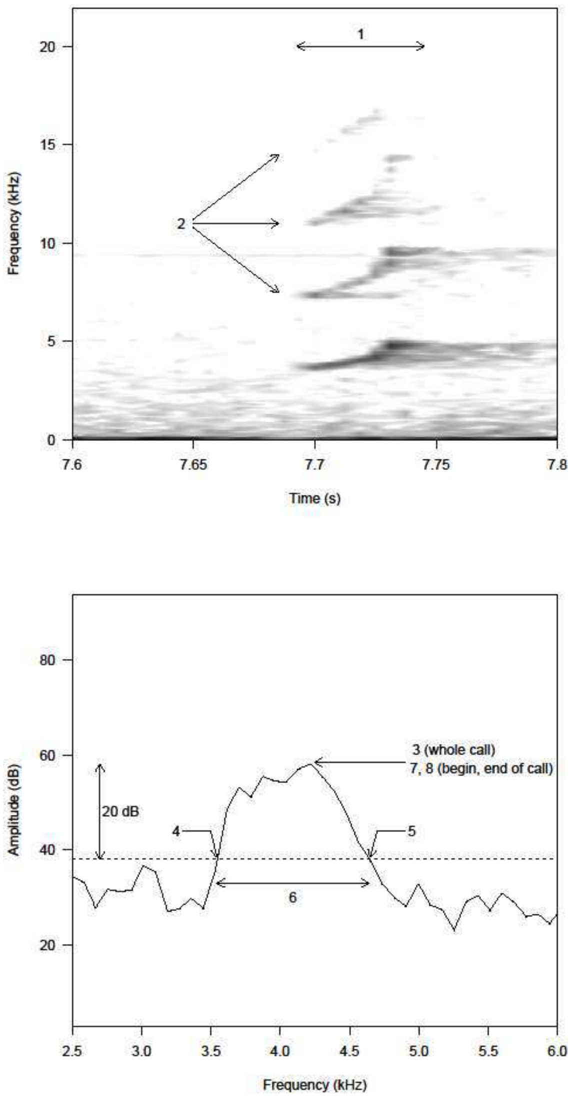

Figure 3.2 Measure of acoustics parameters on a B-call from the ground context, on the spectrogram (top panel) and the power spectrum (bottom panel) with 1: duration, 2: number of harmonics, 3: peak frequency, 4: minimum frequency, 5: maximum frequency, 6: frequency range, 7: first peak, 8: last peak. Figures were drawn using the “seewave” package (Sueur *et al.* 2008)

Statistical Analysis

For each acoustic parameter, I visually inspected histograms and transformed data to approach symmetric distributions (log, square root or fourth root) if necessary. I excluded strongly correlated parameters ($r \geq 0.7$) (Quinn and Keough 2002). Thus, I excluded maximum frequency (both sexes) because it was strongly correlated with the minimum frequency.

I used discriminant function analysis (DFA) to test for acoustic differences between contexts. The aim of this analysis is to determine whether certain objects (here the calls) can be discriminated into classes (caller identity, context) by parameters measured from each object (acoustic parameters). However, a DFA requires independence of data (i.e. it only allows the consideration of a single factor at a time, for example "individual" or "context"), and violating this assumption leads to increased probability of type I errors (Mundry and Sommer 2007). I therefore used permuted discriminant function analysis (pDFA; Mundry and Sommer, 2007), which combines a permutation approach with a DFA. I conducted a crossed pDFA for each sex separately to assess whether the B-calls could be differentiated among contexts based on their acoustic structure. I set "context" as the test factor and "individual" as the control factor to test for contextual differences while controlling for multiple calls of each individual (Mundry and Sommer 2007).

In order to extract the key variables, i.e. the variables that enable discrimination of context in the pDFA, I re-ran 1,000 permuted DFA and recorded those variables that had the highest coefficient of linear discrimination in at least 800 DFAs out of 1,000, i.e. the variables allowing for discrimination in more than 80% of the discrimination tests.

The ICC was conducted with the rptR package (Stoffel *et al.* 2017) in R version 2.14.0 (R Development Core Team 2011). All other tests were conducted in R version 3.4.1 (R Development Core Team 2017). The pDFA was generated using a function kindly provided by R. Mundry, based on the function "lda" of the R package MASS (Venables and Ripley 2002). The R script is provided in the data repository.

Sequential structure

Sequence selection

Responses to predator presence must be rapid, suggesting that alarm signals should convey any potential predator information as early as possible, i.e. once the caller has identified the disturbance. For this reason, I only focused on the first eleven calls of each sequence to measure ten call intervals (mean = 6.69 seconds, SD = 3.38). Hence, what I refer to as "sequence" in the following are the first eleven calls of a sequence.

For the predation context, I only considered sequences of pure B-calls, i.e. with no other alarm call type interspersed (e.g. A-call, Cäsar et al. 2012a). Since B-call sequences can be emitted in synchronization with movements during foraging bouts, I only considered as a new sequence an utterance preceded by at least 30 seconds of silence. As for call selection, I did not consider sequences if given by several individuals at the same time, by immature (infant or juvenile) or unidentified individuals, or if the context could not be determined.

Dataset and analysis

A total of 36 sequences from 12 individuals were considered for this analysis (Table 3.2).

For each sequence, I extracted two features. First, I measured the time interval between two subsequent calls for each of the eleven first calls (i.e. a total of ten duration per sequence). Second, I quantified the level of variability of the call interval for each sequence by calculating the coefficient of variation of the call intervals (CV= standard deviation / mean). A low CV indicates that calls are regularly emitted in the sequence, while a high CV indicates that calls intervals are variable in the sequence, with a mix of longer and shorter intervals. Raw data are provided on a data repository.

Table 3.2 Data sets used for sequence analysis, the numbers indicate the number of sequences each individual provided in each context

	Context	
Ind	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

Statistical analysis

I fitted two generalized linear mixed models (GLMMs). The first one was on the relationship between duration of the call interval and the context of emission with a gamma error structure. The second one was on the relationship between the CV of the sequence and the context of emission, again with a gamma error structure (Payton 1996). For both, I entered context (terrestrial predator vs. ground) and sex of the caller as fixed factors. Identity of the caller was controlled for by including it as a random factor nested within the group identity. I obtained P-values with likelihood ratio tests (LRT) of the full models against the null models, i.e. models without the fixed factor context. The fit of the models was evaluated by the proportion of variance explained (the marginal coefficient of determination R^2_{m}, i.e. the variance accounted for by fixed factors, and the conditional coefficient of determination R^2_{c}, i.e. the variance accounted for by both fixed and random factors) estimated with the delta method for variance estimation described in Nakagawa *et al.*, (2017).

Both GLMM were fitted using the lme4 package (Bates *et al.* 2015) in R version 3.4.1 (R Development Core Team 2017). The R script is provided in the data repository.

RESULTS

Acoustic structure

In females, B-calls could be distinguished on the basis of emission context with 82% of calls correctly classified, significantly higher than the 63% expected by chance (p=0.001) (Figure 3.3). The key parameter allowing for discrimination was the minimum frequency in 937 DFAs out of the 1,000 permutations: minimum frequency was about 0.5 kHz higher in the terrestrial predator context than in the ground context (Figure 3.4).

In males, classification of B-calls to the correct emission context was 69%, which was not significantly higher than the 60% expected by chance (p=0.153).

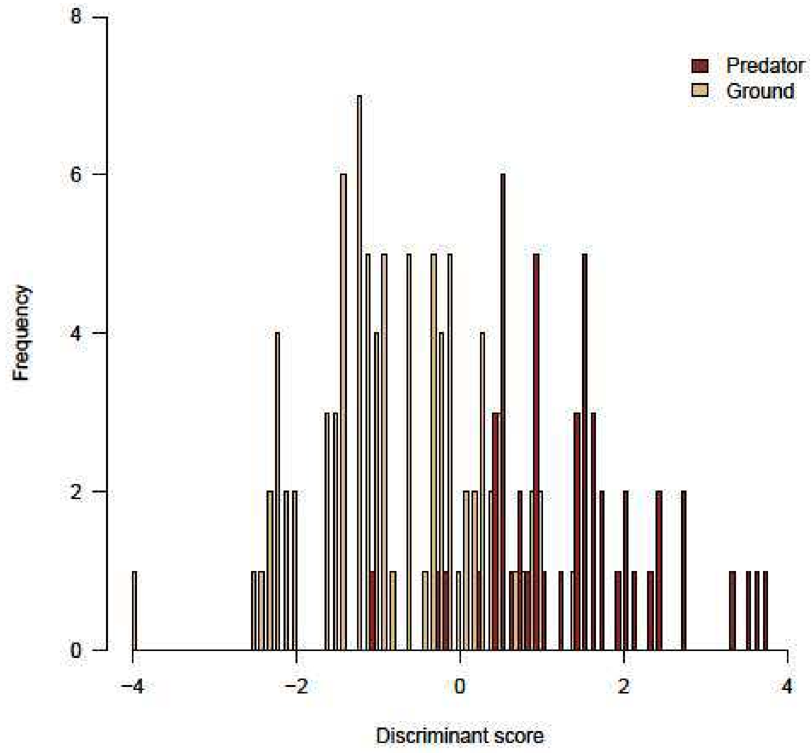

Figure 3.3 Distribution of the discriminant scores of female B-calls given to terrestrial predators and in the ground context. Note that the pDFA does not allow for graphic representation. Hence, this figure is drawn from the results of a DFA, and only serves to illustrate discrimination, but does not represents the results of the actual pDFA

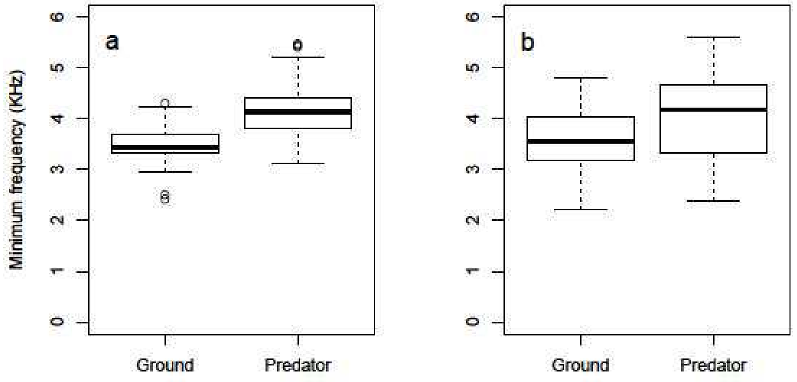

Figure 3.4 Median and quartiles of the minimum frequencies of B-calls in ground and predator context, in females (a) and in males (b)

Sequential structure

Context did not affect significantly the duration of inter-call intervals (LRT: $\chi^2(1)=0.63$, p=0.4252; $R^2_m=0.019$, $R^2_c=0.133$) (Table 3.3, Figure 3.5), but it affected the coefficient of variation 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 inter-call intervals was greater during descent sequences than in sequences in response to terrestrial predators (Table 3.3, Figure 3.5): in the predator context, calls were given with a more regular rhythm than in the ground context calls.

Table 3.3 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

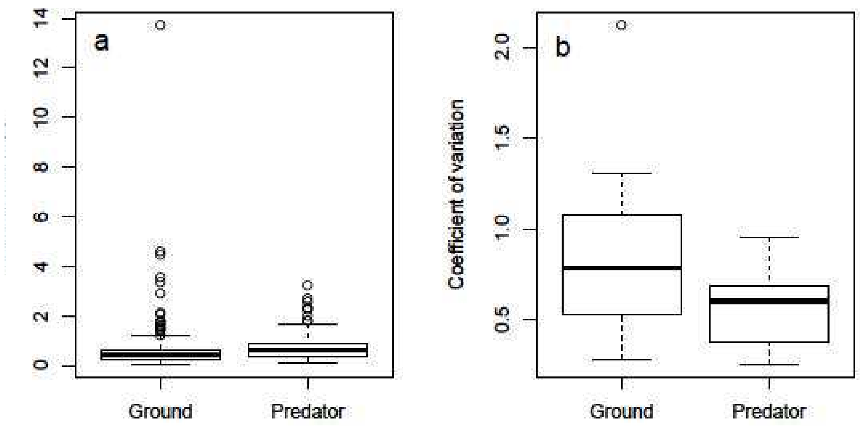

Figure 3.5 Call interval duration of B-sequences between contexts. Median and quartiles of the (a) call interval duration in the ground and predator context, and (b) of the coefficient of variation of the call intervals in the ground and predator context

DISCUSSION

I tested whether B-call sequences to predators and during descent differed in terms of call acoustic structure and/or on the sequential structure level. In female titi monkeys, B-calls could be differentiated probabilistically, mostly based on their minimum frequencies, with the terrestrial predator context being higher-pitched than the ground context (Figure 3.4). B-calls were also typically emitted in more regularly structured sequences during the terrestrial predator compared to the ground context (Figure 3.5). These results suggest that B-call sequences can convey information about the emission context on at least two levels: the acoustic structure of individual calls and the structure of the entire call sequences.

Context-specific acoustic variants within one alarm-call type have also been reported in other primate species, notably Barbary macaques, *Macaca sylvanus*, that produce acoustically different variants depending on the predator type (Fischer *et al.* 1995), and these variants are perceived by receivers (Fischer and Hammerschmidt 2001). This is also the case in chimpanzees *Pan troglodytes*, whose barks are emitted in two different contexts (hunt and snake presence) correlated with two acoustic variants (Crockford and Boesch 2003).

I found acoustic variants in B-calls, but one might consider the classification results as weak. Indeed, the difference between the number of correctly classified calls and the ones expected by chance was only moderately significant in females and not significant in males. These levels of correct classification to the emission context are low compared to other studies (e.g. Price *et al.* 2015), and thus raise the question of whether the differences are biologically relevant and sufficient to allow discrimination by receivers. In the end, playback experiments are needed, but in the meantime it is worth pointing out that the sample sizes were small, the statistical tests were performed on only one call type and B-calls are structurally very simple calls (Figure 3.1), especially if compared to other primate alarm calls (e.g. Crockford and Boesch 2003; Ouattara *et al.* 2009c; Price *et al.* 2015). In this view, it was noteworthy that the classification rate was significant. Moreover, it is possible that sequences emitted in the predator context represent a mix of predatory and ground B-calls because of movements of callers towards the ground to check on the threat. As such, it seems likely that the classification results underestimate the true differences between the two contexts. Therefore, these results suggest the existence of at least two context-specific variants of B-calls, but only future playback experiments will show whether these subtle differences can actually be perceived by receivers.

The minimum frequency was the main parameter allowing for discrimination between the B-call acoustic variants, with the B-calls given to terrestrial predators being higher-pitched than those given in the ground context. Similar increases of minimum frequency with higher arousal have been frequently observed in mammals and birds (Perez *et al.* 2012; Briefer 2012), in line with Morton's (1977) motivation-structural rules. The presence of a predator may be a more stressful situation for the caller, and should result in a higher minimum frequency compared to the arguably less stressful situation of moving towards or near the forest floor.

I found acoustic differences between the alarm and descending contexts in females but not in males. In general, the hypothesis is that pair-living primates, such as titi monkeys, do not show sex differences in vocal repertoires and use their calls in similar ways (Snowdon 2017) in contrast to species with other breeding systems (e.g. Gautier and Gautier-Hion 1982; Stephan and Zuberbühler 2016). Male titi monkeys may indeed produce two acoustic variants but my study failed to show it. In many animal species, males are more engaged in anti-predator behaviour (e.g. van Schaik and van Noordwijk 1989; Brunton 1990), suggesting that male alarm call sequences to terrestrial predators consisted of a mix of predator and ground B-calls, likely emitted while descending near the predator to check on it, more so than in females. This hypothesis needs to be tested in the future with systematic data.

My study also went beyond more traditional analyses insofar as I also analysed differences at the level of the sequential structure. Here, I found that B-calls were emitted more regularly in the predator than in the ground context. Similar effects have been reported in black-capped chickadees (*Poecile atricapilla*), which produce "chick-a-dee" calls with a shorter time interval between the "chick" and "dee" syllables and more "dee" syllable when encountering small, manoeuvrable raptors than large ones (Templeton *et al.* 2005).

Snowdon *et al.* (1997) suggested that non-social calls (e.g. alarm calls) show less variability than calls used in intragroup social interactions (e.g. contact calls) because alarm calls require quick responses from recipients. This has been shown at the spectral level for primates and birds (Charrier *et al.* 2001; Lemasson and Hausberger 2011; Bouchet *et al.* 2012) but to the best of my knowledge has not been tested on call sequence structure. These results can be interpreted such that temporal variability in call sequences is also linked to the degree of social significance of the signal. B-sequences emitted in response to predators may be less socially relevant and thus more regular, than B-sequences when the caller is signalling his movement towards the ground to other members of the group.

Since the coefficient of variation of the call interval is a sequence feature, it may be too costly for receivers to wait until the emission of (at least) three calls to perceive this feature. Thus, differences in acoustic structure may be more important for early decisions about the call-eliciting event, which does not prevent variation in the call interval to convey further information about the context later on. Moreover, although B-call sequences are redundant, call intervals will reassure recipients and enhance discriminability after a few repetitions. However, whether titi monkeys rely on acoustic and/or sequential parameters to attribute meaning about the eliciting context needs be tested with playback experiments.

Alarm calls to predators can have various functions, such as signalling detection to a predator or warning members of the group (see review in Zuberbühler 2009), but the function of the ground B-call sequences are less evident. I can think of several possibilities. First, ground B-calls may signal the caller's own perception of enhanced risk. Foraging in lower strata may be more dangerous, due to higher predation risk (Mourthé *et al.* 2007). B-calls sequences thus provide relatively specific information about the caller whereabouts, which may be relevant to other group members, as also documented in pied babblers *Turdoides bicolor* or Diana monkeys *Cercopithecus diana* (Uster and Zuberbühler 2001; Radford and Ridley 2007). Callers, for example, may elicit higher levels of vigilance from other group members, which increases their own safety. Second, ground B-calls sequences could indicate that no predator is around and that it is safe to forage near the ground, like the "guarding" close calls in meerkats *Suricata suricatta* (Townsend *et al.* 2011). However, I regard this as a less plausible scenario, simply because the two B-call variants are very similar, with a corresponding high risk of misunderstanding, which is also indicated by the less than 100% classification results. Further playbacks are needed to understand the main function of the ground B-call sequences, but it is likely that titi monkeys categorise both event types, going near the ground and terrestrial predator, in similar ways, e.g. as threats (real or feared) related to the ground (Zuberbühler and Neumann 2017). Going down may be perceived as dangerous, simply because terrestrial predators are likely to be encountered (Mourthé *et al.* 2007).

It is a common finding, across many nonhuman primate species, that calls associated with terrestrial disturbances are also given in other contexts (e.g. Fichtel and Kappeler 2002; Wheeler 2010), which has questioned the notion of functionally referential alarm calls (Macedonia and Evans 1993; Fischer and Price 2016). This study adds an additional layer of complexity to this debate, because of context-dependent acoustic and sequential structures in titi monkey "terrestrial alarm" calls. Also relevant is that the production criterion of functional reference is generally difficult to operationalize, since context is always defined by the observer, and this may be different from how animals categorise the world (Zuberbühler and Neumann (2017). Moreover, calls can exhibit different degrees of context-specificity, varying from a classification success of 100% to a statistically significant classification success, like the B-calls of titi monkeys. As such, it appears important that future work explores the concept of context-specificity to get a better understanding of what constitutes context-specific and -unspecific, or better even, to develop a continuous measure of *how* context-specific call types are (Zuberbühler and Neumann 2017; see also Scarantino and Clay 2015). Such research seems essential to understand better the "potentially more complex processes underlying responses to more unspecific calls" (Wheeler and Fischer, 2012, p. 195).

To conclude, titi monkey B-calls seem to have the potential to provide listeners with information about external events, which encourages careful analyses of terrestrial alarm calls and other vocalizations to check for the presence of acoustic and sequential variants. From the recipient's perspective, further experiments are needed to determine whether call variants are discriminated and whether additional contextual cues are taken into account (Scarantino and Clay 2015). Future work on the evolution of referential signalling and its potential roots in primate signalling will need to address these points, notably if callers direct their calls to specific recipients and, in doing so, take their mental states into account.

DATA AVAILABILITY

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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CHAPTER 4. DO TITI MONKEY ALARM SEQUENCES CONVEY INFORMATION ABOUT PREDATOR TYPE AND LOCATION?

ABSTRACT

Black-fronted titi monkeys *Callicebus nigrifrons* emit A-calls to aerial predators and B-calls to terrestrial predators, and previous work has suggested that the two calls can be combined into four different sequences to provide information about predator type (aerial vs. terrestrial) and location (ground vs. canopy). However, previous analyses were preliminary. Here, I collected a large set of titi monkey vocal utterances by exposing six groups at the Santuário do Caraça (Brazil) to two terrestrial predators (tayra *Eira barbara*; oncilla *Leopardus tigrinus*) and one aerial predator (caracara *Caracara plancus*), presented either on the ground or in the canopy. I extracted several metrics from each sequence and modelled what information is encoded by each metric. I found that some metrics encoded for predator type while others encoded for the combination of predator type and location, but no metric encoded for location only. Overall, conveyed information seemed to focus more on the predator type rather than on the predator location. These results are partially congruent with earlier research conducted on the same groups, and confirm that titi monkeys encode both predator type and location in their sequences, but suggest that this process may be different from what has previously been reported.

INTRODUCTION

Animal signals are sometimes investigated to understand how human language, and especially linguistic reference, evolved from an earlier, animal-like communication systems in ancestral humans. One way to investigate whether animals can refer to external events has first been to search for cases of animals using vocal signals in analogous ways to human words (e.g. Struhsaker 1967; Seyfarth *et al.* 1980a, b). Such evidence was found in vervet monkey alarm calls, although it was difficult to determine whether these signals referred to external events, such as the predator type, to the escape strategy, or to internal states of the caller. To remain agnostic about the underlying mental processes, Macedonia and Evans (1993) suggested the term "functionally referential" to designate signals that are context-specific (production criterion) and that elicit specific responses in the receivers even in the absence of the referent (perception criterion).

Most studies investigating animal semantics are focused on call structure, since a lot of information can be conveyed through spectral and temporal features of vocalizations. For example, formant frequency of the corncrake (*Crex crex*) provides information about the identity of the caller (Budka and Osiejuk 2013) and baboon males (*Papio cynocephalus ursinus*) of high quality produce loud "wahoo" calls with a higher fundamental frequency and a longer "hoo" syllable than males of lower quality (Fischer *et al.* 2004).

However, vocal utterances are often organized sequentially, providing an additional layer of information that is often ignored in acoustic analyses of single units. Information can be conveyed through different mechanisms, such as repetition, diversity, combination, ordering and overlapping of vocal units, as well as timing (i.e. temporal spacing of units) (Kershenbaum *et al.* 2014a). For example, European starlings (*Sturnus vulgaris*) produce songs that differ in duration and order of phrases between spring (i.e. the breeding season) and autumn (nonbreeding season) (Alger *et al.* 2016). Males rock hyraxes (*Procavia capensis*) display songs in which the order of calls differs between regions (Kershenbaum *et al.* 2012). Campbell's monkeys (*Cercopithecus campbelli*) produce six different alarm call types and combine them into nine context-specific sequences (Ouattara *et al.* 2009a). However, most of the tools developed to investigate animal sequences were designed for the study of bird and cetacean songs (Kershenbaum and Garland 2015), and have rarely been applied to terrestrial mammals (Kershenbaum *et al.* 2012).

Black-fronted titi monkeys *Callicebus nigrifrons* are well known for their unique alarm system (Cäsar 2011; Cäsar *et al.* 2012a, b, 2013). They produce two alarm calls, the A-call and the B-call, that they combine into complex alarm sequences whose composition and syntax vary with the predator type and location (Cäsar *et al.* 2013), suggesting a preliminary form of zoo-syntax (Cäsar and Zuberbühler 2012). However, the analysis of the sequence structure in Cäsar's study remained simple, suggesting that further investigation using advanced methods for sequence analysis could provide new insights in our understanding of titi monkey semantic capacities.

In this study, I combined an experimental approach with statistical modelling to investigate meaning in titi monkey alarm sequences. Extracting the meaning of acoustic sequences requires to compare sequences within and between contexts and quantify the resulting differences. The extraction of several metrics, a quantitative measure inherent to the sequence itself, allows for the quantitative characterization of a sequence, which then facilitates the comparison. I furthermore developed a method to assess what information is encoded by these metrics.

METRICS

A large number of metrics can be used to compare sequences with each other (Kershenbaum 2014; Kershenbaum *et al.* 2014b, a; Kershenbaum and Garland 2015) but since titi monkey alarm sequences are simple (composed of two call types only), I considered fifteen relevant metrics to compare sequences (Table 4.1). To control for the small size of my sample, I used Bayesian statistics to extract metrics that relied on counts (Table 4.1).

Bayesian probabilities

In sequence studies, results can be biased by rare events or small sample sizes. Using the maximum likelihood paradigm, the occurrence of an event i (e.g. the occurrence of a specific call type) is given by mean = number of i / total number of events. In case of rare events in small sample size, the probability of an event that is not observed would be assigned the value of 0, as if it was an impossible event, while the value of 1 would be assigned to an event that is always observed, as if it was a requirement. To address this issue, I applied Bayesian method to calculate the occurrences of events, as in Alger *et al.* (2016). This paradigm calculates a prior distribution, based on our current knowledge, that is updated to create a biologically relevant posterior distribution of events that are never or always observed. The resulting values may approach but not equal 0 or 1.

I used the Dirichlet distribution as the prior distribution with $\alpha=1$ (see Alger *et al.* 2016 for more details on the technique). The resulting Bayesian posterior mean for the occurrence of i is mean= count of event i + α / (total number of events + $k\alpha$) with k, the number of possible events. In the Bayesian framework, the only probabilities being equal to 0 or 1 are those set by the design from my beliefs.

Table 4.1 Metrics used in the analysis

Metric	Transformation	Model	Error structure
Proportion of A-calls	Bayesian probabilities	GLMM	Beta
Slope of elements	Binary (is the slope 0 or not)	GLMM	Binomial
Mean call interval	Log-transformation	LMM	Gaussian
CV call interval	-	GLMM	Gamma
2-grams AA proportion	Bayesian probabilities	GLMM	Beta
2-grams AB proportion	Bayesian probabilities	GLMM	Beta
2-grams BA proportion	Bayesian probabilities	GLMM	Beta
2-grams BB proportion	Bayesian probabilities	GLMM	Beta
2-grams slope	Binary (is the slope -2.7 or not)	GLMM	Binomial
Shannon entropy	Binary (is the slope negative or not)	GLMM	Binomial
Transition probability Start to A	Bayesian probabilities	GLMM	Beta
Transition probability A to A	Bayesian probabilities	GLMM	Beta
Transition probability A to B	Bayesian probabilities	GLMM	Beta
Transition probability B to A	Bayesian probabilities	GLMM	Beta
Transition probability B to B	Bayesian probabilities	GLMM	Beta

Details about the hypotheses and model formulas are given in Table 4.3

Proportion of A-calls

Here, I calculated the proportion of A-calls in each sequence (number of A-calls / number of calls in the sequence) with the Bayesian method to control for small sample bias. The proportion of call types in the sequence is known to convey information about the predator type in titi monkey alarm sequences (Cäsar *et al.* 2013).

Slope of elements

To highlight structure in the sequence, I calculated the probability of observing an A-call at each place in the sequence. I then graphically represented this metric, performed a linear regression and reported the coefficient of regression. This coefficient represents the slope of the regression line. If the slope equals zero, A-calls are not typically found at specific places in the sequence.

Call interval (mean and coefficient of variation)

I studied the temporal structure of alarm sequences by analysing the silence gap between two calls. To do so, I extracted the mean call intervals of each sequence, as well as a measure of regularity of call emission, the coefficient of variation of the call intervals (CV= standard deviation / mean). A low CV indicates that calls are regularly emitted in the sequence, while a high CV indicates that call intervals are variable in the sequence, with a mix of longer and shorter intervals. Studies have shown that the temporal structure of a sequence can convey information about the context (Warkentin *et al.* 2001; Templeton *et al.* 2005; Berthet *et al.* 2018) (see Chapter 3).

2-grams proportion and 2-grams slope

Repetition of units is an important feature of animal sequences (Kershenbaum 2014; Kershenbaum *et al.* 2014b). A N-gram is a sub-sequence of length N. Here, I investigated 2-grams, i.e. the four possible sub-sequences consisting of two calls, AA, AB, BA and BB. First, I calculated the proportion of each 2-gram in the sequences (i.e. number of each 2-gram / total number of 2-grams) and applied a Bayesian correction to control for small sample size. Second, to quantify the repetition of elements, I extracted the 2-grams distribution (Jin and Kozhevnikov 2011; Kershenbaum and Garland 2015): I graphically represented the probability of appearance by decreasing probability and extracted the coefficient of regression (later referred as 2-grams slope). When the 2-grams slope is different from 0 then one 2-gram is more represented in the sequence.

Shannon entropy

Shannon entropy uses principles of information theory (Shannon 1948) to measure complexity in a sequence, a technique that has been successfully used in animal communication (McCowan *et al.* 1999; Kershenbaum 2014; Ivanitskii *et al.* 2017). Entropy evaluates the unpredictability of a sequence, i.e. the degree of randomness in the sequence. Several values can be considered: the zero-order entropy evaluates the diversity of the vocal repertoire with $H0 = \log_2 N$, where N is the repertoire size; the first-order entropy assesses the proportion of different elements in the sequence, with $H1 = -\sum p(x) \log (x)$, where p(x) is the probability of a syllable x occurring in the sequence. The second-order entropy measure the proportion of different combination of two elements in the sequence, with $H2 = -\sum p(xy) \log (xy)$, where p(xy) is the probability of a syllable y following a syllable x in the sequence. If one plots the entropic values for the different orders (from 0 to 2), the slope provides a measure of organizational complexity (McCowan *et al.* 1999). A large negative slope indicates an important sequential organization, and thus high communication capacities, while a slope of zero indicates a random organization, with a low communicative capacity.

Transition probabilities

Markov chains are often used for sequence order analysis (McCowan *et al.* 1999; Jin and Kozhevnikov 2011; Kershenbaum *et al.* 2014a). The Markov paradigm assumes that probabilities of future events are dependent on a finite number of previous events. A transition matrix M can be derived from this assumption, in which $M_{i,j}$ represents the probability that an event j follows an element i. Chains of events are often represented with a state “Start” at the beginning, and a state “End” in the end (e.g. Alger *et al.* 2016). However, recent analysis suggest that Markov chains are not the most powerful tool to highlight structure in animal sequences (Jin and Kozhevnikov 2011; Kershenbaum *et al.* 2014b). Moreover, Markov chains require exponential distribution of the durations, which is not the case of the current data set. To address this issue, I conducted a semi-Markov analysis (Cane 1959). Semi-Markov analysis requires that distribution of durations of the states is independent of the previous states or its place in the sequence. I verified with graphical assessments that the place of the call in the sequence did not influence its duration.

In this study, a titi sequence can be presented as a chain of events A-call and B-call with an artificial “Start” state at the beginning of the chain but no “End” state in the end, since I did not study the whole sequence. Hence, I extracted the Bayesian transition probabilities from Start to A, A to A, A to B, B to A and B to B for each sequence. Start to B was not considered here since it is negatively correlated with Start to A (Start to B = 1 – Start to A).

2-grams and transition probabilities provide complementary information, the first one describing the probability of occurrence of a 2-calls syllable, the other one describing the probability that one call follows another one. For example, in a sequence AAAAABA, the 2-gram BA has a probability of occurrence of 1/6 while the transition probability from B to A is of 1.

METHODS

Study Subjects and Site

This study was conducted between May 2015 and August 2016 at the “Reserva Particular do Patrimônio Natural Santuário do Caraça”, on the six groups of habituated black-fronted titi monkeys *Callicebus nigrifrons* described in Chapter 2.

Experimental procedure

Predator presentations were conducted using four stuffed predators as stimuli: one aerial predator (two models of caracaras *Caracara plancus*) and two terrestrial predators (one model of tayra *Eira Barbara* and one of oncilla *Leopardus tigrinus*) presented on the ground or in the canopy. Description of the method can be found in Chapter 2. I ran a total of 35 successful trials.

Datasets

Since I focussed on sequences, i.e. utterances of more than one single call, I discarded the three vocal reactions composed of only one call from my dataset.

I included alarm sequences (n=25) from Cäsar *et al.* (2011; 2013). To keep consistent with our data, I discarded sequences in which individuals were already calling at a flying bird before detection of the model (n= 1), when another species gave alarm calls to the observers or to the model just before visual detection (n=1) and vocal reaction composed of only one call (n=1). In sum, I included n=22 sequences from previous research to my n=32 sequences, i.e. the total dataset was composed of n=54 sequences (Table 4.2).

Table 4.2 Description of the sequences dataset, with the year of each experiment and the total number of sequences per group and per condition. Experiments between 2008 and 2010 were carried out by Cäsar et al. (2011; 2013), those from 2015 by MB

	Aerial predator		Terrestrial predators				
	Caracara		Oncilla		Tayra		
Group	Ground	Canopy	Ground	Canopy	Ground	Canopy	Total
A	2010	2008	2009, 2015	2010, 2015	2008, 2016	2016	9
D	2015	2008	2009, 2015	2010, 2015	2008, 2016	2016	9
M	2010, 2015	2009, 2015	2009, 2015	2015	2016	2016	9
P	2010, 2016	2008, 2016	2010, 2015	2010, 2016	2009, 2016	2016	11
R	2010	2008, 2015	2009, 2015	2010, 2015	2009, 2016	2016	10
S	2016	2016	2015	2016	2016	2016	6
Total	8	9	11	10	10	6	54

Metric extraction

I only focused on the first ten calls of each sequence: the duration of emission of the first ten calls emitted during the predator presentations ranges from 3.0 to 133.4 seconds (mean=16.2, s.d.=22.7), which I consider long enough to convey urgent information about a pending threat.

One observer (MB) labelled each of the calls and measured the duration of each call interval, i.e. the silence between each call, by using Praat 5.3.84 (Boersma and Weenink 2009) (Spectrogram, Hanning window, time resolution: 5 ms, frequency resolution: 88 Hz). The 15 metrics previously detailed were extracted from each sequence by using the R software version 3.4.1 (R Development Core Team 2017) and the cfp package (Neumann 2017).

Statistical analysis

I used multi-model inference in an information-theoretic framework (Burnham and Anderson 2002). The aim of this method is to compare relative support for each model in a *set of models* by using the Akaike weight *w* (Anderson 2008; Burnham *et al.* 2011). This weight gives the probability that the model is the best among the set of considered models, ranging for 0 (weak support) to 1 (strong support).

To assess whether titi monkeys encoded information about the predator type and/or location, I created six models for each metric, each model corresponding to a combination of predator type and location. The first model included only the predator type as predictor, which addressed the possibility that the metric encoded for predator type only. The second model only included predator location as predictor, which addressed the possibility that the metric only encoded for predator location. Two more models addressed the possibility that sequences contained information about predator type and location: one model contained both main effects and the other contained in addition the interaction term for location and type. In all models, I controlled for distance of detection (in meter) to avoid a bias due to urgency. For the transition probability from Start to A, it was not possible to create models that addressed the possibility that predator type and location were encoded in the sequence: the first call had only two possibilities, A and B-call, it could only give one information at the time, about either predatory type or location, but not both. Thus, I only created the models testing for predator type only and predator location only for this metric.

Finally, I included two control models: one intercept-only model (later referred as null model), and another one with the distance of detection only (later referred as urgency model). All models were mixed models in which the identity of the group was fitted as random intercept. Description of the general set of models is given in Table 4.3.

Whenever possible, I log-transformed the metric to fit a Gaussian distribution: for these metrics, the five models were linear mixed models (LMMs). For metrics whose distribution did not fit the Gaussian even after transformation, the five models were generalized linear mixed models (GLMMs) with a Beta, Gamma or Binomial error structure (Table 4.1). For each metric I ranked the set of six candidate models and I used Akaike's weight *w* to infer the best model among the set of models (Burnham and Anderson 2002).

To graphically represent statistical uncertainty around the model estimates I used a non-parametric bootstrap procedure. For this, I created 1,000 data sets that were drawn from the original data set by selecting observations with replacement so that each data set comprised as many observations as the original data set. For each data set, I refitted the model and extracted and plotted model results.

All statistics were conducted using the R software version 3.4.1 (R Development Core Team 2017). LMMs were fit using the lme4 package (Bates *et al.* 2015) and GLMMs using the glmmADMB package (Fournier *et al.* 2012), model selection was performed with the MuMIn package (Barton 2016). Collinearity of the variables was controlled for each model using the package car (Fox and Weisberg 2011). Bootstrap were performed with the resamplefunction from the cfp package (Neumann 2018). R scripts of the whole analysis are provided in the data repository.

Table 4.3 Models used to investigate whether information about predator type and/or location is encoded in the metrics. Models details can be found in Table 4.1

Encoded information	Model	Formula	Intercept
Predator type only	Predator type	Metric ~ type + distance	group
Predator location only	Location	Metric ~ location + distance	
Predator type and location	Added type and location	Metric ~ location + type + distance	
	Interaction type and location	Metric ~ location * type + distance	
Neither predator type nor location	Urgency	Metric ~ distance	
	Null	Metric ~	

Interpretation

Two outcomes are possible for the model ranking. First, one of the explanatory models (i.e. models that are not control models) receives unequivocal support ($w_i \geq 0.8$). In this case, I interpret this model as being the best model among all the tested models: the hypothesis expressed by the model is highly supported.

Second, no explanatory model receives unequivocal support, i.e. either one of the control models receives substantial support, or there is no single model that receives high support. In this case, none of the hypotheses expressed by the models is highly supported.

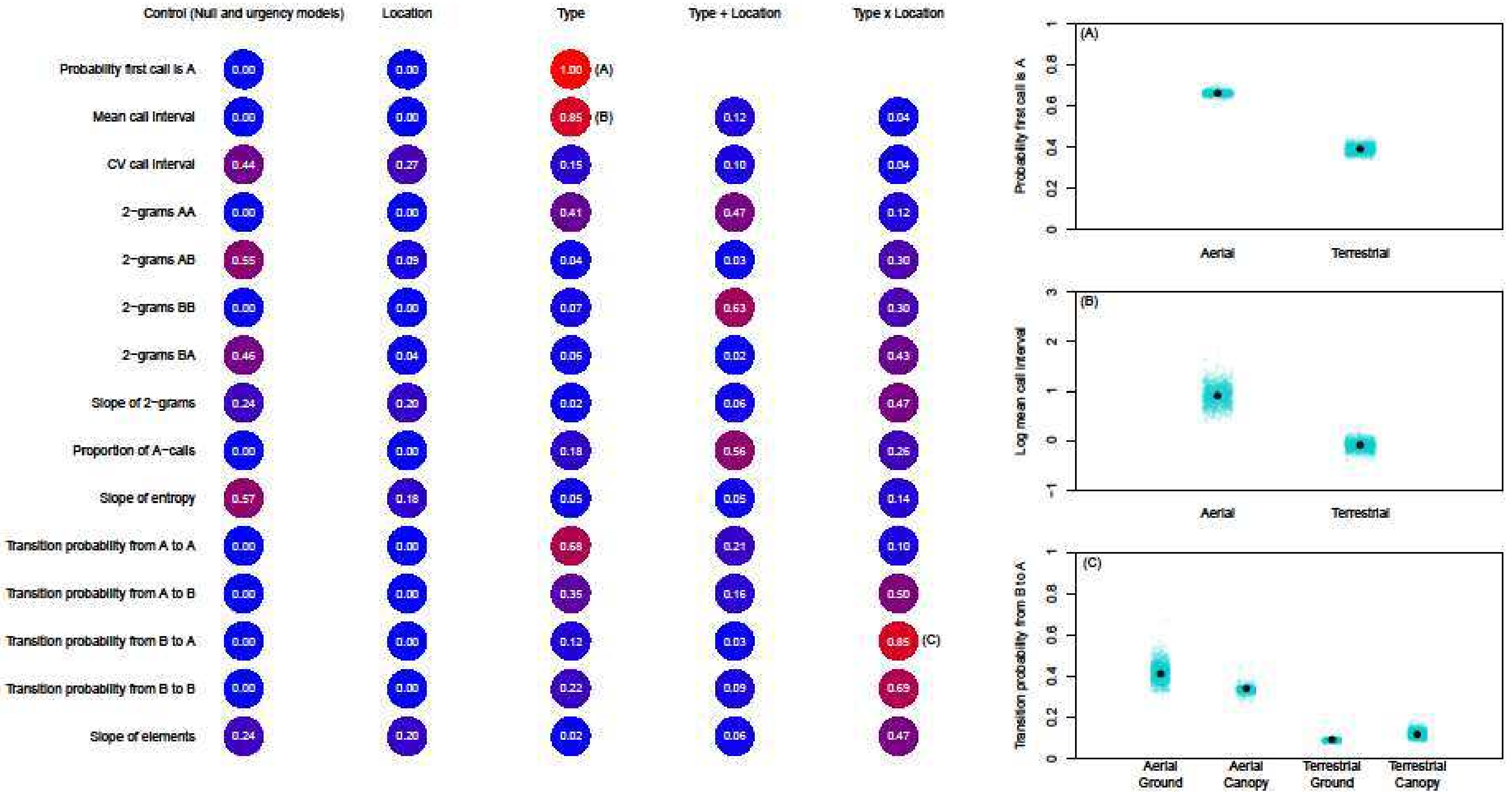
RESULTS

Model selection results indicated that, at the sequence level, titi alarm calls predominantly encoded information about predator type (probability that the sequence starts with an A-call, mean call interval) and predator type in interaction with location (transition probability from B to A-call). None of the investigated metrics encoded for location alone and most metrics encoded neither for type nor location (Figure 4.1).

Closer inspection of the three relevant metrics revealed that the first call was typically an A-call in sequences emitted to aerial predators or a B-call if emitted to terrestrial predators (Figure 4.1). Also calls were emitted faster during encounters with terrestrial than aerial predators (Figure 4.1).

The probability that an A-call followed a B-call provided information about both predator type and location: the transition probability from B to A was higher for aerial than terrestrial predators, but this was higher if given to an aerial predator on the ground than in the canopy, while it was lower if given to a terrestrial predator on the ground than in the canopy (Figure 4.1).

Overall, these results suggest that titi monkeys encode mainly predator type, with some added interactional information about predator location.



	Control (Null and urgency models)	Location	Type	Type + Location	Type x Location
Probability first call is A	0.00	0.00	1.00 (A)		
Mean call interval	0.00	0.00	0.85 (B)	0.12	0.04
CV call interval	0.44	0.27	0.15	0.10	0.04
2−grams AA	0.00	0.00	0.41	0.47	0.12
2−grams AB	0.55	0.09	0.04	0.03	0.30
2−grams BB	0.00	0.00	0.07	0.63	0.30
2−grams BA	0.46	0.04	0.06	0.02	0.43
Slope of 2−grams	0.24	0.20	0.02	0.06	0.47
Proportion of A−calls	0.00	0.00	0.18	0.56	0.26
Slope of entropy	0.57	0.18	0.05	0.05	0.14
Transition probability from A to A	0.00	0.00	0.68	0.21	0.10
Transition probability from A to B	0.00	0.00	0.35	0.16	0.50
Transition probability from B to A	0.00	0.00	0.12	0.03	0.85 (C)
Transition probability from B to B	0.00	0.00	0.22	0.09	0.69
Slope of elements	0.24	0.20	0.02	0.06	0.47

Figure 4.1 Results of the multimodel inference (left) and bootstrapped model estimates of metrics that strongly support a hypothesis (right). Left panel: In rows are each metrics, in columns are each hypothesis, each circle illustrates the weight, i.e. the probability that the metric supports the hypothesis, ranging from blue (*w* = 0, weak support) to red (*w* = 1, strong support). For simplicity of reading, the null and urgency models were merged as "control models" and their weights were added. Right panel: (A) The Bayesian probability that the first call is an A-call encodes for predator type; (B) the mean call interval also encodes for predator type, (C) the Bayesian transition probability from a B-call to an A-call encodes for both predator type and location, in an interaction fashion

DISCUSSION

This analysis shows that titi monkeys encode information about the predator type and, to a lesser degree, its location in their alarm sequences. The type of the first call and the call rate give information about the predator type, while the transition probability from B to A-call gives information about both the predator type and location. Predator type was more strongly encoded than predator location and no metric encoded for predator location only.

The current results thus corroborate Cäsar *et al.* (2013) who also found that titi monkey alarm sequences encode predator location and type, and suggested that sequences were characteristic of predator type with variation indicating an uncharacteristic location, i.e. an aerial predator on the ground or a terrestrial predator in the canopy. However, there are a number of important differences between the current findings and results reported by Cäsar *et al.* (2013). In their study, sequences composed of A-calls were characteristic of aerial predators, and sequences composed of B-calls were characteristics of terrestrial predators, a result not congruent with the current analysis: the variable "proportion of A-calls" did not encode for predator type (model weight of 0.18, see Figure 4.1). Cäsar *et al.* (2013) also suggested that location was indicated by the occurrence of optional switching to B-calls later in the sequence when the aerial predator was on the ground, while location was conveyed by the optional adjunction of a single A-call at the beginning of the B-sequence when the terrestrial predator was in the canopy. This sequence description can be compared to the "transition from A to B-call" metric: if my results were congruent with those of Cäsar, the transition probability from A to B would be close to zero for aerial predators in the canopy, and higher for aerial predator on the ground because of the interspacing of B-calls in the A-sequence. For terrestrial predators, it would be high when it is in the canopy because of the adjunction of A-call at the beginning of the B-sequence, and would be close to 1 for terrestrial predator on the ground, because A-calls should normally not appear in these sequences and thus, the sequence should go back to B-calls very quickly. However, the current results suggest that the transition probability from A to B-call does not encode for added or interaction effects of predator type and location (model weight of 0.16 and 0.5 respectively, Figure 4.1), which is not congruent with Cäsar's results. Finally, Cäsar *et al.* (2013) also showed that the call rate was faster when sequences were emitted to terrestrial than to aerial predator, which is congruent with my results: I showed that the mean call interval coded for predator type (model weight of 0.85) and was lower for terrestrial predators than for aerial predators (Figure 4.1).

Overall, my results suggest that, indeed, titi monkeys encode for both predator type and location in their sequences, but the coding scheme may be different from the one initially described. Differences can be explained firstly by the fact that I only considered the 10 first calls, and not 30 as done in Cäsar *et al.* (2013). However, regarding the fact that a 10-calls sequence last around 15 seconds (see Methods), I considered that most of the relevant information were already present after the emission of 10 calls: in a predatory event, listeners should receive all the relevant information quickly to be able to react adaptively. By limiting my analysis to 10 calls only, I limited bias due to the habituation to the predator presence – especially since the predator is static, which is not a natural situation for monkeys- and other events (e.g. influence of the arrival of other individuals that were not present at the time of the detection), while I made sure that all the relevant information were probably already conveyed.

Second, differences between my study and Cäsar *et al.* (2013) can be explained by the fact that, contrary to them, I did not observe A-call at the beginning of the B-sequences in most of the terrestrial in the canopy conditions (11/16 trials, Table A 1). This difference confirms that it is crucial to replicate experiments to conclude on one species vocal system: small sample can easily misrepresent reality (Munafò *et al.* 2017).

Dual encoding of information has been described in only few species. Meerkats *Suricata suricatta* emit alarm calls whose acoustic structure varies on the level of urgency and the predator type (Manser 2001). Black-capped chickadees *Poecile atricapilla* encode predator behaviour and size in their alarm utterances (Templeton *et al.* 2005), while Carolina chickadees *Poecile carolinensis* encode information about the caller behaviour and location as well as the presence of aerial predators in their chick-a-dee calls (Freeberg 2008). However, it may seem surprising that titi monkeys mainly encode predator type over predator location. Indeed, in a system with dual information encoding like this one, it would seem more adaptive to emphasize location-related information rather than predator-related information, since efficiency of the escape strategy may be greater when knowing where the predator is before knowing what it is. However, it is a common finding in animal vocal systems that predator types are the main – or even only - feature encoded in the vocal utterances (Townsend and Manser 2013), suggesting that escape strategies, and thus, survival success, are mainly relying on the hunting strategy of the predator rather than on their location.

The next step in the analysis is to test how receivers can extract information from these alarm sequences. Indeed, variation in acoustic signals can fail to elicit appropriate reaction from the listeners (e.g. Schibler and Manser 2007), suggesting that listeners do not perceive these variations or do not consider them as relevant. Moreover, playbacks experiments could show what metric is used by listeners: indeed, maybe that the metrics that were highlighted by the model ranking are not the ones that are actually used by the monkeys to extract information.

DATA AVAILABILITY

The datasets generated and the Rscripts used for the current study are available in the following Figshare repository:

https://figshare.com/projects/Probabilistic_meaning_in_titi_monkeys_alarm_call_sequences/30488

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CHAPTER 5. WHAT DO TITI MONKEY ALARM SEQUENCES MEAN?

ABSTRACT

In previous work I have shown that titi monkeys *Callicebus nigrifrons* combine A and B-calls to communicate about predator type (aerial vs. predator) and, to a lesser degree, location (ground vs. canopy). To investigate whether listeners process these sequences, I carried out playback experiments to six free-ranging groups in the Santuário do Caraça, Brazil, to assess what information titi monkeys extracted from the alarm sequences, and to infer what sequence metric listeners attended to. I found that callers extracted information about predator type and location by using the proportion of B-call combinations in the sequences, suggesting that meaning was probabilistic rather than categorical. My results suggest that combinations are a key component of titi monkey vocal communication, and that current theories of animal communication need to integrate more explicitly the probabilistic nature of utterances to understand the evolution of semantics.

INTRODUCTION

The search for meaning in animal communication aims to understand how semantic reference of human words evolved from animal-like signals. Animal vocalizations can convey information about features of the caller (e.g. Ey *et al.* 2007), its internal state (Morton 1977; Briefer 2012; but also see Seyfarth and Cheney 2003 for why calls caused by internal states do not prevent them from conveying information about external events) but the closest equivalent to semantic reference in animals are the functionally referential signals. These signals are specific to external events and elicit adaptive response from the listeners in the absence of context cues (Macedonia and Evans 1993): they are functionally equivalent to human words but since underlying mechanisms of call production remain unclear, it cannot be assessed whether they are cognitively equivalent (Sievers and Gruber 2016).

Information can be encoded in the acoustic structure of the call alone, but also by call combinations. Phonology is the capacity of combining meaningless sounds (phonemes) into meaningful elements (morphemes), while syntax is the capacity of combining morphemes into larger structures, like words or phrases, of which meaning depends on the meaning of its parts and of the rules that combine them. Syntax is found in several species. For example, females Diana monkeys combine two morphological units, one conveying information about the external event and one about the identity of the caller, and receiver can extract this information from the call combinations (Coye *et al.* 2016). In Campbell's monkeys *Cercopithecus campbelli*, adjunction of a suffix "–oo" to a leopard alarm call "Krak" is associated with a general alarm (Ouattara *et al.* 2009a), and make listeners react less strongly than when hearing a "Krak" alone (Coye *et al.* 2015). Pied babblers *Turdoides bicolor* combine alert calls and recruitment calls into a "mobbing sequence" and listeners react more strongly to the mobbing sequences, suggesting that they can extract information about the context from syntactic combinations (Engesser *et al.* 2016). Example of animal phonology are rarer. A classic example is the one of the Chestnut-crowned babbler *Pomatostomus ruficeps* that combines meaningless A and B vocalizations into AB combinations that refer to flight, and BAB combinations that refer to nestling provisioning (Engesser *et al.* 2015).

To conclude that a signal is meaningful, it remains to be empirically assessed how recipients react to them. Several theories explain how signals can modify the behaviour of listeners. First, the manipulative hypothesis argues that the acoustic structure of alarm calls directly induces nervous-system responses in listeners (Owren and Rendall 2001). For example, mammal alarm vocalizations typically exhibit sharp onsets, high frequencies, amplitude fluctuations and high-amplitude noisiness that can impact on receiver attention and emotion and so modify their behaviour. Second, the information theory argues that listeners can learn to associate external events to different call structures and thus adjust their behaviour accordingly when hearing the calls (Seyfarth and Cheney 2003, 2010). For example, after being exposed to the association of a hawk with a neutral tone, Golden-mantled ground squirrels *Spermophilus lateralis* respond similarly to neutral tones than to natural conspecifics hawk alarm calls, suggesting that they learn the association between an acoustic structure and an event (Shriner 1999). Two explanations were proposed. Thompson (1995) suggested that the signal and the event are categorized as similar by the animals because they elicit the same behavioural response. Thus, the signal and the event are functionally equivalent and the association between the two does not require information transfer. A second explanation argued that call structures do not trigger direct instinctive reactions in listeners but rather elicit a mental representation of the referent associated with the call. For example, when primed with snake alarm calls, Japanese tits *Parus minor* become more efficient in finding snake-resembling objects, contrary to when primed with other alarm calls (Suzuki 2018), suggesting that listeners can modify their behaviour not only because of the acoustic structures of the calls but also because the calls elicit mental representation of their referents. This may also be the case of acoustic signals with little context-specificity: since it is not possible for the listeners to establish a strong association between an event and the call, listeners can rely on pragmatics to infer the eliciting context. This is the case for example for putty-nosed monkeys *Cercopithecus nictitans*: playbacks of general calls associated with an external event, like a tree falling, make listeners spend less time looking for the cause of the call than playbacks of general calls alone (Arnold and Zuberbühler 2013), suggesting that listeners try to infer the referent by integrating signals with ongoing contextual cues.

On the opposite, information conveyed in signals can be ignored by animals. This is the case of example for meerkats *Suricata suricatta*. Close calls of meerkats encode a group signature, and their alarm calls encode information about the caller, but listeners do not seem to discriminate between groups or callers (Schibler and Manser 2007; Townsend *et al.* 2010), either because acoustic differences highlighted by statistical analyses are not perceived by animals, or because information is not relevant for them.

All these results suggest that information can be encoded at the call or the sequence level, but that playbacks are crucial to make inferences about the content of encoded information.

I showed earlier that black-fronted titi monkeys *Callicebus nigrifrons* combine A and B-calls into sequences that encode for predator type and location (see Chapter 4) but it remains unknown whether listeners can extract information, leaving it impossible to conclude on semantic abilities of titi monkeys. Thus, in this study, I combined an experimental approach with modelling to investigate meaning in titi monkey alarm sequences. I conducted playback experiments to assess i) whether titi monkeys attend to information encoded in the sequences, and (ii) what sequential metric monkeys attend to.

METHODS

Study Subject and Site

This study was conducted from January to August 2016 at the "Reserva Particular do Patrimônio Natural Santuário do Caraça", on the six groups of titi monkeys already described earlier (see Chapter 2).

Playback stimuli preparation

Broadcasted alarm sequences consisted of 10 calls recorded during predator presentations or during natural predator encounters (see details in Chapter 2). I did not broadcast sequences recorded by Cäsar (Cäsar 2011; Cäsar *et al.* 2012a, 2013) because most of the group members were different or older from those recorded at that time (Table 2.1), which could lead to bias in the experiment.

The first aim of this experiment was to play back the four sequence categories described by Cäsar *et al.* (2013). Thus, since I only recorded two poor-quality sequences corresponding to Cäsar's terrestrial in the canopy sequence out of my twelve trials, I created artificial sequences by adding an A-call from one given individual at the beginning of a B-call sequence from the same individual. The silent time between the single A-call and the nine B-calls was measured on my recorded sequences and on two of Cäsar's sequences and the length of the silent gap for each of the artificial sequences was randomly chosen among these four measures. I sometimes had to replace bad quality calls with other calls from the same sequence and filter background noises in poor quality sequences. I normalized the volume of all the sequences at -1dB. I cut and edited the sequences using Praat 5.3.84 (Boersma and Weenink 2009), Raven 1.5 (Bioacoustics Research Program 2014) and Audacity 2.0.6. (Audacity Team 2014).

In total, I created 22 sequences: 6 Aerial Canopy, 4 Aerial Ground, 6 Terrestrial Canopy and 6 Terrestrial Ground sequences (Table 5.1). However, I removed a Terrestrial Canopy sequence of bad quality from the dataset (see Playback procedure).

Playback procedure

Seven females and seven males were tested (Table 5.2). Each individual was exposed to one set consisting of two stimuli referring to a predator (aerial vs. terrestrial predator) on the ground or in the canopy, i.e., a total of N=28 trials. The presentation of the stimuli was randomised among individuals. No more than two trials were run on the same day within a given group and never for two days in a row to avoid habituation. No stimulus was broadcasted more than twice to limit pseudo-replication.

Table 5.1 Stimulus sequences used for playback experiments

Predator type	Location	Caller	Sequence
Aerial	Canopy	DG	AAAAAAAAAA
		DG	AAAAAAAAAA
		ML	AAAAAAAAAA
		MR	AAAAAAAAAA
		RT	AAAAAAAAAA
		SH	AAAAAAAAAA
	Ground	DG	AAAAAAAAAB
		DG	AAAAAAABAA
		ML	AAAAAABBBB
		SH	AABBAABABB
Terrestrial	Canopy	AR	ABBBBBBBBB
		ML	ABBBBBBBBB
		PC	ABBBBBBBBB
		RK	ABBBBBBBBB
		RT	ABBBBBBBBB*
		SG	ABBBBBBBBB
	Ground	AR	BBBBBBBBBB
		ML	BBBBBBBBBB
		PC	BBBBBBBBBB
		RK	BBBBBBBBBB
		RK	BBBBBBBBBB
		SV	BBBBBBBBBB

* Stimulus removed from the final dataset

Sequences played to a given individual were recorded from a member of the family or a member of a neighbouring group (Table 5.2). In the first case, we made sure that the caller was out of sight of the tested individual, and the speaker was positioned so that the calls came from the direction of the caller. In the second case, we played the stimulus in the overlap area between the territory of the focus group and those of the broadcasted group, to avoid bias due to intrusion by neighbours in the territory (except in one case, a sequence from the D group was played to the R group in the overlap between S' and R' territory).

We monitored the group at least 30 minutes before and after the experiment. During the 30 minutes prior to the trial, we made sure that no duet, group encounter, loud calls from a lost individual, nor predator encounter occurred during this period, otherwise we waited another 30 minutes. We waited for the tested individual to be in low strata (1-8 m high) and in an open area to ensure a good visibility. The angle between the subject, the camera and the speaker was of about 90°, with the subject facing the camera. The speaker was covered with a camouflage net and held at the same height of the tested individual with a perch, or if not possible, at a maximum of 4 meters high. We made sure that no monkey, even if never tested, was able to see the speaker. The reaction of the tested subject was videotaped during twice the length of the broadcasted stimulus. Stimuli were played using an ANCHOR AN-Mini loudspeaker (audio output: 30 W, frequency response: 100 Hz-15 KHz) connected by Bluetooth to an IPHONE 4.2.1. We held the volume of the loudspeaker at a constant level matching natural volume of titi vocalizations to a human hear. To test the setup, the territorial call of a white-shouldered fire-eye (*Pyriglena leucoptera*) was played. This bird call is common in the study area and elicits no reaction from the monkeys.

Table 5.2 Playback experiment schedule. Each subject (Ind) was attributed two stimuli referring to the same predator (Type) in two different locations (Canopy and Ground), recorded from a given individual (Origin). For tests run on the same day, the number in brackets indicates the order of the experiments. If the first letter of the origin is the same as the first letter of the subject, then the stimulus was emitted by a member of the group of the subject, otherwise it is a member of the neighbouring group. Black triangles indicate failed experiments. The stimulus Terrestrial in the Canopy from RT was too bad quality and was removed from the analysis

			Stimulus Canopy		Stimulus Ground	
Ind	Sex	Type	Origin	Date	Origin	Date
DN	F	Aerial	RT DG	2016.05.26 ▲ 2016.08.17	DG	2016.08.09
MR	F	Aerial	ML ML	2016.08.23 ▲ 2016.08.27	ML ML	2016.08.15 ▲ 2016.08.23
SV	F	Aerial	SH	2016.08.03	SH	2016.08.12
AP	M	Aerial	RT	2016.05.03	DG	2016.08.14
PP	M	Aerial	MR	2016.06.01	ML ML	2016.07.12 ▲ 2016.08.18
RF	M	Aerial	DG	2016.08.08	SH	2016.08.28
RT	M	Aerial	DG	2016.08.26	DG	2016.01.07
AU	F	Terrestrial	AR	2016.08.14	AR	2016.08.05
DT	F	Terrestrial	RT RK RK	2016.08.09 ▲ 2016.08.17 ▲ 2016.08.25	RK	2016.03.29
SH	F	Terrestrial	SG	2016.08.19	SV	2016.08.12
RB	F	Terrestrial	RT RK	2016.08.08 ▲ 2016.08.16 (1)	RK	2016.08.16 (2)
MK	M	Terrestrial	ML	2016.08.07	ML	2016.08.15
PS	M	Terrestrial	PC	2016.08.11 (1)	PC	2016.08.11 (2)
RK	M	Terrestrial	SG	2016.08.28	DT AR	2016.03.28 ▲ 2016.08.30

I considered a trial as failed when it was not possible to code most of the gazes of the monkey because it moved during the experiment (n=6) or when the stimulus was too bad quality (n=2, the stimulus was then removed from the analysis). If a trial was failed, I waited at least 8 days to re-run it, except in one case (MR, aerial canopy: only a few calls were played so the subject did not hear the full stimulus) (Table 5.2).

Metric extraction

In the previous analysis, I showed that some metrics encoded for predator type and/or location. To find out what metrics were used by titi monkeys to extract information about a predatory event, I only considered metrics that encoded best for each hypothesis, i.e. the metric with the highest weights for this hypothesis. In other words, I selected the metric with the highest weight from each column of the Figure 4.1. Thus, I selected the probability that the sequence starts with an A-call as being the metric coding best for predator only, the coefficient of variation of the call interval as being the metric coding best for predator location only, the transition probability from a B-call to an A-call as the metric coding best for interaction between predator type and location and the proportion of 2-grams BB for the metric coding best for added predator type and location.

I applied the same procedure to extract metrics from the sequences broadcasted during playbacks than to sequences recorded during predator presentations (see Chapter 4): One observer (MB) labelled each of the calls and measured the duration of each call interval, i.e. the silence between each call, by using Praat 5.3.84 (Boersma and Weenink 2009) (Spectrogram, Hanning window, time resolution: 5 ms, frequency resolution: 88 Hz). The metrics were extracted from each sequence by using the R software version 3.4.1 (R Development Core Team 2017) and the cfp package (Neumann 2017).

Video analysis

The 28 videos recorded from the playback experiments were coded with the software Elan 4.9.4 (Max Planck Institute for Psycholinguistics 2016). The reaction of the caller was analysed during and after the playback experiment, for a total duration of twice the duration of the stimulus (i.e. the duration of the playback plus the same amount of time after the end of the stimulus). I extracted the duration (in seconds) and direction of each gaze, i.e. from the moment the subject looks to one direction until it looks to another direction. Direction of the gaze were categorized as i) upwards: the subject has the head orientated at least at 45° above the horizontal line and looks further than 1 body away from him, ii) downwards: the subject has the head orientated at least at 45° under the horizontal line and looks further than 1 body away from him, iii) towards the speaker: the subject has the head orientated within 45° relative to the line between the subject and the speaker and looks further than 1 body away from him, iv) elsewhere: the subject is looking in another direction or less than 1 body away from him (e.g. food, body part). When the gaze of the subject was not visible, the direction was noted as “Not Visible”.

The proportion of time looking in each direction was calculated from the duration the monkeys spent looking in each direction divided by the time the subject was visible.

Videos were analysed by a coder blind to the experimental conditions (AP). To assess rater reliability, two raters (AP and MB) coded three videos (10% of the total dataset). A Cohen’s kappa coefficient was calculated to control for reliability of the coding of the direction and duration of the gazes. An overlap matrix was created with the conditions (gaze directions) in row and columns (Holle and Rein 2015). Agreements were tailed on the table diagonal (same duration and same direction), disagreements on off-diagonal cells: When one coder noted a duration as one gaze bout (e.g. “Elsewhere” from 12 to 13 seconds, coder 1) and the other coded two (or more) gaze bouts for the same duration (e.g. “Elsewhere” from 12 to 12.5 seconds and

“Down” from 12.5 to 13 seconds, coder 2), the gaze bout of the first coder was cut into two bouts to facilitate comparison with the other coder’s results (e.g. “Elsewhere” from 12 to 12.5 seconds and “Elsewhere” from 12.5 to 13 seconds for coder 1; “Elsewhere” from 12 to 12.5 seconds and “Down” from 12.5 to 13 seconds for coder 2; there is agreement from 12 to 12.5 and disagreement from 12.5 to 13). The level of between-rater agreement was considered as substantial (k = 0.79, Landis and Koch 1977), but it should be stressed that this method has limits since a long agreement of several seconds counts as much as a short disagreement of half a second, so the statistical agreement is lower than reality. I thus considered that the inter-rater agreement was good.

Statistical analysis

I used the same method as detailed in Chapter 4, based on multimodel inference (Burnham and Anderson 2002): I compared relative support for each model in a set of models by using the Akaike weight w, ranging for 0 (weak support) to 1 (strong support) (Anderson 2008; Burnham *et al.* 2011).

To graphically represent statistical uncertainty around the model estimates I used a non-parametric bootstrap procedure (see Chapter 4).

All statistics were conducted using the R software version 3.4.1 (R Development Core Team 2017). GLMMs were fitted using the glmmADMB package (Fournier *et al.* 2012), model selection was performed with the MuMIn package (Barton 2016). Collinearity of the variables was controlled for each model using the package car (Fox and Weisberg 2011). Bootstrap were performed with the resamplefunction from the cfp package (Neumann 2018). R scripts of the whole analysis can be found on a Figshare repository.

Do titi monkeys attend to information about predator type and location?

I investigated the effect of the triggering stimulus that elicited the broadcasted sequence (i.e. the type and location of the predator) on the behaviour of the caller. Importantly, here I ignored the quantifiable differences between sequences given in different conditions, and simply used the eliciting situation as explanatory.

I created six models, each one assessing how the combination of eliciting predator type and location affected the time that the listeners spent looking in each direction. The first model only included the eliciting predator type as predictor, which addressed the possibility that listeners only attend to the type of predator that elicited the alarm sequence. The second model included the eliciting location of the predator only, which addressed the possibility that listeners attend to the location of the eliciting predator only. Two more models addressed the possibility that listeners attend to information about predator type and location: one model contained both main effects and the other contained in addition the interaction term for location and type. In all models I controlled for the height of the listeners (i.e., the distance between the individual and the ground, in meter) to avoid a bias due to urgency. Finally, I included two control models, one with the direction of gaze only (null model), and another one with the height and direction of the gaze of the listener only (urgency model). All models were mixed models in which the identity of the listener and the broadcasted sequence were fitted as random intercept.

All models were GLMMs fitting a binary error structure, details are given in Table 5.3. I ranked the set of six candidate models using Akaike’s weight *w* (Burnham and Anderson 2002).

Table 5.3 Models used to infer whether titi monkeys extract information about predator type and location. Models are GLMMs with a binary error structure, the dependent variable is the proportion of time spent looking in each direction

Extracted information	Model	Fixed factors	Intercept
Predator type only	Predator type	direction * (type + height)	id + stimulus
Predator location only	Location	direction * (location + height)	
Predator type and location	Added type and location	direction * (type * location + height)	
	Interaction type and location	direction * (type + location + height)	
Neither predator type nor location	Urgency	direction * height	
	Null	direction	

Do titi monkeys attend to sequential metrics?

A second analysis was also run on the reaction of receivers during the playbacks, but this time I investigated the effect of the *metrics* of the broadcasted sequence on the behaviour of the listeners, regardless of the eliciting stimulus. If the previous analysis shows that titi monkeys extract information, I expect that they attend to the metric coding best for this information.

I created six models, each assessing if sequential metric affected the time listeners spent looking in one of the four directions (upwards, downwards, towards the speaker, elsewhere). The first model only included the metric coding best for predator type as the predictor (probability that the sequence starts with an A-call). The second model included the metric coding for predator location only as the predictor (coefficient of variation of call interval). Two models addressed the possibility that listeners attended to the metric coding best for predator type and location: one model contained the metric that coded best for both main effects (i.e. the proportion of 2-grams BB) and the other one contained the metric that coded best for the interaction between predator location and type (i.e. the transition probability from B to A-call). Here again, I controlled for the height of the listeners in all the models. Finally, I included two control models, one with the direction of gaze only (null model), and another one with the height and gaze direction of the listener only (urgency model). All models were mixed models in which the identity of the listener and the broadcasted sequence were fitted as random intercepts.

Details on the models can be found in Table 5.4; the metrics used for this analysis were transformed to be consistent with the transformations performed on those metrics in the previous analysis (Table 4.1). I ranked the set of six candidate models using Akaike's weight *w* (Burnham and Anderson 2002).

Table 5.4 Models used to infer what metric was used by titi monkeys when attending to conspecific alarm call sequences. Models were GLMMs with binary error structures, the dependent variables were the proportion of time spent looking in each direction. To be consistent with the analysis performed on vocal reactions to predator presentations, metrics used as factors were transformed as described in Table 4.1

Metric	Model	Fixed factors	Intercept
Starts with A	Predator type	direction * (metric "type only" + height)	id + stimulus
CV call interval	Location	direction * (metric "location only" + height)	
2-grams BB	Added type and location	direction * (metric "location x type" + height)	
Transition B-A	Interaction type and location	direction * (metric "location + type" + height)	
None of the tested metrics	Urgency	direction * height	
	Null	direction	

RESULTS

Do titi monkeys attend to information about predator type and location?

In this analysis, I was agnostic with regards to the sequence metrics and just considered stimulus type as explaining factor for gaze reactions to playbacks of different alarm sequences.

I found that monkeys most likely attended to information about predator type and location (model with both effects, w = 0.86) while the next-best model with only predator type had a weight of 0.13. Other models representing the extraction of information about predator location only and no extraction of predator type or location had a combined weight of less than 0.01 (Table 5.5).

Visual assessment of the model results showed that the reactions of the listeners were different depending on the type and location of the predator that elicited the vocal sequence (Figure 5.1). When hearing a sequence recorded from an encounter with an aerial predator, titi monkeys looked more upwards and less towards the speaker than when the sequence was recorded from an encounter with a terrestrial predator. In the same way, sequences recorded from encounters with predators in the canopy elicited more gaze upwards and less towards the speaker than sequences recorded from predators on the ground. These results suggest that titi monkey can extract information about both the predator type and location in an additive fashion from alarm sequences.

Table 5.5 Results of the model ranking investigating what information is extracted from the alarm sequence by listeners. Models are ordered by their AICc scores

Hypothesis of information extraction	AICc	ΔAICc	Weight
Addition type and location	431.73		0.86
Predator type only	435.47	3.75	0.13
Interaction type and predator	440.68	8.96	0.01
Urgency	477.36	45.64	0.00
Predator location only	479.29	47.57	0.00
Null	502.07	70.34	0.00

AICc: Akaike's information criterion correction for small sample size; ΔAICc: difference in AICc scores between the best model and each model; Weight: likelihood of the model

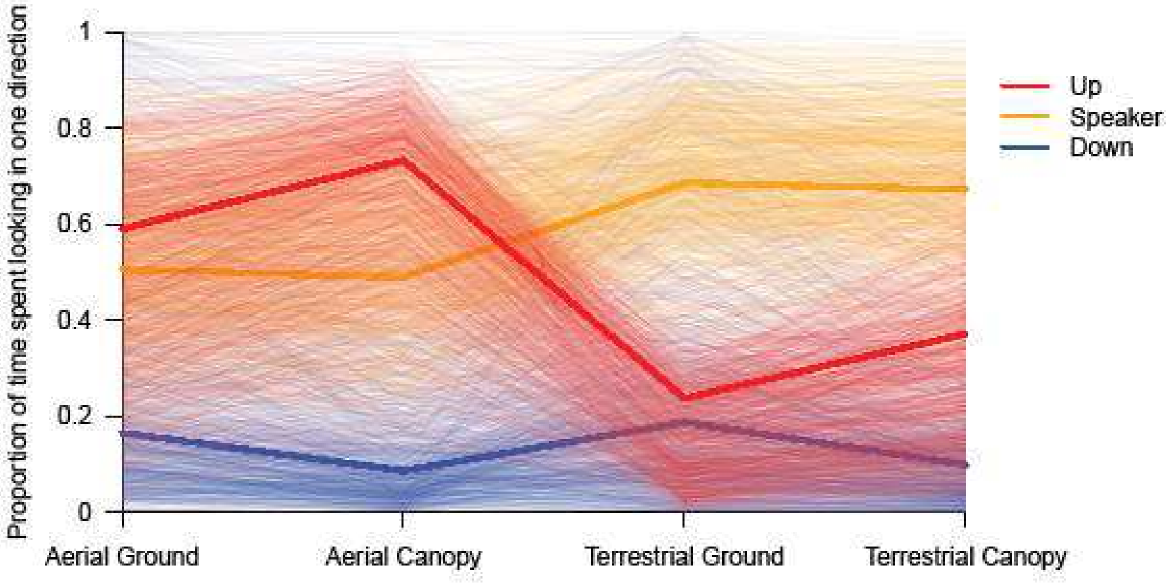

Figure 5.1 Proportion of time the listener spent looking in each direction depending on the experimental condition. Plotted model estimates were obtained from 1,000 bootstraps. Subjects looked more upwards (red) and less towards the speaker (yellow) when they were tested with sequences elicited by an aerial predator or by a predator in the canopy

Do titi monkeys attend to sequential metrics?

In this analysis, I was agnostic with regards to the stimulus that elicited the vocal reaction and only considered sequence metrics as explaining factors for reactions to playbacks.

Results of model ranking showed that the model representing the hypothesis that titi monkeys extracted information from the proportion of 2-grams BB in the sequence had the highest probability of being the best one (*w* = 0.999). All other models had a combined weight of 0.001 (Table 5.6).

Table 5.6 Results of model ranking investigating how information is extracted by the listeners from the alarm sequence. Models are sorted by their AICc scores

Hypothesis of metric extraction	Metric tested	AICc	ΔAICc	Weight
Metric coding for predator type + location	Probability 2-gram BB	432.13		1.00
Metric coding for predator type x location	Transition probability BA	445.37	13.24	0.00
Metric coding for predator type only	Probability begins with A-call	457.98	25.85	0.00
None of the tested metrics	No metric – Urgency	477.36	45.24	0.00
Metric coding for predator location only	CV call interval	479.32	47.2	0.00
None of the tested metrics	No metric - Null	502.06	69.94	0.00

AICc: Akaike's information criterion correction for small sample size; ΔAICc: difference in AICc scores between the best model and each model; weight: likelihood of the model

The proportion of 2-grams BB was the metric that encoded best for added effects of predator type and location. Indeed, it was lower for aerial than terrestrial predators and, within predator types, the proportion of 2-grams BB was slightly greater when presented on the ground than in the canopy (Figure 5.2). These results showed that, when the proportion of 2-grams BB in a sequence increased, listeners spent more time looking towards the speaker and less time looking upwards (Figure 5.3), suggesting that titi monkeys considered the relative proportion of 2-grams BB to extract information about the predator type and location.

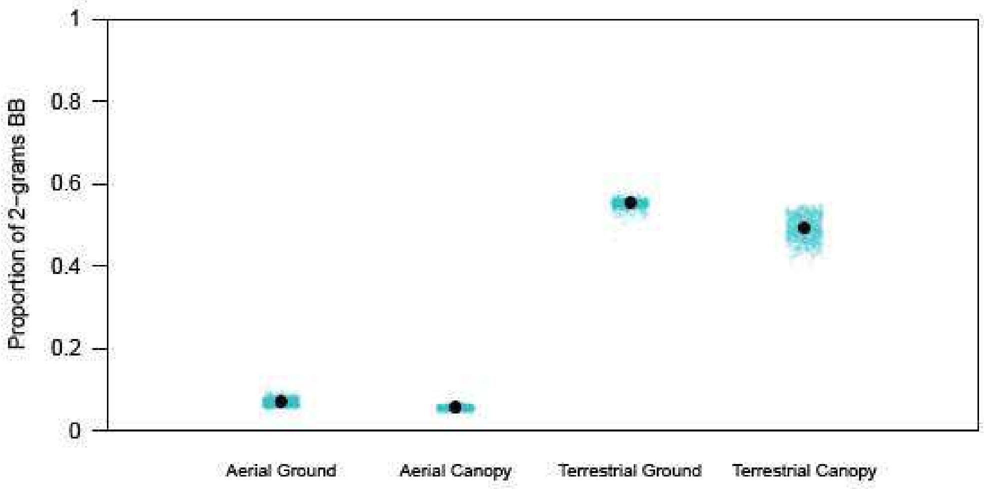

Figure 5.2 Bayesian proportion of 2-grams BB depending on the eliciting stimulus. Plotted model estimates were obtained with 1,000 bootstraps of the results from Chapter 4. The proportion of 2-grams BB was greater for terrestrial than aerial predators, and slightly greater for predators on the ground than in the canopy

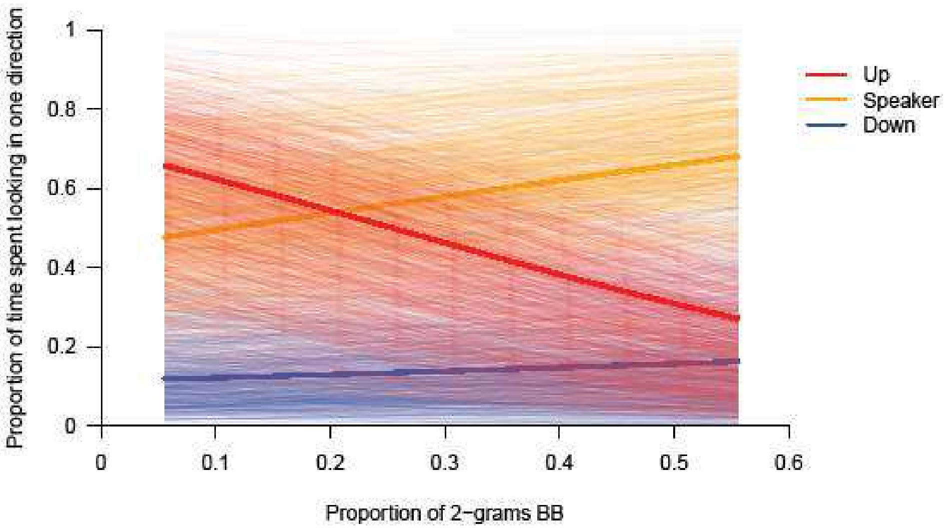

Figure 5.3 Proportion of time listeners spent looking in each direction depending on the Bayesian proportion of 2-grams BB. Plotted model estimates were obtained after 1,000 bootstraps. Listeners spent more time looking towards the speaker (yellow) and less time looking upwards (red) with an increasing number of 2-grams BB in the sequence

DISCUSSION

In this analysis, I investigated what information titi monkeys extracted from alarm sequences and what metric they attended to. First, model ranking showed that listeners' reaction was dependant on the predator type and location that elicited the alarm sequence. Second, model ranking showed that reaction of listeners depended on the proportion of combinations of two B-calls in the sequence (later referred as B-call duplets): the greater the proportion of B-calls duplets was, the more listeners looked upwards.

These results suggests that alarm sequences of titi monkeys are meaningful because listeners can extract information about both predator type and location, but the meaning seems to be more probabilistic than categorical: indeed, instead of coding directly for predator type and location, the sequences appear to encode both aspects of the predator encounter as a continuum: the greater the proportion of 2-grams BB is, the more likely it is a terrestrial predator on the ground over an aerial predator in the canopy.

Categorical meanings have long been the focus of research in animal communication, but my results suggest that it may be crucial to also consider the notion of probabilistic meanings. In a seminal paper, Seyfarth and Cheney (1997) were puzzled by the enigma that animals appear to have so few semantic labels, mostly limited to predator classes and a few social events. However, this may be partly caused by the fact that context is almost always defined categorically and related to human concepts and their perception of the world. Indeed, in one definition of context-specificity, Macedonia and Evans stated that "…all eliciting stimuli must belong to a common category" (p. 179). However, human perception of the world may be different from the natural categorisation taking place by an animal's mind (Zuberbühler and Neumann 2017) and concepts may be more fuzzy. For example, bonobos *Pan paniscus* use a non-categorical, probabilistic encoding of quality in their vocal sequences given to discovering food (Clay and Zuberbühler 2011) and black-capped chickadees *Poecile atricapilla* probabilistically encode information about predator size in their "chick-a-dee" calls (Templeton *et al.* 2005). Even humans can extract meaning probabilistically from non-linguistic utterances of babies (Kersken *et al.* 2017), which suggests that even for us, categorical classification and labelling may not always be the default mechanism in communication.

While it seems difficult, from a human point of view, to conceptualise the logic behind the titi monkeys' probabilistic alarm system, in which at least two sources of information are encoded as a probabilistic measure, my results suggest that it is relevant but also crucial to extend the framework of animal communication from categorical to probabilistic meaning. Indeed, encoding graded information could be more common that previously thought in animal communication and could reflect different cognitive capacities and conceptual systems in animals than those investigated so far.

My results show that titi monkeys concatenate calls to create meanings. Indeed, surprisingly, the metric that titi monkeys appear to use is the proportion of B-calls duplets, rather than, for example, the proportion of B-calls. By combining vocalizations, titi monkeys expend the size of their vocal repertoire (Zuberbühler and Lemasson 2014) but also exhibit a syntactic capacity (Russell and Townsend 2017) also found in other taxa including birds (Engesser *et al.* 2016; Suzuki *et al.* 2017), non-primate mammals (Manser *et al.* 2014) and primates (Arnold and Zuberbühler 2006; Ouattara *et al.* 2009b, a; Schlenker *et al.* 2016b). I already showed that sequences of B-calls are context-specific, with the B-calls being emitted more regularly to predators than when the monkeys are advertising that they are moving near the ground (Berthet *et al.* 2018) (see Chapter 3). Both results suggest that combination of calls into sequences is a key component of titi monkey vocal communication.

Sequences are widespread in animal communication and several techniques exist to analyse them (McCowan *et al.* 1999; Kershenbaum 2014; Kershenbaum *et al.* 2014b, a; Kershenbaum and Garland 2015; Alger *et al.* 2016). However, there is a relative paucity of research on long vocal sequences, probably because this requires more complex mathematical tools. Here, I developed a relatively simple analysis tool to investigate information transfer between callers and receivers by relatively complex sequences. My results have demonstrated that the proportion of 2-grams BB encoded best for both predator type and location (Chapter 4), while playback experiments suggested that receivers extracted information using this feature (this chapter). Interestingly, the metric that is used by titi monkeys to extract information is not one of the three metrics that had the highest weight in the previous analysis (Chapter 4: probability that the sequence starts with an A-call, mean call interval, transition probability from B to A), suggesting that animals do not necessarily communicate information using the most salient features. Naturally, it is also possible that titi monkeys used further metrics simultaneously to extract information from the alarm sequences, a topic for further research.

DATA AVAILABILITY

The datasets generated and the Rscripts used for the current study are available in the following Figshare repository:

https://figshare.com/projects/Probabilistic_meaning_in_titi_monkeys_alarm_call_sequences/30488

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CHAPTER 6. DISCUSSION

SUMMARY OF RESULTS

Cäsar *et al.* (2012a, b) suggested that black-fronted titi monkey A-calls referred to predators in the canopy and B-calls to general disturbances on the ground. Based on their research, Cäsar *et al.* (2013) further proposed that titi monkey alarm sequences exhibited a sophisticated syntactic system, in which the predator type and location were encoded in the following way: (1) aerial predators elicited sequences of A-calls, pure if discovered within the canopy and interspersed with B-calls if discovered on the ground; (2) terrestrial predators elicited B-calls, pure if discovered on the ground and introduced with a single A-call if discovered in the canopy. However, these results were preliminary since i) no acoustic analysis was carried out on B-calls, ii) sequence analyses were relatively simple and did not include more complex sequential features and iii) no playbacks were run to assess whether listeners could extract information from the alarm sequences.

The research reported here sought to address these shortcomings. In doing so, I was able to show that B-calls are communicatively more complex than suggested before, since context was encoded both at the call structure and sequence level (Chapter 3). B-calls emitted to predators had a higher minimum frequency than those emitted when the caller was moving towards the ground, at least in females, and B-calls were emitted more regularly in predatory sequences than in ground sequences. Further playbacks are needed to verify if recipients are able to attend to these features.

I then conducted predator presentations to infer what information was encoded by different metrics that characterised each sequence. Here, I showed that most conveyed information was about predator type, with some variation due to location (Chapter 4), which confirmed Cäsar *et al.*'s theory that both predatory type and location were encoded, albeit by different encoding mechanisms.

Finally, I conducted playbacks experiments that showed that titi monkeys could extract information about both predator type and location, and that they did so with the relative proportion of B-calls duplets in the sequence (Chapter 5) which encoded both information as one continuum, suggesting that information was conveyed in a probabilistic way.

SEMANTIC CONTENT OF TITI MONKEY ALARM SEQUENCES

Meaningful vocal utterances have been found in a number of New World primate species. Tufted capuchin monkeys *Cebus appela nigritus* and Geoffroys' marmosets *Callithrix geoffroyi* emit calls that convey information about the presence of food (Di Bitetti 2003; Kitzmann and Caine 2009). Tufted capuchins also produce "bark" calls that refer to aerial predators (Wheeler 2010). Finally, saddleback and moustache tamarins *Saguinus fuscicollis* and *Saguinus mystax* and Geoffroy's marmosets emit alarm calls that convey reliable information about the type of predator (aerial vs. terrestrial) (Kirchhof and Hammerschmidt 2006; Petracca and Caine 2013). Overall, however, evidence for meaningful vocalisations are relatively scarce in New World monkeys, mainly because most relevant studies have focussed on Old World monkeys and Great apes. New World monkeys vocal capacities, however, have contributed in other important ways, such as for questions about vocal flexibility in call production, like babbling and call convergence (Snowdon 2009). In the current study, I have expanded the literature on New World primate communication, by showing that titi monkeys encoded and extracted information about predator type and location in probabilistic ways, mainly by the number of B-calls duplets in the sequence (the higher number, the more the caller is responding to terrestrial predator on the ground; the lower the number, the more it is responding to an aerial predator in the canopy). New World monkeys, in conclusion, are promising models to study the evolution of primate vocal capacities, and it is crucial to maintain research efforts on this group of primates.

To my knowledge, probabilistic encoding of information has never been shown before in New World monkeys. Similar behaviour has been documented before in other primates, but mainly as a mean to encode differences in urgency (e.g. distance to the predator), for example by gradually varying the rate of delivery or fundamental frequency of vocalizations. For example, red-fronted lemurs (*Eulemur fulvus rufus*) increase frequency and amplitude of their "woof" calls in high arousal contexts (i.e. contexts of agitated behaviours), like during group encounters, compared to low arousal contexts, like when a dog is present (Fichtel and Hammerschmidt 2002). Yellow-bellied marmots *Marmota flaviventris* increase the rate of delivery of their alarm calls when the threat approaches, i.e., as the risk experienced by the caller increases (Blumstein and Armitage 1997). This reflects emotional component of animal vocalizations as described by Morton (1977) and then Briefer (2012), who showed that contexts with a negative valence and high arousal (i.e. contexts of fear) elicit emission of high-frequency calls at a higher rate.

As mentioned before, probabilistic meaning is also found in non-urgency-related contexts, like in the food calls of bonobos, who vary the relative proportion of call types depending on the perceived quality of the food: some call types are mainly given, but not only, to preferred food, while other call types are mainly given, but not only, to less preferred food, and these information are extracted by listeners (Clay and Zuberbühler 2011).

It is often considered that probabilistically encoded information are not context-specific, and thus, are semantically vacuous: historically, studies of animal meaning searched for the equivalent of human words in animal vocalizations (Seyfarth *et al.* 1980b) and this was usually done by focussing on vocal signals elicited only by a narrow range of situations (Seyfarth and Cheney 2003). Macedonia and Evans (1993), for example, wrote that "…all eliciting stimuli must belong to a common category" (p. 179), such that "…ground squirrel alarm calls thus provide only probabilistic information about predator identity and cannot be considered functionally referential" (p. 184). Finally, Seyfarth and Cheney (1997) wrote that they were

“…left with an enigma: why do monkeys (and other animals) apparently have so few semantic labels?” (p. 181). It could be argued that classification schemes of animals are different from those of humans, and that context-specificity should not be defined as a set of stimuli belonging to a common category, to allow for integration of probabilistic meaning as evidence of semantic capacities of animals. Thus, one proposal is that context-specificity should be redefined as a correlation between vocal signals and features of the external events (Dezecache and Berthet, in prep.). This definition does not prevent urgency-based systems from possessing semantics, contrary to traditional views in comparative cognition (e.g. Macedonia and Evans 1993). Indeed, call production referring to internal states of the caller (affective production) has long been opposed to call production referring to external events (referential production) (Marler *et al.* 1992). However, as argued by Seyfarth and Cheney (2003), semantics of calls are mainly defined by the ability of the listener to extract meaning from the signal than by the mechanisms underlying the signal production in the caller. Thus, the definition of context-specificity proposed by Dezecache and Berthet does not prevent urgency-based related systems to be semantically rich, as long as listeners can extract meaning from it. As shown in chapter 5, titi monkeys appear to encode both predator type and location as a continuum, from aerial predator in the canopy to terrestrial predator on the ground (Figure 5.2, Figure 5.3). Hence any attempt to classify titi monkey sequences in dual categorization terms (predator type and location) does not appear to reflect their natural perception of the threat. With the definition of context-specificity proposed by Dezecache and Berthet, the proportion of B-calls is correlated to the predator type and its location, without classifying the context into categories. If information is reliability extracted from the sequences by listeners, it is difficult to argue against the semantics content of such utterances.

CALL COMBINATIONS IN TITI MONKEYS

Titi monkeys alarm sequences are characterised by different call combinations. For B-calls, the acoustic structure differs depending on the context of emission, but these small acoustic differences may be strengthened by the regularity of emission of the calls, suggesting that combinations of B-calls into sequences may be an important mechanism to increase discrimination of eliciting contexts by listeners, but this remains to be tested experimentally. Titi monkey alarm sequences encode for predator type and location using the relative proportion of B-calls duplets in the sequence. Interestingly, the proportion of A and B-calls in the sequence did not carry information about the predatory event, while the combination of two B-calls did (Chapter 5). Moreover, titi monkeys produced long loud call sequences during predatory events, territorial defence or when isolated from the group, and the order and proportion of call types in these sequences may play an important role in context discrimination (Robinson 1979a; Cäsar 2011; Caselli *et al.* 2014), although this feature was not explored in my study. All these results show that syntactic call combination is indeed an essential feature of titi monkey vocal communication.

Nonhuman primates have fixed vocal repertoires. Early experiments showed that squirrel monkeys raised in absence of species-specific vocalizations, (i.e. isolated from conspecifics, raised by a mute mother, or deaf infants) developed a vocal repertoire identical to that of normally raised squirrel monkeys (Winter *et al.* 1973). However, further research showed that nonhuman primates exhibit higher flexibility in call usage and perception than production (Snowdon 2009), and call combination is one mechanism used by animals to extend the size of their limited vocal repertoires (Zuberbühler and Lemasson 2014), especially when the number of events to refer is larger than the number of call available (Nowak *et al.* 2000). Moreover, Griesser (2008) suggested that species living in kin groups, like titi monkeys, tend to develop more complex antipredator vocal systems because they are more vulnerable to predators due to the small size of the groups, but also to avoid the death of relatives.

Several New World primates combine calls: this is the case of howler monkeys, famous for their loud call sequences produced at dusk to defend their territories (e.g. Horwich and Gebhard 1983). Cotton-top tamarins *Saguinus oedipus oedipus* combine an alarm call with a contact call into a "Inverted U+ whistle" that seems to function as a "all-clear" signal (Cleveland and Snowdon 1982). Overall, these studies mainly focussed on the flexibility of the sequences rather than their semantic content, which remains to be explored by future research.

Titi monkeys possess a rich and complex vocal repertoire (Moynihan 1966) consisting of alarm and loud call sequences, as well as food and social calls also emitted in sequences (personal observations). Further research may reveal rich and unexpected syntactic and semantics capacities in these unexplored domains of vocal communication.

MECHANISMS OF CALL PRODUCTION

Titi monkey alarm sequences encode rich and complex content but it is unknown whether callers also intend to provide such information. Indeed, Schlenker *et al.* (2016b, a) analysing Cäsar *et al.*'s (2012a, 2013) data with linguistic methods, suggested that titi monkey alarm utterances may not be considered syntactic but that each call may be uttered as independent from the others, referring to the momentary situation during production. In this view, A-calls may refer to serious non-ground events and B-calls to any type of noteworthy events. Thus, for aerial predators in the canopy, titi monkeys would only emit A-calls (referring to a serious non-ground predator), and they would only emit B-calls to terrestrial predators on the ground (serious ground predators). When the terrestrial predator is in the canopy, titi monkeys would emit one A-call (serious non-ground predator, since the predator is in the canopy and is dangerous). Since it has been shown that felids stop hunting after deterrence (Zuberbühler et al.

1999b), titi monkeys would then switch to B-calls (non-serious non-ground predator). Finally, for an aerial predator on the ground, they would emit A-calls (serious non-ground predator) but would then switch to B-calls when realizing that the bird in not in a hunting position while on the ground (non-serious non-ground predator).

This view is interesting and deserves to be refined in light of the new predator presentation and playback results I presented here. Indeed, since I did not observe exactly the same encoding pattern in the sequences than Cäsar *et al.* (2013), notably because I did not observe a single A-call at the beginning of the sequence in most of the terrestrial predator in the canopy conditions, and since I showed that B-calls potentially convey information about the eliciting context, two complementary theories to that of Cäsar *et al.* and Schlenker *et al.* can be proposed.

First, as I discussed before, titi monkeys may encode predator type and location probabilistically using the relative proportion of B-calls duplets. This seems to be the easiest and most direct explanation of my results. However, my analysis could not integrate the fact that B-calls exhibit two acoustic variants (Chapter 3): I considered the B-calls in the mixed alarm sequences as one call type, while finer acoustic analysis could have revealed that maybe two different B-calls are used, increasing the complexity of the vocal system of titi monkeys.

Second, it could be that each call indeed referred to the situation at the time it was uttered, as suggested by Schlenker *et al.* (2016a). In this view, A-calls refer to non-ground predators, regardless of the seriousness of it, and B-calls fall into two acoustic variants: the B1-call, referring to ground predators, and the B2-call, referring to the movement of the caller towards the ground. For an aerial predator in the canopy, titi monkeys should then emit A-calls only (non-ground predator). For the terrestrial on the ground, they should emit B1-calls only (ground predator), and B2-calls only if moving to the ground to forage. However, for terrestrial predators in the canopy, they should only emit B1-calls (ground predator), as observed in most of my trials (Table A 1). The few trials for which a single A-call was observed at the beginning of the sequence (see Cäsar *et al.* 2013) could be explained as identification mistakes by the caller. Indeed, most of the predators found in the canopy are raptors or capuchins, and they typically elicit A-calls (Cäsar 2011; Cäsar *et al.* 2012a), while tayras and oncillas climb with ease but are mostly terrestrial (Asensio and Gómez-Marín 2002; Sunquist and Sunquist 2002). Thus, it could be argued that the most adaptive reaction when spotting a non-identified shape in the trees is to react as if it was a non-ground predator (A-call). When the caller manages to identify the predators, it would switch to B-calls (non-ground predator). This could explain why I observed two sequence types during my trials: in some cases, titi monkeys immediately identified the predator (B1-calls only), in other cases they did not (A-call then B1-calls). Finally, aerial predators on the ground would elicit A-calls (non-ground predators) but since the models are not moving and are in an unusual position, titi monkeys would descent to check on the weird predator (personal observations) and emit B2-calls (movement of the caller towards the ground) within the A-call sequence. These theories need to be experimentally tested to conclude on the encoding mechanisms of titi monkeys' alarm sequences.

CONCLUSION

Call combination is a key component of titi monkey vocal system. Callers can combine B-calls into sequences whose temporal parameters have the potential to convey information about the context. They also combine A and B-calls into sequences that convey reliable information about predator type and location. This complex vocal system may have been favoured by the kin-based social system of this species (Griesser 2008), as well as the need to communicate a large number of events from their environment (Nowak *et al.* 2000). Although titi monkeys are not the closest relatives of human (Perelman *et al.* 2011), their unique communication system provides interesting insights on the selection pressures that may have shape the evolution of human language.

Fischer (2017) showed that the continuity with human language may rely on the capacity of listeners to process the signals rather than on the vocal production learning. This statement strengthens the need for further investigation of the cognitive capacities of listeners in titi monkeys, for example to assess whether they can discriminate between the context-specific B-sequences. However, Schlenker *et al.* (2016b) argued that the syntax and semantic properties of primate vocal utterances should be analysed with formal linguistic methods to be fully understood and comparable with human language. Therefore, further interdisciplinary research, combining linguistics and primatological approaches, is crucial to conclude on the fascinating question of semantic capacities of titi monkeys.

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ANNEXES

ANNEXE 1 RESULTS OF PREDATOR PRESENTATIONS

Table A 1 Alarm sequences produced in each predator presentation experiment (raw data) run by Cristiane Cäsar (Cäsar 2011; Cäsar et al. 2013) and me

Predator type	Experimenter	Group	Ground	Canopy
Aerial	CC	A	AAAAAAAAAA	AAAAAAAAAA
		D		AAAAAAAAAB
		M	AAAAAAAAAA	AAAAAAAAAA
		P	AAAABAAAAA	AAAAAAAAAA
		R	AAAAAAAAAA	AAAAAAAAAA
	MB	D	AAAAAAAABA	
		M	AAAAAAAAAA	AAAAAAAAAA
		P	AABAABACBA	AAAAAACACC
		R		AAAAAAAAAA
		S	AAAAAAAAAA	AAAAAAAAAA
Terrestrial	CC	A	BBBBBBBBBB	ABBBBBBBBB
		A	BBBBBBBBBB	
		D	BBBBBBBBBB	ABBBBBBBBB
		D	BBBBBBBBBB	
		M	BBBBBBBBBB	
		P	BBBBBBBBBB	BBBBBBBBBB
		P	BBBBBBBBBB	
		R	BBBBBBBBBB	AAABAAAABB
		R	BBBBBBBBBB	
	MB	A	BBBBBBBBBB	BBBBBBBBBB
		A	BBBBBBBBBB	ABBBBBBBBB
		D	BBBBBBBBBB	BBBBABBBBB
		D	BBBBBBBBBB	BBBBBBBBBB
		M	BBBBBBBBBB	BBBBBBBBBB
		M	BBBBBBBBBB	BBBBBBBBBB
		P	ABBBBBBBBB	BBBBBBBBBB
		P	BBBBBBBBBB	BBBBBBBBBB
		R	BBBBBBBBBB	BAABBBBBBB
		R	BBBBBBBBBB	ABBBBBBBBB
		S	BBBBBBBBBB	BBBBBBBBBB
		S	BBBBBBBBBB	BBBBBBBBBA

ANNEXE 2 DEMOGRAPHIC DATA

All groups gave birth between October 2015 and August 2016, most of the time during the dry season (see details in Table A 2).

In 2015, important civil engineering was performed on the dam of Tanque Grande. The noise level was high for a year (May 2015 to June 2016), due to the trucks crossing the forest several times per day, the workers talking loudly and the work itself (jackhammers, for example). Monkeys of the four groups inhabiting the forest of Tanque Grande (A, D, R and S) seemed very stressed by this event, regularly alarm calling and fleeing when hearing the trucks, avoiding the areas close to the dam, and it is very interesting to note that 3 out of these 4 groups did not gave birth this year while both Cascatinha groups (M and P) did, and the only Tanque Grande group that gave birth (R group) did not manage to keep the infant alive (see below). This suggests that other environmental factors (food, climate) probably did not limit fertility, but that the stress generated by the work may have.

On the 29th of September 2015, after a three-month interruption of monitoring, the R group was seen with two infants seemingly of between one and two months old (Fragaszy *et al.* 1982). They were both carried by the father and were climbing and playing on branches proximate to the male's back. On the 7th of October 2015, only one infant was still present in the group, and on the 9th of October 2015, none. Twinning is very rare in titi monkeys and in any case one of the twin dies within a few days after birth (Knogge and Heymann 1995; Valeggia *et al.* 1999). It would seem unlikely that the R group gave birth to twins, since the two infants were at least a month old when seen together and because adoption from neighbouring groups are possible (Cäsar and Young 2008). In any case, both infants did not survive the twinning or adoption event.

Table A 2 Demographic events observed between October 2014 and August 2016 in the six habituated groups. At the beginning of the study, the groups D, M and P had an infant, hence born around August-September of 2014

Event	Goup	Estimate date	Observation
Birth	A	2014.11.04-12	Female
	D	2016.07.19-2016.08.01	Unknown sex
	M	Soon before 2015.10.07	Male
	M	2016.08.22-23	Unknown sex
	P	Soon before 2015.10.07	Female
	P	2016.07.29- 2016.08.11	Unknown sex
	R	2016.07.15-2016.08.02	Unknown sex
	S	2016.07.15-2016.08.03	Unknown sex
Two infants in the same group	R	September 2015	2015.09.27-2015.10.06: disappearance of the 1st 2015.10.06-2015.10.12: disappearance of the 2nd
Disappearance	D	June-October 2015	One female of 3 years old
	M	June-October 2015	Two males, 3 years old and 3-4 years old
Death	P	2015.10.05-2015.10.07	One male of 3 years old
New individual	S	2016.08.19-30	One adult male

Four young adults disappeared from three groups between June and October 2015, but one body was found on the 7th of October 2015 in the core area of the P group. It was already too decayed to recognize it and know whether it died due to predation, but we assumed that it was the missing young adult. We assume that the three other missing adults have dispersed.

One new adult male was observed in the S group in the end of the study. At this time, the group was composed of two mated adults (male: Serge and female: Sahel), a young adult male of 4 years old (Suco), a young adult female of around 3 years old (Savi) and an infant. The group was observed once on the 30th of August 2016, from 0820 until 0900, during an intergroup encounter with the R group that we were monitoring on this day. Sahel and Savi were duetting with a new, non-habituated male who seemed stressed by our presence. Serge, Suco and the baby were out of sight. On the 1st of September, we monitored the S group from 1010 until 1140 and observed that Sahel and the new male were physically close to each other, while Serge, who was carrying the infant, was keeping distance from the couple. It is possible that the new individual replaced the mated male in the group, but unfortunately, we were not able to collect more data on this event since it occurred on the last day of this study. Dispersion mechanisms in titi monkeys are not well known, but cases of replacement of one of the mate have already been reported (Cäsar 2011).

ANNEXE 3 COPULATIONS

We observed 23 copulations from all groups but the P and M groups. Five of these copulations were extra-pair, i.e. between one of the mated adult and another individual, while all the other involved the mating adult pair. Extra-pair copulations have been already observed in titi monkeys (Cubicciotti III and Mason 1978), however the five extra-pair copulations we observed were all performed with a young adult from the group (Table A 3). Since the groups were not monitored between 2011 and October 2014, and because we do not have genetic data, it is not possible to conclude whether these copulations were incestuous, i.e. between parent and offspring, or if the young adults were genetically different from the adult they mated with. As far as I know, these copulations are the first report of within-group sexual activity by young adults, since the only study on sexual activity of titi monkeys reported that "none of the mature females who were still living with their parents when they were between 2.5 and 3.6 years of age became pregnant or showed signs of sexual/reproductive activity", with no information about young males (Valeggia *et al.* 1999).

Table A 3 Extra-pair copulationsobserved between two members of the same group

Date	Group	Mated adult	Other individual
2016.06.14	S	Male	3 years old female
2016.06.23 (once), 2016.06.29 (3 times)	R	Female	3 years old male

ANNEXE 4 TERRITORIES

Calculation from GPS data recorded during the study period showed that territories of these groups range from 6.8 to 8.5 ha (mean ± s.e. = 7.6 ± 0.3, Table 2.1). Territories overlap with adjacent territories (Figure 2.2): each group shares a part of his territory with another habituated group, but also with non-habituated groups, when the geographical landscape allowed it (not shown on the map).

Territories of these six groups are much smaller than those described in the literature (around 20 ha, Duarte *et al.* 2018). This could be explained by the density of titi monkeys in the Reserve: Black-fronted titi monkeys seem to inhabit every fragments of Atlantic forest in Caraça (Cäsar 2011), which can create a high competition for resources (Caselli *et al.* 2015), hence leading to the reduction of territories size. This high population density could also explain the copulations we observed between young adults and one of the paired adult: dispersion mechanisms of titi monkeys are little-known, but high-density may delay dispersion, due to few mating and territory opportunities (Bossuyt 2002; Bicca-Marques and Heymann 2013), thus leading to extra-pair copulations with young adults.

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