Chimpanzee Culture in Cantanhez National Park, Guinea-Bissau

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ABSTRACT

The existence of non-human animal (hereafter animal) culture, i.e., behaviours that are group-typical and shared by members of a community through social learning, is now generally accepted. Its presence has been identified in many vertebrate, and even invertebrate, species. Much like in humans, culture is present in the most diverse aspects of an animal's life, from complex material culture, communication, and social behaviours, to foraging, travel, and migratory routes. Importantly, culture can be a source of adaptive behaviour whereby individuals can more readily discover the solution to a problem if they attend to or copy the behaviour of other individuals facing the same problem, allowing individuals to better exploit their natural and social environment. Of all animal species, chimpanzees (*Pan troglodytes*) have the largest known cultural repertoire. However, despite several long-term field studies, large gaps remain in our knowledge of the behavioural repertoire of and behavioural variation across the majority of Africa's chimpanzees. Furthermore, few studies have compared the behaviour of neighbouring chimpanzee communities, despite such comparisons promising to provide the strongest evidence for culture, and few have studied communities inhabiting anthropogenic landscapes, although their culture is in the most imminent danger of disappearing.

The aim of this thesis was to study behavioural variation across four unhabituated neighbouring chimpanzee (*Pan troglodytes verus*) communities, inhabiting an agroforest habitat matrix in Cantanhez National Park (CNP), Guinea-Bissau. As these critically endangered chimpanzees regularly encounter local people and a loss of fear of humans might drive aggressive interactions, habituation was never a possibility. Therefore, a combination of direct opportunist observations, but mostly indirect methods of data collection (e.g., camera traps, abandoned tools, nests) were employed. Additionally, a combination of data analysis methodologies – automated behavioural annotation, primate archaeology techniques, and stable isotope analysis – were used to tackle the large data set collected, which included 4197 chimpanzee videos, 1747 indirect chimpanzee traces, 125 chimpanzee hair samples, and 390 plant samples.

In Chapter 2 I compile a list of 18 putative cultural behaviours for CNP's chimpanzees, some of which had never been described for Guinea-Bissau, or even the rest of tropical Africa, and describe evidence of inter-community variation that spans tool use, communication, and social behaviour. In Chapter 3 I focus on a subset of these cultural behaviours – honey dipping – and describe inter-community variation as well as variation in tools used to collect the honey from different insect species. Employing primate archaeological techniques, I assign putative functions to the collected tools and suggest that different tools are used as part of a tool set. In Chapter 4, with the aid of an automated, machine learning based drumming annotation software, I examine a chimpanzee 'universal' – buttress drumming – and identify inter-community variation in drumming duration. Through exploring the contexts in which drumming occurs, I further suggest that the duration of a drumming bout might be part of community-specific signalling in particular contexts, such as travel. Finally, in Chapter 5 I use stable isotope analysis and find significant inter-community variation in the diets of these neighbouring chimpanzees, that point towards previously unknown differences in feeding behaviour.

Overall, this thesis presents the first long-term study focussed on chimpanzee behavioural variation in CNP, highlighting the importance and feasibility of simultaneously studying multiple neighbouring communities, without the need for habituation. Technological advances have not only changed the way we collect data but also the type and amount of data we can collect and analyse, allowing us to closely examine many aspects of an animal's life. Finally, this study underlines how different communities facing similar environmental pressures can adopt different adaptations, even at a local scale. Given the rate of habitat destruction and climate change that wild species are facing, there has never been a more relevant time to study how different communities behave and adapt to life in the Anthropocene.

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AUTHOR CONTRIBUTIONS

All work in this thesis is primarily my own.

This thesis is presented as a set of independent data chapters laid out in scientific journal format, with an additional general introduction and discussion. Chapter 2 is in press in *Royal Society Open Science* and Chapter 3 is published in *Frontiers in Ecology and Evolution*.

Dora Biro and Kimberley Hockings, my supervisors, helped conceive the methodologies used, contributed their ideas and expertise, and provided feedback on all chapters.

Max Bain, Arsha Nagrani and Andrew Zisserman developed the automated behavioural recognition software used to analyse part of the buttress drumming data in Chapter 4.

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CHAPTER 1

General Introduction

1.1 Animal Culture

Culture permeates every aspect of human life. It encompasses a range of phenomena, from language, law, and religion, to technology, art, and social customs; all are socially transmitted and vary greatly between and even within human societies. Culture has greatly shaped our evolution, and it has allowed humans to explore, settle, and exploit all corners of the Earth. The existence and nature of non-human animal (henceforth, animal) culture, on the other hand, has for decades been a controversial topic of study. Researchers from a diverse range of disciplines (anthropology, psychology, archaeology, and zoology) have attempted to define culture, providing criteria that were to various degrees exclusive or inclusive (see McGrew, 2004 for a review): for some, culture is uniquely human and tightly connected to our capacity for language, symbols and ethics, while for others, evidence of any inheritance that is not purely genetic can be sufficient to deem a species cultural (Laland and Hoppitt, 2003).

It has been suggested that a broader definition of culture will be more stimulating for researchers studying it, particularly if seeking the evolutionary foundations of the phenomenon (Laland and Hoppitt, 2003). The general consensus is that for a behaviour to qualify as "cultural", there must first be **innovation** or modification of a pre-existing behaviour, then this behaviour has to **diffuse** through a group of individuals through **social learning** (i.e., "learning that is influenced by observation of, or interaction with, another animal (typically a conspecific) or its products" (Heyes, 1994)). This means that genetically inherited behaviours as well as knowledge that individuals acquire on their own (asocial learning) cannot be considered cultural (e.g., Laland and Hoppitt, 2003; McGrew, 2004; Laland *et al.*, 2009). Additionally, several definitions also posit that this socially learnt and transmitted information should underpin group-typical behaviour patterns and thus explain **continuity** of behaviours within groups over time, as well as behavioural **variations** between groups (Laland and Hoppitt, 2003; Laland and Janik, 2006; Laland *et al*., 2009).

It is important to note that despite social learning being paramount for cultural transmission (Fragaszy and Perry, 2003), in most situations, social learning will not result in culture, since most socially learned information is transient (Whiten, 2017; Whiten *et al*., 2017). Given the wide acceptance of these criteria, for the present thesis I will be following the relatively broad, and hence more inclusive, definition of culture proposed by Laland and Hoppitt (2003:151) "group-typical behaviour patterns, shared by members of a community that rely on socially learned and transmitted information". Despite the general acceptance of these guidelines, their interpretation is not devoid of contention. For some authors, the exclusion of ecological and genetic factors as drivers of inter-community behavioural variation should be sufficient to demonstrate culture (Wrangham *et al*., 1994; Whiten *et al*., 1999); however, for others if social learning is not undoubtably demonstrated, then that behaviour cannot be considered cultural (Fragaszy and Perry, 2003). Furthermore, it is important to mention that consensus on the existence of animal culture has yet to be reached. Some researchers suggest that another major characteristic of (human) culture is its capacity for building upon previous knowledge resulting in the gradual accumulation of cultural modification, a phenomenon known as **cumulative culture** (Tomasello, 1994, 1996). The process that allows for culture to accumulate, "**the ratchet effect**", allows new modifications to be introduced and passed on to other individuals through **high fidelity transmission,** that, according to the authors, is only possible in humans through a unique combination of advanced cognitive abilities including imitation, language, and teaching (Tomasello, 1994; Tennie *et al*., 2009; Dean *et al*., 2014). By contrast, animal (although the authors focus mainly on chimpanzees) culture arises and is maintained by a form of "non-form-copying social learning", i.e., animal cultural traits consist instead of "latent solutions" that can be individually re-invented when internal and external conditions are optimal – a proposition known as the **Zone of Latent Solutions** hypothesis (ZLS) (Tennie *et al*., 2009, 2020). Once an individual in a group invents a behaviour, their activities can make it much easier for other group members to adopt the behaviour too, through emulation, or local or stimulus enhancement (Tennie *et al*., 2009, 2020). These processes, however, do not necessarily preserve the form of the behaviour. Only the faithful transmission of information that humans are capable of can prevent the loss of modifications and result in cultural accumulation, such that what is being transmitted goes beyond what a single individual could have invented on its own in a single lifetime (Tomasello, 1994; Boyd and Richerson, 1996). For example, writing this thesis on a laptop would have been impossible had there not been thousands (or even millions) of years of technological evolution on which the invention and manufacture of computers builds.

Although the existence of cumulative culture in animals continues to be hotly debated, a number of recent studies suggest that animals are in fact capable of building upon previous knowledge. These range from experimental evidence of gradual improvement in the efficiency of pigeons' homing routes over artificially created "generations" (Sasaki and Biro, 2017), to long term field studies on translocated bighorn sheep showing the emergence, over multiple decades, of migratory patterns that increasingly better match the seasonal emergence of vegetation ("green wave surfing") (Jesmer *et al*., 2018).

But why is the study of animal culture relevant to biologists? Cultural transmission is a source of adaptive behaviour; individuals can more readily discover the solution to a problem if they pay attention to or copy the behaviour of other individuals facing the same problem, allowing individuals to better exploit their natural environment (Laland *et al*., 2009; Whiten *et al*., 2011).

This adaptation through social learning is also much faster (sometimes intragenerational) than adaptation through genetic change (Whiten *et al*., 2011). Nonetheless, such copying may allow for arbitrary or even maladaptive behaviours to be maintained in the group, even when there are changes in the environment (Boyd and Richerson, 1996). Cultural transmission can even violate the premises of natural selection, since it can spread behaviours that are disconnected from the animals' environment. Furthermore, cultural traditions can influence evolutionary rates and trajectories by homogenising behaviours within a population and allowing quick changes in a population's behavioural repertoire. Overall, the study of animal culture can inform researchers of different ways animals adapt and evolve (Laland *et al*., 2009). Finally, given how quickly habitats are changing due to human encroachment and climate change, it has never been timelier to study how and how quickly animals respond to new environmental challenges. In turn, these can inform targeted conservation strategies that might assist the continuity of species.

1.2 Approaches to studying animal culture

Some of the most impressive examples of animal culture, but perhaps some of the most criticised, are a product of the **ethnographic method** (Wrangham *et al*., 1994), or **method of exclusion** (Krützen *et al*., 2007). This method compares the behavioural repertoire at a species or subspecies level of several different long-running study sites, cataloguing behaviours that are present in certain communities but absent at others. The inference is that, for any behavioural differences that cannot be explained by either ecological or genetic differences between groups, must, by default, be socially learnt and therefore cultural. One of the most cited examples of such an "ethnographic" study is by Whiten and colleagues (1999) that analysed the presence/absence of a list of 65 candidate behaviours in six chimpanzee (*Pan troglodytes*) communities. The study found that 39 of these behaviours could not be explained by either genetic or ecological differences between the different chimpanzee populations, and therefore must be cultural. This study was followed by similar ones in orangutans (Van Schaik *et al*., 2003; Krützen *et al*., 2011), gorillas (Robbins *et al*., 2016), and spider monkeys (Santorelli *et al*., 2011). This approach soon received criticism for being too simplistic: how can one categorically exclude all ecological and genetic influence on behavioural variation when these factors are inexorably interlinked (Laland and Janik, 2006; Laland *et al*., 2009; Koops *et al*., 2013)? Some degree of correlation between culture and ecology is to be expected, since culture is a source of adaptation to a group's environment. Additionally, some degree of correlation between culture and genetics is expected since the capacity for learning and transmission of knowledge is influenced by an animal's biological predisposition for these. Hence it should be evident that ecology, genetics, and social learning can all influence, to some degree, behavioural variation (Laland and Janik, 2006; Humle, 2010). In addition, some critics of the method (e.g., Laland and Janik, 2006) have argued that if rigorously applied, it might reject many possible cases of animal culture. Despite some of the limitations of this method, these large-scale, long-term, and systematic studies have undeniably provided us with an invaluable foundation for future exploration (Laland and Janik, 2006; Whiten, 2017).

As a counterpoint to the observational nature of the ethnographic method, many authors advocate a complementary, experimental approach to testing for culture, for example through **translocation experiments** (Laland and Janik, 2006; Laland *et al*., 2009). Such experiments require individuals to be moved from one population to another or that whole populations are moved between sites, resulting in the complete elimination of ecological or genetic influences on behaviour (Laland *et al*., 2009). The broad concept is that, if a translocated animal adopts the behaviour of the resident population a genetic explanation can be excluded, but if an entire population is replaced by a new one and the new population still exhibits different behaviours to the original population, then an ecological explanation is excluded. These types of experiments were successful in demonstrating that the choice of routes and schooling sites in French grunts (*Haemulon flavolineatum*) (Helfman and Schultz, 1984) as well as choice of mating sites of Bluehead wrasse (*Thalassoma bifasciatum*) (Warner, 1988) were socially learnt. However, while some taxa, such as fish, are amenable to these kinds of experimental manipulations, for most vertebrate species they are problematic: not only is the feasibility of capturing and transporting large animals an issue, but the ethics of translocating animals living in stable, long-term groups that are hostile to unfamiliar individuals are also questionable (e.g., McGrew, 2004). Additionally, the same criticism can be made here as for the ethnographic method: attempts to completely rule out ecology and genetic influence might result in missing out on many possible cases of animal culture (e.g., Laland and Janik, 2006).

A better complement to observational experiments, where social learning is difficult to demonstrate, are perhaps **diffusion experiments** (Whiten and Mesoudi, 2008). In captivity and under controlled conditions researcher can easily introduce new stimuli, resources or information, and trace their spread (or lack thereof) through a group (e.g., in captive chimpanzees: Rawlings *et al*., 2014; Van Leeuwen *et al*., 2014). For example, Whiten *et al*. (2005) tested three captive groups of chimpanzees in a new foraging task, the “Pan-pipe”. To access a food reward, chimpanzees had to resort to the use of a tool in one of two different ways. Two high ranking females were taken out of two of the experimental groups and each was trained in one of the solutions to the “Pan-pipe”. Once they were proficient in solving the task they were re-introduced to their groups, and after some time, where only the expert females were given access to the apparatus while the others could observe them, all individuals of the three groups were given access to it. In the groups with an expert female, the majority of the other group members adopted their expert's technique to solve the task, whereas the control group (without an expert) was not able to obtain the food reward. This elegant experiment clearly demonstrates chimpanzees' capacity to copy behaviours with sufficient fidelity to create group-typical traditions (Whiten *et al*., 2005). Studies like this, give us valuable insight into a species' capacity for innovation and social learning, nonetheless, if the intent is to study animal culture in the wild, and to understand the role of culture in the natural lives of these animals, observational methods might remain the preferable option (Whiten, 2019).

More compelling evidence for animal culture using observational methods *in situ* might come from studies that compare only communities of the same subspecies (e.g., Laland and Janik, 2006), or, better yet, **neighbouring communities** (McGrew, 2004; Whiten, 2017, 2019). The latter experience genetic cross-mixing through migration and are likely to inhabit habitat continuums or at least very similar habitats, which would reduce any genetic or ecological variation respectively (e.g., in meerkats: Thornton *et al*., 2010; in chimpanzees: Luncz *et al*., 2012; Koops *et al*., 2015; Pascual-Garrido, 2019). Additionally, conducting studies at a smaller scale allows researchers to not only record the presence or absence of a behaviour, but indeed to look for more subtle variation in superficially shared behaviours. Illustrating this approach, Luncz and colleagues (2012) compared the selection of wooden and stone hammers for coula (*Coula edulis)* nut-cracking in three neighbouring chimpanzee communities in Tai National Park (Côte d'Ivoire). Even though these neighbouring communities inhabit the same forest habitat and ecological variation is minimal, the study showed that there was still marked variation in hammer size and raw material preferences between communities (see further examples in section 1.3).

One of the biggest benefits of studying animal groups for long periods of time is that opportunities for witnessing the invention, diffusion, and maintenance of behaviours in real time drastically increases with study duration. Studying the **natural transfer of individuals** between groups (migration) is a golden opportunity to document whether an immigrant individual conforms to their new groups' culture, or, conversely, if a newcomer's behaviour is adopted by the community they migrated to (e.g., in chimpanzees: Biro, 2011; Luncz and Boesch, 2014). In fact, such migration events are the natural equivalents of a translocation experiment. A great example of such a natural translocation 'experiment' is described by Luncz and Boesch (2014) where an immigrant female chimpanzee at Tai National Park (Côte d'Ivoire) adopted the local variant in nut-cracking technique over a period of several weeks after arriving in her new community. Given its complexity, the acquisition of nut-cracking behaviour is thought to be restricted to a sensitive period between 3 and 7 years of age; after that an individual will no longer be able to learn the technique (Inoue-Nakamura and Matsuzawa, 1997; Biro *et al.*, 2003). However, Luncz and Boesch's (2014) results support the idea that cultural transmission can still occur between adults with an already fully developed behavioural repertoire (albeit concerning the detailed form of the behaviour, rather than the acquisition of novel behaviour from scratch), and this can contribute to the persistence of traditions over time.

Another way of studying behavioural variation in the wild is through **field experiments** (e.g., in birds: Aplin *et al.*, 2013, 2015; in chimpanzees: Biro *et al.*, 2003; Gruber *et al.*, 2009; Koops *et al.*, 2022). For example, in Bossou (Guinea) where chimpanzees are habitual oil-palm nutcrackers, researchers introduced two novel nut species that were known to be cracked in neighbouring communities (Biro *et al.* 2003). One of these nuts was the previously mentioned coula nut, and, to the researchers' surprise, one female started cracking it proficiently and without any initial exploratory behaviour. She was the only individual to do so with such signs of familiarity, hypothesised to be a result of her having migrated from a neighbouring community that traditionally cracked these nuts (Biro *et al*. 2003). Genetic data later appeared to lend support to this hypothesis (Shimada *et al*., 2004), revealing that this female was indeed likely an immigrant. Over time, more and more individuals of the Bossou community started to crack the novel coula nuts, probably because this female served as a salient model for learning (Biro, 2011). In a complementary vein, field experiments can also demonstrate how complex behaviours, such as nut cracking, cannot be individually learned or learned through local enhancement (Koops *et al*., 2022), as is proposed by the ZLS hypothesis (e.g., Tennie *et al*., 2009, see section 1.1). Koops and colleagues (2022) provided a non-nut-cracking chimpanzee community in Seringbara (Nimba Mountains, Guinea), for over a year, with intact nuts, stones, and nuts previously cracked with stones, to test if Seringbara chimpanzees were capable of individually inventing nut cracking. Despite some chimpanzees interacting with the nuts, no individual tried to use the stones provided to crack them. According to the authors this lack of (re-)invention of the behaviour over such a long period of time, that included both periods of high and low fruit availability, provides strong support for the hypothesis that this specific form of tool use is socially learned (Koops *et al*., 2022). Additionally, a longer exposure to these nuts and tools is unlikely to have changed the results, since chimpanzee visits to these "outdoor laboratories" declined over time (Koops *et al*., 2022).

Other field experiments go a step further and observe in real time the diffusion through social learning of a new resource or information that has been artificially introduced into a wild community (e.g., in blue tits: Aplin *et al*., 2013; in vervet monkeys: van de Waal *et al*., 2013). This type of experiment can test for capacities for cultural diffusion within a population, but does not inform us on how new behaviours *naturally* appear and diffuse in the population (Thornton *et al*., 2010). Nonetheless, in some "lucky" instances (more likely to occur at long-term study sites) researchers can witness the invention or change of a specific behaviour in real time, and chart its spread through the population over time. Statistical tools, particularly **network-based diffusion analysis (NBDA)** (Hoppitt *et al*., 2010), can then be used to confirm whether the behaviour's spread is likely to be driven by social learning (rather than a series of independent individual inventions), lending support to a cultural interpretation. This was the case with the newly invented "moss-sponging" (Hobaiter *et al*., 2014) technique by chimpanzees of the Sonso community (Budongo Forest, Uganda): both the behaviour's invention and its later spread was witnessed by researchers, and the sequence of the latter was confirmed to align with channels available for transmission (social network links) within the community. Another excellent example of using NBDA to examine the role of social transmission in the spread of a new behaviour is the case of "lobtail feeding" by humpback whales (Allen *et al*., 2013) (see section 1.3 for details).

Lastly, methodological tools from **archaeology** can be a valuable addition to the study of animal culture. Archaeological excavations in the Tai Forest revealed that chimpanzees have been nut-cracking in the same location as contemporaneous chimpanzees for at least 4,300 years, demonstrating their capacity to maintain long-term traditions (Mercader *et al*., 2007). Similar methods have uncovered stone tools used by capuchin monkeys (*Sapajus libidinosus*) in Serra da Capivara (Brazil) 2,400-3,000 years ago, showing evidence of not only long-term use but also tool variation through the centuries (Falótico *et al*., 2019). This type of data is incredibly valuable for understanding the time-depth of animal culture; however, these archaeological approaches are constrained to behaviours that result in the making and/or use of non-perishable artefacts.

1.3 Animal culture in the wild

The first suggestions of cultural phenomena in animals were put forward over 70 years ago. Arguably the earliest of these concerned the transmission of "milk-bottle opening" by titmice

(*Parus* sp.), a newly invented behaviour that was tracked across the United Kingdom and across different bird species (Fisher and Hinde, 1949). Thereafter, expanding the list of putative examples of animal culture, human-provisioned Japanese macaques (*Macaca fuscata*) were seen inventing new food processing techniques (e.g., sweet potato washing) that quickly spread through kin networks (Kawai, 1965), and regional birdsong dialects were discovered in white-crowned sparrows (*Zonotrichia leucophrys*) (Marler and Tamura, 1964). Since then, the field of animal culture has flourished, and evidence of social learning and culture now exists for all major classes of vertebrates, as well as some invertebrates, both in the wild and in captivity (Whiten, 2017; 2021). Additionally, and perhaps more importantly, there is increasing evidence of culture across varied domains, including communication, social customs, tool use, foraging and food processing, migration and navigation patterns, mate choice and predator avoidance. In the previous section (1.2) some of these examples were used to illustrate different approaches to the study of animal culture. In this section I will focus exclusively on examples from the wild, to showcase the diversity of species that have been studied and the range of contexts in which cultural processes have been identified.

Some of the most impressive and compelling examples of culture in the wild come from **communication** in the ocean. Male humpback whales (*Megaptera novaeangliae*) sing long and elaborate songs in their winter breeding grounds, where the same song is shared by all males of the same population. Each year, part of the song undergoes changes that are quickly adopted by all members of the population (Payne and Payne, 1985). Furthermore, migration events can bring about even more profound changes: for example, in the east coast of Australia the arrival of a few individuals from the west coast triggered what scientists called a “cultural revolution”, where the eastern population's song was rapidly and completely replaced by that of the west (Noad *et al*., 2000).

The study of cetaceans has not only given us invaluable examples of culture in communication. As previously mentioned, the diffusion of lobtail feeding by humpback whales represents a well-known example of innovation and diffusion of a new **foraging** technique. Lobtail feeding ("striking the water's surface one to four times with the ventral side of the fluke, followed by a bubble-feeding sequence" Allen *et al*., 2013:485) was first observed being innovated by a single individual in 1980, which, over a period of 27 years was seen to spread to others in the population through social learning (Allen *et al*., 2013).

In other taxa, cultural processes are at play in yet other behavioural contexts. For example, over an 11-year study of meerkat (*Suricata suricatta)* **sleeping patterns,** Thornton and colleagues (2010) found that neighbouring groups differed in the times they emerged from their burrow in the morning. Each group consistently emerged at the same time each morning, but the intergroup differences were prominent despite overlapping territory (or even sleeping borrows), group turnover over time and inter-group migration. It is therefore suggested that morning emergence time is culturally transmitted within meerkat groups, rather than dictated by the environment or genetics. Other cultural behaviours appear to have more of a social function, i.e., **social customs**, as is the case with white-faced capuchins (*Cebus capucinus)* in Costa Rica. By comparing different groups, researchers have identified six behaviours argued to be cultural traditions: "bond testing rituals" that include hand-sniffing, prolonged sucking of body parts, and eye-poking (Perry *et al*., 2003; Perry, 2011).

But the best-studied case of animal culture comes, perhaps unsurprisingly, from the chimpanzee (e.g., McGrew, 1992; Nishida *et al*., 1999; Whiten *et al*., 1999; Biro *et al*., 2003; Schöning *et al*., 2008; Luncz and Boesch, 2014; Kühl *et al*., 2016). Chimpanzee culture is considered prolific, since it exhibits extensive variation across several different behavioural domains (e.g., McGrew, 1992). Examples abound of chimpanzee **tool use**, including the already mentioned nut cracking (e.g., Luncz *et al*., 2012) as well as termite fishing (e.g., Pascual-Garrido, 2019), honey gathering (e.g., Sanz and Morgan, 2009), leaf and moss sponging (Hobaiter *et al*., 2014), spear hunting (Pruetz and Bertolani, 2007), and algae scooping (e.g., Boesch *et al*., 2017). But not all chimpanzee tool use is extractive in nature. Leaf-clipping (i.e., ripping apart one or more leaves, using the mouth, and/or fingers, producing a conspicuous and distinctive ripping sound) for example, is performed with different functions by different communities, to initiate play behaviour (e.g., Nishida, 1980) or as part of a drumming display (e.g., Boesch, 1995). Furthermore, there are many more examples of chimpanzee cultural variation beyond the context of tool use. Whiten *et al*.'s (1999) original catalogue, as well as the recent paper by Kühl *et al*. (2019), reveal a vast range from cave use (Pruetz, 2007) to accumulative stone throwing (Kühl *et al*., 2016), hand-clasp grooming (e.g., Van Leeuwen *et al*., 2012) and even ground nesting (e.g., Koops *et al*., 2007).

1.4 Gathering data on behavioural variation in the wild

In section 1.2 I focussed on the methodological approaches employed to study animal culture both in the wild and in captivity, using both observational and experimental methodologies. In this section I will be focussing on specific methodologies that can be used to collect behavioural data in the wild.

In situ studies allow researchers to observe not only the behavioural repertoire of a species but also understand the conditions (ecological or social) in which the behaviours happen, which provide the raw information with which research questions and hypotheses can be produced (Bateson and Martin, 2021). Most long-term field studies rely on **habituation** of the studies' subjects to human presence in a way that allows for observers to be close enough to the subjects in order to distinguish individuals, observe their behaviour, as well as record their subtle interactions with the environment and with others (Gruen *et al.*, 2013). Studies of this kind have provided us with invaluable accounts of communities' behaviours and, through comparisons with other well-studied groups, their culture (e.g., Whiten *et al.*, 1999). However, there are many limitations associated with habituating wild animals. Not only does it require a major long-term investment of time, money, labour and sometimes risk for the observer, but it can also be a source of disease transmission to the subjects (e.g., Woodford *et al.*, 2002), a source of unwanted behavioural change (e.g., due to provisioning: Foroughirad and Mann, 2013), and leave unafraid animals vulnerable to poaching or other negative human interactions (e.g., Robbins and Boesch, 2011). However, most unhabituated animals are afraid of human observers which makes their observation nearly impossible (Boyer-Ontl and Pruetz, 2014).

When direct observation is impossible or opportunistic, researchers must rely on other methods of data collection - **indirect methods**. These can include the collection and macro-analysis of trace evidence, such as scats and feeding remains (e.g., McGrew *et al.*, 1988; McLennan, 2013), nesting sites (e.g., Sousa *et al.*, 2011), or tool use artefacts (e.g., Carvalho and Almeida-Warren, 2019). Other, more technologically sophisticated methods such as DNA barcoding and metabarcoding of scats (e.g., Hartvig *et al.*, 2021), or isotope analysis of biological samples such as hairs (e.g., Crowley *et al.*, 2014) can also be valuable sources of behavioural information, by informing on specific foods an individual ate, or by giving information of dietary patterns over long periods of time, respectively. Indirect methods can provide researchers with valuable and detailed information of specific behaviours (e.g., tool use, resource exploitation); however, much of the behavioural repertoire of the group, particularly behaviours that do not leave any material trace behind (e.g., social customs) will be completely or partially missed. Other methodologies such as the use of drones, satellite imagery, and bio-loggers can be excellent tools to study the movement and migration of species (Hughey *et al*., 2018). The recent proliferation of camera traps in behavioural studies has proven to be particularly promising when seeking to study the overall species repertoire, since cameras can provide researchers with a rich source of information on specific behaviours, social interactions, individual identification and much more. The use of camera traps might also allow for a more accurate sampling of a group's behaviour: it is not biased against individuals that traditional methods of behavioural observation might occasionally overlook, such as more timid group members (females, infants or lower ranking individuals) who also might be at a higher risk from habituation altogether (e.g., Boyer-Ontl and Pruetz, 2014). The combined use of some of these indirect methods of study can provide us with rich behavioural information about a community, especially if they are employed over extended periods of time. Additionally, given how much less time is required to collect indirect data compared to the process of habituating and following a given group, indirect data collection is particularly amenable to studying multiple communities concurrently (see Kühl *et al*., 2019), or better yet, to comparing neighbouring communities. Long term habituation and daily observation might provide us with the best methodology to study animal behaviour and its variation; however, ultimately, the safety and conservation of the species that researchers want to study and protect should always be the priority (Gruen *et al*., 2013).

My thesis employs a combination of direct and indirect methods to explore the behavioural repertoire of four unhabituated neighbouring chimpanzee communities, and to assess inter-community behavioural variation at a local, subspecies and species level. In the next section, I describe the study species, the research setting and its particularities, highlighting how it provides a particularly informative context in which to examine the emergence and maintenance of behavioural – and potentially cultural – variation in our closest living relative.

1.5 The study species and site

Chimpanzees are the most abundant and widespread of all Great Ape species (Humle *et al*., 2016a). The most recent predictions estimate the total population size to be between 172,700–299,700 individuals (Butynski, 2003). Chimpanzees are found in a variety of habitats across the African forest belt occupying primary forest, secondary forest, swamp forest, dry forest, submontane and montane forest, savanna woodland, farmland and fallow-agricultural matrixes dominated by oil palms (Humle *et al.*, 2016a). However, despite their widespread distribution, chimpanzees face many threats such as poaching, disease transmission, habitat loss and degradation, and have been classified as endangered by the IUCN since 1996 (Butynski, 2003; Humle *et al*., 2016a).

Chimpanzees are highly social and live in communities ranging in size from 12 (Bossou, Guinea: Hockings *et al.*, 2012) to 150 individuals (Kanyawara, Uganda: Potts *et al.*, 2011). These communities are multimale-multifemale, and exhibit a fission-fusion dynamic – i.e., the community splits ("fission") into smaller groups, and merges ("fusion") throughout the course of one or more days (Kummer, 1971). Female chimpanzees tend to leave their natal community once they reach reproductive age (*c.* 10 years of age), and males are generally philopatric (Williamson *et al*., 2013). Chimpanzees' maximum life span is thought to be *c.* 50 years, with slow reproductive rates due to the complete dependency of infants during the first five years of age *(Williamson et* *al.*, 2013). A female will typically give birth every six years and have on average three to four offspring during her lifetime (Inskipp, 2005). Estimated chimpanzee generation time is 25 years (Langergraber *et al.*, 2012). Chimpanzees build nests every night, and occasionally during the day. Nests are usually arboreal, but ground nests are common in some communities (e.g., Nimba, Guinea: Koops *et al.*, 2007). They are considered ripe fruit specialists but have an omnivorous diet that includes many other plant organs (i.e., flowers, leaves, nuts, seeds, tubers, and bark), as well as non-plant foods including honey, invertebrates and occasionally vertebrate meat (e.g., Morgan and Sanz, 2006; Potts *et al.*, 2011; Bessa *et al.*, 2015). Chimpanzees are, together with bonobos (*Pan paniscus*), our closest living relatives, sharing approximately 99% of our DNA (Mikkelsen *et al.*, 2005; Prüfer *et al.*, 2012). This close genetic similarity, together with chimpanzees' behavioural diversity and ecology make the species a good living model of our last common ancestor, giving researchers valuable insights into the study of human evolution (e.g., McGrew, 2010).

Generally, four chimpanzee subspecies are recognised: the Eastern Chimpanzee (*P. t. schweinfurthii*); the Central Chimpanzee (*P. t. troglodytes*); the Nigeria-Cameroon Chimpanzee (*P. t. ellioti*); and the Western Chimpanzee (*P. t. verus*). In this thesis I will be focussing on the study of the Western Chimpanzee. In the past 50 years, this subspecies has experienced a significant population decrease and was categorised as critically endangered in 2016 (Humle *et al.*, 2016b; IUCN SSC Primate Specialist Group, 2020). *P. t. verus* is present in eight countries – Senegal, Guinea, Guinea-Bissau, Ghana, Liberia, Côte d'Ivoire, Mali and Sierra Leone – and the population size has been most recently estimated to be around 52,800 individuals (range 17,600–96,600) (Heinicke *et al.*, 2019; IUCN SSC Primate Specialist Group, 2020). Most western chimpanzees inhabit savannah-dominated habitats, followed by forest-dominated habitats and cropland. Mosaic habitats have a lower density of individuals than habitats of contiguous forest, while at least 40% of all western chimpanzees live within 5km of human settlements and c. 60% live within 5km of a road (Heinicke *et al*., 2019; IUCN SSC Primate Specialist Group, 2020).

All of the work in this thesis took part in Guinea-Bissau, where until 1988 chimpanzees were thought to be extinct (Lee *et al*., 1988; Scott, 1992). Currently, the estimated number of chimpanzees in Guinea-Bissau is 1,908 (range: 923–6,121) (Heinicke et al., 2019). It was only after their (re-)discovery that systematic research was launched in the region (Gippoliti *et al*., 2003), and only recently have more in-depth studies been conducted in the four Guinea-Bissau National Parks that are known to harbour chimpanzees – Cantanhez NP (e.g., Hockings and Sousa, 2013; Bessa *et al*., 2015; Vieira *et al*., 2019; Bersacola *et al*., 2021), Lagoas de Cufada NP (Carvalho *et al*., 2015), Boé NP (Kühl *et al*., 2016, 2019) and Dulombi NP (Bersacola *et al*., 2018).

In this thesis I concentrated my study efforts in Cantanhez National Park (CNP) (N11° 14.287' W15° 02.281'), located in the Tombali region of south-west Guinea-Bissau. CNP has an area of 1057 km^2 and it has been a National Park since 2008. It is a mosaic of settlements, agricultural fields, sub-humid forest, secondary forest, palm groves, mangroves, woodland, and open savannahs. Recent work has revealed that despite the large percentage of forest cover across the habitat matrix, these forest areas are highly fragmented and interspersed with areas cleared for agriculture (Bersacola, 2019). Forest edges often remain around agricultural fields and, together with fallow land and orchards, work as corridors for some wildlife (Bersacola, 2019). CNP holds 110 villages with an estimated population size of 25,000 people (Bersacola, 2019) that include many ethnic groups: the Nalu (traditional owners of the land in Cantanhez (Temudo, 2012)), Fula, Balanta, Mandinga, Sussu, Tanda, Papel and others. This means that throughout the National Park people and wildlife live in close proximity. CNP is extremely rich in wildlife: it is home to six species of diurnal primates – the critically endangered western chimpanzee (*Pan troglodytes verus*), the endangered Temminck's red colobus (*Piliocolobus badius temminckii*), the vulnerable King colobus (*Colobus polykomos)*, the Guinea baboon (*Papio papio*), the green monkey (*Chlorocebus sabaeus*), the Campbell's monkey (*Cercopithecus campbelli*) - and at least one species of nocturnal primate, the Demidoff's galago (*Galagoides demidoff)* (Bersacola, 2019). It is estimated that 10 to 12 chimpanzee communities inhabit CNP (Bersacola, 2019). These include the four neighbouring communities studied in this thesis: Caiquene-Cadique, Lautchandé, Madina and Cambeque. Previous surveys, genetic and ecological research, local knowledge, and the presence of natural barriers all suggest that these are independent communities (Hockings and Sousa, 2013; Sá, 2013; Bessa *et al*., 2015; Bersacola, 2019; Vieira *et al*., 2019; Hockings *et al*., 2020, 2021). These four neighbouring communities are unhabituated to researchers and only limited information is known about them. Nonetheless, previous work estimates that the Caiquene-Cadique community has a minimum of 49 individuals and Madina 48 individuals (Marina pers. comm; Vieira *et al*., 2019). The same information is lacking for the two other communities (Lautchandé and Cambeque) at present, but it is likely that these communities have similar group sizes. Recent work (which included data collected for this thesis) has confirmed the presence of leprosy (*Mycobacterium leprae)* in three (Caiquene-Cadique, Lautchandé, and Cambeque) of the studied communities (Hockings *et al*., 2021, Appendix A).

There are two marked seasons in CNP, the dry-season (November to mid-May) and the rainy season (mid-May to October). Data collection took place over the course of 23 consecutive months, between February 2017 and December 2018; covering both dry and rainy seasons. During 2017, annual rainfall was 2351 mm with an average temperature of 26.3ºC (15.6ºC min to 38.6ºC max). Direct and indirect data was collected by me together with a group of local field assistants

(Mamadú Cassamá, Iaia Camará, Adulai Camará, Fernando N'Dafa and Idrissa Galiza), and camera traps deployed throughout the four study communities continuously recorded video data throughout the study period.

Very little is known about the CNP or even the Guinea-Bissau chimpanzees' behavioural repertories. Bessa *et al*. (2015) studied the feeding ecology of the chimpanzee community of Caiquene-Cadique (CNP), where leaf sponging, and honey consumption (without tools) were identified. The authors also suggest, based on indirect evidence, the possibility of these chimpanzees consuming giant African snails, cracking them against wooden anvils (Bessa *et al*., 2015). Additionally, a recent cross-African study, that included a chimpanzee community in Boé NP, described a newly discovered behaviour in the region – accumulative stone throwing (Kühl *et al*., 2016). This same behaviour was confirmed in the region of Paia Lumba (Dulombi NP), during a pilot study in 2015 (unpublished data). Despite their scarcity so far, these new findings for the country, together with the local habitat diversity representing an unusual ecological setting among chimpanzee populations, suggest that any new research done in this territory could yield interesting results. Additionally, due to the increased encroachment on their habitat, these chimpanzees are facing rapid changes in their available resources, forest connectivity and rate of human encounters. These consequences are all suggested to potentially drive the loss of cultural traits (Kühl *et al*., 2019) but also their modification as an adaptive response (Gruber *et al*., 2019; Kalan *et al*., 2020). This highlights the importance of surveying chimpanzee communities inhabiting anthropogenically impacted areas, such as those of CNP.

1.6 Thesis outline

In addition to this introductory chapter, the thesis includes four research chapters that are self-contained and can be read independently, as well as a final general discussion chapter. Supplementary material for each data chapter can be found at the end of the chapter, and Appendix can be found at the end of the thesis.

Chapter 2 describes and compares the cultural repertoire of the four previously unstudied, unhabituated neighbouring chimpanzee communities in CNP (Guinea-Bissau). During a two-year period, behavioural data was continuously and simultaneously collected at the four study sites through opportunistic observations and indirect methods that included extensive use of camera traps. In total, I collected and analysed 4197 chimpanzee videos and 1747 indirect chimpanzee traces, and compiled a list of 18 putative cultural behaviours, some of which had never been described for Guinea-Bissau, or even the rest of Africa. Additionally, by creating preliminary behavioural profiles for each neighbouring community, I was able to find significant evidence of inter-community variation that spanned tool use, communication, and social behaviour.

Chapter 3 focuses on inter-community differences in a specific set of behaviours identified in Chapter 2 - extractive tool-use for honey dipping. I collected a total of 204 individual stick tools, used for honey dipping, in those two of the four study communities where this behaviour was present. Using methods from the field of primate archaeology, I analysed all collected tools and assigned them putative functions based on different patterns of use-wear. Additionally, I compared the characteristics of tools used (i) to extract different types of honey, and (ii) by members of the two communities and found significant differences in both. The data collected also suggests the use of tool-sets, i.e., tools with different functions used sequentially towards the same goal; as well as possible multi-function tools, never before described for this subspecies.

Chapter 4 examines a chimpanzee ‘universal’ (i.e., a behaviour present in all communities studied to date) – buttress drumming. Despite its universal nature, there are some suggestions in the literature that this long-distance communication signal might carry important community-specific information. With the help of automated drumming annotation created using a deep convolutional neural network (CNN) approach, I analysed 1207 buttress drumming videos in relation to context, community, age and sex, as well as the incorporation of other behaviours (pant hoot vocalizations, loud self-scratching and leaf-clipping) into the drumming sequence. These analyses revealed that, drumming duration varied between communities, age and sex classes, context and with the presence/absence of pant hoot vocalisation. More importantly, my data suggests that buttress drumming duration might be part of community-specific signalling in specific contexts, such as travel, but that such specificity loses its relevance in contexts where long-distance communication is not the main function of drumming, such as agonistic displays.

Chapter 5 uses stable isotope analysis to compare the diet composition of all four chimpanzee communities studied. I collected and analysed a total of 125 chimpanzee hair samples as well as 390 plant samples from the four communities. $\delta^{13}C$ and $\delta^{15}N$ chimpanzee and plant values were compared between study communities and with previously published chimpanzee isotope data, in order to investigate possible isotope differences that could point us towards previously unknown behavioural variation. These isotope analyses confirmed a surprisingly high inter-community variation of both $\delta^{13}C$ and $\delta^{15}N$ chimpanzee values in CNP, as well as significant differences from previously studied chimpanzee communities inhabiting similar anthropogenic environments.

These results suggest that communities facing similar pressures may adopt different feeding strategies in response to increased human encroachment, even at small regional level.

Chapter 6 presents a General Discussion. Here, I synthesise my findings, discuss their implications for the field of animal culture, outline my work's limitations and provide recommendations for future research.

Overall, my thesis presents the first long-term study focussed on chimpanzee behaviour and behavioural variation in Cantanhez National Park (Guinea-Bissau), and, more importantly, it is the first to collect continuous and simultaneous data on four unhabituated neighbouring communities using a combination of direct and indirect methodologies and yielding a substantial amount of behavioural data, some of it unique, for a critically endangered species.

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CHAPTER 2

Inter-community behavioural variation confirmed through indirect methods in four neighbouring chimpanzee communities in Cantanhez NP, Guinea-Bissau

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Supplementary material can be found at the end of the chapter

2.1 Abstract

Culture, while long viewed as exclusively human, has now been demonstrated across diverse taxa and contexts. However, most animal culture data are constrained to well-studied, habituated groups. This is the case for chimpanzees, arguably the most 'cultural' non-human species. While much progress has been made charting wild chimpanzees' cultural repertoire, large gaps remain in our knowledge of the majority of the continent's chimpanzees. Furthermore, few studies have compared neighbouring communities, despite such comparisons providing the strongest evidence for culture, and few have studied communities living in anthropogenic habitats although their culture is in imminent danger of disappearing. Here we combine direct, indirect and remote methods, including camera-traps, to study, over two years, four unhabituated neighbouring chimpanzee communities inhabiting human-impacted habitats in Cantanhez NP, Guinea-Bissau. From traces collected during 1089 km of reconnaissance walks, and 4197 videos from 56 camera-trap locations, we identified 18 putative cultural traits. These included some noteworthy novel behaviours for these communities, and behaviours possibly new to the species. We created preliminary behavioural profiles for each community, and found inter-community differences spanning tool use, communication, and social behaviour, demonstrating the importance of comparing neighbouring communities and of studying previously neglected communities including those inhabiting anthropogenic landscapes.

2.2 Introduction

Animal culture, defined as patterns of behaviour that are group-typical and transmitted, at least in part, through social learning (Laland and Hoppitt, 2003), is argued to be a source of adaptive behaviour: individuals can more readily discover the solution to a problem if they attend to or copy the behaviour of other individuals facing the same problem, allowing them to better exploit their natural and social environment. Furthermore, cultural traditions can influence evolutionary rates and trajectories by homogenising behaviours within a population and allowing rapid changes in a population's behavioural repertoire (Feldman and Laland, 1996). Overall, the study of animal culture can inform researchers of different ways animals adapt to their environment (e.g., foraging techniques, migratory patterns, communication of information), and might be a valuable tool when planning conservation strategies (Laland *et al*., 2009; Gruber *et al*., 2019; Kühl *et al*., 2019; Brakes *et al*., 2021). The first evidence for animal culture was described over 70 years ago where the transmission of a new behaviour (milk-bottle opening) by titmice (*Parus sp*) was tracked across the United Kingdom (Fisher and Hinde, 1949). Soon after, novel foraging behaviours that spread through kin networks were found in Japanese macaques (*Macaca fuscata*) (e.g., sweet potato washing) (Kawai, 1965), and regional birdsong dialects were discovered in white-crowned sparrows (*Zonotrichia leucophrys*) (Marler and Tamura, 1964). Since then, evidence of animal culture has been identified in numerous taxa, from fish to meerkats, cetaceans and apes (e.g., Helfman and Schultz, 1984; Thornton *et al*., 2010; Luncz *et al*., 2012; Mann *et al*., 2012; Allen *et al*., 2013), both in the wild and in captivity.

Multiple approaches to studying animal culture have been proposed. Among these, the one most widely used in the wild to date is the ethnographic method (Wrangham *et al*., 1994) or "method of exclusion" (Krützen *et al*., 2007) that identifies culture by ruling out possible ecological or genetic explanations for inter-group behavioural variation documented. A paradigmatic example of this method was the first large-scale chimpanzee cross-population study that extracted 39 candidate behavioural traits from six habituated chimpanzee communities in West and East Africa (Whiten *et* *al*., 1999). These included habitual or customary behaviours that could not be explained by ecological or genetic differences between populations, hence by exclusion were considered cultural variants. Some experimental methods rely on a similar rationale: for example, translocation experiments, where individuals are moved from one population to another or whole populations are exchanged between sites (Helfman and Schultz, 1984; Warner, 1988) seek to establish whether existing behavioural variation is more likely to be traditional than due to genetic or environmental influences. Nonetheless, many argue that methods of exclusion, if rigorously applied, might erroneously reject cases of animal culture (e.g., Wrangham *et al*., 1994; Laland and Janik, 2006; Laland *et al*., 2009), and in fact, ecology, genetics, and social learning are inexorably interlinked and can all influence, to some degree, behavioural variation (Laland and Janik, 2006; Humle, 2010). For more direct demonstrations of cultural processes, some researchers have employed elegant field experiments where new resources or information are artificially introduced into a wild community to observe, in real time, their diffusion through social learning (e.g., Thornton and Malapert, 2009; Aplin *et al*., 2013). Nonetheless, although these studies allow us to confirm that new behaviours can spread through groups through social learning, they do not inform us about the nature and spread of naturally occurring behavioural variation (Thornton *et al*., 2010). Statistical approaches try to tackle this problem: for example, network-based diffusion analysis (NBDA) has been used to study the transmission of newly invented behaviours in chimpanzees (Hobaiter *et al*., 2014), humpback whales (Allen *et al*., 2013) and bottlenose dolphins (Wild *et al*., 2019). While powerful when the right data are available, one limitation of the NBDA approach is that it requires long-term data collection on the same population, as well as some degree of luck in witnessing the natural emergence and transmission of a novel behaviour.

While large-scale ethnographic studies have given us valuable insights into species-level variation in putatively cultural behaviours (Whiten *et al*., 1999, 2001), it has been suggested that more compelling evidence for culture might come from studying the same subspecies (e.g., Laland and Janik, 2006;

Luncz *et al*., 2012). For example, while all four subspecies of *Pan troglodytes,* but not all known communities, engage in army ant (*Dorylus* spp.) dipping, there is variation in the technology employed even among different communities of the same subspecies (Schöning *et al*., 2008). Some of this variation can be linked to the aggressiveness of the ant species exploited, but in some cases such an ecological account does not fully explain the variation found (Humle and Matsuzawa, 2004; Kühl *et al*., 2019). Therefore, many have recently suggested that the study of neighbouring communities might be the most informative approach to studying animal culture in the wild (e.g., Thornton *et al*., 2010; Luncz *et al*., 2012; Koops *et al*., 2015; Pascual-Garrido, 2019): comparing communities where groups broadly face the same ecological constraints and individuals migrate between communities, make ecological or genetic explanations for behavioural variation less likely or important compared to a cultural explanation. Illustrating this approach, Luncz and colleagues (2012) compared the selection of wooden and stone hammers for coula (*Coula edulis*) nut-cracking in three habituated neighbouring chimpanzee communities in Tai National Park, Ivory Coast. Even though these neighbouring communities inhabit the same forest habitat with minimal ecological variation between their home ranges, the study showed that there was marked inter-community variation in hammer size and raw material preferences (Luncz *et al*., 2012). Similarly, Thornton and colleagues (2010) have shown that neighbouring groups of meerkats show consistent differences in their time of emergence from their sleeping burrows, despite overlaps in burrow use and extensive gene flow between groups. In sum, it is clear that different methods have their pros and cons and their feasibility is dependent on species, environment, financial and ethical constraints, among others (Brakes *et al*., 2021), but many of the examples here described emphasise the need for more comparative studies of behavioural variation at a local scale, both in terms of the presence/absence of specific behaviours across communities and their detailed descriptions that may reveal subtle variation in form.

In chimpanzees, arguably the most "cultural" among non-human species, much effort has focused on building a comprehensive catalogue of the species' cultural repertoire (Whiten *et al*., 1999, 2001). Yet, while much of the species' range has been studied (McGrew, 2004; Sanz *et al*., 2004; Pruetz, 2007; Gruber *et al*., 2015; Hockings *et al*., 2015a; Kühl *et al*., 2016, 2019), new behaviours and behavioural variants keep being identified, suggesting that gaps still remain to be filled. At the same time, increasing human disturbance, and consequent fragmentation, degradation and change of habitats and available resources, are suggested to have the potential to bring about both the loss of existing behavioural variants (Kühl *et al*., 2019) and their modification as an adaptive response to environmental changes (Hockings *et al*., 2015a; Gruber *et al*., 2019; Kalan *et al*., 2020). This highlights the importance of surveying communities inhabiting anthropogenically impacted areas. Specifically, little is known about the westernmost populations of the species' distribution. This means that not only are we lacking detailed information about potential regional cultural variation, but we may have a limited time in which to chart it: behaviours may go extinct, and we may lose our ability to document how chimpanzees respond to life in the Anthropocene (Hockings *et al*., 2015b).

The present study aims to fill these gaps by being the first to explore cultural variation between neighbouring chimpanzee communities inhabiting Cantanhez National Park (CNP), Guinea-Bissau. CNP is thought to be home to approx. 10-12 chimpanzee communities (Bersacola, 2019) inhabiting a mosaic of habitats. Bessa *et al*. (2015) studied the feeding ecology of the chimpanzee community of Caiquene-Cadique in CNP for nine consecutive months, where apart from the use of leaf sponges, a universal chimpanzee behavioural trait, no other type of tool use was confirmed (unpublished data). A recent study, however, has found evidence of honey-dipping tools in other CNP communities (Bessa *et al*., 2021); Bessa *et al*. (2015) also suggest, based on indirect data (i.e., traces such as accumulation of snail shells, use-wear marks in wooden anvils, and bite marks in soft tissue of discarded snails), the possibility of the Caiquene-Cadique chimpanzees cracking giant African snails (*Achatina achatina*) against wooden anvils and eating them (a behaviour first described in a recent study of Bili-Uéré's (DRC) chimpanzees, though direct evidence is yet to be found (Hicks *et al.*, 2019)). Additionally, a recently discovered behaviour – accumulative stone throwing (Kühl *et al.*, 2016; Kalan *et al.*, 2019) – has also been confirmed in Boé National Park, Guinea-Bissau. Overall, preliminary work indicates the presence of a potentially unique cultural profile for the poorly studied Guinea-Bissau chimpanzees.

We collected behavioural data on four unhabituated neighbouring chimpanzee communities in central CNP. Due to the communities' close proximity to local people, we chose not to habituate chimpanzees, therefore any direct observation was merely opportunistic, and the majority of data collection relied on a combination of indirect methods and camera traps. As such, our aim was also to demonstrate the feasibility of using this combination of methods to achieve our three primary goals: 1) to document new behaviours for the studied communities, expanding our to-date limited knowledge of their behavioural repertoire; 2) to identify behavioural variation among communities; and 3) to contribute to continental-level comparisons in the ever-growing chimpanzee behavioural repertoire. Our indirect data collection methods (see below) were particularly suitable for behaviours that leave trace evidence (extractive tool use, resource consumption), while the camera trap data was our principal source of information for behaviours that are more ephemeral/do not leave traces (communication and display, social behaviour). We structure our results according to these categories.

2.3 Methods

Study Site

CNP is located in the Tombali region of south-west Guinea-Bissau. CNP has an area of 1057 km^2 and is a mosaic of settlements, agricultural fields, sub-humid forest, secondary forest, palm groves, mangrove, and savannah. Recent work has revealed that a large percentage of forest cover remains across the habitat matrix, but these forest areas are highly fragmented and interspersed with cultivated land. Areas cleared for agriculture often have remnant forest edges that together with fallow land and orchards can work as corridors for some wildlife (Bersacola, 2019) (Fig. 2.1). Data collection took place in four neighbouring unhabituated chimpanzee communities at CNP: Caiquene-Cadique, Lautchandé, Madina and Cambeque, over the course of 23 consecutive months (February 2017 - December 2018). We identified different communities based on previous research, including genetic studies, local knowledge, and the presence of natural and anthropogenic barriers (e.g., villages, roads, rivers and estuary branches that form small peninsulas) (Hockings and Sousa, 2013; Sá, 2013, 201; Bessa *et al*., 2015; Bersacola, 2019; Vieira *et al*., 2019; Hockings *et al*., 2020). The size of each community's ranging area was estimated using minimum convex polygons: Caiquene-Cadique 14.8 km^2, Madina 19.0 km^2, Cambeque 7.1 km^2, and Lautchandé 8.4 km^2. Additionally, previous work had estimated the minimum community size in Caiquene-Cadique as 49 individuals (Ramon, pers comm) and Madina as 48 individuals (Vieira *et al*., 2019), for Lautchandé or Cambeque we did not possess sufficient information to estimate community sizes.

Data collection

As the chimpanzees of CNP are unhabituated to researchers, a combination of direct, indirect and remote data collection methods were employed. To ensure that data collection was systematic and comparable to other study sites across Africa, methods were adapted from the Pan African Programme guidelines (Arandjelovic *et al*., 2014; Kühl *et al*., 2019) and are explained in detail below. Study efforts across the four sites are summarised in Table 2.1.

Indirect data

Reconnaissance walks ("recces", which followed given compass bearings while walking the path of least resistance (Arandjelovic *et al*., 2014)) were conducted at each study site by following chimpanzee paths and forest trails that covered as many different habitat types as possible. This method was chosen over systematic transects in order to minimise disturbance to an already highly fragmented habitat, and to avoid opening up new trails for hunters. Recces were walked in rotation between communities, from February to July 2017 and from February to July 2018 spanning across the second half of the dry season and the first half of the wet season (see Table 2.1 for number of recces and total distances covered per community). During recces, data on direct encounters (i.e., group size, composition, location, and behaviours) and indirect signs of chimpanzee presence and behaviour, including faeces, feeding traces, nests, tool-use sites, and foot and knuckle prints, were collected. These data were used to estimate chimpanzee core ranging areas and to find the best locations to set up camera traps. Any plant and animal species associated with chimpanzee behaviour (e.g., feeding traces, tools used in extractive foraging) that were not identified in situ were collected for later identification by local people or, when necessary, by consulting the relevant literature (see Catarino *et al*., 2006, 2020). Every behaviour that was identified in one community was added to a list of candidate behaviours for inter-community variation, and efforts were made to confirm the presence of each in all of the other communities.

Remote data

Camera traps (Bushnell Trophy Cam HD Aggressor No-glow) were set up at 56 different locations in CNP (see Table 2.1 for a more detailed breakdown). Camera traps were motion triggered and recorded 1-minute videos, and were set up in places habitually used by chimpanzees and where (1) tools had been found and/or tool use behaviour was expected to take place (e.g., stingless bee hives; natural water sources), and (2) where there was evidence of habitual presence of chimpanzees and where other behaviours of interest could occur (e.g., trees with large buttress roots with clear signs of wear). To maximise the chances of capturing behaviours of interest, some cameras were moved during the study to account for seasonal changes in chimpanzee ranging patterns, and to capture previously undocumented behaviours.

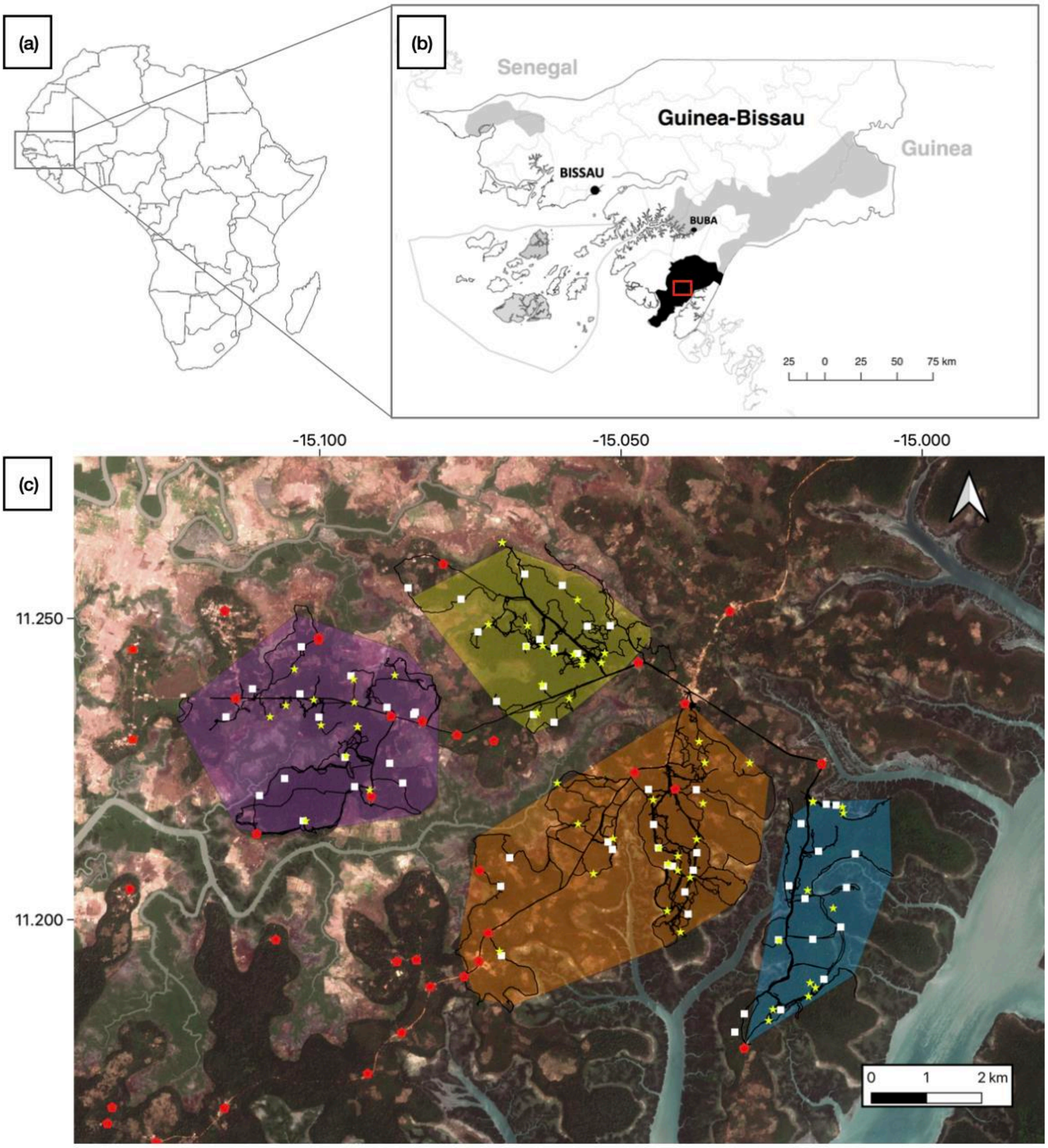

Figure 2.1. Locations of research sites and study communities' core home ranges. (a) Map of Africa showing the location of Guinea-Bissau. (b) Map of Guinea-Bissau showing the locations of Cantanhez National Park (black) and other protected areas (light grey). (c)Aerial image showing the locations of the four study sites in CNP. The core home ranges of the four chimpanzee communities, minimum bounding polygons estimated from direct and indirect chimpanzee data points, are illustrated in different colours: [CC] Caiquene-Cadique (purple), [LA] Lautchandé (yellow), [MA] Madina (orange), and [CB] Cambeque (blue); The minimum bounding polygon for the core home range of this chimpanzee community provides only a rough estimate given the paucity of direct and indirect data points available. Black lines indicate recces walked, red pentagons correspond to locations villages and other human settlements, white squares correspond to 50x50 plots, and yellow stars correspond to camera trap locations. Sentinel-2 imagery was downloaded from the Sentinel Hub, Sinergise Ltd (https://www.sentinel-hub.com/). All maps were created using QGIS version 3.12 (https://www.qgis.org).

Resource availability data

During recces, data on the presence of specific resources were recorded ad libitum: nut bearing trees, movable stones, beehives, termite and army ant nests and trails, and giant African snails. These resources were selected since they are associated with specific putative cultural behaviours, mostly tool use, in other studied communities. Additionally, 15 50x50 m plots were established at random locations in each of the four communities' ranging areas to assess the presence/absence of the same resources. When movable stones were found they were tested for hardness and sturdiness by hitting them twice with significant force against a wooden or stone substrate; if the stone did not fragment it was considered potential tool raw material (Arandjelovic *et al.*, 2014).

Data analysis

We compiled a list of behaviours present based on the data collected (including camera trap footage) and categorised observations as either species-typical behaviours (i.e., present universally across all habituated chimpanzee populations), or putatively cultural (i.e., present at some or all of our study sites but not across the whole species range, or present at at least one, but not all of our study sites). The categorisation was done based on previously published data on chimpanzee culture and behavioural variation (following Whiten *et al.*, 1999; Kühl *et al.*, 2019). Definitions for each behaviour are given in Table 2.2 (adapted from Whiten *et al.*, 1999; Nishida *et al.*, 2010; Kühl *et al.*, 2019). Given that these are previously unstudied and unhabituated communities, data were insufficient to categorise behaviours as customary (occurs in all or most members of at least one age-sex class) or habitual (not customary but seen repeatedly in several individuals), as defined in Whiten *et al.*, (2001)). Therefore, the following categories were used to describe our knowledge of the prevalence of each behaviour: Confirmed (C) - the behaviour was clearly identified in the community; Unconfirmed (U) – the behaviour was not yet recorded in the community, but this may be explained by insufficient observation opportunities.

Table 2.1. Summary of study effort at each of the study sites, in Cantanhez National Park in Guinea-Bissau. All relevant resources were confirmed in all studied communities apart from Melipona sp, only confirmed in Cambeque and Madina. Information is provided on number of recces and kilometres (km) walked; camera trap (CT) number, operational period (first day and last day active) and total number of days active and functional; total number of videos recorded that contained chimpanzees. estimated ranging area; indirect data points collected; number of confirmed behaviours; Behaviours and their categorization (C – Presence confirmed through indirect data; C*- Presence confirmed through direct evidence (video or observation); U – Unconfirmed) at the four study sites in CNP. Additionally, the total number of indirect data (I) and camera trap videos (V) of each behaviour is given for each community after the behaviour categorization (I,V).

		CHIMPANZEE COMMUNITY			
		Caiquene-Cadique	**Cambeque**	**Lautchandé**	**Madina**
RECCES	**No. of recces**	48	49	48	50
	Distance Walked (km)	230.8	260.4	236.6	361.5
CAMERA TRAP DEPLOYMENTS	**No. of CTs**	12	11	15	18
	First CT Day	05/04/2017	07/03/2017	06/05/2017	20/02/2017
	Last CT Day	08/12/2018	22/11/2018	10/12/2018	04/12/2018
	Total CT Days	2367	2391	2109	2828
	Total no. chimpanzee videos	2254	828	468	647
DATA	**Estimate ranging areas (km^2)**	14.8	7.1	8.4	19.0
	No. of indirect traces	538	230	230	749
	No. of confirmed behaviours	17	14	9	18
BEHAVIOUR	**Fluid-dip**	U (0,0)	U (0,0)	**C (1,0)**	U (0,0)
	Honey-dip	U (0,0)	U (0,0)	U (0,0)	**C (2,0)**
	Honey-dip large stingless bees	U (0,0)	**C* (63,2)**	U (0,0)	**C (1,0)**
	Honey-dip small stingless bees	U (0,0)	**C (38,0)**	U (0,0)	**C* (100,8)**
	Leaf-sponge	**C (6,0)**	**C (6,0)**	**C (5,0)**	**C* (4,3)**
	Honey-feed, no tools	**C* (10,4)**	**C (3,0)**	**C (5,0)**	**C (2,0)**
	Mangrove-eat	**C* (6,7)**	**C (2,0)**	U (0,0)	**C (7,0)**
	Saltwater-drink	**C* (0,4)**	U (0,0)	U (0,0)	**C* (0,4)**
	Aimed-throw	**C* (0,4)**	U (0,0)	U (0,0)	U (0,0)
	Branch-drag	U (0,0)	U (0,0)	U (0,0)	**C* (0,1)**
	Branch-shake	**C* (0,10)**	**C* (0,2)**	**C* (0,2)**	**C* (0,8)**
	Buttress-drum	**C* (15,624)**	**C*(8,110)**	**C* (11,141)**	**C* (32,171)**
	Ground-slap	**C* (0,3)**	**C* (0,2)**	U (0,0)	**C* (0,1)**
	Ground-slap knuckles	**C* (0,1)**	**C* (0,1)**	U (0,0)	**C* (0,1)**
	Leaf-clip, fingers	**C* (0,33)**	**C* (0,1)**	**C* (0,3)**	**C* (0,4)**
	Leaf-clip, mouth	**C* (0,34)**	**C* (0,4)**	**C* (0,7)**	**C* (0,19)**
	Leaf-drag	**C* (0,11)**	U (0,0)	**C* (0,2)**	U (0,0)
	Leaf-pull, finger	**C* (0,10)**	**C* (0,1)**	U (0,0)	**C* (0,5)**
	Leaf-pull, mouth	**C* (0,2)**	**C* (0,1)**	**C* (0,1)**	**C* (0,3)**
	Rain-dance	**C* (0,13)**	U (0,0)	U (0,0)	U (0,0)
	Raspberry	**C* (0,3)**	**C* (0,1)**	U (0,0)	**C* (0,14)**
	Food-share	**C* (0,7)**	U (0,0)	U (0,0)	**C* (0,13)**

Table 2.2. Definition of behaviours recorded in the present study that show potential variation among study sites (adapted from Whiten *et al.*, 1999 and Nishida *et al.,* 2010).

Behaviour	**Definition**
Extractive tool use	
Fluid-dip	Manufacturing a probe from a twig to extract fluid.
Honey-dip	Manufacturing a probe from a twig, to extract honey bee (*Apis mellifera*) honey from nest.
Honey-dip large stingless bee forest	Manufacturing a probe from a twig, to extract large stingless bee honey (*Melipona sp*) from nest, generally found in open secondary forest.in forest setting.
Honey-dip small stingless bee mangrove	Manufacturing a probe from a twig, to extract small stingless bee honey (*Meliplebeia sp*) from nest, generally found in mangroves.
Leaf-sponge	Bundling leaves/vegetation, chewing or folding, to collect water and squeeze into the mouth.
Resource consumption Feeding and habitat use	
Honey-feed, no tools	Feeding on bee honey without a tool, employing snatch and run approach.
Mangrove-eat	Collecting salty leaves of *Avicennia germinans* (found exclusively in mangrove areas) from tree, either ingesting or chewing and spitting out (wadge).
Saltwater-drink	Drinking mangrove salt water that collects in puddles.
Communication and display	
Aimed-throw	Aiming and throwing of object.
Branch-drag	Dragging a large branch as part of a display.
Branch-shake	Shaking of branch, producing a conspicuous sound, prior to a buttress-drumming display.
Buttress-drum	Beating/drumming with hands or feet on buttress or trunk of a tree, normally preceded by pant-hoot vocalization.
Ground-slap	Striking substrate with open hands/feet or alternate hands/feet during display, sometimes followed by pant hoot vocalization.
Ground-slap, knuckles	As above, but substrate is struck with the knuckles instead of open hands.
Leaf-clip, fingers	Ripping apart of one or more, normally dried, leaves from the ground using the thumb and index fingers, one by one, producing a conspicuous and distinctive ripping sound. Typically precedes buttress-drumming display.
Leaf-clip, mouth	As above, but clipping is performed with mouth. Typically precedes buttress-drumming display.
Leaf-drag	Walking forward fast quadrupedally with head down and shoulders hunched, while pushing dry leaves with hands and feet, producing distinctive sound. Sometimes performed before and/or after buttress drumming.
Leaf-pull, fingers	Pulling of leaves, one by one, from a shrub or a twig, with index finger and thumb. Typically precedes buttress-drumming display.
Leaf-pull, mouth	As above, but pulling is performed with the mouth. Typically precedes buttress-drumming display.

Behaviour	Definition
Rain-dance	Performing vigorous charging displays at the start of heavy rain. May include slow as well as rapid charges, and may involve a variety of display patterns (e.g., ground slap, branch drag, branch shake, throw).
Raspberry	Producing a spluttering sound by pressing air and saliva through lips.
Social behaviours	
Food-share	Feeding by two or more individuals simultaneously on an item of food obtained by one of the individuals. Theft is excluded.

2.4 Results

During the study period a total of 1089 km were walked over 195 recces, and camera traps were set up in 56 locations, for a combined total of 9695 days, yielding a total of 4197 videos of chimpanzees (Table 2.1). During recces, 1747 indirect chimpanzee traces were recorded, including 204 extractive stick tools. For detailed information on study effort and data collected in each community see Table 2.1.

Our surveys of resource availability revealed the following results. At all sites the availability of three species of nut-producing trees (*Elaeis guineensis, Detarium senegalense* and *Parinari excelsa*) was confirmed. Termites (*Macrotermes* sp. and *Cubitermes* sp.) were present at all study sites, as were army ants (*Dorylus* sp.). At other chimpanzee research sites, these resources are exploited with the use of tools (i.e., nut-cracking and termite and ant fishing/dipping) (see Whiten *et al*., 2001). Nonetheless, no evidence of tool-assisted nut-cracking or insectivory was confirmed for CNP; additionally, a previous study that analysed hundreds of faecal samples in Caiquene-Cadique found no macroscopic evidence of insectivory (Bessa *et al*., 2015). Movable stones were present in all CNP communities but infrequently encountered. The stones found were fragile and broke easily, and no evidence of their use was found. Giant African snails (*Achatina achatina*) were present in all CNP communities' home range, nonetheless no conclusive evidence (e.g., direct observation) of the exploitation of snails was found. Honeybees (*Apis mellifera*) were present at all five study sites and a species of stingless bee (*Meliplebeia* sp.) was confirmed for all four CNP sites. A second species of stingless bee (*Meliponula* sp.) was only confirmed at Cambeque and Madina. Tool-use evidence as well as trace evidence of discarded honeycombs with teeth marks confirmed that each CNP community fed on at least one of the different types of honey (see Bessa *et al*., 2021).

A total of 22 behaviours of interest were identified during the study period; Figure 2.2 and Table 2.1 show their distribution across the study communities, and Figure 2.3 charts how their cumulative number varied with study effort (number of months of data collection). Four of these behaviours - Leaf-sponge, Buttress-drum, Branch-drag and Branch-shake - are universal for the species (Whiten *et al*., 2001), while the remaining 18 behaviours are putatively cultural. Four of the putatively cultural behaviours (Leaf-clipping with mouth and with fingers, Leaf-pulling with fingers, and Honey consumption without the use of tools) appear to be universal for our four CNP study sites. Five other behaviours were only confirmed at one of the study sites: these included Rain-dance (Caiquene-Cadique), Aimed-throw (Caiquene-Cadique), Branch-drag (Madina), Honey-dip (Madina), Fluid-dip (Lautchandé). The site with the greatest number of behaviours confirmed was Madina (18), while Lautchandé had the fewest (9). The following sections describe variation in different behavioural domains across the four sites in more detail.

Extractive Tool Use

A total of 204 dipping tools and 16 leaf sponges were found across the CNP study sites. The pattern of tool recovery exhibited some marked variation among the four neighbouring communities. No evidence of extractive dipping tools to access fluid (including honey) were found in Caiquene-Cadique, while the extraction of different honey types with tools appeared to be frequent in Cambeque (100 tools) and Madina (103 tools) (see Table 2.1). Video evidence of extractive tool use was recorded in Madina and Cambeque.

Feeding and Mangrove Resource Use

The use of mangroves and its resources (saltwater and salty leaves) was confirmed in three of the CNP communities. Chimpanzees at Caiquene-Cadique and Madina were seen Saltwater-drinking, and they, as well as Cambeque chimpanzees, were confirmed to chew on the salty leaves of black mangrove trees, *Avicennia germinans* (Mangrove-eat). No evidence of mangrove use was found at Lautchandé even though mangroves are present within the chimpanzees' known ranging area. Camera trap evidence of Mangrove-use was recorded for Madina and Caiquene-Cadique.

Communication and Display

Leaf-clipping, a type of non-extractive tool use, was observed in all four CNP communities. All Leaf-clipping was confirmed through camera trapping and performed in association with Buttress-drumming. Leaf-pulling in association with drumming was also recorded in all CNP communities. Raspberry vocalisation was captured on camera in three of the CNP communities, but not at Lautchandé. Additionally, in Caiquene-Cadique male chimpanzees were caught on camera throwing the giant fruit of *Treculia africana* during drumming displays.

Social Behaviours

Fruit-sharing of large *T. africana* fruits was recorded on camera in Caiquene-Cadique and in Madina, between a mother and her dependent offspring as well as between adults. Rain-dance display was only confirmed at Caiquene-Cadique.

Figure 2.2. Distribution of behaviours across the five study sites (Caiquene-Cadique, Lautchandé, Madina, and Cambeque). Coloured icons represent confirmed behaviours (C), and faded icons represent behaviours for which presence is still unconfirmed (U). See figure key for correspondence of icons to specific behaviours, described in detail in Table 2.2. Green squares represent known species universals, and blue squares represent universals for the CNP study sites. The Sentinel-2 imagery was downloaded from the Sentinel Hub, Sinergise Ltd (https://www.sentinel-hub.com/). All maps were created using QGIS version 3.10.5 (https://www.qgis.org)

2.5 Discussion

An essential component of identifying cultural variation in non-human species is documenting behavioural variation across populations. However, studying behaviour and its variation in groups of unhabituated wild animals is a notoriously difficult and long process (e.g., Hicks *et al*., 2019). Verifying the presence of specific behaviours and identifying inter-community variation are hampered by the patchy nature of available data, where the length of study and the methods employed have implications on the amount and nature of data that can be collected. Behaviours that leave behind material evidence, specific artefacts or modifications in the environment (e.g., feeding traces, tools, constructions) are easier to detect, while behaviours that leave no trace evidence (e.g., social or communicative behaviours) and/or are infrequent or rare in nature are impossible to document without direct in situ or remote observation. It is also evident that the longer a study runs the more researchers can learn about a population's behavioural repertoire, not simply because data accumulate over time but also because these studies may end up habituating or semi-habituating the individuals to researchers. Nonetheless, despite the constraints of working with unhabituated populations, the present study was able to compile, through a combination of direct and indirect methods, and remote monitoring, a list of behaviours and potential behavioural variations in four neighbouring chimpanzee communities in Guinea-Bissau. Furthermore, we were able to identify some noteworthy novel behaviours for these communities, as well as behaviours that are possibly new to the species as a whole, thus filling gaps in our knowledge of the chimpanzee behavioural repertoire at a continental scale.

We identified 22 distinct behaviours, some of which had previously been described as universal at a species level, such as Buttress-drumming and Leaf-sponging (Whiten *et al*., 1999), and therefore will not be considered candidates for cultural behaviours. Even though we did not find all the universal behaviours in all the study communities we cautiously assume that this was an artefact of sampling time and methodology, and that with increased study effort these will, in time, be identified for all communities. Several other behaviours appear universal at a local level in CNP such as Leaf-clipping or Honey-feeding (without tools), but when put into context at a subspecies or species level, are good candidates for cultural behaviour. This is either because the behaviour is known to be absent at least at some other study sites (e.g., Honey-feeding), the behaviour is exhibited in different contexts (e.g., Leaf-clipping), or there is as yet no evidence of the behaviour existing elsewhere (e.g., Mangrove-eat), suggesting variation across the species' range. Given our limitations (in terms of both data collection methods and study duration) it is not possible to conclusively confirm the absence of most of the studied behaviours, but considering the encounter rates of certain behaviours some putative variation seems likely. Perhaps the most striking case in point is Honey-dipping. While we confirmed the presence of at least one type of stingless bee (*Meliplebeia* sp.) in all four CNP communities' ranges and the presence of honey bee (*Apis mellifera*) at all four study sites, evidence of dipping for honey was only found in two of the four CNP communities. Although we cannot conclude with certainty that dipping for honey is absent in Caiquene-Cadique and Lautchandé – and indeed the picture may change as research efforts continue – it is likely that it occurs with at least greater frequency in the communities of Cambeque and Madina (for further discussion see Bessa *et al*., 2021). We chose to separate honey dipping into three types depending on the type of honey exploited (stinging bee, small stingless bee and large stingless bee honeys) since a previous study found significant differences in the number and types of tools used to extract these different types of honey (Bessa *et al*., 2021). We succeeded in confirming not only extractive tool use, but also non-extractive tool use. This type of behaviour does not produce recognisable artefacts and can only be documented through observation. Yet, despite working with unhabituated chimpanzees, we were able to remotely observe this behaviour through the use of camera traps. Leaf-clipping/pulling behaviours were confirmed in all CNP communities, and even though their function is as of yet unknown, they appear to play a role in Buttress-drumming displays. Hence, they could be considered examples of communicative or social tool use (Shumaker *et al*., 2011). Additionally, there appears to be variation in Leaf-clipping and Leaf-pulling behaviours, and through further analyses we will be able to characterise these variations in more detail (manuscript in preparation). For example, in some cases a single leaf is carefully clipped, in others several leaves are clipped simultaneously; on some occasions both varieties of leaf-clipping happen simultaneously (i.e., with mouth and hand) or leaf clipping and pulling are combined during the same event. Interestingly, such variation contrasts with what has been described at Tai Forest, the only other community known to Leaf-clip prior to drumming: there, according to Boesch (Boesch, 1995), the behaviour is highly stereotyped among all the individuals of the group, unlike the within-community variation we observed. Also associated with buttress drumming we identified a Raspberry vocalization that is commonly heard in captivity and has been described as species atypical behaviour (Hopkins *et al*., 2007). This behaviour has only been described in the wild once, in the Ngogo community (Uganda), but in the context of grooming (Pika, 2014).

One group of behaviours, which occurred exclusively in the mangrove habitat, is of particular interest. To our knowledge the use of mangroves has only been described in Loango (Gabon) (Head *et al*., 2019, but see Garriga *et al*., 2019) for another potential example, although this study did not report whether chimpanzees used the mangroves within their habitat. Mangroves are an integral part of the Cantanhez landscape and evidence of chimpanzees utilising resources within was confirmed for three of the four study sites. At the fourth, Lautchandé, we were not able to confirm any mangrove-related behaviours despite the presence of mangroves in the community's habitat. It may be the case that with the increase of study effort this will change; however, similarly to honey dipping, it is possible that different communities rely more on this habitat type than others. In the sense that mangrove-related behaviours are so clearly dependent on the environment, they can be compared to cave use by chimpanzees at other sites in West Africa (e.g., Fongoli in Senegal, Pruetz, 2007). Ecology can easily explain the absence of the behaviour in many communities, yet it can still vary between communities where ecological characteristics alone cannot account for such variation, and, as such, cave use was considered a cultural behaviour by Kühl and colleagues (2019). This affirms the value of incorporating ecology and habitat in studies of behavioural variation, and illustrates why it is fruitful to explore new areas and habitat types when studying chimpanzee behaviour. In a similar vein, our findings regarding some less common social behaviours, such as Food-sharing and Rain-dance, detected in at least one of the studied communities, demonstrates the importance not only of study duration but also of diversification in the range of possible behaviours that researchers should look for in unhabituated communities.

It is worth noting that despite the study effort being similar across the four sites (see Table 2.1), the number of videos and indirect traces collected varied substantially between communities. This may in part have been due to the fact that at the beginning of our study we had a better baseline knowledge of the movements of the Caiquene-Cadique community than any of the other three communities, and hence were able to record a much larger number of chimpanzee videos at Caiquene-Cadique. However, this would not explain why in the first month of the study we in fact confirmed many more behaviours in Cambeque than in the other communities (see Fig. 2.3). Additionally, the differences in ranging areas (with chimpanzees at Madina and Caiquene-Cadique estimated to range over larger areas than those at Cambeque and Lautchandé) could also help account for some of the disparity in number of indirect traces found: larger ranging areas may correspond to larger population sizes,

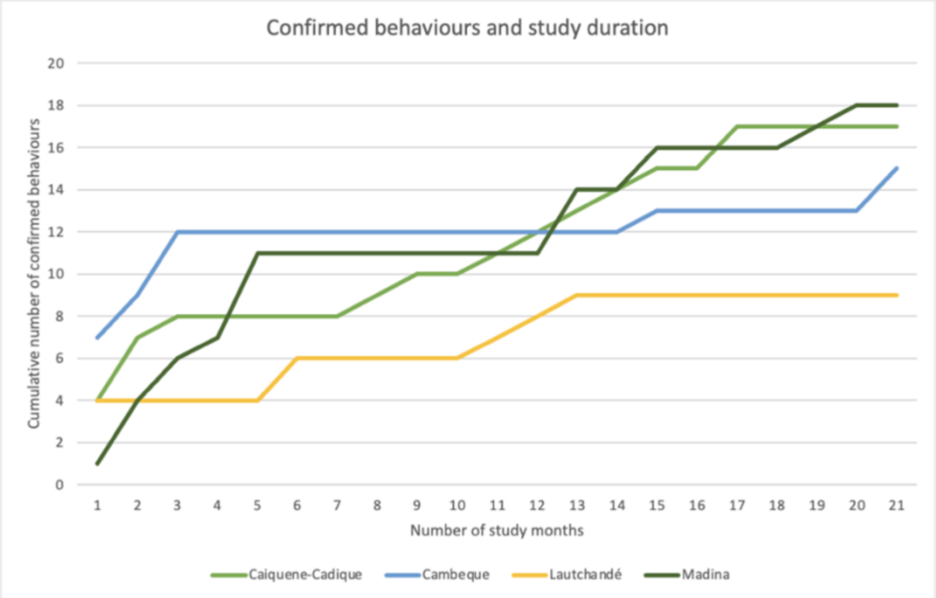

Figure 2.3. Cumulative number of confirmed behaviours found in each study community over the study period.

which, in turn, may produce larger numbers of traces. It is likely that with an increase in study effort better parity in data volume across the four communities would be achieved, which may further clarify how much of the observed inter-community variation found is due to behavioural differences.

Notwithstanding, in two years of research, we were able to compile an extensive list of wild chimpanzee behaviours never before described for CNP, for Guinea-Bissau, or (in the case of some behaviours) for the rest of Africa. We concede that this list is likely to be far from comprehensive, since it is clear that longevity of study and close, direct observation of individuals are key factors that influence the size of the behavioural repertoire assembled for each chimpanzee community, as is evident through the increase in the number of confirmed behaviours over time (see Fig. 2.3). The recent study by Kühl and colleagues (2019) comparing the presence/absence of 31 behaviours amongst 144 chimpanzee communities (46 previously largely unstudied communities along with information from published literature on an additional 106 communities) concluded that these behaviours had on average 88% less chance of occurring in chimpanzee communities inhabiting highly anthropogenically impacted areas compared to low impacted areas (Kühl *et al.*, 2019). This would thus seem to discourage the study of communities that, like ours, inhabit fragmented forest mosaics (Hockings and McLennan, 2019). However, our results show that chimpanzee communities that inhabit human-impacted environments demonstrate rich behavioural repertoires. In CNP we were able to confirm 9 of the 65 behaviours described by Whiten and colleagues (2001) and 7 of the 31 behaviours described in Kühl *et al.* (2019). These numbers might seem low, but given that the original lists only include, by definition, behaviours that show variation across communities, it is in fact expected that we will only encounter some of these behaviours in newly studied communities. Examining the data presented by Kühl and colleagues (2019), even communities that are fully habituated and have been studied for over 40 years, only show a subset of the 31 behaviours analysed (e.g., 18 for Bossou, Guinea and 17 for Gombe, Tanzania).

Thus, our research shows that studying the behaviour of neighbouring primate communities inhabiting human-impacted areas can be a useful source of information in studies of animal culture, and re-affirms the value of using a combination of direct and indirect methodologies to document the behaviour of unhabituated communities. The fact that over the course of our relatively short study we identified behaviours which do not leave noticeable material evidence behind and that are rare, seasonal, or absent in other known chimpanzee communities, provides further justification for the validity of this approach. Additionally, and contrary to some previous studies, our approach was not constrained to a pre-selected list of behaviours. This allowed us to, for example, explore behaviours specific to unusual habitat types, such as mangroves, and identify previously unknown behaviours that we would have missed otherwise. At the same time, it is important to remember that behavioural repertoires are not static and should not be seen as such: behaviours can disappear (Nishida *et al*., 2009), resurface after years of absence (Kalan and Boesch, 2018), change (Hockings *et al*., 2015a) or be, as far as we know, newly invented (Hobaiter *et al*., 2014). In fact, the ever-changing conditions that chimpanzees inhabiting disturbed habitats face might translate into a need to rapidly adapt through changes in established behaviours or through innovation (Gruber *et al*., 2019). This means that while some behavioural variants might disappear (Kühl *et al*., 2019), given the flexible nature of chimpanzee behaviour others might arise anew and be passed down to the next generation via social learning (Gruber *et al*., 2019). Furthermore, human-chimpanzee dynamics are different in areas where local people have prolonged sympatry or exposure to wildlife and more gradually encroach into wildlife habitats, than those where human encroachment is more rapid and chimpanzees are killed for food or as ‘pests’ (McLennan and Hockings, 2014; Gruber *et al*., 2019). Thus, chimpanzees that for generations have been facing human disturbances and are tolerated by local people, such as our study communities, might display a rich behavioural repertoire that has allowed them not to become dependent on specific foods or habitat types (e.g., primary forest) to survive, and therefore might be better equipped for a continued existence in human-disturbed areas. In a related vein, it is increasingly evident that in addition to comprehensive and up-to-date information on the species’ and subspecies’ status, distribution and population trends, genetic and cultural diversity are also important to guide effective conservation activities (Gruber *et al*., 2019). Our research helps build baselines for chimpanzee cultural diversity in CNP and for the species as a whole which has the potential - if done carefully (Carvalho *et al*., 2022) - to be integrated into existing, evidence-based conservation frameworks.

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CHAPTER 3

First evidence of chimpanzee extractive tool use in Cantanhez, Guinea-Bissau: cross community variation in honey dipping

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Supplementary material can be found at the end of the chapter

3.1 Abstract

Wild chimpanzee tool use is highly diverse and, in many cases, exhibits cultural variation: tool-use behaviours and techniques differ between communities and are passed down generations through social learning. Honey dipping – the use of sticks or leaves to extract honey from hives – has been identified across the whole species' range. Nonetheless, there seems to be marked variation in honey dipping at a species level, with most descriptions originating from Central Africa, and involving the use of complex tool sets, or even multifunctional tools. In West Africa, while honey consumption is common, in most cases tools are not used. We document, for the first time, the use of honey dipping tools in unhabituated chimpanzee (*Pan troglodytes verus*) communities at Cantanhez National Park (CNP), Guinea-Bissau. Over a 23-month period we employed a combination of direct (camera traps, n=1944 camera trap days) and indirect (1000 km of reconnaissance walks, collection of abandoned tools) methods to study four neighbouring communities in central CNP. Fluid dipping tools were found in three of the four communities; here we analyse 204 individual stick tools from the 70 tool-use ateliers found. In addition to documenting individual tool dimensions and raw materials, we adopt methods from primate archaeology to describe the typology of different tools based on use-wear patterns. We describe differences in tools used for different honey types, between communities, and tools and tool kits that show an unexpected degree of complexity. Our data also suggest the use of tool sets, i.e., tools with different functions used sequentially towards the same goal; as well as possible multifunction tools (pounding and dipping), never before described for western chimpanzees. Our study fills gaps in our knowledge of the wild chimpanzee cultural repertoire and highlights how chimpanzee tool manufacture and use can vary even at local scales.

3.2 Introduction

Apart from humans, chimpanzees show the greatest diversity of tool use in the animal kingdom, making and using a variety of complex tools as part of their daily lives (McGrew, 2004). In addition, different communities exhibit different tool-use repertoires (Goodall, 1986; Whiten *et al*., 1999; Boesch and Boesch-Achermann, 2000; McGrew, 2004; Matsuzawa *et al*., 2011; Pruetz *et al*., 2015) – a phenomenon commonly ascribed to cultural variation. West African chimpanzees, for example, are known to crack nuts using wooden or stone hammers, whereas this behaviour is entirely absent from East Africa despite the presence of the necessary resources and raw materials within East-African chimpanzees' ranges (Boesch and Boesch-Achermann, 2000; Matsuzawa *et al*., 2011). How such regional differences emerge and are maintained are key questions in understanding the spread of cultural traits, and, given chimpanzees' close evolutionary proximity to humans, are also relevant to understanding the origins of hominin technology and culture. Several different methods to identify culture have been suggested, most prominently those applying the "method of exclusion" (e.g., Whiten *et al*., 1999) and those positing a tri-dimensional approach to traditions (Fragaszy and Perry, 2003). The former proposes the explicit exclusion of ecological or genetic factors as drivers of inter-population behavioural variation within a species (Wrangham *et al*., 1994; Whiten *et al*., 1999), whereas the latter places the strongest emphasis on demonstrating social learning, without which a behaviour cannot be considered cultural (Fragaszy and Perry, 2003). Subsequent work argued that ecology, genetics and social learning are in fact inexorably interlinked and can all influence, to some degree, behavioural variation (Laland and Janik, 2006; Koops *et al*., 2013), thus leading to a useful convergence between the two main frameworks.

The past two decades have seen further refinement of these methodologies in the study of wild primates. First, while early works on chimpanzee culture conducted comparisons at a species-wide scale (Whiten *et al*., 1999, 2001; Schöning *et al*., 2008), it has been suggested that more compelling evidence for culture in chimpanzees might come from the study of behavioural variation within the same subspecies (e.g., Laland and Janik, 2006; Luncz *et al*., 2012). In particular, comparisons of neighbouring communities, where habitat types are similar and individuals broadly face the same ecological constraints and migrate between communities, make ecological or genetic explanations for behavioural differences less likely compared to a cultural explanation (i.e., one based on local innovation and subsequent diffusion through social learning). Illustrating this approach, Luncz and colleagues (2012) compared the selection of wooden and stone hammers for coula (*Coula edulis)* nut-cracking in three neighbouring chimpanzee communities in Taï National Park (Ivory Coast). Even though these neighbouring communities inhabit the same forest habitat and ecological variation is minimal, the study showed that there was still marked variation in hammer size and raw material preferences between communities (Luncz *et al*., 2012). This confirms that studying nuanced differences in details of the *same* behaviour between communities that exist in close proximity can yield tantalising evidence for subtle behavioural variation. Second, direct evidence for social learning being involved in the maintenance of specific behaviours has become available through observations of natural immigration and the emergence of subsequent conformity (Luncz and Boesch, 2014). Furthermore, novel social-network-based analyses have also confirmed the socially mediated diffusion of a newly invented behavioural variant in wild chimpanzees (Hobaiter *et al*., 2014). Taken together, these studies elegantly bridge the gap between the method of exclusion and the tri-dimensional approach to the study of animal traditions.

Honey dipping behaviour – chimpanzees' use of sticks or leaves to extract honey from hives – has been identified across the whole species' range (Boesch and Boesch, 1990; Tutin *et al*., 1995; Ohashi, 2006; Fowler and Sommer, 2007; Sanz and Morgan, 2009; McLennan, 2011). However, it is from Central Africa that most descriptions of honey dipping of arboreal and terrestrial honey from different stinging and stingless beehives seem to originate. Central African chimpanzees (*Pan troglodytes troglodytes*) use complex tool sets, sometimes composed of up to five tools with different functions used in sequence to gain access to and extract honey, and have even been described using multifunctional tools, i.e., tools where a single object can have different functions (Bermejo and Illera, 1999; Sanz and Morgan, 2007; Boesch *et al*., 2009). In East Africa, the use of stick tool sets to access honey by chimpanzees is rare. However, chimpanzees at Bulindi in Uganda use tool sets, including both digging sticks and more slender sticks to probe the stingless bees' narrow underground entry tubes (McLennan, 2011; McLennan *et al*., 2019). In West Africa, honey consumption also occurs frequently, but in many cases no tools are used to extract the honey (Boesch and Boesch, 1990). This might be because this subspecies of chimpanzee (*Pan troglodytes verus*) feeds more frequently on the honey of stinging bees (*Apis* sp.) whose painful sting does not allow individuals to spend long enough near hives to use tools, meaning they must instead adopt other approaches (such as using hands only; Boesch and Boesch, 1990). Nonetheless, in the Ivory Coast the chimpanzees inhabiting Comoé National Park have been described to frequently use dipping stick tools not only to access honey but also water. These chimpanzees were even observed modifying their tools by chewing on their end to create a 'brush tip' prior to use (Lapuente *et al*., 2017). Variations in honey dipping thus appear to exist between subspecies and within the same subspecies, hence both genetic and environmental explanations might be at play. Hence, it is of particular interest to study this behaviour between neighbouring communities inhabiting similar habitats, where variation due to the latter two influences is expected to be minimal. Furthermore, we still lack a complete picture of the full chimpanzee cultural repertoire, despite long-term study across much of the species' range, and the addition of new sites of unhabituated chimpanzee communities identifying new behaviours and behavioural variants (e.g., Sanz *et al*., 2004; Pruetz and Bertolani, 2007; Gruber *et al*., 2015; Hockings *et al*., 2015; Kühl *et al*., 2016; IUCN, 2020). Specifically, no long-term studies have yet reached the westernmost populations of the species' distribution. Recent work in Cantanhez National Park (CNP), Guinea-Bissau, has started to fill this gap (Sousa *et al*., 2011; Hockings and Sousa, 2012; Bessa *et al*., 2015; Vieira *et al*., 2019; Hockings *et al*., 2020). For example, a 9-month study at CNP found, through the analysis of faecal samples and other indirect data, that these chimpanzees fed on wild bee honey with some degree of frequency, however no tools were ever found (Bessa *et al*., 2015). In more recent surveys, however, evidence of dipping tools began to emerge (E. Bersacola, personal communication), confirming the presence of the behaviour at CNP.

In the present study, we employ a combination of direct and indirect methods to systematically survey CNP for honey-dipping tools, and compare four neighbouring chimpanzee communities unhabituated to the presence of researchers. Specifically, we aim to 1) identify the presence of honey dipping tools in the four communities' home ranges, 2) compare the characteristics of tools used to exploit different honey sources, and 3) compare the characteristics of tools across those communities that use them.

3.3 Methods

Study site

Cantanhez National Park (CNP, N11º14.287’ W15º 02.281’) is located in the Tombali region of south-west Guinea-Bissau. CNP is a mosaic of settlements, cropland, sub-humid forest, secondary forest, mangrove, and savannah (Catarino and Palminha, 2014). There are two marked seasons in Guinea-Bissau: dry season (November to mid-May) and rainy season (mid-May to October). During 2017, annual rainfall was 2351 mm with an average temperature of 26.3ºC (15.6ºC min to 38.6ºC max). It is estimated that there are 10-12 chimpanzee communities in CNP as a whole (Bersacola, 2019). In the forested areas of central-southern CNP, genetic, behavioural and ecological research support the presence of seven different chimpanzee communities (Hockings and Sousa, 2013; Sá, 2013; Bessa *et al*., 2015; Bersacola, 2019; Vieira *et al*., 2019; Hockings *et al*., 2020); these include the four studied communities: Caiquene-Cadique, Lautchandé, Madina and Cambeque. Due to the unhabituated nature of these communities, at present little is known about their community sizes and compositions; nonetheless, previous workers estimate that the communities’ range between 35-60 individuals (Bessa *et al*., 2015; Vieira *et al*., 2019).

Data collection

Data collection took place over the course of 23 consecutive months (February 2017 - December 2018). Since the main aim of this study was to assess the presence of and potential inter-group variation in honey-dipping behaviour in neighbouring chimpanzee communities, where none of the studied communities were habituated to researchers, a combination of direct and indirect methods of data collection were employed. A total of 187 reconnaissance walks (“recces”) were walked, covering just over 1,000 km. Since several neighbouring communities were being studied, five consecutive recces in each were initially performed to assess preliminary core ranging area and habitat composition. After obtaining this information, recces were walked 6 days a week. These were performed in rotation across communities, accumulating information that would help maximise data collection at each, while also ensuring that all communities were sampled equally across different months/seasons. All data were collected by JB who was accompanied by two field assistants at all times. Camera traps were set up by JB during recces and checked every two-weeks by JB or one of the trained field assistants. Supplementary Table 3.1 presents a summary of the cumulative study effort in each of the communities.

Resource availability

Data on resource availability were collected during recces at each study site by following chimpanzee paths and forest trails that covered as many different habitat types as possible. This method was chosen over systematic transects in order to minimise disturbance to an already highly fragmented habitat, and to avoid opening up new trails for hunters. Honey availability was assessed during recces *ad libitum*: every time a hive of honey bees or stingless bees was encountered, a GPS point of its location was taken, the habitat was classified (dry forest, riparian forest, woodland, palm grove, mangroves, fallows, croplands, savannah woodland and grassland (Catarino *et al.*, 2020)), and hive type (e.g., arboreal, subterranean) and bee species (local name and scientific name when possible) were recorded. Honey is an important subsistence resource for the local human communities, therefore, hives were usually easily located by one of the field assistants. A careful visual search was conducted to locate honey bee hives, as well as the small tubular entrances of stingless bee hives. Honey bee hives were also located through the sound of the swarm. Initially, local honey harvesters were contacted for information about the potential location of hives; other than hives that were impossible to deplete, most of the locations identified in this way were already depleted (or destroyed), or were likely to become so in the near future.

Indirect data collection: Home ranges, Evidence of honey consumption, and Tools

Chimpanzee ranging areas were estimated using minimum bounding polygons from direct chimpanzee encounters and camera trap data, as well as indirect (e.g., nests, feeding traces, faecal samples, abandoned tools) data points, continuously collected during reconnaissance walks from February 2017 to July 2018. Additionally, the highly fragmented nature of the chimpanzees' habitat, with human settlements, roads, cultivated areas as well as many mangrove estuaries acting as natural and artificial boundaries, was helpful when estimating the home ranges. Data points that were collected in areas where there might be overlap between communities were excluded.

Hives and their surroundings were inspected for evidence of honey consumption by chimpanzees: detached wax from the hive's entrance, honeycomb traces, tools discarded by the hive, detached fresh green leaves (debris from tool manufacture), freshly snapped branches, or any other chimpanzee signs (e.g., prints, faecal or feeding traces).

When a tool use atelier - a location where tools were used to extract a resource, and were then left behind - was encountered, we recorded its exact location by GPS, photographed the site with the tools *in situ,* and photographed the individual tools. We registered the bee species associated with the tool use atelier, the species of tree in which the hive was located, whether it was an arboreal or terrestrial hive, and its distance from the ground (using a tape measure or a rangefinder depending on its height).Only sticks that showed clear signs of modification, such as stripped bark, lateral branches removed, frayed or blunt ends, or signs of honey or wax at one or both extremities, were considered tools (e.g., Hernandez-Aguilar *et al*., 2007; Lapuente *et al*., 2017). We then collected each tool, gave it a label, recorded its species, and, when possible, we measured its distance from the source by refitting (i.e., finding the presence of a scar left in a plant as a result of the chimpanzees harvesting the raw material) the tool to its original source (methods adapted from Koops *et al.*, 2015; Pascual-Garrido, 2018). If more than one tool was found by the same hive we grouped the tools into age categories depending on colour, pliability and degree of decay (new – still green and pliable; recent – browning in colour and less pliable; old – dry appearance, no pliability/fragile, with possible signs of decay; adapted from Pascual-Garrido, 2018). Collected tools were photographed, measured (length; mid, proximal and distal diameter; length of fray), and all modifications were recorded (fragmented/detached from substrate; percentage of bark left; stripped ends; attachments removed; signs of use on extremities; bite marks; and presence of honey/wax). Additionally, we recorded use-wear patterns on the extremities, categorised into three types: brushed/frayed (significantly frayed with long and separated wood fibres), blunt/mashed (minimal to no rounding with significant fringing and lateral/backward bending of terminal wood fibres) and fragmented (broken end with sharp edges) (adapted from Heaton and Pickering, 2006; Boesch *et al.*, 2009). In order to record whether the same hive was repeatedly exploited, the tool use sites were revisited every week, unless it could be confirmed that the hive had been depleted and the bees had abandoned it.

Remote data collection: Camera trap sampling

Since the chimpanzee communities were unhabituated to researchers' presence, the opportunities to observe them directly were few. However, eight camera traps (Bushnell Trophy Cam HD Aggressor No-glow) provided observational data in the form of video footage. The camera traps were set up in chosen locations, on video mode, and programmed to film for 1 minute when triggered by movement. These camera traps were set up in places where tools had been found and the resource (honey) had not been totally depleted (i.e., was likely to be revisited) and/or tool use behaviour was likely to take place due to the presence of beehives. To maximise the chances of capturing behaviours of interest on video, some of the cameras were moved during the study period, for example if bees had abandoned a particular hive, if water and/or salt had disabled a camera in a mangrove location, or if the chimpanzees were known to be utilising new travel routes due to seasonal changes in their habitat. 12 camera traps (three in Caiquene-Cadique, three in Cambeque, two Lautchandé and four in Madina) were operating for a cumulative total of 1923 days (399 days in Caiquene-Cadique, 648 days in Cambeque, 363 in Lautchandé and 513 in Madina), from February 2017 to December 2018 (See Supplementary table 3.1).

Data analysis

All statistical tests were performed in R (version 1.1.463), using t-tests and Chi-square tests. Given that data were based on indirect evidence, we assumed that each event was an independent event (but see Discussion). Data were also tested for normal distribution using the Shapiro-Wilk's method, and for homogeneity of variance using Bartlett's test. Two-sample t-tests and Welch's two-sample t-tests were used when comparing datasets with equal and unequal variance, respectively.

Our principal comparisons of interest focused on the dimensions (e.g., length) and characteristics (e.g., modifications) of tools used to collect different types of honey, and of tools used to collect the same type of honey but by different communities. Given the nature of the data collected, where most hives had less than five tools found associated with them, we were not able to use a generalized linear model. Instead, we compared tool dimensions and characteristics using t-tests. For descriptive characteristics we employed Chi-square tests.

3.4 Results

Based on the 4293 direct chimpanzee encounters and camera trap data, as well as 1796 indirect data collected during reconnaissance walks, the four communities' ranging areas surveyed in the present study were estimated using minimum convex polygons: Caiquene-Cadique 14.8 km^2, Madina 19.0 km^2, Cambeque 7.1 km^2, and Lautchandé 8.4 km^2 (Fig 3.1).

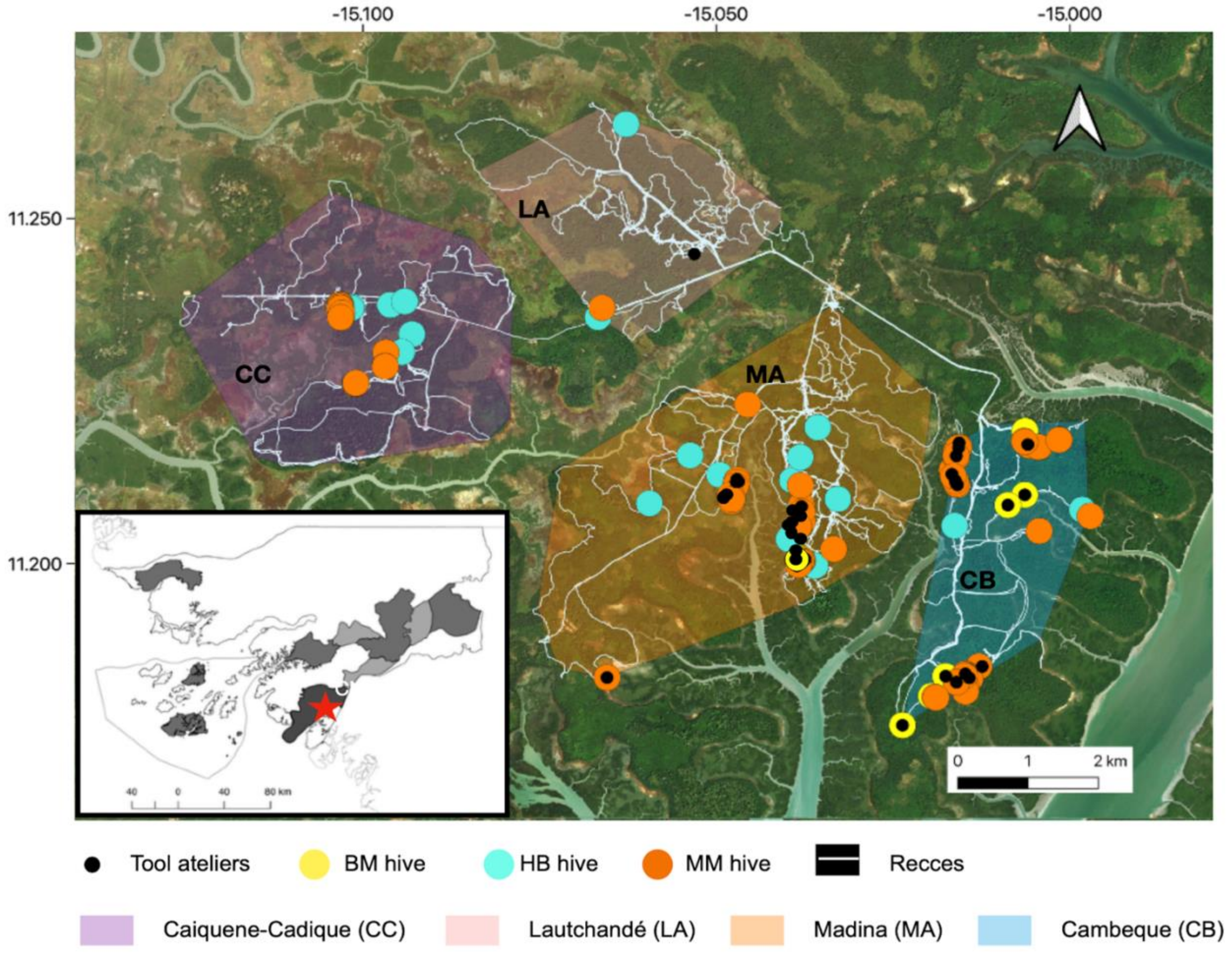

Figure 3.1. Map of the study site showing each of the study communities' estimated core home range. CNP is marked by a red start, Caiquene-Cadique is shown in purple, Lautchandé in pink, Madina in orange and Cambeque in blue. Ranging areas during the study period were estimated using minimum bounding polygon from 4293 direct (chimpanzee encounters and camera trap data) and 1796 indirect (nests, feeding traces and faecal samples) data points, collected during reconnaissance walks from February 2017 to July 2018.

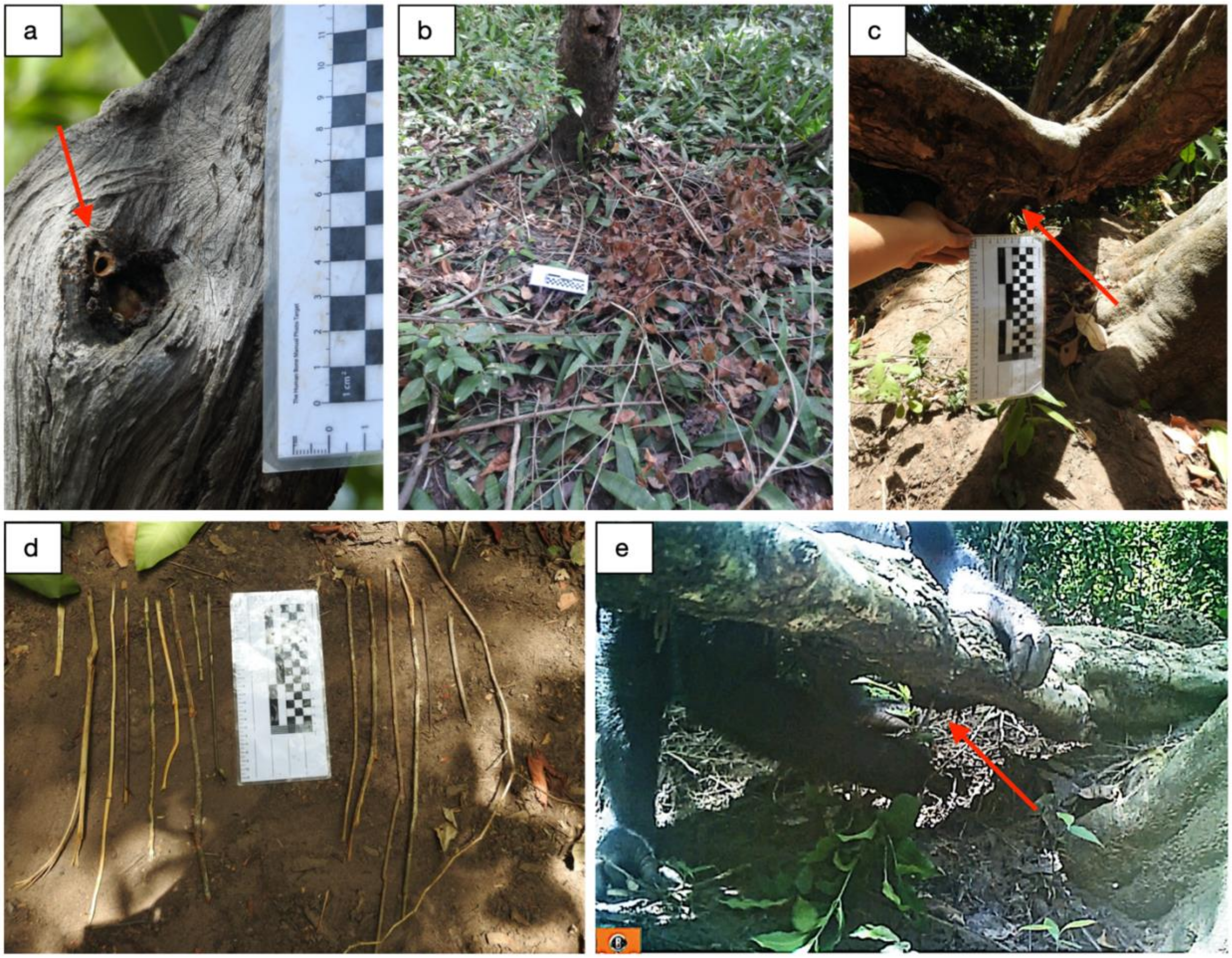

Figure 3.2. Beehives and tools used to extract honey by chimpanzees in CNP. (a). A small stingless bee (Meliplebeia sp.; MM) hive located in the mangrove area at Madina. The arrow points to the entrance tube of the stingless bees' hive; the hive's entrance covered by wax has been exposed, tools were found next to the hive. (b). A large stingless bee (*Meliponula sp*.; BM) hive exploited by chimpanzees in secondary open forest at Cambeque, with tools in situ (c). A large stingless bee (*Meliponula sp*.; BM) hive located in secondary forest at Cambeque. The arrow points to the entrance tube of the stingless bees' hive; the hive's entrance covered by wax has been exposed. (d). Tools found by hive c with two different estimated ages of use – one-day-old (new) on the left, approx. 1-week-old (recent) on the right. (e). Screenshot from camera trap footage showing a juvenile chimpanzee inserting a manufactured tool into the hive shown in (c) in Cambeque secondary open forest (tool indicated by red arrow) (see Supplementary material 3.3, Observation 2).

Resource availability

All beehives were arboreal, at varying distances from the ground (15-350 cm), and could normally only be accessed by a very small opening covered with dry hard wax (see Fig 3.2a and 3.2c for examples). No subterranean hives were found. Three types of bees and respective hives were identified: one species of stinging honey bee (local name bagueira, scientific name *Apis mellifera,* hereafter BH) and two species of stingless or sweat bees, large stingless bees (local name bagueira mudo, scientific name *Meliponula sp,* hereafter BM) and small stingless bees (local name mosca mel, *Meliplebeia sp*, hereafter MM). Hive characteristics varied between and within bee species. *Apis mellifera* were normally found inside live trees with varying entry sizes; *Meliplebeia* sp. were always found in live trees with very small entries and only a single entrance tube (see Fig 3.2a); and *Meliponula* sp. were found both in dead and live trees, with generally large entries in dead trees (see Fig 3.2b) and generally small entries in live trees (see Fig 3.2c and 3.2e).

Of all bee species, the hives of MM were most commonly encountered during recces (N=80). They were present in all study communities but in different numbers and at different densities: 40 in Cambeque, 22 in Madina, 17 in Caiquene-Cadique and one in Lautchandé. The next most common hive was that of BH (N=23): 10 in Madina, seven in Caiquene-Cadique, four in Cambeque, and two in Lautchandé. BM (N=7) was only encountered six times in Cambeque and once in Madina. Supplementary Table 3.2 presents these densities standardized against home range size and km of recces walked.

Hives were found across different habitats. MM was almost exclusively found in the mangrove area (97.5%) with the remainder in woodland, BM mostly in woodland (71.4%) with the remainder in the mangrove area and cropland. Most BH was found in dry forest (60.9%), followed by mangrove and cropland.

Figure 3.3. Examples of tools found abandoned in tool use ateliers. (a). Brush tips; (b). blunt tool ends; (c). fragmented tool ends; (d). tool ends with honey residue (indicated by the red arrows); (e). potential multifunction tools with fray/brush ends and blunt/mash ends at opposite tool ends.

Indirect evidence of honey consumption without tools

We found indirect traces for the consumption of BH, without tools, in all four study communities. These traces consisted of honeycomb or wax that was discarded after it had been exploited. They presented distinct tooth marks or were left behind in the form of wadges.

Tools collected

In total we collected 204 individual stick tools from 70 tool use ateliers (see Fig 3.2b and 3.2d for examples). Stick tools were found in three of the four study communities' home ranges, although at different frequencies. 50% of all tools were found in Madina (N=103) and 49% in Cambeque (N=100), with only a single tool found in Lautchandé, and none in Caiquene-Cadique. These tools were associated with four types of fluid extraction: MM, BM, and BH honey, and, on a single occasion, an unidentified fluid (UF). Tools were mainly recovered from mangrove areas (67%), followed by closed secondary forest (30%), open secondary forest (2%) and an agricultural field (1%). In Madina we found tools associated with the extraction of MM (97%), BH (1.9%), and BM (1%), while in Cambeque tools were only associated with the extraction of BM (62%) and MM (38%). In Lautchandé the single tool found was associated with UF. By grouping tools found by the same hive into age classes as well as revisiting hives that were not depleted after the first raiding event we were able to confirm their repeated exploitation, with the use of tools, on separate occasions. One BM hive in Cambeque was successfully raided twice and on two other occasions chimpanzees attempted to exploit it without success (recorded on video). At least seven MM hives were exploited more than once by chimpanzees in Madina.

Table 3.1 presents descriptive statistics for all tools and tool characteristics. Overall, the mean number of tools found per atelier was 3.05 (± 2.75). Mean tool length was 40.79 (± 15.39) cm and mean mid diameter 6.46 (± 15.3) mm. 90.7% of all tools had four to six modifications. Tools were found with distinct wear patterns – brushed/frayed, blunt/mashed and fragmented (see Fig 3.3a-c for examples of each type of wear pattern).

112 (54.9%) of 204 tools were found to have at least one frayed end and 106 (52%) tools had at least one blunt end. 14.7% (n=30) of tools had two different wear patterns at opposite extremities, suggesting possible multifunction for those tools (Fig. 3.3e). Sticks that had both ends fragmented or without a clear wear pattern (i.e., absence of brush or blunt end) were only considered tools if they had traces of honey on at least one of the extremities (n=15, 7.4% tools). Honey residue was found on 132 tool extremities: on 66 (51.2%) brush tips, 36 (35.6%) blunt ends, and 30 (26.5%) fragmented ends (Fig.3.3d).

Of the tools with at least one frayed end, 63.4% (n=71) had a frayed proximal end and the mean fray length was 26.31 (±17.46) mm. Of the tools with at least one blunt end 71.7% (n=76) had blunt distal ends. 62.4% (n=128) of all tools had signs of wear at only one extremity, of these 56.3% (n=72) had proximal wear patterns. 109 (53.4%) tools presented signs of honey/wax/insects and 20 (9.8%) had distinct bite marks.

Tools were made out of fresh twigs from tree species typically found no further than 5 m from the hive (mean±SD =0.436±1.026), with most being sourced from the same tree where the hive was located. In the mangroves one species was used exclusively as raw material, the mangrove tree (*Avicennia germinans*). Similarly, in cropland only one species was used, the orange tree (*Citrus sinensis*). In dry forest, several different species were chosen as raw material, including *Strombosia pustulata, Sarcocephalus latifolius, Vitex doniana, Dialium guineense, Ceiba pentandra, Antiaris toxicaria, Trichilia monodelpha and Albizia ferruginea.*

Table 3.1. Summary of tools and tool characteristics as a function of type of fluid exploited. MM – *Meliplebeia* sp., small stingless bee; BM – *Meliponula* sp., large stingless bee; BH – *Apis mellifera*, honey bee; UF – Unknown fluid.

Type of fluid	Number of tool ateliers	Number of tools	Mean number of tools per atelier	Tool characteristics						
				Mean length (cm)	Mean diameter (mm)	Mean number of modifications	Mean length of fray (mm)	Only 1 end used	Both ends used	Possible multifunction tools
MM	59	138	2.53 ± 1.79 [1-8]	40.4 ± 12.1 [13.4-78.8]	6.2 ± 1.5 [3.3-11.2]	4.87 ± 0.94 [2-6]	22.4 ± 11.0 [3.4-65.5]	98 (71.1%)	40 (28.9%)	20
BM	9	64	7.86 ± 4.71 [1-14]	42.58 ± 20.8 [6.8-133]	6.9 ± 2.6 [3.2-15.1]	4.9 ± 0.98 [2-6]	35.6 ± 25.7 [4.9-113.0]	30 (46.8%)	23 (35.9%)	10
BH	1	2	2	34.15 ± 14.8 [23.7-44.6]	5.0 ± 2.0 [3.6-6.4]	6	18.0 ± 10.3 [4.3-27.8]	0	2	0
UF	1	1	1	32.8	10.4	6	33.5	0	1	0
TOTAL	**70**	**204**	**3.08±2.75 [1-14]**	**40.79±15.39 [6.8-133]**	**6.46±15.3 [6.8-133]**	**4.9±0.95 [2-6]**	**26.31±17.46 [3.4-118.0]**	**128**	**76**	**30**

Comparison of tools by site and by resource exploited

Table 3.1 further breaks down tools and tool characteristics by the type of fluid exploited. Of the four sources of fluid exploited, MM was associated with the highest number of tools and tool use ateliers encountered (138 and 59, respectively), followed by BM with 64 tools and 9 ateliers, BH with two tools and one atelier, and UF with a single tool recovered.

Given the low frequency of tools recovered in association with BH and UF these will not be used in subsequent comparisons. Additionally, the single tool found in Madina associated with BM extraction was not included in the comparisons. Given the structure of the dataset that remains, in which only one chimpanzee community repeatedly exploited more than one type of fluid, and only one type of fluid repeatedly exploited by more than one community, we first focus on potential differences in tool use associated with two different resources frequently consumed at one particular site, then compare tool use at two different sites associated with a single resource.

Comparison of tools found in Cambeque for the extraction of BM and MM

Cambeque was the only site at which more than one type of honey (BM and MM) was frequently exploited with the use of tools (Table 3.2). The mean number of tools found per atelier was significantly higher for BM (7.88± 4.36) compared to MM (2.47 ± 1.99) (Welch's two-sample t-test: *t*=3.33, *df*=8.6, *p*=0.009) (Fig. 3.4a). Mean tool length was similar for MM (43.88 ± 13.10 cm) and BM (40.93 ± 15.47 cm), confirmed by a non significant test result (Welch's two-sample t-test: *t*=0.48, *df*=97.9, *p*=0.633) (Fig. 3.4b). The mean mid diameter of BM tools was higher than that of MM tools but this difference was also not significant (6.88 ± 1.9 mm and 6.25± 1.31 mm, respectively; Welch's two-sample t-test: *t*=1.52, *df*=95.2, *p*=0.131). MM and BM tools had a similar number of modifications: on average 4.84 and 4.90 respectively. The percentage of bark left after modification was significantly lower in BM (60.47 ± 33.94%) than MM (79.45 ± 23.30%) tools (Welch's two-sample t-test: *t*=3.31, *df*=96.6, *p*=0.001) (Fig. 3.4c).

Table 3.2. Summary of tools and tool characteristics as a function of chimpanzee community. Shown, are tools associated with the exploitation of small stingless bee honey (MM), for the two communities (Cambeque and Madina) as well as large stingless bee honey (BM), for one community (Cambeque).

Community	**Number of tool ateliers**	**Number of tools**	**Mean number of tools per site**	**Mean length (cm)**	**Mean diameter (mm)**	**Mean number of modifications**	**Average length of fray (mm)**	**Only 1 end used**	**Both ends used**	**Possible multifunction tools**
Cambeque MM	16	38	2.47 ± 1.99 [1-8]	43.9±13.1 [21.2-78.8]	6.3±1.3 [3.3-15.1]	4.84 [3-6]	25.2±11.9 [8-46.2]	32 (84.2%)	5 (13.1%)	2
Cambeque BM	8	63	7.88 ± 4.36 [1-14]	40.93±15.47 [6.8-133]	6.88 ± 1.9 [3.2-15.1]	4.9 ± 0.96 [2-6]	35.4 ± 25.5 [4.9-113.0]	30 (47.6%)	22 (34.9%)	10
Madina MM	43	100	2.40 ±1.53 [1-6]	39.1±11.51 [13.4-71.5]	6.2±1.5 [3.3-11.2]	4.89 [2-6]	21.8±10.5 [3.4-65.5]	66 (66%)	32 (32%)	18

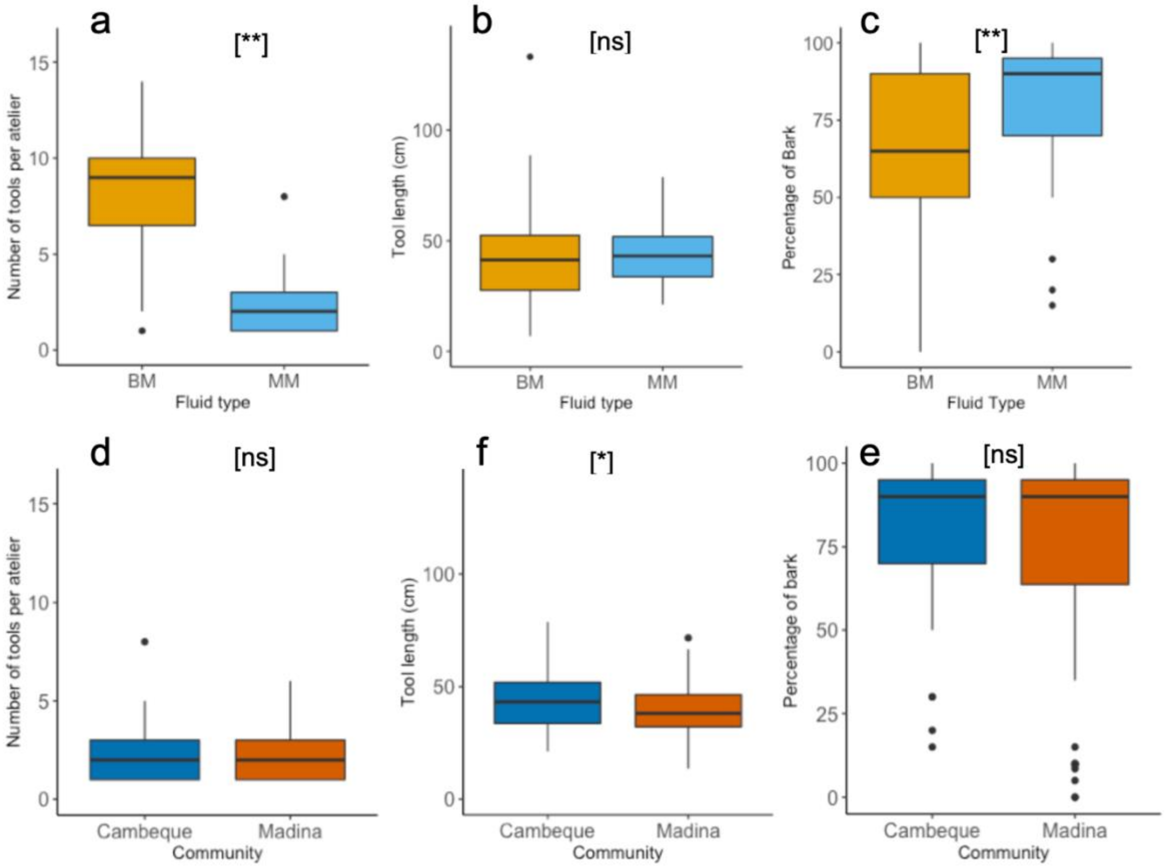

Figure 3.4. Honey dipping tool characteristics. Tools per atelier, tool length (cm) and percentage of bark remaining, compared between fluid types BM and MM at Cambeque (a, b, c) and between two neighbouring communities, Cambeque and Madina, exploiting MM (d, e, f). Boxplots indicate upper and lower quartile; thick horizontal lines represent median. Maximum and minimum data range indicated by whiskers; black dots show individual outliers. P-values are indicated by [ns] – p>0.05; [*] – p≤0.05; [**] – p≤0.01.

51% (n=19) of MM tools and 49% (n=31) of BM tools had at least one frayed end. Blunt ends were present in 47% (n=18) and 46% (n=29) of MM and BM tools, respectively. 17% of all BM tools and 3.2% of MM tools had fragmented ends without any further signs of wear (i.e., no blunt or frayed ends). There was a significant difference when comparing tools that had signs of wear on both ends: this was the case for 13% (n=5) of MM tools and 35% (n=22) of BM tools (Chi-square test: X^2_2=11.34, p=0.001). Of these tools 45% (n=10) BM and 40% (n=2) MM tools had different wear types at the two tool ends, i.e., they were potential multifunction tools. Finally, the average length of fray on frayed ends was significantly longer on BM tools (35.44 ± 25.48 mm) than MM tools (25.16 ± 11.91 mm) (Welch's two-sample t-test: t=2,09, df=55.5, p=0.041).

Comparison of tools used to exploit MM in Cambeque and Madina

MM honey was the overall most frequently exploited resource with the use of stick tools; however, no evidence was found in Caiquene-Cadique and Lautchandé despite the presence of MM hives at those two sites. We therefore compare MM tool characteristics between the communities of Cambeque and Madina only (Table 3.2).

In Madina we found 43 ateliers with a total of 100 tools, while in Cambeque 16 ateliers provided 38 tools. The mean number of tools found per atelier was similar between the two communities, with 2.47 and 2.40 tools in Cambeque and Madina, respectively (two-sample t-test: *t*=0.04, *df*=51, *p*=0.970) (Fig. 3.4d). Mean tool length was significantly higher in Cambeque (43.9 ± 13.1 cm) compared to Madina (39.1 ±11.53 cm) (two-sample t-test: *t*= 2.12, *df*=136, *p*=0.036) (Fig. 3.4e), but there was no significant difference in mean mid diameter (two-sample t-test: *t*=0.18, *df*=136, *p*=0.850). The percentage of bark left after modification was not significantly different between communities (two-sample t-test: *t*=0.68, *df*=136, *p* =0.496) (Fig. 3.4f). All tools found in both communities were sourced from the same tree species of mangrove (*Avicennia germinans*).

In Cambeque 51% (n=19) and in Madina 58% (n=58) of all tools had at least one frayed end. Blunt ends were present in 47% (n=18) and 56% (n=56) of all tools in Cambeque and Madina respectively. 5.4% of tools in Cambeque and 4.0% Madina had no signs of wear at either extremity. Tools with signs of wear at both ends were significantly more frequent in Madina (n=32, 32%) compared to Cambeque (n=5, 15.8%) (Chi-square test: X^2_2 =4.495, *p*=0.034); of these 56% and 40% tools had different wear patterns at the two ends for Madina and Cambeque, respectively. The average length of fray on frayed ends was longer in Cambeque (25.16 ± 11.91mm) than Madina

(21.81± 10.54mm), but the difference was not significant (two-sample t-test: t=1.20, df=82, p=0.233).

Remote camera trap observations of tool use and honey extraction

Camera traps provided footage of chimpanzees extracting or attempting to extract MM, BM and HB. In total, 6977 videos were captured, of which 386 (5.5%) were of chimpanzees. Of the 386 chimpanzee videos, 12 (3.1%) videos provided evidence of tool use to extract MM in Madina and BM in Cambeque (Fig 3.2e). These 12 videos corresponded to seven independent events (where an event started when an individual approached a hive and finished when the individual left and did not return into the camera's view). See supplementary material 3.3 where we describe each tool-use event.

Additionally, camera traps captured chimpanzees on three separate occasions in Caiquene-Cadique raiding a natural HB hive, by inserting their hands deep inside a tree trunk. As soon as these chimpanzees removed their hands from the active hive, swarms of bees emerged, and the chimpanzees ran off holding honeycomb. After the hive was abandoned by the bees (no more bees could be seen or heard in the camera trap footage) one individual inspected it using the same method but was seen leaving calmly and empty handed. All individuals were adult males. In Madina a natural HB hive that had been exploited the previous day by humans was checked by an adult female chimpanzee. She looked inside and inserted her hand into the tree trunk, but no honey was extracted.

3.5 Discussion

Our study examined the use of dipping tools to access different types of honey in four neighbouring chimpanzee communities in central CNP, Guinea-Bissau. This is the first evidence of honey dipping tool use by chimpanzees in Guinea-Bissau. Dipping tools were found in three of the four studied communities, but only in two of these (Madina and Cambeque) were we able to positively identify the tools as honey dipping tools. These results are puzzling since honey is present in the home ranges of all four studied communities, and a previous study on the feeding ecology of CNP chimpanzees, based on indirect data collection and faecal sample analyses, have shown that honey is an important part of the diet at Caiquene-Cadique (Bessa *et al.*, 2015), one of the communities where dipping tools were not found. This discrepancy might be due to a number of methodological, ecological or behavioural factors.

Firstly, these communities are unhabituated to human researchers – we therefore had to rely on a combination of indirect methods and camera traps to study their tool use behaviour. Thus, it is possible that tool use related to honey extraction is present at Caiquene-Cadique as well, but we simply failed to find evidence for it. In a similar vein, we were also unable to attribute tools found to specific individuals, and as such, one or a few individuals could have been responsible for the manufacturing of a large proportion of tools found at a given site. (Note, however, that our video footage, albeit limited, supports the idea that within a given community there are indeed different individuals making and using tools.). Most of our knowledge on wild chimpanzee behaviour comes from communities that can be followed daily and their behaviour studied directly (e.g., Goodall, 1986; Boesch and Boesch-Achermann, 2000; Matsuzawa *et al.*, 2011; McLennan *et al.*, 2019). Hence, when studying unhabituated communities, much of the subjects' behavioural repertoire remains inaccessible to the researcher. Nonetheless, studies on these communities are beginning to gain traction, partly because of a reduction in efforts to habituate new communities given that so many already live in fragmented habitats and in close proximity to humans, where the loss of fear of humans could be counter-productive to conservation and welfare. It is also worth noting that the number of communities already habituated to researchers is extremely low compared to the species' total population size. In West Africa, for example, only five chimpanzee communities are fully habituated to researchers (one at Bossou, Guinea, Matsuzawa *et al*., 2011; three in the Taï Forest, Ivory Coast, Boesch and Boesch-Achermann, 2000; and one in Fongoli, Senegal, Pruetz and Bertolani, 2007) representing a total of approximately 200 individuals out of an estimated 52,811 (CI 17,577-96,564) chimpanzees in the region (Heinicke *et al*., 2019). As such, our current knowledge of chimpanzee behaviour and behavioural variation - that is biased towards those communities that can be followed - may represent only a small fraction of the full picture. On the other hand, despite the limitations of studying unhabituated communities, studies have already successfully discovered new behaviours and behavioural variation through a combination of suitable methods that did not rely on habituation, (e.g., Kühl *et al*., 2019). Furthermore, in our research, we employed the same methodology and level of effort in each of the four studied communities, suggesting that the variation we found is unlikely to be a function of differential research effort or observational bias.

Another possible explanation for the differences we found may be rooted in differential resource availability. Honey was recorded in all the studied communities' home ranges; however, in Lautchandé honey was not encountered frequently and chimpanzees did not seem to utilize the mangrove areas where stingless bee honey is commonly found. This might explain the low incidence of tool use (with only a single tool found) at Lautchandé. Nonetheless, honey was encountered frequently in Caiquene-Cadique, where the chimpanzees often use mangroves to access different parts of their home range and pass many stingless bee hives along their routes. This suggests that occasions for honey extraction are plentiful at Caiquene-Cadique, hence lack of opportunity cannot solely explain the complete absence of tools there.

A third, related explanation for the apparent lack of tool use for honey-extraction in Caiquene-Cadique might be that chimpanzees feed on honey from stinging honey bees (*Apis mellifera*) more frequently, either from natural or artificial hives (Bessa *et al*., 2015). To exploit this particular resource, chimpanzees have been observed employing a different approach that is quicker than tool use and therefore less likely to subject them to painful stings from the bees: they perform rapid hit-and-run raids on the hives.

Lastly, the differences in the numbers of tools recovered at the four sites might be due to variation in material culture between communities. Many studies have shown that different chimpanzee communities exhibit different behaviours, including the use of tools, even without obvious ecological or genetic determinants on these behaviours (Luncz *et al*., 2012; Whiten *et al*., 1999). This has been described as cultural variation, i.e., the emergence and maintenance of behavioural variants through local innovation and subsequent social learning. While the validity of a genetics-ecology-culture trichotomy has rightly been called into question (Laland and Hoppitt, 2003; Laland *et al*., 2009; Koops *et al*., 2013, 2014), evidence of behavioural variation even across neighbouring chimpanzee communities that share migrants and that inhabit very similar habitats has provided some of the most convincing evidence so far of cultural processes at work (Luncz *et al*., 2012; Koops *et al*., 2015; Pascual-Garrido, 2019). Nonetheless, it is also important to note that even in cases where ecological or genetic differences exist, intergroup variation in behaviour may still be attributable to culture, as long as the given behaviour is acquired at least in part by social learning. In fact, if different communities exhibit the same behavioural pattern, but these behaviours are socially learnt, they still qualify as cultural. Although we cannot state with certainty that honey dipping tools are absent in Caiquene-Cadique and Lautchandé, from the evidence gathered in this study their use certainly appears less frequent than that of Cambeque and Madina chimpanzees. Due to the spatial proximity of these four communities, and the resulting exchange of migrants and similarities in ecology and resource availability in at least three of the studied communities, we may therefore hypothesize that these differences in honey exploitation are, at least in part, cultural in nature. Nonetheless, direct evidence of social learning, as emphasised particularly strongly by the tridimensional model of animal traditions (Fragaszy and Perry 2003), would be necessary for this hypothesis to stand. Collecting such evidence - which typically requires extended periods of direct observation - was beyond the scope of our current study.

At a more fine-grained level, when comparing tools used to exploit different types of stingless bee honey, some further notable differences were found. BM tool ateliers tended to have significantly more tools associated with each honey extraction event than did MM or BH tool ateliers, tools where both the proximal and distal ends were used were more frequently found in BM ateliers, and, when ends were frayed, the fray length tended to be significantly longer for BM tools. These differences might be due to the type of habitat where the different bee species' hives are normally found. MM are mostly found in mangrove areas, an open landscape where shelter is limited and where humans frequently pass. Given that BM is normally found in the forest where the likelihood of encountering humans is much lower, chimpanzees might have the opportunity to spend longer periods of time exploiting them. The hives' location and structure might be related to these differences as well. Most MM hives found were located high up in live trees and had extremely small entrances, while some BM nests were found in dead or fragile tree trunks which meant that the small hive opening could be enlarged more easily, or the trunk could be fragmented. A larger opening will allow increased access to honey and therefore more time spent at the site. This may in turn translate to more repetitions of using the same tool (thus making the fray longer), until it has to be substituted by using the other tool end (leading to more tools with both the distal and proximal ends used) and/or by manufacturing a new tool (leaving behind a higher number of tools at the site).

Additional differences were also found when comparing the tools used for MM extraction in Madina and Cambeque. In Cambeque, tools were significantly longer than in Madina, and in Madina there were significantly more tools found with both ends showing signs of use or modifications. Importantly, at both sites tools were used to exploit the same resource, the raw material used was the same (*Avicennia germinans*), and the hives were exclusively arboreal, located in live trees with small entryways. Hence, the differences in tools cannot easily be explained through environmental differences, and a genetic explanation is unlikely given the documented gene flow between communities (Sá, 2013). Therefore, again a cultural explanation is likely, this time pertaining to subtle differences in the characteristics of tool manufacture and use between the Cambeque and Madina communities.

When analysing the CNP chimpanzees' dipping tool kit as a whole, some important patterns were found. Many of the tools collected had one or both ends frayed. Frayed/brush ends have been described in many other chimpanzee communities for fluid dipping (Stanford *et al*., 2000; Fowler and Sommer, 2007; Boesch *et al*., 2009; Sommer *et al*., 2012; Lapuente *et al*., 2017) or termite fishing (e.g., Sanz and Morgan, 2007). In Comoé National Park (Ivory Coast) chimpanzees have been seen biting the ends of tools to loosen the fibres creating a brush (Lapuente *et al*., 2017), and at Goualougo (Republic of Congo) a similar manufacture process to create brushed ends for termite fishing tools is associated with increased termite harvest (Sanz and Morgan, 2007). However, in other cases brushed ends have been linked to the fibre structure of the raw material that, when broken, might naturally form a brush (Takemoto *et al*., 2005). In the case of CNP we do not know if the fray is a simple by-product of use or if it is specifically added by chimpanzees prior to use. Notably, we found all three types of wear (brush, blunt and fragmented) on tools made out of multiple different species of raw material. Furthermore, all tools used to exploit MM were made out of the same raw material but exhibited all three types of wear. Taken together, these observations are more supportive of a pattern of production rather than fraying being merely a by-product of the fibre structure. Interestingly, the human communities that live alongside the CNP chimpanzees also exploit the same types of honey opportunistically: when they encounter a stingless bee hive they will often enlarge the opening of the hive, for easier access to the honey, and chimpanzee tools are sometimes encountered by these trees (JB, personal observation). Human traces, however, are clearly distinct from those of chimpanzees given that they present clean cuts made by a knife or machete on the end opposite to the brush, all side branches are sliced off, and the bark is peeled off using the same cutting tool, while the hole of the hive will also present signs of having been enlarged by a machete. As such, the likelihood that we misclassified human tools as chimpanzee tools is very low. Nonetheless, these observations raise important questions about how human activities such as honey harvesting and traditional apiculture might impact chimpanzee tool use (see Hockings *et al*., 2015 for similar research), and should be the focus of future research.

As tools with different wear patterns were found associated with the same hives, including subsets that were the same approximate age (see, for example, Fig 3.2d), it is possible they were all used during the same honey extraction episode, in a potential sequence. This suggests the use of tool sets by chimpanzees at CNP. When comparing the patterns of wear encountered in CNP with other published data it is possible that the CNP tool set has at least three types of tools. The first are exploratory probes, where very little modification is present. On some occasions (as confirmed by camera trap footage) chimpanzees simply procure a small twig, remove some side branches with leaves, and use it in a delicate motion to test if there is any honey present in the hive to collect. The second are pounding tools, where one or both tool ends present blunt or mashed ends, suggesting a pounding motion, possibly to break or separate the hard wax in the hive. Finally, the third type represent extraction tools – these have brush ends that are either a by-product of use or a deliberate modification (see Boesch *et al*., 2009). Given the scarcity of video evidence to date, we can only speculate that these tools serve these specific functions and that they may have been used in sequence, nonetheless the fact that tools that were made of the same raw material presented such distinct wear patterns gives us some degree of confidence that they were used for different functions. Additionally, comparing our indirect data to direct evidence collected in Central Africa, where tool sets of up to five different tool types are used in sequence (e.g., Loango NP, Gabon, Boesch *et al*., 2009; Moukalaba-Doudou NP, Gabon, Wilfried and Yamagiwa, 2014), strengthens our hypothesis that a similar sequence of use could be present in CNP.

Finally, we describe another characteristic of the CNP honey-dipping tool kit. In a few cases, tools showed different types of wear patterns at opposite ends, one frayed and one blunt. This suggests that these tools may have had more than one function, i.e., they were multifunctional tools, used both for pounding and for extraction. Such tools have been described for honey extraction in Central Africa (at Goualougo, by Sanz and Morgan, 2009 and at Loango, by Boesch *et al*., 2009). Multifunctional tools were once thought to be unique to humans, manifestations of highly sophisticated and complex technology. While we now know them to also be present within the wild chimpanzee tool kit, they have never until now been described for West African chimpanzees. Indeed, the use of tools to collect honey within Western chimpanzees has been regarded as less common than among Central African chimpanzees (Boesch and Boesch, 1990; Bermejo and Illera, 1999; Ohashi, 2006; Lapuente *et al*., 2017). It is important to note that given the lack of direct evidence of tool manufacture, we cannot completely discount the possibility that different wear patterns on the same tool resulted from an individual re-using another individual's tool for a different function.

Our study illustrates the importance of research on previously unstudied chimpanzee communities, including chimpanzees inhabiting human-impacted areas. They allow us to fill some gaps in our knowledge of the chimpanzee behavioural repertoire, revealing interesting new behaviours, and adding to the list of habitat types that we now know to be exploited by wild chimpanzees. CNP chimpanzees' use of the mangrove habitat is one such example (note that previous, albeit rare, reports of mangroves being part of chimpanzee home ranges did not describe their use by the resident communities – e.g., Loango chimpanzees in Head *et al*., 2019). Over 67% of the tools we recovered were found in mangroves, which suggests that this habitat type may have great significance for chimpanzees inhabiting the westernmost limit of the species' distribution. Our results also suggest potential cultural variation between neighbouring communities, and, the fact that our evidence was gathered through a combination of indirect and direct methods confirms that multifaceted methodologies are able to provide meaningful data even when studying populations where habituation is not possible or appropriate. It is clear, like in any other study of wild animal behaviour, that the longer the study continues the more we will learn about the lives and behaviour of these chimpanzees. It is, therefore, imperative that studies like ours continue long term, not only informing us about chimpanzee behaviour but also about chimpanzee behavioural variation and behavioural plasticity that might aid future conservation strategies, for example by identifying key chimpanzee resources, adaptations to anthropogenic changes, or even cryptic behaviours in response to added land pressures.

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3.9 Supplementary Materials

Supplementary Table 3.1. Summary of study effort at each of the study site in Cantanhez National Park, Guinea-Bissau. Information is provided on camera trap (CT) number, operational period and days active; total number of videos recorded that contained chimpanzees; number of recces and kilometres (km) walked; indirect data points collected; number of extractive tools collected; number of confirmed behaviours.

	Recce study effort			**Indirect data collection**		**Camera Trap study effort**					
Chimpanzee community	No. of recces	Distance Walked (km)	Estimate ranging areas (km^2)	No. of indirect traces	No. of tools	No. of CTs Deployed	First CT Day	Last CT Day	Total CT Days	Total Videos with Chimpanzees	Total videos of tool use
Caiquene-Cadique	48	230.8	14.8	538	0	3	16/04/2017	10/03/2018	399	179	0
Cambeque	49	260.4	7.1	230	100	3	13/03/2017	24/09/2018	648	25	2
Lautchandé	48	236.6	8.4	230	1	3	18/04/2017	04/11/2018	363	105	0
Madina	50	361.5	19.0	749	103	4	20/02/2017	29/09/2018	513	77	10
Total	**195**	**1089.3**	**49.3**	**1747**	**204**	**13**	**20/02/2017**	**04/11/2018**	**1923**	**386**	**12**

Supplementary Table 3.2. Total number of MM, BH and BM hives found in each chimpanzee community, and standardised densities against home range size and kilometres of recces walked. MM – *Meliplebeia sp*, small stingless bee; BM – *Meliponula* sp., large stingless bee; BH – *Apis mellifera*,

	Hives								
	MM			**HB**			**BM**		
Chimpanzee community	**Total Number**	**Number per Km walked**	**Number per Km^2 of home range**	**Total Number**	**Number per Km walked**	**Number per Km^2 of home range**	**Total Number**	**Number per Km walked**	**Number per Km^2 of home range**
Caiquene-Cadique	17	0.07	1.1	7	0.03	0.5	0	0	0
Cambeque	40	0.15	5.3	4	0.02	0.6	6	0.02	0.8
Lautchandé	1	0.004	0.1	2	0.01	0.2	0	0	0
Madina	22	0.06	1.2	10	0.03	0.5	1	0.003	0.05

Supplementary material 3.3 – Description of each tool use event captured via camera traps.

Observation 1 (18/07/2017, 14:10:44) Cambeque (secondary open forest). Adolescent male inspects a BM hive, inserts his finger into the small opening, pulls it out and smells it, looks around, with his right hand breaks a twig with many attached leaves, puts the proximal end in his mouth, inserts the same end into the hive, brings the tool back to his mouth, smells it, inserts it again, this time deeper, inspects it again, discards tool, tries to widen the hive's entrance with his middle finger, extracts some of the wax, smells finger and leaves.

Observation 2 (06/08/2017, 13:47:59) Cambeque (secondary open forest). Juvenile individual (sex undetermined) approaches an abandoned BM hive with a small green stem, with some attached leaves, in his/her mouth. With the right hand, the individual removes the leaves and inserts the proximal tool end into the hive's narrow entrance, moves it around in a circular motion, takes it out, smells the tool end, inserts it again now with greater force, pushes it as far as it will go, pulls it out, looks at it and leaves. See video S1.

Observation 3 (18/11/2017, 14:23:14) Madina (mangrove). Adolescent female approaches tree with MM hive, climbs up, breaks a twig with attached leaves, holds it in her mouth and climbs further until she is out of view (the camera trap has been knocked out of position and its view is no longer centred on the hive). The distal end of the tool (with leaves) is seen falling towards the ground.

Observation 4 (04/12/2017, 12:12:16) Madina (mangrove). Adult female with an infant climbs up tree, sits down, breaks a branch with her right hand, and climbs up carrying it towards the hive, out of view. Leaves are seen falling.

Observation 5 (04/04/2018, 10:01:32) Madina (mangrove). Young adult female is seen sitting next to hive, she stands up, breaks off a twig with no attachments with her right hand, quickly inserts the proximal end into the hive with back-and-forth and circular motions, takes the stick out, puts proximal end into her mouth and leaves.

Observation 6 (08/04/2018, 07:07:40) Madina (mangrove). Adult female is seen dangling from tree branches (approximately 4m from the ground), breaks twig with right hand while holding herself with left, inserts proximal end into hive entrance, brings it to mouth, discards tool, rapidly descends and runs away.

Observation 7 (18/06/2018, 16:44:42) Madina (mangrove). Juvenile female is seen in front of hive inserting a small stick into hive's entrance, puts the whole stick in her mouth, breaks a larger twig with attached leaves, descends the tree carrying larger twig in her mouth. A second adolescent female approaches the same location carrying a broken twig with attached leaves, inserts the proximal end into the hive's entrance with considerable force, pulls it out, inspects it and discards it.

CHAPTER 4

Culture in communication: inter-community variation in buttress drumming by wild chimpanzees

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Supplementary material can be found at the end of the chapter

4.1 Abstract

Some of the most widely accepted examples of animal culture involve vocal variation in birds and cetaceans. Even though chimpanzees (*Pan troglodytes*) have a vast cultural repertoire, evidence of group-specific communication is largely lacking for the species, and the small body of evidence that exists is highly contested. Apart from a wide range of vocalizations, chimpanzees communicate with one another through gestures, facial expressions, body postures, object manipulation and even through the production of rhythmic acoustic non-vocal sounds. The current study focusses on the last example, in the form of a universal chimpanzee behaviour – buttress drumming. Buttress drumming is used by individuals to communicate their location, facilitate group gathering and cohesion, and can also be used as part of agonistic displays. Previous studies have found evidence of variation in many aspects of drumming (e.g., bout duration, number of beats per bout), some at the individual level and others at group level; however, a consensus about how and why drumming might vary has yet to be reached. Here we analyse 1207 buttress drumming videos obtained through camera traps deployed between February 2017 and December 2018 in four neighbouring chimpanzee (*Pan troglodytes verus*) communities in Cantanhez National Park, Guinea-Bissau. We examine variation in drumming bout duration at the community level, as well as in terms of context (travel, feeding, agonistic display, and play), age and sex, and the production of accompanying pant hoot, leaf-clipping and loud self-scratching. We find that drumming duration varies with community, context, age and sex as well as the use of accompanying pant hoot vocalization. Our research suggests that buttress drumming duration might be part of community-specific signalling in specific contexts, such as travel, but that such specificity loses its relevance in contexts where long-distance communication is not the main function of drumming, such as agonistic displays.

4.2 Introduction

One of the earliest reports of animal culture, i.e., "group-typical behaviour patterns, shared by members of a community that rely on socially learned and transmitted information” (Laland and Hoppitt, 2003:151), was the discovery of regional song dialects in white-crowned sparrows (*Zonotrichia leucophrys*) (Marler and Tamura, 1964). Since then, some of the most widely accepted and compelling evidence of animal culture has come from examining vocal traditions (Laland and Janik, 2006; Lameira, 2017) in cetaceans (e.g., Helweg *et al*., 2005; Noad *et al*., 2000) and birds (e.g., Williams and Lachlan, 2022). This is perhaps explained by the fact that intergroup variation found in communication or other vocal behaviour is less likely to be affected by ecology as other types of behaviour (e.g., foraging, travel, tool use), and therefore in the absence of a genetic explanation can more readily be considered cultural (Lameira, 2017). Nonetheless, despite the vast body of evidence for animal culture across vertebrate taxa (Whiten 2021), the clearest examples for vocal culture come from birds, cetaceans, pinnipeds, elephants and bats (Janik and Knörnschild, 2021).

Primates have been key targets of vocal learning studies, driven in a large part by motivations to explore the origins and evolution of language in humans (e.g., Fouts *et al*., 1982; Hayes and Hayes, 1951). However, these studies have had limited success, primarily because primates appear to have seemingly inflexible vocal repertoires, that are species-specific and context dependent (e.g., Schel *et al*., 2013; Seyfarth *et al*., 1980; Slocombe and Zuberbühler, 2007). It is still not clear if these context specific vocalizations are innate and driven by arousal, or if, at least in part, they are socially learnt. It is generally accepted that primates lack the ability to generate new vocalizations outside of their limited, genetically determined species-specific repertoire (Slocombe, 2015; Townsend *et al*., 2020). Nonetheless primates have the ability to combine existing vocalizations, with specific meaning, to create a different message (Ouattara *et al.*, 2009; Cäsar *et al.*, 2013) and recent studies point towards the possibility of primates having greater vocal flexibility than previously thought (Townsend *et al.*, 2020). Additionally, some studies have reported the "invention" of new vocalizations: for example, the novel production of a species-atypical vocalization, the "raspberry" has been reported from both captive (Hopkins *et al.*, 2007) and wild chimpanzees (Pika, 2014; Bessa *et al.*, 2022). In fact, evidence from captivity shows that raspberries can be socially transmitted from mother to offspring (Taglialatela *et al.*, 2012).

Many primate vocal studies have focussed on great apes, especially on one of our two closest living relatives, the chimpanzee (*Pan troglodytes*) (e.g., Arcadi *et al.*, 1998; Crockford *et al.*, 2004; Desai *et al.*, 2021; Mitani *et al.*, 1992). Some studies have shown the structural modification of certain species-specific vocalizations, such as the pant hoot vocalization. This species-typical vocalization functions to communicate location (Goodall, 1968; Mitani and Brandt, 1994), facilitate social bonds (Fedurek *et al.*, 2013) and group gathering (Wrangham, 1977; Mitani and Nishida, 1993; Fedurek *et al.*, 2014), as well as to advertise numerical strength to neighbouring communities (Wilson *et al.*, 2001, 2002; Herbinger *et al.*, 2009) during patrols (Wilson, 2007). Therefore, researchers have argued that callers (and listeners) might benefit from having a community-specific signature in their pant hoots. Crockford *et al.* (2004) compared the pant hoots of four communities in the Tai Forest and found evidence of variation between neighbouring communities. The authors attributed these differences to social learning and suggest that it is the necessity of locating members of their own group as well as the need to distinguish the pant hoots of neighbouring communities, that explain these community-specific signatures (Crockford *et al.*, 2004). However, when a similar study was performed in Gombe National Park (Tanzania) and in Kibale National Park (Uganda), researchers found no evidence of variation between neighbouring communities, and instead found evidence of intra-community variation (Desai *et al.*, 2021). The authors suggest that the lack of community-specific differences found could be due to specific features of Gombe chimpanzees, a different subspecies; alternatively, the previous findings by Crockford *et al.* (2004) might have resulted from statistical artifacts and thus do not support the suggestion that social learning plays a role in the development of pant hoots (Desai *et al.*, 2021).

Similarly, there is controversy surrounding another promising case of learnt group-specific vocalisation in captive chimpanzees: “referential food grunts” (Watson *et al.*, 2015b). Food grunts are highly context dependent, and exclusively used during feeding or when arriving to a feeding patch (Schel *et al.*, 2013a). Despite being context dependent, there is a high degree of variation in the vocalization, related to the value of food being eaten/encountered (Slocombe and Zuberbühler, 2006; Kalan *et al.*, 2015). When a group of zoo chimpanzees was moved from the Netherlands and integrated with a group at Edinburgh zoo, Watson and colleagues (2015b) reported that after two years the structure of the call of most “Dutch” chimpanzees produced in response to apples had changed to match that of the resident “Scottish” group. According to the authors, this was a clear case of cultural conformity through vocal learning; however, Fischer and colleagues (2015) have argued that the results may instead have been an outcome of the gradual reduction in stress levels and arousal of the “Dutch” chimpanzees over the experimental period. In a counterargument, Watson *et al.* (2015a) dismiss this possibility since the “Scottish” chimpanzees also had to have experienced high levels of stress and arousal from seeing their territory “invaded” by a new group; nonetheless, they did not show any evidence of vocal change. Given the lack of agreement over this and other cases, chimpanzees’ capacity to modify their vocal calls through learning remains hotly debated.

In addition to vocal communication, primates communicate in many other ways that do not involve vocalizations, including gestures, object manipulation (e.g., leaf-clipping: Kalan and Boesch, 2018) or even rhythmic beats (e.g., chest beats: Salmi and Muñoz, 2020; drumming: Arcadi *et al*., 1998). Chimpanzees can generate rhythmic acoustic non-vocal sounds using their hands and/or feet to hit their own chests, the ground, buttress roots of trees or even manmade objects (Reynolds and Reynolds, 1965; Goodall, 1968; Nishida, 2011; Babiszewska *et al*., 2015). Of these behaviours, buttress drumming (hereafter drumming) appears to be universal and frequently used across all known chimpanzee communities (Whiten *et al*., 1999; Kühl *et al*., 2019). Chimpanzees drum more often with their feet, while grabbing the crest of the buttress, rather than drumming with their hands, and the sound produced can be audible for at least 1 km through a forest (Boesch, 1991; Arcadi *et al*., 1998; Arcadi and Wallauer, 2013). In general, drumming ranges from a single beat to brief sequences (or 'bouts') of beats, averaging three to six beats; in general drumming bouts last 0.5 to 1.5, seconds, but in some cases might last up to 5.5 seconds (Reynolds and Reynolds, 1965; Goodall, 1968; Arcadi *et al*., 1998, 2004; Arcadi and Wallauer, 2013; Babiszewska *et al*., 2015). Drumming typically occurs in conjunction with the species-typical pant hoot vocalization (Reynolds and Reynolds, 1965; Goodall, 1968; Arcadi *et al*., 1998, 2004), and, much like charging displays, is predominantly a male activity (Goodall, 1968; Arcadi *et al*., 1998). However, drumming occurs frequently during travel by large mixed parties, where encounters with specific favoured trees appears to trigger drumming displays by many members of the party, including females and juveniles (Goodall, 1968).

Drumming appears to serve several functions. Since the drumming sound can travel long distances (Boesch, 1991; Arcadi *et al*., 1998), even further than pant hoots (Crockford *et al*., 2004), it functions to enable long-distance communication and the coordinated movement of different parties (Reynolds and Reynolds, 1965; Goodall, 1968; Mitani and Nishida, 1993; Wrangham *et al.*, 1996; Arcadi *et al.*, 1998; Nishida, 2011). It has also been suggested that drumming might be used to call for help during conflict (Slocombe and Zuberbühler, 2007), and that it might convey information about travel direction, although this is, at least so far, based only on anecdotal evidence (Boesch, 1991; Boesch and Boesch-Achermann, 2000). Drumming can also be performed as part of a charging display that includes piloerection, bipedal walking and swaying, dragging of branches, swaying of small branches, throwing of rocks or other objects, ground-slapping and stamping. These displays may be accompanied by vocalizations which are normally not directed at a specific subject, or can be silent and directed at a specific individual and more likely to end in an attack (Goodall, 1968; Nishida, 2011). Therefore, it has been suggested that a vocal charging display accompanied by drumming might serve as a show of strength, to maintain or challenge the social hierarchy of the group without necessarily engaging in direct physical aggression (Goodall, 1968; Nishida, 2011).

During territorial boundary patrols chimpanzees tend to engage in very loud displays, with several drumming bouts occurring in sequence, accompanied by climax screams and longer pant hoots. In these situations, females frequently join the drumming displays. Presuming that chimpanzees from neighbouring territories can assess the size of each other's groups based on the sound of their drumming, and that female and male drumming is indistinguishable to a listener, Boesch and Boesch-Achermann (2000) propose that female drumming is used to exaggerate the strength of the group and thus to discourage direct confrontation. However, given that drumming appears to be mostly restricted to males and it is an activity that requires a considerable amount of strength and physical fitness to be executed, Babiszewska and colleagues (2015) hypothesised that drumming could act as a signal of the drummer's social status. Nonetheless, by looking at the frequency of drumming, the authors found no support for this hypothesis in Budongo (Uganda), since it appears that group composition, presence of maximally swollen females and allies, as well as the social status of the drummer, did not act as predictors of the occurrence of drumming. The authors did nonetheless find evidence that older males drummed more frequently than younger individuals, which was attributed to the skill and strength required to hit a buttress and produce sound (Babiszewska *et al.*, 2015). More interestingly, they also found individual differences in the duration of drumming, number of beats, inter-beat duration and use of double beats during travel, which might convey information about the drummer's identity (Babiszewska *et al.*, 2015); however, the authors did not explore the possible relationship between these individual differences and the individual's social status.

A previous study by Arcadi and colleagues (1998) in Tai Forest (Côte d'Ivoire) also found significant differences between individuals in mean number of beats, bout duration and duration of inter-beat intervals. However, in a follow-up study in Kanyawara (Uganda), no significant differences between males' drumming patterns were found (Arcadi *et al.*, 2004). Additional individual differences were found in Tai when looking at the integration of drumming into the accompanying pant hoot phrase sequence, with some individuals restricting drumming to the pause between the build-up and climax phases, and others not (Arcadi *et al.*, 1998). Finally, in Mahale (Tanzania) individual drumming patterns have been described when different individuals drummed on the walls of a research building, with one individual using one hand, another using two hands, and yet another using two feet (Nishida *et al.*, 2009). Explanations for individual differences in drumming have been proposed. For example, Babiszewska *et al.* (2015), suggested that the reverberatory qualities of the buttress (i.e., tree species, size, age, etc) might provide a simple explanation for many of these differences, and hence the context of drumming should be carefully taken into account. On the other hand, Arcadi and Wallauer (2013) showed that the galloping gait of chimpanzees can influence their pattern of drumming, and could also be responsible for the occurrence of a double beat.

Group-specific patterns of drumming – as well as inter-community differences in these patterns – have also been proposed. When comparing drumming duration, Tai chimpanzees were found to drum for longer and with more beats per bout than Kanyawara chimpanzees, although this difference was attributed to the fact that Tai chimpanzees accompanied their drumming with pant hoots more frequently than Kanyawara chimpanzees (Arcadi *et al*., 2004). In terms of the integration of drumming into the pant hoot, Kanyawara chimpanzees tended to start drumming further into the vocalization than Tai chimpanzees, and a greater proportion of their drumming bouts started after the pant hoot had already ended (Arcadi *et al*., 2004). Contrary to the individual differences found by Babiszewska *et al*. (2015), these community-specific differences between the geographically-distant Tai and Kanyawara chimpanzees (Arcadi *et al*., 2004) could be an indication that some degree of social learning and transmission is involved in the development of drumming. However, some caution is needed when interpreting these results since the authors are comparing two different chimpanzee subspecies, which could account for at least part of the variation found. Individual differences in drumming appear to be present in two of the three communities in which this behaviour has been studied, and it is still not clear what mechanisms are behind community-specific variation. This is not surprising given the small number of studied sites as well as sample sizes. However, given the different possible functions of drumming, could it be that community or individual differences may be function/context dependent? For example, might we expect community differences when the function is long distance communication, but individual differences when the function is intimidation during an aggressive display?

Thus, more research is needed to understand the functions of chimpanzee communication via drumming, the role of learning in its development at the individual level, and the nature and source of variation exhibited at both the individual and group level. However, as previous studies have shown, assembling a representative amount of data is not easy and normally requires years of following habituated communities. In this study, we approach data collection in a different way, by using targeted camera traps for a period of two years to capture buttress drumming in four neighbouring unhabituated chimpanzee (*P. t. verus*) communities in Cantanhez National Park (CNP), Guinea-Bissau. We suggest that the use of camera traps might provide more accurate sampling of the communities' drumming than the more traditional method of collecting recordings live during 'group follows' of habituated communities in which the drumming of solitary males or all-female parties might be overlooked. We compare chimpanzee drumming duration in our four neighbouring communities and assess factors that might underlie any variation found. We make the following predictions: i) we expect to find differences between age and sex classes in rate of drumming, given that this is a prominent feature of previous studies' findings; ii) we expect drumming accompanied by pant hoots to be longer in duration, given previous findings; iii) we expect that pant hoots will be used more frequently (and hence drumming will be longer) during travel, given the pant hoot's role in long distance communication; iv) we expect to find significant inter-community variation in drumming duration in contexts where long distance communication is the main function of drumming (e.g., travel).

4.3 Methods

Study site and study communities

CNP is located in the Tombali region of south-west Guinea-Bissau. CNP is a mosaic of settlements, agricultural fields, sub-humid forest, secondary forest, palm groves, mangrove, and savannah (Catarino *et al*., 2006). It is estimated that 10 to 12 chimpanzee (*P. t. verus*) communities inhabit CNP (Bersacola, 2019). Data collection took place in four neighbouring unhabituated communities: Caiquene-Cadique, Lautchandé, Madina and Cambeque (Fig 4.1), over the course of 23 consecutive months (February 2017 – December 2018). Different communities were identified based on previous research, including genetic studies, local knowledge, and the presence of natural and anthropogenic barriers (e.g., villages, roads, rivers and estuary branches that form small peninsulas) (Hockings and Sousa, 2013; Sá, 2013; Bessa *et al*., 2015, 2021; Bersacola *et al*., 2018). Each community's ranging areas were estimated based on opportunistic sightings and indirect evidence (faeces, feeding traces, nests, tool-use sites, and foot and knuckle prints; see (Bessa *et al*., 2022), using minimum convex polygons: Caiquene-Cadique 14.8 km^2, Madina 19.0 km^2, Cambeque 7.1 km^2, and Lautchandé 8.4 km^2. Even though these communities are unhabituated to researchers, previous work has estimated that there are at least 49 individuals in Caiquene-Cadique (M. Ramon, pers. comm.) and 48 in Madina (Vieira *et al*., 2019).

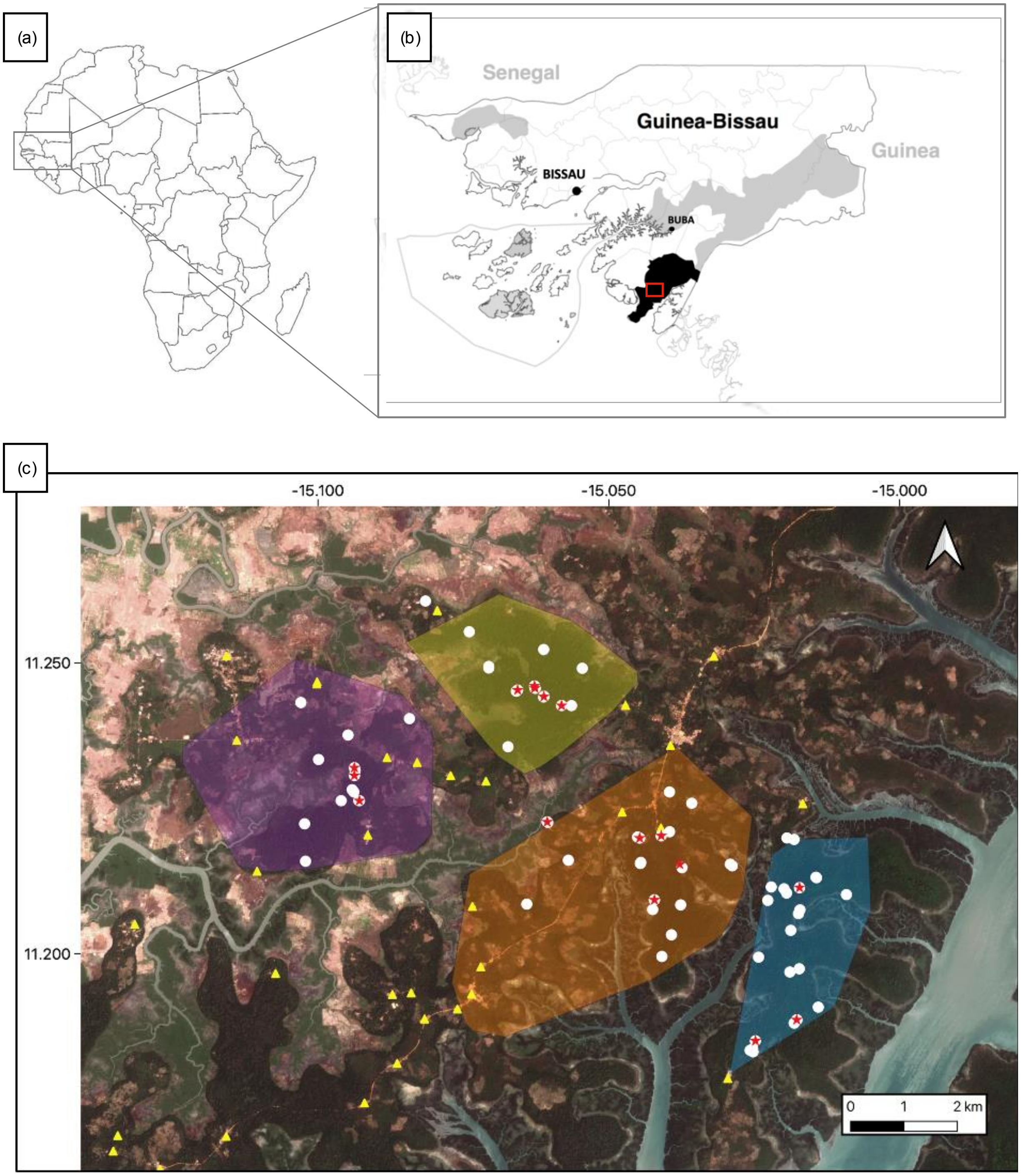

Figure 4.1 Locations of research sites and study communities' core home ranges. (a) Map of Africa showing the location of Guinea-Bissau. (b) Map of Guinea-Bissau showing the locations of Cantanhez National Park (black) and other protected areas (light grey). The red rectangle in (b) corresponds to the region depicted in panel (c). (c) Aerial image showing the locations of four chimpanzee study communities and their core home ranges in Cantanhez National Park, Guinea-Bissau. Core home ranges are shown as minimum bounding polygons estimated from direct (i.e. observations) and indirect evidence (i.e. faecal and feeding traces, prints, nest, discarded tools), and are illustrated in different colours: Caiquene-Cadique (purple), Lautchandé (yellow), Madina (orange), and Cambeque (blue). Yellow triangles correspond to villages and other human settlements, red stars correspond to camera trap locations and white circles correspond to drumming trees. Sentinel-2 imagery was downloaded from the Sentinel Hub, Sinergise Ltd (https://www.sentinel-hub.com/). All maps were created using QGIS version 3.12 (https://www.qgis.org)

Data collection and analysis

During reconnaissance walks within the chimpanzees' home ranges, drumming trees were identified based on the presence of large buttress roots, indirect evidence such as use-wear patterns on the buttresses (i.e., smoothing of the crest), as well as by following the sound of buttress drumming. A total of 46 drumming trees were identified (9 in Caiquene-Cadique; 10 in Lautchandé; 14 in Madina; 13 in Cambeque), of five different species (*Ceiba pentandra, Parinari excelsa, Antiaris toxicaria, Treculia africana, Ficus* sp.*).* A subset of these trees was selected in each community (see Table 4.1), based on location within the home range (e.g., close to main paths and in areas where the chimpanzees were frequently heard buttress drumming), and on the degree of use-wear of the buttress crest. Camera traps (Bushnell Trophy Cam HD Aggressor No-glow) were set up at these selected locations, each equipped with a 32 GB SD card, 8 AA batteries and a silica pack to protect them from humidity. Camera traps were motion triggered and recorded 1-minute videos, and were set up pointing towards the part of the buttress with the most prominent signs of wear. They were positioned at a distance of approximately 3m from the buttress to record any other behaviour that might take place before or after the drumming, as well as to film as many individuals present as possible. Given that these were unhabituated communities, with seasonal changes to their home range use, some cameras had to be moved to other locations to maximise the chances of capturing drumming behaviour.

After deployment, camera traps were checked after a week of recording to assess the functioning of the camera, its positioning, lighting, and success (i.e., whether chimpanzees were indeed captured using the drumming tree). If problems were encountered, the camera was adjusted, or in case of malfunction it was replaced by a new camera. After the first check-up, cameras were checked at intervals of two weeks. During each check, any small vegetation that might trigger the camera or obstruct filming was cut down and any other debris (e.g., fallen branch) was cleared away. The cameras were wiped clean, the silica pack was exchanged, the battery level was checked, and batteries replaced when necessary, and SD cards were swapped. At the base camp all SD cards were downloaded and backed up on two external hard drives.

Given that the cameras were set up in central areas of the chimpanzees' home ranges, many of the videos recorded contained chimpanzees but did not contain drumming, many were triggered by other animals or local people, and others were false triggers. In total we collected 14,215 videos corresponding to over 236 hours of footage. To streamline annotation time, every time an SD card was swapped, JB checked through all videos and separated them into those containing chimpanzees and those not containing chimpanzees. In total 3158 chimpanzee videos (with and without drumming) were recorded, corresponding to over 54 hours of footage.

To expedite the labour-intensive and time-consuming nature of manual video analysis, part of the subsequent annotation was done in collaboration with the Visual Geometry Group (University of Oxford). A system of automated drumming annotation was created, using a deep convolutional neural network (CNN) approach, consisting of four steps: audio action detection, visual detection and tracking, audio-visual action recognition, and action-specific implementation details. First, a selected group of videos was manually annotated for drumming, and these were used to train the CNN model. The automated process separated videos with drumming from those without auditorily, and selected drumming sequences in each video automatically by visually detection "when at least one hand or foot was in contact with the buttress, and auditorily, when the distinct beat sound was heard." (Bain *et al.*, 2021). The drumming sequences were defined as beginning when the first beat was detected (visually or auditorily) and ending with the last beat. The automated beat detection predicted drumming duration with 0.205 s mean and 0.131 s median errors. Given the short duration of most drumming bouts, we opted for manually correcting drumming duration after automated detection, which was nonetheless far more efficient than annotating all drumming bouts manually without the help of the automated method. If more than 2 seconds occurred between clusters of beats, these were not considered part of the same drumming bout (Arcadi *et al*., 2004). Other behaviours, including pant hoots, that occurred before the first beat or after the last beat were not automatically detected. For further information on the automated pipeline for behavioural recognition see Appendix D.

Table 4.1. Summary of study effort at each of the four study sites in Cantanhez National Park, Guinea-Bissau. Information is provided for each site on number of camera traps set; number of camera trap active days; total number of videos recorded; total number of chimpanzee videos recorded; total number of drumming videos; and the average number of drumming videos per active camera trap days.

Camera Trap ID	Community	Camera trap – active days	Total number of videos	Total number of chimpanzee videos	Total number of drumming videos	Daily average of drumming videos
CNPCqCd1	Caiquene-Cadique	466	1619	727	168	0.4
CNPCqCd2	Caiquene-Cadique	502	1234	1048	541	1.1
CNPCqCd5	Caiquene-Cadique	359	1083	306	30	0.1
Total Cameras	**Caiquene-Cadique**	**1327**	**3936**	**2081**	**739**	**0.6**
CNPCB7	Cambeque	287	2097	116	55	0.2
CNPCB8	Cambeque	299	1164	149	65	0.2
CNPCB11	Cambeque	19	88	26	9	0.5
Total Cameras	**Cambeque**	**605**	**3349**	**291**	**129**	**0.3**
CNPL1	Lautchandé	464	1423	127	50	0.1
CNPL3	Lautchandé	414	1154	28	17	0.0
CNPL5	Lautchandé	122	161	104	51	0.4
CNPL10	Lautchandé	100	76	25	9	0.1
CNPL12	Lautchandé	64	202	30	22	0.3
Total Cameras	**Lautchandé**	**1164**	**3016**	**314**	**149**	**0.1**
CNPM7	Madina	241	545	15	6	0.0
CNPM10	Madina	388	2295	268	123	0.3
CNPM11	Madina	235	477	108	35	0.1
CNPM13	Madina	119	443	44	13	0.1
CNPM14	Madina	76	154	37	13	0.2
Total Cameras	**Madina**	**1059**	**3914**	**472**	**190**	**0.2**
ALL CAMERAS		**4155**	**14215**	**3158**	**1207**	**0.3**

Apart from the automated drumming annotation, manual annotation was conducted to collect data on group size and composition (i.e., number of adult males, adult females including maximally swollen females and females carrying dependent offspring, and immature individuals), individual identity (at present this was only possible for a small number of individuals due to the unhabituated nature of the communities and large group sizes), duration of pant hoot vocalization, and context. Context was separated into four broad categories: a) travel, where an individual was moving through camera view. As some individuals waited for group members whilst travelling in a party, we classified the context as travel when the individual did not stop for more than 5 minutes; b) feeding, where the individual or group was actively processing or carrying food, or had just joined a feeding party, producing food grunts; c) agonistic display, where an individual alone or in a group drummed as part of a charging display, combining drumming with running, bipedal walking or swaying, dragging branches, swaying branches, throwing rocks or other objects, ground slapping or stamping, piloerection or direct aggression to other individuals; and d) play, where an individual (or a group) approached a buttress tree and began drumming without producing much sound, normally in combination with other play and exploratory behaviours. Additionally, leaf-clipping (i.e., ripping apart one or more leaves from the ground using the mouth or fingers, one by one, producing a conspicuous and distinctive ripping sound) and loud self-scratching (i.e., audible scratching of oneself, typically used to indicate frustration) were also annotated. Manual annotation was done by JB and JG using the VGG Image Annotator (VIA) software (see Bain *et al.*, 2021).

Statistical analyses

All statistical tests were performed in R (version 1.1.463), and data were tested for normality using the Shapiro-Wilk's method, and for homogeneity of variance using Bartlett's test. Non-parametric

Kruskal-Wallis test and generalized linear mixed models (GLMMs) were the main statistical tools used in the analyses. GLMMs were used to investigate the influence of categorical variables (context, age and sex class, community, presence or absence of pant hoot, leaf-clipping and loud self-scratching) on drumming duration. Drumming duration was log-transformed to meet the assumption of the parametric test. When exploring the influence of mixed explanatory factors, we used Akaike's Information Criterion (AIC) to select the best explanatory model. A lower AIC value indicated better support for the model (Akaike, 1974), with terms considered to improve the model's fit only if their exclusion from the model inflated AIC values by more than two units (Burnham and Anderson, 2004).

4.4 Results

In total we recorded 1207 videos containing drumming; of these, 929 contained complete drumming bouts. The remainder contained only partial bouts, with the video either starting after the drumming had begun or ending before the bout had finished. There were differences in the number of drumming videos collected for each community: the largest number was collected in Caiquene-Cadique (N=739), followed by Madina (N=190), Lautchandé (N=149) and Cambeque (N=129). Standardised against active camera trap days in each community, on average 0.6 ± 0.5 drumming videos were recorded daily in Caiquene-Cadique, followed by 0.3 ± 0.1 in Cambeque, 0.1 ± 0.1 in Lautchandé and 0.2 ± 0.1 in Madina. Table 4.2 shows the drumming videos collected in each community, broken down by context as well as age and sex class.

Across the four communities, most drumming was performed by males (84.8%, N=1023), followed by females (6.9%, N=83) and immature individuals of either sex (4.6%, N=56). Due to low visibility or drumming occurring outside the camera's view, we were not able to determine the age and sex of the drummer in 3.7% of videos (N=45). Of female drummers, 26.5% were maximally swollen females and 48.2% were carrying dependent offspring. Most drumming occurred in the context of travel (64.6%, N=780), followed by agonistic display (21.6%, N=261), feeding (10.7%, N=129) and play/exploratory context (3.1%, N=37) (see Supplementary Table 4.1).

Pant hoot vocalization accompanied 88% (N=788) of all complete drumming videos. Male drumming was accompanied by pant hoot in 93.7% (N=711) of complete videos, and female drumming in 73.7% (N=42) of complete videos. Broken down by context, complete drumming bouts were accompanied by pant hoots during 96.8% (N=90) when feeding, 93% (N=517) when travelling, 82% (N=173) when engaged in agonistic displays and 22.9% (N=8) when playing. Apart from pant hoots, loud self-scratching occurred before drumming in 23.1% (N=207) of all complete drumming bouts, with males (23.8%, N=181) loud self-scratching more often than females (15.8%, N=9). Loud self-scratch was most often associated with a feeding (27.9%, N=26) context for drumming, followed by travel (24.6%, N=137), play (22.8%, N=8), and agonistic display (16.6%, N=35). Leaf-clipping preceded 7.7% (N=69) of all complete drumming bouts (8.7%, N=66 of male drumming, and 3.5%, N=2 of female drumming). Leaf-clipping was most often associated with a feeding (9.7%, N=9) context, followed by travel (8%, N=45), agonistic display (6.6%, N=14) and play (2.8%, N=1). Data on pant hoots, loud self-scratching and leaf-clipping are summarized in Table 4.2.

Across the 895 complete drumming videos where the drummer's age/sex class could be determined (adult males, adult females, or immature individuals), the mean ± SD duration of a drumming bout was 2.23 ± 1.72 s (range: 0.21-19.48 s).

Table 4.2. Percentage of complete drumming videos with pant hoot (PH), loud self-scratching (LS), and leaf-clipping (LC). Videos are categorised by community, context, and age and sex class. AM – adult male; AF – adult female; IMM – immature.

		Caiquene-Cadique			**Madina**			**Lautchandé**			**Cambeque**		
		PH(%)	**LS(%)**	**LC(%)**	**PH(%)**	**LS(%)**	**LC(%)**	**PH(%)**	**LS(%)**	**LC(%)**	**PH(%)**	**LS(%)**	**LC(%)**
AM	Play	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Travel	98.6	36.0	10.1	92.1	9.6	9.6	97.5	10.0	2.5	89.9	6.1	4.1
	Agonistic	90.3	22.4	6.9	57.1	7.1	0.0	71.9	6.3	6.3	91.7	12.0	0.0
	Feeding	98.5	26.7	9.3	100	50.0	0.0	100	25.0	16.7	NA	NA	NA
	Total	**96.6**	**31.4**	**9.2**	**88.5**	**9.9**	**8.4**	**88.1**	**84.0**	**6.0**	**90.4**	**8.1**	**2.7**
AF	Play	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Travel	68.2	18.5	0.0	100	0.0	0.0	63.6	18.2	0.0	0.0	0.0	0.0
	Agonistic	75	20.0	20.0	100	0.0	0.0	66.7	0.0	0.0	100	0.0	0.0
	Feeding	100	0.0	0.0	0.0	NA	NA	0.0	NA	NA	0.0	NA	NA
	Total	**75**	**15.6**	**4.4**	**100**	**0.0**	**0.0**	**64.7**	**11.8**	**0.0**	**100**	**0.0**	**0.0**
IMM	Play	16	24.0	0.0	33.3	0.0	16.7	0.0	50.0	0.0	33.3	33.3	0.0
	Travel	25	25.0	0.0	0.0	NA	NA	16.6	6.0	0.0	0.0	0.0	NA
	Agonistic	0.0	0.0	0.0	100	0.0	0.0	0.0	0.0	NA	0.0	0.0	NA
	Feeding	100	0.0	0.0	0.0	NA	NA	0.0	0.0	NA	0.0	0.0	NA
	Total	**21.1**	**21.2**	**0.0**	**42.8**	**0.0**	**14.3**	**12.5**	**12.5**	**0.0**	33.3	**33.3**	**3.0**
ALL	Play	16	24.0	0.0	33.3	0.0	16.7	0.0	50.0	0.0	66.7	33.3	0.0
	Travel	89.6	33.4	8.9	89.9	9.2	9.2	82.4	10.5	1.8	80	5.5	3.6
	Agonistic	79.4	20.6	14.0	58.8	5.9	17.0	67.5	5.0	5.0	85.2	11.1	0.0
	Feeding	83.1	22.5	7.9	100	50.0	2.0	92.3	23.1	15.4	0.0	NA	NA
	Total	**83.2**	**28.4**	**8.0**	**84**	**9.0**	**8.3**	**76.8**	**10.7**	**4.5**	**79.3**	**8.2**	**2.4**

Given the number of independent variables that could have had an influence on drumming duration (dependent variable), we conducted GLMM analyses. All possible GLMM combinations with the explanatory variables "age/sex class" (Adult Male, Adult Female, Immature), "community" (Caiquene-Cadique, Lautchandé, Cambeque, Madina), "context" (travel, feeding, agonistic display, play), “pant-hoot” (yes, no), “loud self-scratch” (yes, no), “leaf-clipping” (yes, no) were run. AIC values were calculated for each model and the best fit model was selected (see Supplementary Table 4.2). Leaf-clipping, and loud self-scratching did not appear to improve the explanatory power of the model. The optimal model contained community, context, age/sex class, and pant hoot, with all variables significantly affecting duration (GLMM, $R2= 0.049$, $F(10, 884) = 5.702$, $p< 0.001$).

Drumming duration varied with community, with Caiquene-Cadique’s 2.14 ± 1.46 s (range: 0.21-12.25)) average duration being shorter than Lautchandé (2.34 ± 2.93 s (range: 0.23-19.48)), Madina (2.38 ± 1.39 s (range: 0.54-7.67)) and Cambeque (2.38 ± 1.63 s (range: 0.56-10.88)) (Fig 4.2a). Additional significant differences were found between contexts, where duration was shorter during feeding (1.69 ± 1.16 s (range: 0.27-6.45)) compared to display (2.26 ± 2.50 s (range: 0.21-19.48)), travel (2.29 ± 1.32 s (range: 0.23-8.57)) and play (2.54 ± 2.26 s (range: 0.47-12.01)) (Fig 4.2b). Adult females performed on average longer drumming bouts (2.74 ± 1.77 s (range: 0.25-7.94)) than immatures (2.54 ± 2.26 s (range: 0.47-12.01)), and adult males (2.21 ± 1.66 s (range: 0.21-19.48)) performed the shortest drumming bouts (Fig 4.2c). Additionally, drumming bouts that included a pant hoot vocalization were longer (2.68 ± 1.64 s (range: 0.21-19.48)) than those that did not (2.03 ± 1.79 s, range: 0.33-12.02)) (Fig 4.2d). Finally, drumming preceded by loud self-scratching (2.18 ± 1.31 s (range: 0.21-7.94)) was on average shorter than drumming without loud self-scratching (2.25 ± 1.81 s (range: 0.23-19.48)) (Fig 4.2e), and drumming preceded by leaf-clipping (2.25 ± 1.74 s (range: 0.21-19.48)) was on average longer than without leaf-clipping (2.08 ± 1.20 s (range: 0.39-5.37)) (Fig 4.2f).

Given that 83% of complete drumming bouts were performed by males, we also conducted a separate analysis on male drumming bouts only (N=744). The same dependent and explanatory variables (except age/sex class) as in the previous analysis were tested. AIC values were calculated for each model and the best fit model was selected (see Supplementary Table 4.3). Leaf-clipping did not improve the explanatory power of the model, and the optimal model contained context, pant hoot, community, as well as loud self-scratching, with all variables significantly affecting duration (GLMM, R2= 0.052, F(8, 750) = 6.154, p<0.001). Drumming bouts that included a pant hoot vocalization were longer (2.68 ± 1.64 s (range: 0.21-19.48)) than those that did not (2.03 ± 1.79 s (range: 0.33-12.02)). Mean male drumming duration was longest (2.28 ± 1.29 s (range: 0.23-7.65)) during travel, followed by display (2.23 ± 2.51 s (range: 0.23-18.48)), and was shortest during feeding (1.69 ± 1.48 s (range: 0.28-6.46)). Mean male drumming duration was shortest in Caiquene-Cadique (1.98 ± 1.26 s (range: 0.21-12.25)), followed by Madina (2.40 ± 1.36 s (range: 0.54-7.67)), Cambeque (2.44 ± 1.65 s (range: 0.56-10.88)) and Lautchandé (2.45 ± 3.16 s (range: 0.23-19.48)). Finally, drumming preceded by loud self-scratching (2.16 ± 1.27 s (range: 0.21-7.67)) was on average shorter than drumming without loud self-scratching (2.19 ± 1.79 s (range: 0.23-19.48)).

Finally, we further explored the effect of context by analysing only those male drumming bouts that were accompanied by a pant hoot. Duration varied significantly with context (H(2)=25.706, p<0.001), with travel (2.23 ± 1.80 s, range: 0.37–3.29) longer in duration than display (1.49 ± 1.34 s, range: 0.33–7.91). Additionally, we separated male drumming bouts that occurred in the context of agonistic display and those occurring during travel. Kruskal-Wallis tests showed that there was no significant difference in duration when comparing drumming during agnostic displays between communities (H(3)=1.3816, p=0.709), whereas the same comparison in the context of travel showed significant differences between communities (H(3)=11.784, p=0.008); see Figure 4.3.

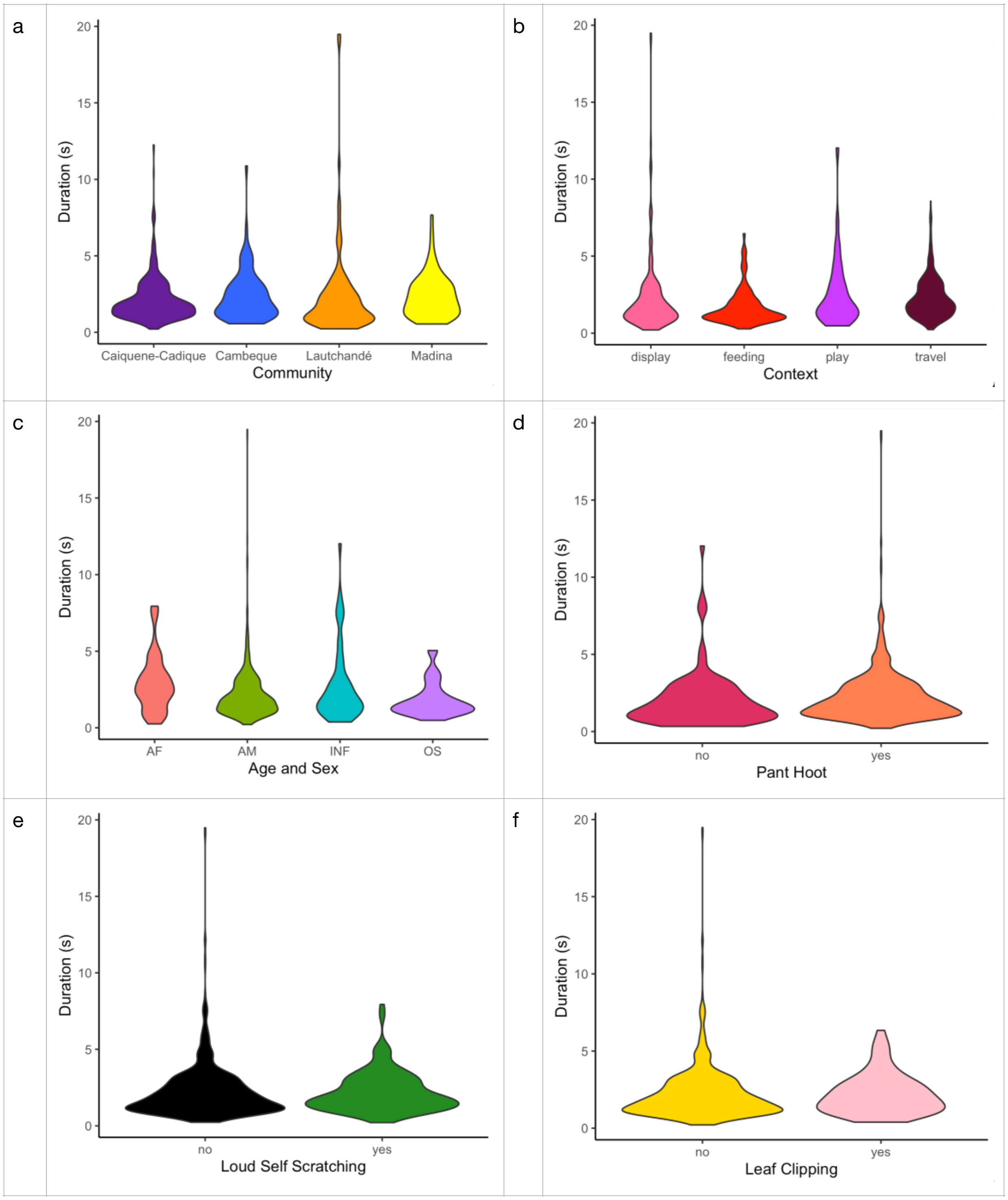

Figure 4.2 Complete drumming bout durations as a function of (a) age and sex class, (b) context, (c) community, (d) presence or absence of accompanying pant hoot vocalization, (e) presence or absence of loud self-scratching, and (f) presence or absence of leaf-clipping.

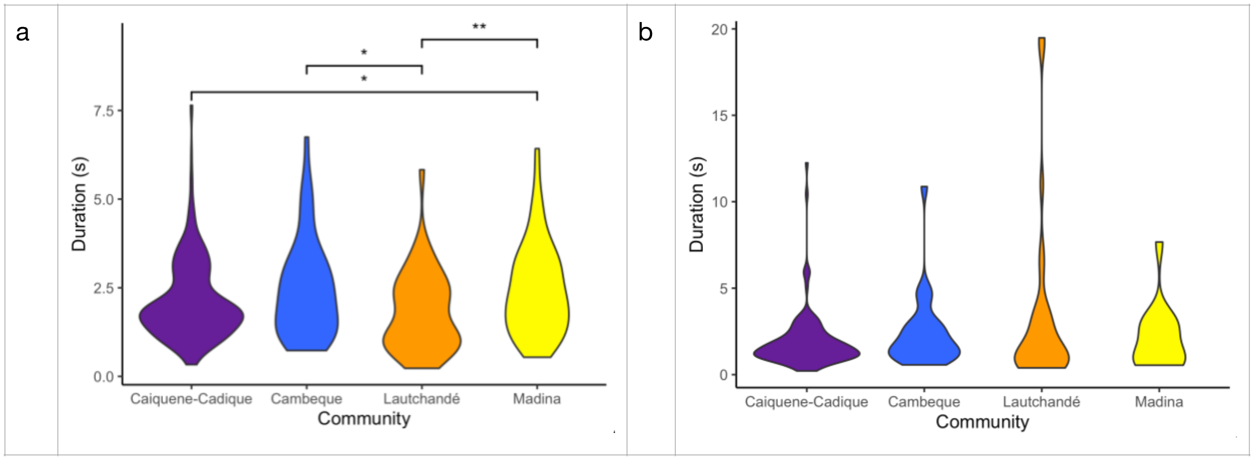

Figure 4.3. Duration of complete male drumming bouts with accompanying pant hoot vocalization, categorised by community during (a) travel and during (b) agonistic display. P-values for significant differences are indicated by * $p < 0.05$; ** $p \leq 0.01$.

4.5 Discussion

Culture plays an important role in many aspects of an animal's life. Through social learning an individual can adopt group-specific behaviours that can enhance their fitness (e.g., tool use: Pascual-Garrido, 2019; foraging specialization: Van Schaik *et al*., 2003; travel routes: Jesmer *et al*., 2018; communication: Noad *et al*., 2000). Of all non-human species, chimpanzees have the largest cultural repertoire (e.g., Whiten *et al*., 1999; McGrew, 2004); however when it comes to communication, despite their large repertoire of vocalizations (e.g., Crockford, 2019), gestures (e.g., Hobaiter and Byrne, 2011), facial expressions (e.g., Parr and Waller, 2006), and even communication through object manipulation (e.g., leaf-clipping: Kalan and Boesch, 2018), it is still not clear if chimpanzees possess group-specific signals. In this study we focus on buttress drumming, a species-specific form of non-vocal communication universal to all known chimpanzee communities (Whiten *et al*., 1999), but whose development, function, and variation are still not fully understood. This two-year study is the first to use remote video collection to document, over a continuous period, buttress drumming in four wild, unhabituated, neighbouring chimpanzee communities. Our main question concerned the potential existence of inter-community differences in drumming duration, and whether drumming duration is dependent on the drumming's function, and therefore context. We successfully collected a large and representative data set, that included all age and sex classes from the four studied communities, and streamlined video annotation through an automated audio-visual behavioural recognition pipeline to detect and track chimpanzee drumming behaviour (Bain *et al*., 2021).

The majority of chimpanzee drumming bouts we recorded were accompanied by pant hoot vocalizations, and drumming occurred when chimpanzees were either travelling, feeding, playing or during agonistic displays. Mean drumming duration in CNP was 2.23 s, which is consistent with other study sites (Reynolds and Reynolds, 1965; Arcadi *et al*., 1998, 2004; Arcadi and Wallauer, 2013; Babiszewska *et al*., 2015); however, the maximum duration we encountered (19 s at Lautchandé during an agonistic display), far exceeded previous reports. In all of our studied communities we found evidence of leaf-clipping performed prior to drumming bouts, similar to what has been described for other western chimpanzees at Tai Forest (Kalan and Boesch, 2018). We do not fully understand the exact function of this behaviour, but it could work as a mechanism to alleviate stress, similarly to the loud self-scratching that frequently preceded drumming bouts.

We found that drumming duration varied significantly with community, behavioural context, age and sex class, as well as the presence/absence of an accompanying pant hoot vocalization, but no significant differences were seen as a function of whether loud self-scratching or leaf-clipping preceded a drumming bout. Previous work (Arcadi *et al*., 2004; Babiszewska *et al*., 2015) has been divided over the question of whether or not there are community-specific-signatures for drumming. Nonetheless, and given how contradictory previous reports have been, we decided to explore our data further. Following the lead of previous work (e.g., Arcadi *et al*. 2004; Babiszewska *et al*. 2015), we decided to test male only drumming. Once again, community, context, and the presence of pant hoot all increased the model's explanatory power; in addition, loud self-scratching also emerged as a significant predictor of drumming duration. As predicted based on previous work (e.g., Boesch and Boesch-Achermann, 2000; Arcadi *et al.*, 2004; Babiszewska *et al.*, 2015), male chimpanzees drummed more frequently than females and immature individuals. However, males had significantly shorter drumming bouts than females, a phenomenon that has never been reported, possibly due to a bias in research towards adult male drumming behaviour (Arcadi *et al.*, 1998, 2004; Arcadi and Wallauer, 2013; Babiszewska *et al.*, 2015). In fact, previous work suggested that female and male drumming were indistinguishable to a listener and therefore female drumming could be used to exaggerate a groups' strength to neighbouring communities (Boesch and Boesch-Achermann, 2000). To what extent this is indeed the case remains to be determined.

Drumming with accompanying pant hoots was significantly longer than drumming preformed silently, both when analysing all drumming bouts and male-only drumming bouts, which is consistent with previous published work (Arcadi *et al.*, 2004). Additionally, drumming during travel was also on average longer than during agonistic display. Moreover, when analysing only male drumming bouts with pant hoots, inter-context variation was maintained. These results suggest that contextual differences in drumming duration cannot be explained by the presence/absence of a pant hoot vocalization.

Given these significant differences in context, we next tested for inter-community differences during the two most recorded contexts: travel and agonistic display. As predicted, inter-community variation was significant during travel, but no significant variation was found during agonistic displays. These results are consistent with the different functions of drumming. During travel, drumming is thought to be used as long-distance communication between parties of the same community (Reynolds and Reynolds, 1965; Goodall, 1968; Mitani and Nishida, 1993; Wrangham *et al*., 1996; Arcadi *et al*., 1998; Nishida, 2011) which can be heard at distances of at least 1 km (Boesch, 1991; Arcadi *et al*., 1998). When individuals cannot see or hear each other vocalise, a community-specific drumming signature could be more informative than an individual one, since it can be heard not only by members of the same community but also members of neighbouring ones. Given the fission-fusion nature of chimpanzee communities, the most relevant information that a party could obtain from listening to another party's drumming would be clues that allow them to discriminate own-group from 'foreign' drumming, as this would reduce the likelihood of a potentially dangerous encounter with a neighbouring community. On the other hand, during agonistic displays, drumming has a different function (Goodall, 1968; Nishida, 2011). In this case, and together with other intimidation behaviours (e.g., bipedal swagger, leaf dragging), drumming is used to show dominance or power to a nearby audience – instead of giving information on the location of a group/individual, it is a show of strength, which could perhaps also explain the above-average duration of some of the drumming bouts recorded during this context. Additionally, during intimidation of nearby conspecifics, a community-specific signature would carry no relevant information – indeed, it may even be replaced by an individual one. Given that we are working with unhabituated communities, and all data were collected remotely, we were unable to include individual variation in our analyses. Therefore, at present we are not able to test this latter hypothesis, but with ongoing data collection and progress with individual identification we expect to be able to do so in future.

Apart from the lack of individual identification being an important limitation of our study, it is also worth noting that the number of videos collected in each community varied. This happened despite the study effort being similar across the four sites (see Table 4.1), and it may in part have been due to the fact that at the beginning of our data collection we had a better baseline knowledge of the movements of the Caiquene-Cadique community than any of the other three communities, and therefore were better able to identify focal drumming trees in their home range. It is also possible that the Madina, Lautchandé and Cambeque communities use their home ranges in different ways, changing their main routes more frequently or having a larger number of important drumming points spread out through their territory, which were not covered by our camera traps. It is likely that an increase in study effort, along with a better understanding of the communities' movements, will provide us with uniformity in data volume across the four communities, which may further clarify how much of the observed inter-community variation found is indeed due to learnt (i.e., cultural) differences.

Despite these limitations, our study suggests that drumming duration might be part of community-specific signalling in the context of long-distance communication, but that such specificity loses its relevance (and hence is no longer maintained) in contexts where long-distance communication is not the main function of drumming. These results could also suggest that when investigating inter-community differences in other forms of communication, such as vocalizations, we should take context into consideration.

In conclusion, our study confirmed that the use of camera traps is a suitable way of collecting large data sets on buttress drumming in unhabituated chimpanzee communities. Importantly, our method provided valuable and rare observations of drumming by females and immatures – both of whom have been largely overlooked in previous research. Furthermore, our combination of this methodology with state-of-the-art machine learning tools in the processing of large volumes of video data shows that it is now possible to collect and analyse the type of longitudinal data that will provide us with much-needed insight into the learning and development of drumming behaviour (Arcadi *et al.*, 2004; Babiszewska *et al.*, 2015), as well as into more cryptic aspects of drumming such as its combination with leaf-clipping (Kalan and Boesch, 2018) and accumulative stone throwing (Kühl *et al*., 2016).

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4.9 Supplementary Material

Supplementary table 4.1. Summary of all chimpanzee drumming videos collected in the four study communities in Cantanhez National Park, Guinea-Bissau. Cells show total number of videos as well as complete-bout videos categorised by community, context, and age and sex class. AM – adult male; AF – adult female; IMM – immature; OV – out of view.

		Caiquene-Cadique		**Madina**		**Lautchandé**		**Cambeque**		**Total**	
		All	**Complete**	**All**	**Complete**	**All**	**Complete**	**All**	**Complete**	**All**	**Complete**
AM	Play	0	0	0	0	0	0	0	0	**0**	**0**
	Travel	387	297	159	115	61	40	83	49	**690**	**501**
	Agonistic	136	116	14	14	39	32	32	25	**221**	**187**
	Feeding	93	75	2	2	16	12	1	0	**112**	**89**
	Total	**616**	**488**	**175**	**131**	**116**	**84**	**116**	**74**	**1023**	**777**
AF	Play	0	0	0	0	0	0	0	0	**0**	**0**
	Travel	37	27	3	1	11	11	1	1	**52**	**40**
	Agonistic	14	10	1	1	6	6	1	1	**22**	**18**
	Feeding	9	8	0	0	0	0	0	0	**9**	**8**
	Total	**60**	**45**	**4**	**2**	**17**	**17**	**2**	**2**	**83**	**66**
IMM	Play	26	25	6	6	2	2	3	3	**37**	**36**
	Travel	8	4	0	0	6	6	0	0	**14**	**10**
	Agonistic	2	2	1	1	0	0	0	0	**3**	**3**
	Feeding	2	2	0	0	0	0	0	0	**2**	**2**
	Total	**38**	**33**	**7**	**7**	**8**	**8**	**3**	**3**	**56**	**51**
OV	Play	0	0	0	0	0	0	0	0	**0**	**0**
	Travel	13	10	3	3	3	0	5	5	**24**	**18**
	Agonistic	8	8	1	1	4	2	2	1	**15**	**12**
	Feeding	4	4	0	0	1	1	1	0	**6**	**5**
	Total	**25**	**22**	**4**	**4**	**8**	**3**	**8**	**6**	**45**	**35**
ALL	Play	26	25	6	6	2	2	3	3	**37**	**36**
	Travel	445	338	165	119	81	57	89	55	**780**	**569**
	Agonistic	160	136	17	17	49	40	35	27	**261**	**220**
	Feeding	108	89	2	2	17	13	2	0	**129**	**104**
	Total	**739**	**588**	**190**	**144**	**149**	**112**	**129**	**85**	**1207**	**929**

Supplementary table 4.2. AIC values describing the explanatory power of the independent variables on the response variable (drumming duration) in all complete drumming bouts

Factor	AIC value
Community, Context, Sex and Age, Pant Hoot	1714.9
Community, Context, Sex and Age, Pant Hoot, Self-scratch	1716.3
Context, Sex and Age, Pant Hoot	1717.7
Sex and Age, Context, Pant Hoot	1717.7
Community, Context, Sex and Age, Pant Hoot, Leaf-clipping	1718.4
Full model	1720.1
Context, Sex and Age, Pant Hoot, Leaf-clipping, Self-scratch	1724.5
Community, Context, Pant Hoot, Self-scratch	1725.8
Community, Context, Sex and Age	1727.0
Community, Sex and Age, Pant Hoot	1727.4
Community, Context, Pant Hoot, Leaf-clipping	1727.4
Community, Context, Pant Hoot, Leaf-clipping, Self-scratch	1729.4
Context, Pant Hoot, Leaf-clipping, Self-scratch	1729.9
Community, Context	1730.8
Context	1731.5
Community, Context, Sex and Age, Leaf-clipping, Self-scratch	1732.1
Community, Sex and Age, Pant Hoot, Leaf-clipping, Self-scratch	1732.2
Sex and Age, Pant Hoot	1735.5
Pant Hoot	1744.9
Community	1745.3
Community, Pant Hoot, Leaf-clipping, Self-scratch	1745.6
Sex and Age	1750.1
Pant Hoot, Leaf-clipping, Self-scratch	1751.9
Self-scratch	1753.8
Leaf-clipping	1754.5
Leaf-clipping, Self-scratch	1757.6

Supplementary Table 4.3. AIC values describing the explanatory power of the independent variables on the response variable (drumming duration) in all complete male drumming bouts

Factor	AIC value
Community, Context, Pant hoot, Self-scratch	1414.1
Community, Context, Pant hoot, Leaf-clipping	1416.3
Full model	1417.8
Context, Pant hoot, Leaf-clipping, Self-scratch	1421.6
Context	1426.2
Community, Context	1426.9
Community, Pant hoot, Leaf-clipping, Self-scratch	1429.4
Pant hoot	1432.9
Pant hoot, Leaf-clipping, Self-scratch	1440.3
Community	1441.2
Community	1441.2
Self-scratch	1449.5
Leaf-clipping	1450.1
Leaf-clipping, Self-scratch	1453.3

CHAPTER 5

Stable isotope analysis reveals dietary variation in four unhabituated neighbouring chimpanzee communities

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Supplementary material can be found at the end of the chapter

5.1 Abstract

Animal culture can be a source of adaptive behaviour, allowing rapid changes in group-specific behaviours such as the exploitation of novel food sources or the adoption of new foraging strategies in response to natural or anthropogenic changes to the environment. However, to what extent inter-group differences in feeding behaviours are driven by cultural processes rather than resource availability is a key question in animal culture studies. In this context, comparing feeding behaviour in neighbouring groups that inhabit similar environments and have access to broadly the same resources can be particularly informative. While multiple methods are available to study feeding behaviour (observation, faecal analyses, camera trapping, DNA barcoding), these typically only provide researchers with short-term dietary information. On the other hand, analysing stable isotope values present in animal tissue can provide us with an estimate of dietary patterns through time. Here, we compare the stable isotope composition of four unhabituated neighbouring chimpanzee communities inhabiting a highly human-impacted landscape in the Cantanhez National Park, situated in Guinea-Bissau. From February to June 2018, we measured $\delta^{13}C$ and $\delta^{15}N$ values in 125 chimpanzee hair samples and 390 plant samples, from 57 chimpanzee plant foods, in order to create an isotope baseline. Our results show inter-community variation of $\delta^{15}N$ plant values as well as a surprisingly high inter-community variation of both $\delta^{13}C$ and $\delta^{15}N$ chimpanzee values, suggesting that these communities may have adopted different feeding strategies in response to increased human encroachment. Additionally, our results indicate that these chimpanzees' stable isotope values differ from those reported for other chimpanzee communities inhabiting similar environments. The level of inter-community variation found across such a small geographic scale highlights the importance of studying neighbouring communities and supports the use of stable isotope studies in the study of animal culture.

5.2 Introduction

Animal culture, i.e., "group-typical behaviour patterns, shared by members of a community that rely on socially learned and transmitted information" (Laland and Hoppitt, 2003:151), has been identified in a large number of vertebrate and invertebrate species (Whiten, 2021). Much like it is in humans, culture is present in the most diverse aspects of non-human animals' lives, from complex material culture (e.g., chimpanzees (*Pan troglodytes*): Whiten *et al*., 1999), communication (e.g., humpback whales (*Megaptera novaeangliae*): Allen *et al*., 2018) and social behaviours (e.g., white-faced capuchin *(Cebus capucinus)*: Perry *et al*., 2003), to travel (homing pigeons (*Columba livia*): Sasaki and Biro, 2017) and migratory routes (e.g., Bighorn sheep (*Ovis canadensis*): Jesmer *et al*., 2018), and foraging behaviour (e.g., Sulphur-crested cockatoos (*Cacatua galerita*): Klump *et al*., 2021). An interesting aspect of culture is that it can serve as a source of adaptive behaviours. Through social learning, individuals can more rapidly acquire novel solutions to problems than through individual exploration and learning (Laland *et al*., 2009). This is the case for foraging, where the social transmission of knowledge and skills regarding the selection and processing of food can create group-specific foraging strategies. Some well-studied examples include milk-bottle opening by titmice (*Parus* sp.) (Fisher and Hinde, 1949), sweet potato washing by Japanese macaques (*Macaca fuscata*) (Kawai, 1965), lob tail fishing in humpback whales (e.g., Allen *et al*., 2013), sponging in bottlenose dolphins (*Tursiops* sp.) (e.g., Mann *et al*., 2012), nut cracking in western chimpanzees (*P. t. verus*) (e.g., Carvalho *et al*., 2008) and stone tool use to access encased foods by bearded capuchins (*Cebus capucinus*) (e.g., Barret *et al*., 2018). Additionally, the behaviours propagated are, at least partially, independent from the environment (Laland *et al*., 2009). This means that even when different groups face very similar environmental challenges, they can still develop different adaptations (e.g., *C. capucinus: Panger et al., 2002*; *Pongo pygmaeus wrumbii* : Bastian *et al*., 2010)

Multiple methods are available for the study of wild animals' feeding strategies. Traditionally, and perhaps being the gold standard, this relies on intense behavioural observation of subjects that are habituated to human observers (e.g., Bateson and Martin, 2021), but this approach is time consuming and requires data collection over months and even years to account for temporal differences in food availability. If animals are unhabituated, macroanalysis of faecal samples (e.g., wolves (*Canis lupus*): Gade-Jorgensen and Stagegaard, 2000) and foraging traces (e.g., chimpanzees: McGrew *et al*., 1988) can inform us about feeding behaviour and dietary variation over time. Molecular methods like DNA barcoding (e.g., European bison (*Bison bonasus*): Hartvig *et al*., 2021), as well as the more efficient and precise DNA metabarcoding (e.g., lake fish: Hänfling *et al*., 2016) allow for the identification at a species level of all consumed foods, through the analyses of DNA strains found in faecal samples. This method, however, is expensive and time consuming, and each sample can only inform on what an individual has been eating over a period of days. More recently, camera traps have been used to document feeding behaviour (e.g., moor macaques (*Macaca maura*): Zak and Riley, 2017) but this method is better suited for foods found in dense clumps, such as human cultivated crops, and provides little information on feeding behaviour more broadly.

In the last decade, an increasing amount of research has been conducted on reconstructing animal diets through examining stable isotope compositions (e.g., cougar (*Puma concolor)*: Henaux *et al*., 2011; Reef fish: Wyatt *et al*., 2012; chimpanzees: Sponheimer *et al*., 2006; seagulls (*Larus* sp.) : Lato *et al*., 2021) and has become a well-established approach in wildlife ecology (Newsome *et al*., 2007; Yeakel *et al*., 2016), human evolution (Lee-Thorp *et al*., 2007; Sponheimer *et al*., 2013), and studies of present-day human populations (Correia *et al*., 2019). In more recent years, it has been increasingly used to reconstruct the diet of non-human primates (hereafter primates), especially populations that are difficult to observe via traditional methods because they are nocturnal (Schoeninger *et al*., 1999; Crowley *et al*., 2014) or engage in cryptic behaviours (Loudon *et al*., 2014). In the present study, we used stable isotope analysis to compare the diets of four neighbouring communities of wild chimpanzees, with a view to elucidating the roles of resource availability and potential cultural factors in their foraging behaviour.

Stable isotope analysis in the reconstruction of animal diets

Stable isotope values recorded from animal tissues including hair, tooth bioapatite, bone collagen and excreta (i.e., faeces and urine) can provide researchers with an estimate of a species' dietary patterns (Crowley, 2012; Sandberg *et al*., 2012). These tissues permanently record dietary data in terms of the relative abundance of several stable isotopes. Of these, carbon and nitrogen are the most frequently used for understanding feeding ecology and reconstructing diets (Sandberg *et al*., 2012). In the study of free-ranging mammals, hair samples are often used, since they are relatively easy to collect non-invasively (e.g., from night nests, or sticky traps) and their composition will remain relatively unaltered for long periods of time. Hair may be particularly informative given that it records dietary data through time, as it grows. The time frame available to examine stable isotope data from hair is linked to the length of the strand(s). This typically allows for examinations over several months, as 1 cm of hair corresponds to approximately one month (Tobin, 2005; Oelze *et al*., 2014).

In terrestrial ecosystems, plants exhibit three photosynthesis pathways (i.e., C_3, CAM, and C_4) resulting in different stable carbon isotope ($\delta^{13}C$) values. Trees and shrubs utilize a C_3 pathway and have $\delta^{13}C$ values that are lower than those found among tropical C_4 grasses and CAM plants, which are predominantly succulents (Sandberg *et al.*, 2012). In C_3 plants, stable carbon isotope variation is largely linked to forest structure, light intensity, and forest canopy cover. In relatively undisturbed forests, plants that grow under a dense canopy cover have lower $\delta^{13}C$ values than those growing in more open areas due a "canopy effect" (Ehleringer *et al.*, 1987). Typically, plants growing closer to the ground in forests with continuous canopies will be more ^{13}C-depleted (Van der Merwe and Medina, 1991) compared to plants growing at higher heights. Different plant parts may also vary in their $\delta^{13}C$ values, and non-photosynthetic tissues (i.e., flowers and fruits) tend to have higher $\delta^{13}C$ values than leaves, and older leaves can be even more ^{13}C-depleted compared to immature leaves (Cernusak *et al.*, 2009).

Stable isotope nitrogen values ($\delta^{15}N$) are commonly related to an animal's trophic position in an ecosystem and there is a stepwise enrichment of ^{15}N (~3‰) at each trophic level (DeNiro and Epstein, 1981; Schoeninger *et al.*, 1983). Despite this well documented trophic effect, some animals may exhibit comparatively low $\delta^{15}N$ values (Sponheimer *et al.*, 2005; Sandberg *et al.*, 2012). Furthermore, individuals experiencing prolonged nutritional stress may catabolize their tissues resulting in higher $\delta^{15}N$ values (Cherel *et al.*, 2005; Loudon *et al.*, 2007). In tropical ecosystems, nitrogen tends to be highly available or in excess in tropical forests (Martinelli *et al.*, 1999). However, the baseline environmental $\delta^{15}N$ in anthropogenically-disturbed habitats, characterized by livestock grazing and/or the application of organic fertilizers, may be high (Bateman and Kelly, 2007). Additionally, wild and cultivated legumes (e.g., beans and peanuts) frequently exhibit comparatively low $\delta^{15}N$ values due to the reliance on N_2 fixating bacteria (Shearer and Kohl, 1994). Therefore, caution is needed when interpreting animals' stable nitrogen values, and baseline stable nitrogen values of plants and soils should be included in these interpretations.

Although the studies of stable isotopic compositions of an animal tissues' are not straightforward, the number of published studies to date provide a good comparative framework, especially for the genus *Pan*. This, together with the fact that single strands of hair, collected non-invasively, can provide us with months of dietary information, make stable isotope analyses a very valuable tool to study chimpanzee feeding behaviour, especially among unhabituated communities.

Chimpanzee feeding ecology and stable isotope studies

Chimpanzees (*Pan troglodytes*) are considered ripe-fruit specialists, but exhibit an omnivorous diet that includes a wide array of other plant organs (i.e., flowers, leaves, nuts, and seeds) and non-plant foods including honey, invertebrates, and occasionally vertebrate meat (e.g., Morgan and Sanz, 2006; Potts *et al*., 2011; Watts *et al*., 2012; Bessa *et al*., 2015). Dietary variability across chimpanzee communities reflects the different environments they inhabit and the distribution of the food resources that are available. This frequently results in dietary differences among chimpanzees across spatial and temporal scales (Pruetz, 2006; Potts *et al*., 2011; Bi and Wittig, 2019). The habitats that many chimpanzee communities currently occupy are continuously becoming more degraded due to anthropogenic pressure, which significantly impacts chimpanzees' food availability and patterns of habitat use (Hockings and McLennan, 2012). When fruit is scarce, chimpanzee communities are forced to adapt their feeding strategies in different ways. An array of behavioural adaptations may be employed, including increasing the diversity of foods they eat (Fawcett, 2000), expanding their foraging ranges (Tutin *et al*., 1997; Fawcett, 2000; Morgan and Sanz, 2006), ingesting fallback foods or increasing the consumption of non-fruit foods

(Harrison and Marshall, 2011; Chancellor *et al*., 2012), relying on human crops (Hockings and McLennan, 2012; McLennan, 2013) or increasing the consumption of invertebrates (Koops *et al*., 2013). Understanding chimpanzees' feeding behaviour and how different communities respond to the dynamic conditions of their environment, be it seasonal or not, is essential to understanding how today's chimpanzees will adapt and survive in the Anthropocene.

To date, numerous stable isotope studies have explored how different chimpanzee populations adapt their diet to the ecosystems they inhabit. Chimpanzees living in habitats where anthropogenic influence is high, tend to have higher $\delta^{13}C$ than communities inhabiting forested habitats (Loudon *et al*., 2016). These higher $\delta^{13}C$ values are probably linked to the consumption of cultivated C_3 plants grown in open fields with no canopy effect and C_4 crops, which typically include maize, sorghum, and millet. Chimpanzees inhabiting savannah habitats tend to have higher $\delta^{13}C$ values linked to the consumption of C_3 plants growing in open landscapes (Schoeninger *et al*., 1999; Sponheimer *et al*., 2006). Stable isotope analyses of savannah-dwelling chimpanzee communities have demonstrated that even though they live in environments that may be dominated by C_4 vegetation, they preferentially consume C_3 resources growing in forested microhabitats on the landscapes they utilize (Schoeninger *et al*., 1999). Chimpanzee communities living in environments where ripe fruit is scarce, might exhibit lower $\delta^{13}C$ values, reflecting higher levels of folivory, since leaves are generally ^{13}C-depleted compared to fruits (Oelze *et al*., 2016).

Although some studies have demonstrated a relationship between chimpanzee $\delta^{13}C$ values in relation to habitat openness and anthropogenic impacts, the same does not appear to be true for $\delta^{15}N$ values. Chimpanzee communities known to consume higher quantities of vertebrate prey, such as those in the Tai Forest in Côte d'Ivoire (Fahy *et al*., 2013), exhibit the highest $\delta^{15}N$ values among *Pan* (Loudon *et al*., 2019). In contrast, high rates of legume consumption have been linked to the low δ^{15}N values of some chimpanzee communities (Schoeninger *et al*., 1997, 1999; Ometto *et al*., 2006). The consumption of large amounts of termites has also been suggested as a possible explanation for low δ^{15}N values among some chimpanzee communities (Loudon *et al*., 2016), making it evident that other sources of data including behavioural observations, faecal sample analyses, or even discarded tools are important to explain δ^{15}N values (Loudon *et al*., 2016).

Stable isotope comparisons of *Pan* communities that rely solely on the isotopic values of animal tissues might lead scientists into erroneous conclusions, given that plant isotopic baseline values vary between habitats and regions, and are impacted by biotic and abiotic factors in the soil and plant physiologies (Amundson *et al*., 2003; Casey and Post, 2011; Oelze *et al*., 2016; Wessling *et al*., 2019). Oelze *et al*. (2016) investigated the relationship between vegetation baselines and climatic variables, and suggested that researchers should correct the apes' stable isotope data to a standardized plant baseline from each respective site which is represented with the uppercase delta symbol (Δ). Plant baseline values allow for the comparison of isotopic fractionation, which describes the trophic enrichment from diet to tissue, rather than simply comparing the animal tissue isotopic values of different communities or species (Crowley *et al*., 2014; Oelze *et al*., 2016).

From stable isotopes to intra- and inter-community behavioural variation

Even though there are still many constraints when analysing the isotopic composition of chimpanzees' tissues in the absence of any behavioural data, correcting for baseline vegetation data might help to narrow down on specific behaviours that could explain isotope values, especially when comparing them to previously published isotope values. Stable isotope studies of habituated communities, where specific individuals of different ages and sexes can be sampled, and where behavioural variation within and between communities is well documented, have been of particular importance to the overall study of chimpanzees' isotopic values. In these studies, stable isotope values can be explained by understanding the behaviour of an individual or group which provides us with invaluable comparative data. For example, in the Tai Forest, male and female chimpanzees have significantly different $\delta^{15}N$ values. This has been linked to previously described behavioural variation in this community, where male chimpanzees hunt and share meat with each other much more frequently than females do (Boesch and Boesch-Achermann, 2000). Therefore, female values are used as a good proxy for low levels of animal protein ingestion and male values for high levels of hunting (Fahy *et al.*, 2013). Oelze and colleagues (2016) compared corrected stable nitrogen values of chimpanzees from the unhabituated Sapo community in Liberia with those of the Tai Forest chimpanzees and found that the chimpanzees of Sapo might hunt even more frequently than the males of Tai, showing how informative stable isotope studies can be, even for unhabituated communities.

Here we present stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) data from hair collected from four neighbouring communities of chimpanzees (*P. troglodytes verus*) inhabiting Cantanhez National Park (CNP) in Guinea-Bissau. The feeding ecology and dietary patterns of these four communities are comparatively understudied and each community remains unhabituated. To assist our interpretations, we also examined the $\delta^{13}C$ and $\delta^{15}N$ data of the plants that the chimpanzees at this site are known to consume. We sampled chimpanzees in the Caiquene-Cadique, Lautchandé, Madina, and Cambeque communities which inhabit fragmented forests, face high levels of anthropogenic disturbance, and exhibit variation in their group-specific behavioural repertoires (Bessa *et al.*, 2022). A previous nine-month study analysed 377 faecal samples collected from the Caiquene-Cadique chimpanzee community resulting in a list of plant food species (wild and cultivated) providing a glimpse of the diet of these groups (Bessa *et al.*, 2015). The study revealed traces of honey in the faeces, but no evidence of the consumption of social insects or vertebrate hunting was observed. As such, the chimpanzee $\delta^{13}C$ and $\delta^{15}N$ data we analyse here will be primarily driven by the values of the plants they consume. However, recent genetic analysis of a small number of chimpanzee faecal samples revealed that at least two species of vertebrates (*Genetta pardina* and *Potamocherus porcus*) are consumed in Caiquene-Cadique and one species of primate (Mona monkey, *Cercopithecus campbelli*) is hunted in at least two of the other neighbouring communities (Lautchandé and Cambeque) (Hockings *et al*., 2021). Nonetheless, these data do not allow us to estimate the frequency of meat eating in any of these communities.

Given how little we know about these CNP communities, our main aim was to shed some new light on their feeding ecology, possible adaptations to their highly fragmented habitat as well as potential inter-community variation. Firstly, we present the first stable isotope data collected for Guinea-Bissau's plants and chimpanzees. Given that all CNP communities inhabit similar habitats within a small geographic range, we did not expect plant isotope values to vary between communities. Further, as all four communities inhabit neighbouring agro-forest mosaics where crops grow in open fields and C_4 crops are available, we predicted chimpanzee $\delta^{13}C$ values to be high. However, considering the lack of information we have on the feeding behaviour of these communities it proved difficult to make predictions about these communities' $\delta^{15}N$ values. Secondly, we compare the isotope composition of all four neighbouring communities. Despite living in close geographic proximity, in similar environments with access to broadly the same food resources, previous work has revealed some degree of behavioural variation between the studied communities, therefore we expect to find some inter-community variation in stable isotope values. Lastly, we integrate our data with previously published chimpanzee isotope analyses, to test the prediction that communities inhabiting other anthropogenic environments or savannah woodlands will have isotope signatures more similar to CNP than those inhabiting primary forests.

5.3 Methods

Study site and subjects

Cantanhez National Park (CNP) is located in the Tombali region of south-west Guinea-Bissau. CNP is a mosaic of settlements, agricultural fields, sub-humid forest, secondary forest, palm groves, mangrove, and savannah (Catarino *et al*., 2006). This region is characterized by two marked seasons: dry season (November to mid-May) and rainy season (mid-May to October). CNP's annual rainfall for 2017 was 2351 mm with an average temperature of 25.3C (14.6C min to 38.6C max) (Bessa *et al*., 2021). The majority of local people in CNP are subsistence farmers practicing slash and burn agriculture, and some of the most commonly cultivated crops are peanuts (*Arachis hypogaea),* rice (*Oryza glaberrima)*, maize (*Zea mays*)*,* sugar cane (*Saccharum officinarum*), pigeon pea (*Cajanus cajan*), cassava (*Manihot esculenta*) and several different varieties of beans (*Phaseolus vulgaris*). Within or along the village edge several species of fruit trees are planted, including mango (*Mangifera indica*), orange (*Citrus sinensis*), lime (*Citrus auratifolia*), baobab (*Adansonia digitata*) and papaya (*Carica papaya*); some of these species are also found in abandoned villages within the forest. Recently, cashew (*Anacardium occidentale*) is being grown in large orchards as a cash crop, accounting for c. 9% of the terrestrial land use in the central CNP (Bersacola, 2019).

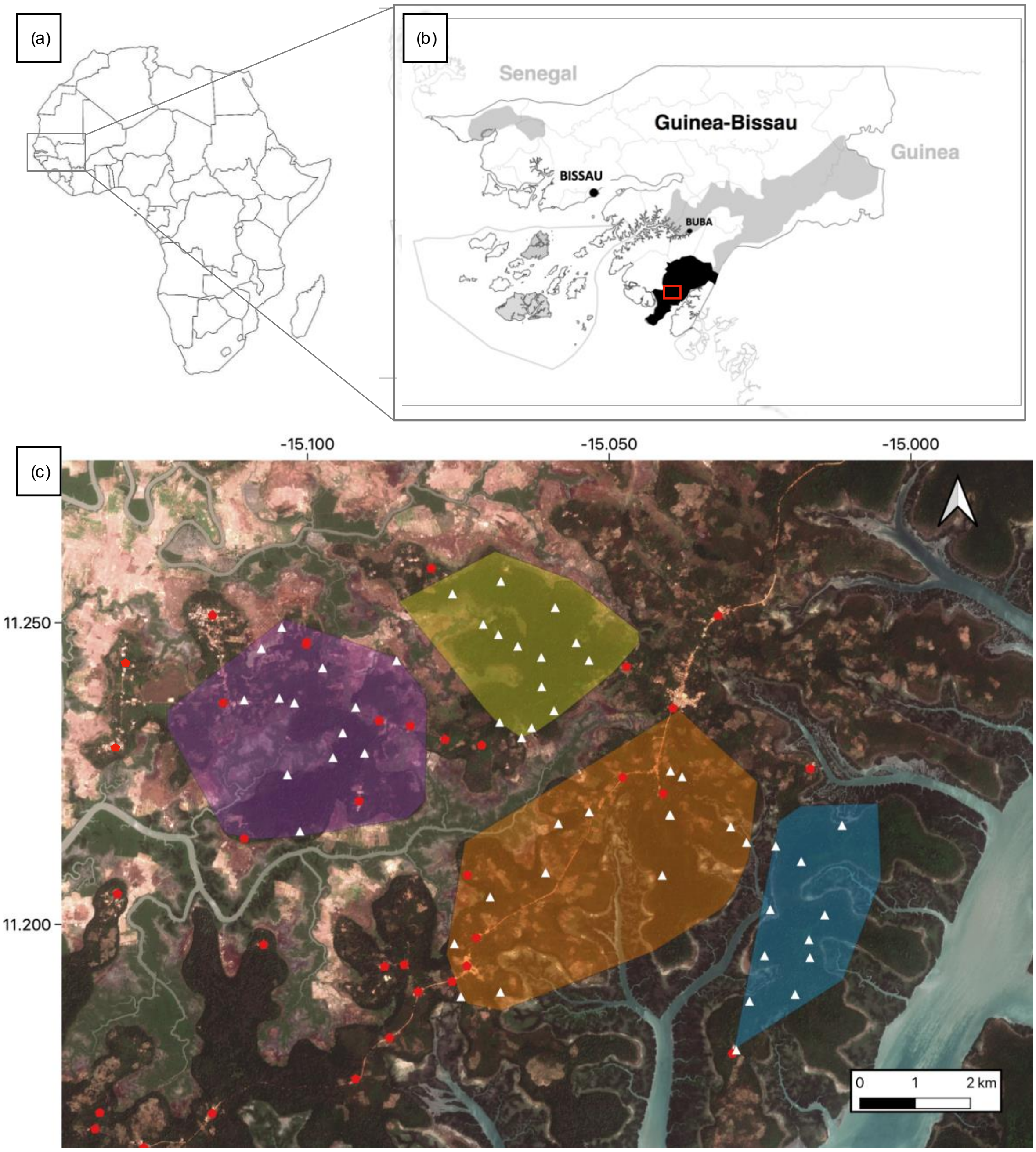

Figure 5.1. Location of study sites and sampling locations. Maps show location of (a) Guinea-Bissau, and of (b) CNP within Guinea-Bissau as the solid black shaded region. The red rectangle in (b) corresponds to the region depicted in panel (c). (c) Aerial image showing the locations of the four study sites in CNP and the core home ranges of the four chimpanzee communities. Core home ranges were estimated using minimum bounding polygons based on direct and indirect chimpanzee data points, and are illustrated in different colours: Caiquene-Cadique (purple), Lautchandé (yellow), Madina (orange), and Cambeque (blue). Red pentagons correspond to villages and other human settlements and white triangles correspond to locations where chimpanzee hair samples were collected for each community. Sentinel-2 imagery was downloaded from the Sentinel Hub, Sinergise Ltd (https://www.sentinel-hub.com/). All maps were created using QGIS version 3.12 (https://www.qgis.org)

It is estimated that 10 to 12 chimpanzee (*Pan troglodytes verus*) communities inhabit CNP (Bersacola, 2019), which include our four study communities: Caiquene-Cadique, Lautchandé, Madina and Cambeque. Previous genetic, behavioural and ecological research as well as local knowledge and the presence of natural barriers suggest that these are independent communities (Hockings and Sousa, 2013; Sá, 2013; Bessa *et al*., 2015; Bersacola, 2019; Vieira *et al*., 2019; Hockings *et al*., 2020; Bessa *et al*., 2021). These four neighbouring communities are unhabituated to researchers and little is known about their group sizes and compositions. Nonetheless, previous work estimates that the Caiquene-Cadique community has a minimum of 49 individuals and the Madina community consist of 48 individuals (M. Ramon, pers comm; Vieira *et al*., 2019). Such information is lacking for the two other communities (Lautchandé and Cambeque), but it is likely that these communities have similar group sizes. All four neighbouring sites have a similar degree of anthropogenic impact. Figure 5.1 shows the location of each community and their estimated minimum ranging area.

Data collection

Chimpanzee hair and plant food samples were collected from all four neighbouring chimpanzee communities during February-June 2018. Collection procedures and the size and content of the datasets are described in detail below.

Chimpanzee hair samples

A total of 125 chimpanzee hair samples were collected, however due to sample quality only 123 were analysed. This includes 36 hair samples from Caiquene-Cadique, 32 hair samples from Lautchandé, 27 hair samples from Cambeque, and 30 hair samples from Madina (see

Supplementary Table 5.1). All hair samples were collected non-invasively. During the first half of the project, hair samples (n= 49) were collected opportunistically during reconnaissance walks ("recces") in areas where the chimpanzees were recently observed or known to frequent. This included portions of each community's home range, where the chimpanzees had recently been feeding, under trees frequently used for buttress-drumming, or from fresh faecal samples. On these occasions, one single hair was collected from each location. During the second half of data collection, hair samples were collected more systematically, directly from arboreal overnight nests the morning after the chimpanzees had slept (Oelze *et al*., 2014; Loudon *et al*., 2016). At CNP, most chimpanzee nests are constructed in the tall *Elaeis guineensis* palm trees, therefore all climbing, and retrieval of hair samples was conducted by experienced local palm climbers. When a large new nest cluster was found, the climber climbed up several nests and carefully looked for and collected the chimpanzees' hair samples. Given how challenging it is to locate fresh nests of unhabituated communities, as well as how strenuous the climbing was, only a limited number of nests could be climbed in a given day. Once collected, the hair samples were stored and labelled in paper envelopes in an airtight container with silica gel (Reitsema, 2015).

Chimpanzee plant food samples

A total of 390 plant samples were collected, and 197 samples were analysed for this study, including all fruit and leaf samples as well as bark and flower samples confirmed to be eaten by at least one of the four chimpanzee communities. Before the plant sample collection, a database of wild and cultivated chimpanzee plant food species was compiled (Supplementary Table 5.2). We used previously published data from a nine-month chimpanzee feeding ecology study that took place in Caiquene-Cadique (Bessa *et al*., 2015), together with dietary reconstructions from faecal samples, and discarded chimpanzee feeding traces found during recces, as well as local knowledge of the dietary patterns of the chimpanzees. Whenever possible, the flowers, fruits, and leaves of each plant species were collected from each of the four chimpanzee communities' ranges. In some instances, this was not possible due to seasonality or if a plant species was not found in the home range of our study communities of chimpanzees (Supplementary Table 5.2). In total, 45 wild plant species and 12 cultivated species, were collected from at least one of the study sites. All plants collected followed a C_3 photosynthetic pathway (i.e., with lower $\delta^{13}C$ values than those that would be found in C_4 and CAM plants). Of these and based on previous work by Bessa *et al.* (2015), 10 were considered important (i.e., found to be present in at least 50% of faecal samples in one or more months or in more than10% of all faecal samples) wild foods and 7 considered important cultivated species. These 17 species are listed in Table 5.1, along with their availability in the four communities' home ranges. Plant samples were transported to the basecamp labelled with scientific and local name, date of collection, and collection location. Samples were cleaned of exogenous debris, and cut up when necessary (e.g., fruits). All plant samples were placed between newspaper sheets in a wooden plant press and exposed to the sun. Given the high humidity, the newspapers were changed every two days until the samples were dry. Once fully desiccated, each plant sample was stored in individual paper envelopes with silica pouches, for transport and stable isotope analyses.

Table 5.1. List of important wild and cultivated chimpanzee food species collected during the study period. "x" corresponds to species present and collected from the study community; "+" corresponds to species present in the study community but not collected (due to seasonality, or resource depletion); "-" corresponds to species whose presence was not confirmed for the study community.

Plant species	Caiquene-Cadique	Cambeque	Lautchandé	Madina	Wild/Cultivated
Ceiba pentandra	x	x	x	x	wild
Dialium guineense	x	x	x	x	wild
Elaeis guineensis	x	x	x	x	wild
Ficus sp.	x	x	x	x	wild
Ficus sur	x	+	x	+	wild
Landolphia heudelotii	x	x	x	x	wild
Parinari excelsa	x	x	x	x	wild
Saba senegalensis	x	x	x	x	wild
Spondias mombin	x	x	+	x	wild
Treculia africana	x	x	x	+	wild
Adansonia digitata	x	x	x	x	cultivated
Anacardium occidentale	x	x	x	x	cultivated
Carica papaya	x	x	x	x	cultivated
Citrus aurantifolia	x	x	x	x	cultivated
Citrus sinensis	x	x	+	x	cultivated
Citrus sp.	x	x	-	x	cultivated
Mangifera indica	x	x	x	x	cultivated

Statistical analyses

All statistical analyses were performed using R (version 1.1.463). Data were tested for normality using the Shapiro-Wilk's method, and for homogeneity of variance using Bartlett's test. Given unequal variation, Welch's ANOVA was used to compare the plant and hair $\delta^{13}C$ and $\delta^{15}N$ values as well as $\Delta^{13}C$ and $\Delta^{15}N$ averages for each CNP community. Additionally, Post-hoc Tukey test was used to explore significant variance between communities.

Ethics statement

The research was reviewed and approved by the Instituto da Biodiversidade e Áreas Protegidas (IBAP) in Guinea-Bissau. All data collection was non-invasive and purely indirect, strictly adhering to ethics guidelines detailed by the Association for the Study of Animal Behaviour (United Kingdom). Chimpanzee hair samples were exported from Guinea-Bissau under the CITES permit 1630633 issued by Ministério do Ambiente e Biodiversidade, Órgão Nacional de Gestão CITES, Republic of Guinea-Bissau.

5.4 Results

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values: Plants

Mean plant $\delta^{13}C$ values ranged from -29.9‰ to -29.3‰ across the four sites, and mean plant $\delta^{15}N$ values ranged from 2.4‰ and 4.5‰ (Table 5.2). There were no significant inter-site plant differences in the $\delta^{13}C$ values (Welch's ANOVA, $F_{3, 106.5} = 1.0$, p=0.389). However, there were significant inter-site differences in the $\delta^{15}N$ plant values (Welch's ANOVA, $F_{3, 1067.9} = 8.7$, p<0.001). Different plant organs' $\delta^{13}C$ values varied significantly (Welch's ANOVA, $F_{2, 79.8} = 18.9$, p<0.001) with leaves exhibiting the lowest mean $\delta^{13}C$ values (-30.3‰) compared to flowers and fruits. $\delta^{15}N$ values did not vary significantly between the three plant organs (Welch's ANOVA, $F_{2, 63.31} = 2.7$, p=0.073), and ranged from 2.9‰ (leaves) to 4.1‰ (flowers) (Table 5.2). Comparisons between wild and cultivated plants at CNP did not reveal significant differences in $\delta^{13}C$ values (Welch's ANOVA, $F_{1, 163.5} = 2.7$, p=0.104). Mean $\delta^{13}C$ values for cultivated foods were -29.2‰ and -29.8‰ for wild foods. There were significant differences in $\delta^{15}N$ values of cultivated vs. wild foods at CNP (Welch's ANOVA, $F_{1, 125.9} = 22.5$, p<0.001) and the mean $\delta^{15}N$ value for cultivated plants was 4.1‰ and 2.5‰ for wild plants.

Table 5.2. Plant baseline $\delta^{13}C$ and $\delta^{15}N$ in ‰, from four sites in Cantanhez National Park, Guinea-Bissau. Means and standard deviations (SD) were calculated based on N samples (total number of samples = 201).

	Cultivated				**Wild**				**All plants**			
	$\delta^{13}C$ (±SD)	N	$\delta^{15}N$ (±SD)	N	$\delta^{13}C$(±SD)	N	$\delta^{15}N$(±SDD)	N	$\delta^{13}C$(±SD)	N	$\delta^{15}N$(±SD)	N
Caiquene-Cadique												
flower	-30.4±0.4	5	5.8±2.2	5	-28.5±1.5	4	4.9±2.7	4	-29.6±1.4	9	5.4±2.3	9
fruit	-28.3±2.1	8	6±1.8	8	-29.9±1.8	8	3.7±1.7	8	-29.1±2.1	16	4.9±2.1	16
leaf	-29.9±1.5	10	4.7±2	10	-31.1±2.7	15	3.4±2.6	15	-30.6±2.3	25	3.9±2.4	25
All plant parts	**-29.5±1.8**	**23**	**5.4±2**	**23**	**-30.3±2.4**	**27**	**3.7±2.4**	**27**	**29.9±2.2**	**50**	**4.5±2.3**	**50**
Cambeque												
flower	-31.8	1	2.6	1	-27.2±0.6	3	2.8±0.4	3	-28.3±2.4	4	2.7±0.4	4
fruit	-29.3±3.5	4	4.1±3.7	4	-28.5±1.9	15	1.8±1.7	15	-28.7±2.2	19	2.3±2.3	19
leaf	-30.3±2.9	7	3.9±2.5	7	-30.9±1.8	19	1.9±1.6	19	-30.7±2.1	26	2.4±2.1	26
All plant parts	**-30.1±2.9**	**12**	**3.9±2.7**	**12**	**-29.6±2.2**	**37**	**1.9±1.6**	**37**	**-29.7±2.4**	**49**	**2.4±2.1**	**49**
Lautchandé												
flower	-29.8	1	2.7	1	-28.4±1.4	5	3.6±1.7	5	-28.6±1.4	6	3.4±1.5	6
fruit	-27.9±1.3	6	3.3±2.7	6	-28.8±2	15	2.5±1.6	16	-28.5±1.8	21	2.7±1.9	22
leaf	-30.9±0.3	4	3.3±2.8	4	-30±2.8	17	2.5±1	17	-30.2±2.5	21	2.6±1.4	21
All plant parts	**-29.2±1.8**	**11**	**3.2±2.5**	**11**	**-29.3±2.4**	**37**	**2.6±1.4**	**38**	**-29.3±2.2**	**48**	**2.8±1.7**	**49**
Madina												
flower	-27.9±0.2	2	3.8±4.4	2	-27.6±1	2	2.9±0.4	2	-27.7±0.6	4	3.3±2.6	4
fruit	-28.1±1.7	12	3.3±2.1	12	-28.6±2.1	8	1.8±3	8	-28.3±1.8	20	2.7±2.6	20
leaf	-29.4±2	11	3.5±2.4	11	-31.2±2.2	14	1.7±2.5	14	-30.4±2.3	25	2.5±2.6	25
All plant parts	**-28.7±1.9**	**25**	**3.4±2.3**	**25**	**-30±2.5**	**24**	**1.8±2.5**	**24**	**-29.3±2.3**	**49**	**2.6±2.5**	**49**

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values: Chimpanzees

The mean $\delta^{13}C$ values for the four chimpanzee communities ranged from -24.7 to -24.0‰, and mean $\delta^{15}N$ values ranged from 4.2 to 4.6‰ (Table 5.3). Significant inter-community differences were found in $\delta^{13}C$ values (Welch's ANOVA, $F_{3,62.2} = 23.0$, $p<0.0001$) and $\delta^{15}N$ values (Welch's ANOVA, $F_{3,\ 61.9} = 6.7$, $p<0.001$). Additionally, a post hoc Tukey test showed that Caiquene-Cadique's $\delta^{13}C$ values were significantly lower compared to the other study communities ($p<0.0001$). The post hoc Tukey test also revealed that the $\delta^{15}N$ values of the Cambeque community were higher than those of the Caiquene-Cadique ($p<0.001$) and Lautchandé ($p=0.01$)

communities and the δ^{15}N values of the Madina community were higher than those of the Caiquene-Cadique community ($p<0.05$).

When accounting for plant baseline data at each site, Δ^{13}C averages ranged from 4.7‰ (Madina) to 5.7‰ (Cambeque) and Δ^{15}N averages ranged from -0.3‰ (Caiquene-Cadique) tom2.2‰ (Cambeque). Significant inter-community differences in Δ^{13}C values (Welch's ANOVA, $F_{3,\,63.3} = 15.572$, $p<0.001$) and Δ^{15}N values (Welch's ANOVA, $F_{3,\,65.5} = 193.52$, $p<0.001$) were found. Additionally, a post hoc Tukey test showed that Cambeque's Δ^{13}C values were significantly higher compared to the other study communities ($p<0.0001$), and that Madina's Δ^{13}C values were significantly higher than Lautchandé's ($p=0.02$). The post hoc Tukey test also revealed that the Δ^{15}N values of the Caiquene-Cadique community were lower than all other three communities ($p<0.001$), the Δ^{15}N values of the Cambeque community were significantly higher than those of Lautchandé ($p<0.001$) and Madina ($p<0.05$) communities, and Madina's Δ^{15}N values were significantly higher than Lautchandé's.

Table 5.3. Descriptive statistics for 123 chimpanzee hair sample δ^{13}C and δ^{15}N values in ‰, from four neighbouring communities in Cantanhez National Park, Guinea-Bissau. Also shown are site mean Δ^{13}C and Δ^{15}N in comparison with plant baseline data from the corresponding site.

Site	**N**	**δ^{13}C(±SD)**	**δ^{13}C (Min-Max)**	**δ^{15}N (±SD)**	**δ^{15}N (Min-Max)**	**Δ^{13}C**	**Δ^{15}N**
Caiquene-Cadique	35	-24.7±0.3	-25.5, -24.1	4.2±0.4	2.8, 5.6	5.3	-0.3
Lautchandé	32	-24.2±0.4	-25.0, -23.2	4.2±0.5	3.5, 5.3	5.0	1.4
Madina	30	-24.0±0.4	-24.5, -22.6	4.4±0.5	3.5, 5.6	5.3	1.8
Cambeque	26	-24.0±0.4	-25.5, -23.4	4.6±0.4	4.1, 6,5	5.8	2.2
CNP - all samples	123	-24.2±0.5	-25.5, -22.6	4.4±0.5	2.8, 6.5	5.4	1.3

Variation in chimpanzee stable isotope values across African study sites

Table 5.4 presents published chimpanzee stable carbon and nitrogen isotope data, covering 13 study sites from 8 publications, corrected for plant baseline data compared to the CNP values in this study. Inter-site comparison shows marked variation between different study sites, including the four CNP sites. Values varied between 2.1‰ to 7.2‰ for $\Delta^{13}C$ and -0.3‰ to 4.1‰ for $\Delta^{15}N$. Among the communities examined, the highest $\Delta^{13}C$ values were found at Hérémakhono (Senegal) and lowest at Ishasha (Uganda) and the highest $\Delta^{15}N$ values were found in Sapo (Liberia) and lowest in Caiquene-Cadique (Guinea-Bissau; present study).

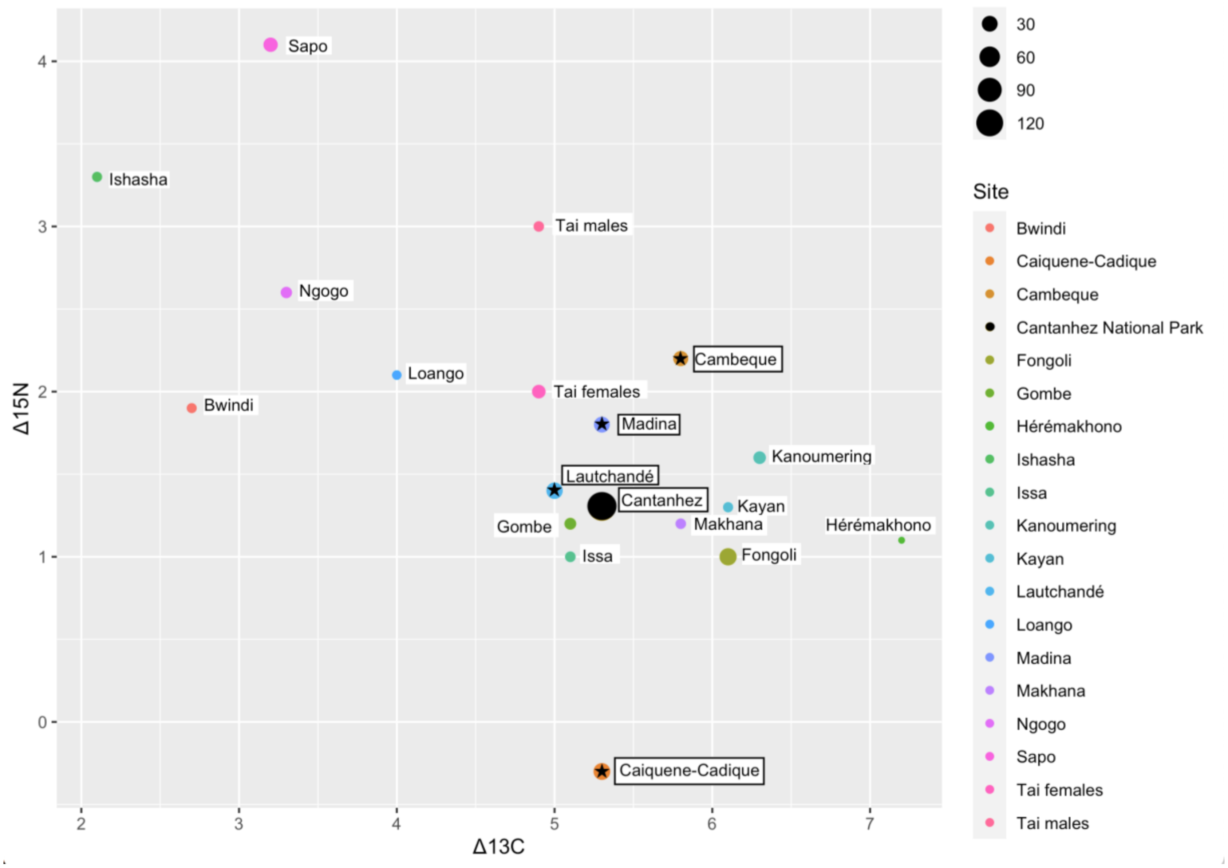

Figure 5.2. Mean $\Delta^{13}C$ and $\Delta^{15}N$ reported for different *Pan* research sites. Size of dot denotes number of hair samples (range: 7-123). Dots with stars indicate the four neighbouring chimpanzee communities of the present study; solid black dot ("Cantanhez") indicates the mean across these four communities. Δ values are calculated based on the following hair and plant datasets: present study, Schoeninger *et al*., 1999, 2016; Fahy *et al*., 2013; Oelze *et al*., 2014, 2016; Nockerts *et al*., 2016; van Casteren *et al*., 2018; Wessling *et al*., 2019

Table 5. 4. Descriptive statistics for 325 chimpanzee hair samples from 17 communities across Africa (present study, Schoeninger *et al*., 1999, 2016; Fahy *et al*., 2013; Oelze *et al*., 2014, 2016; Nockerts *et al*., 2016; van Casteren *et al*., 2018; Wessling *et al*., 2019) with site mean Δ^{13}C and Δ^{15}N in comparison with plant baseline of each site.

Site	**Country**	**Subspecies**	**N**	**Habitat type**	**Δ^{13}C**	**Δ^{15}N**	**References**
Cantanhez	*Guinea-Bissau*	*P. t. verus*	123	Anthropogenic mosaic	5.4	1.3	Present study
Caiquene-Cadique	*Guinea-Bissau*	*P. t. verus*	35	Anthropogenic mosaic	5.3	-0.3	Present study
Lautchandé	*Guinea-Bissau*	*P. t. verus*	32	Anthropogenic mosaic	5.0	1.4	Present study
Madina	*Guinea-Bissau*	*P. t. verus*	30	Anthropogenic mosaic	5.3	1.8	Present study
Cambeque	*Guinea-Bissau*	*P. t. verus*	26	Anthropogenic mosaic	5.8	2.2	Present study
Tai males	*Côte d'Ivoire*	*P. t. verus*	11	Primary forest	4.9	3.0	Fahy *et al*. 2013 ; Oelze *et al*. 2016
Tai females	*Côte d'Ivoire*	*P. t. verus*	20	Primary forest	4.9	2.0	Fahy *et al*. 2013 ; Oelze *et al*. 2016
Sapo	*Liberia*	*P. t. verus*	23	Primary forest	3.2	4.1	Oelze *et al*. 2016
Fongoli	*Senegal*	*P. t. verus*	37	Savannah-woodland	6.1	1.0	Wessling *et al*. 2019
Kanoumering	*Senegal*	*P. t. verus*	16	Savannah-woodland	6.3	1.6	Wessling *et al*. 2019
Makhana	*Senegal*	*P. t. verus*	11	Savannah-woodland	5.8	1.2	Wessling *et al*. 2019
Kayan	*Senegal*	*P. t. verus*	10	Savannah-woodland	6.1	1.3	Wessling *et al*. 2019
Hérémakhono	*Senegal*	*P. t. verus*	7	Savannah-woodland	7.2	1.1	Wessling *et al*. 2019
Gombe	*Tanzania*	*P. t. schweinfurthii*	14	Primary forest	5.1	1.2	Schoeninger *et al*. 2016: Nockerts *et al*. 2016
Ishasha	*Uganda*	*P. t. schweinfurthii*	10	Savannah-woodland	2.1	3.3	Schoeninger *et al*. 1999
Ngogo	*Uganda*	*P. t. schweinfurthii*	13	Primary forest	3.3	2.6	Casteren *et al*. 2018
Issa	*Tanzania*	*P. t. schweinfurthii*	11	Savannah-woodland	5.1	1.0	Casteren *et al*. 2018
Bwindi	*Uganda*	*P. t. schweinfurthii*	10	Mountain forest	2.7	1.9	Oelze *et al*. 2016
Loango	*Gabon*	*P. t. troglodytes*	9	Mosaic	4.0	2.1	Oelze *et al*. 2014; 2016

5.5 Discussion

The present study is the first to examine the stable isotope ecology of four neighbouring communities of chimpanzees (*Pan troglodytes verus*) which inhabit environments that are heavily impacted by human activities. We found inter-community variation in plant $\delta^{15}N$ as well as inter-community variation in chimpanzee $\delta^{13}C$ and $\delta^{15}N$ values as well as $\Delta^{15}N$ and $\Delta^{13}C$ averages.

To contextualize the chimpanzee stable isotopic hair values at Cantanhez National Park (CNP), we analysed the $\delta^{13}C$ and $\delta^{15}N$ values of the wild and cultivated plants that were available to each of the four communities. As predicted, the plant $\delta^{13}C$ values were not significantly different between the four CNP communities. However, contrary to our predictions, there were significant differences in the $\delta^{15}N$ values of the plants at the study sites. The $\delta^{15}N$ plant values for the Caiquene-Cadique community were higher compared to the remaining three study communities. Additionally, and given the anthropogenic nature of the environment and availability of cultivated crops, we investigated the isotopic differences between wild and cultivated plants and, as expected, found significant differences in $\delta^{15}N$ values, with crops having higher $\delta^{15}N$ values compare to wild plant species.

There were significant inter-community differences both in $\delta^{13}C$ and $\delta^{15}N$ values, despite the four communities at CNP occupying similar habitat types and having similar access to the same species of wild and cultivated plants. On average, Caiquene-Cadique had significantly lower $\delta^{13}C$ values compared to other communities, and Cambeque had significantly higher $\delta^{15}N$ values than the Caiquene-Cadique and Lautchandé communities. The differences in the $\delta^{13}C$ values are unlikely to be explained by canopy effect (i.e., plants that grow under a dense canopy cover have lower $\delta^{13}C$ values than plants growing in more open areas (Ehleringer *et al.*, 1987)) since most human crops available to the chimpanzee communities at CNP were cultivated in areas of low canopy coverage under direct sunlight. Controlling for plant $\delta^{13}C$ and $\delta^{15}N$ baseline values makes accounting for behavioural differences or other physiological influences on the variation of isotopes more straightforward (Wessling *et al.*, 2019).

Analysing $\Delta^{15}N$, inter-community variation at CNP revealed that the Caiquene-Cadique and Cambeque communities were the largest outliers. For example, Caiquene-Cadique exhibited extremely low $\Delta^{15}N$ (-0.3‰) values and Cambeque exhibited high $\Delta^{15}N$ values (2.2‰). Despite having higher $\Delta^{15}N$ values compared to the other three communities, the Cambeque $\Delta^{15}N$ values still averaged lower than what would be expected for a trophic level increase (~ 3 ‰) and therefore it is unlikely that frequent meat consumption is responsible for the $\delta^{15}N$ variation found among these communities (Minagawa and Wada, 1984; Wessling *et al.*, 2019). Higher $\Delta^{15}N$ could be explained partially by the CNP chimpanzees' preferences for cultivated foods which were enriched with ^{15}N. The low Caiquene-Cadique $\Delta^{15}N$ values are harder to explain. Insectivorous diets may influence $\Delta^{15}N$ values, and the consumption of ^{15}N-depleted termites has been suggested to explain low $\delta^{15}N$ values found among chimpanzees at Fongoli, Senegal (Loudon *et al.*, 2016). However, no direct or indirect evidence of insectivory, including through macro analysis of faecal samples (Bessa *et al.*, 2015), has been found for the Caiquene-Cadique community. Additionally, the Caiquene-Cadique chimpanzees frequently consume at least nine species of crops (Bessa *et al.*, 2015) which would drive the $\Delta^{15}N$ values higher, as opposed to lower. It is possible that the Caiquene-Cadique chimpanzees relied more on wild foods which exhibited lower $\delta^{15}N$ values compared to the cultivated plants. Nonetheless, this dietary preference alone may not account for the variation in the $\delta^{15}N$ values that were found in this study. Discussions with local farmers have revealed that the Caiquene-Cadique chimpanzees had recently started expanding their consumption of cultivated foods to legumes (peanuts and beans). The consumption of only one species of cultivated legume (pigeon bean, *Cajanus cajan*) had previously been documented for Caiquene-Cadique (Hockings and McLennan, 2012); however, it was not considered an important species for the chimpanzees. Legumes are ^{15}N-depleted (Shearer and Kohl, 1994) and heavy consumption of leguminous plants could partially explain some of the inter-community variation in the δ^{15}N values among the four communities of chimpanzees at CNP. As such, the δ^{15}N values may not reflect less reliance on cultivated foods that were utilized by the Caiquene-Cadique chimpanzees, and instead may demonstrate an expansion of their consumption of cultivated species by incorporating legumes, as suggested by the local reports.

With regards to Δ^{13}C values at CNP, the Cambeque chimpanzee community had the highest values (5.8‰) and Lautchandé the lowest (5.0‰). Δ^{13}C differences among the four chimpanzee communities at CNP were not as profound as the Δ^{15}N values detected, but could potentially reveal behavioural differences between communities associated with feeding. For example, Lautchandé's lower Δ^{13}C values could reveal a preference for feeding in areas with higher canopy coverage, a preference for wild foods over cultivated foods, or higher levels of folivory. It is possible that some of the chimpanzee communities are responding to shifts in ripe fruit availability by increasing the consumption of crops, hunting more frequently (Cambeque), including new foods into their diet (Caiquene-Cadique) or resorting to higher levels of folivory (Lautchandé). Given the indirect nature of our data collection and the relatively few observational feeding data we have collected to date, it is only possible to speculate about the roots of the dietary differences we have documented. Nonetheless, the stable isotope data analysed in this study can further clarify any small behavioural differences exhibited by the CNP chimpanzees that we would be unlikely to document otherwise. Additionally, the growing number of isotope studies of habituated and unhabituated chimpanzee communities inhabiting a variety of different habitat types and exhibiting diverse feeding behaviours (e.g., Fahy *et al*., 2013; Oelze *et al*., 2016; Wessling *et al*., 2019) allows us to further compare our results at a larger scale and perhaps discover indications of what kinds of behavioural variations may be linked to the isotopic differences reported in this study. Therefore, we compiled and compared the $\Delta^{13}C$ and $\Delta^{15}N$ data from *P. troglodytes* stable isotope studies across Africa.

Comparisons of $\Delta^{13}C$ and $\Delta^{15}N$ values from chimpanzee communities across Africa revealed that the four CNP communities examined in this study exhibited relatively low $\Delta^{13}C$ and $\Delta^{15}N$ values. The $\Delta^{13}C$ values at CNP are somewhat unexpected given the fragmented nature of the environment and year-round access to cultivated foods, both factors that can drive ^{13}C-enrichment. Surprisingly, the $\Delta^{13}C$ values at CNP aligned more with the values found at sites with low anthropogenic impact, including the Tai National Forest, Côte d'Ivoire (Fahy *et al*., 2013) and Gombe, Tanzania (Schoeninger *et al*., 2016) where chimpanzees inhabit primary forests with dense canopy coverage. Of the four CNP sites, the Cambeque community had the highest $\Delta^{13}C$ values, but still lower than most savannah dwelling chimpanzee communities (Wessling *et al*., 2019). Despite anthropogenic disturbances to the local ecosystems and the availability of C_4 crops including sugar cane and maize, the values at CNP suggest that the chimpanzees are not relying heavily on ^{13}C-enriched foods, which is contrary to what is expected of chimpanzees inhabiting anthropogenically-disturbed habitats (e.g., Loudon *et al*., 2016). The CNP communities might continue to preferentially feed in forest fragments with more continuous canopy coverage, relying primarily on wild plant foods, including ^{13}C-depleted leaves. Humans and chimpanzees have been living in sympatry in CNP for generations; however, human encroachment has been rapidly increasing (Sousa and Frazão-Moreira, 2010). Even though chimpanzees are not hunted for meat, there are aggressive interactions between the two species that include humans chasing chimpanzees away from their cultivated fields and occasional retaliatory killings (Bersacola *et al*., 2021a). A recent study by Bersacola *et al* (2021b) reported that chimpanzees in Caiquene-Cadique increase their presence in anthropogenic areas with crops when wild resources are scarce, and it is possible that the consequent increase in aggressive interactions between humans and chimpanzees is, in turn, driving chimpanzees away from the villages and back into the forest for most of the year. On the other hand, low $\Delta^{15}N$ may indicate low hunting rates, similarly to sites such as Hérémakhono, Senegal (Wessling *et al*., 2019) or Gombe (Schoeninger *et al*., 2016). Low levels of hunting could possibly be explained by low availability of prey, since despite it being illegal, hunting of other non-human primates such as Temminck's red colobus, Campbell's monkey and guinea baboons, is commonplace in CNP (Da Silva *et al*., 2014; Minhós *et al*., 2016). When comparing all these communities it is particularly noticeable how Caiquene-Cadique $\Delta^{15}N$ values are substantially different. To date, these appear to be the lowest values of $\Delta^{15}N$ values even when including savannah habitats where chimpanzees habitually consume ^{15}N-depleted termites (Wessling *et al*., 2019). These results are puzzling, however a possible explanation could be that the Caiquene-Cadique chimpanzees are changing their diets more rapidly than the other communities, by incorporating new foods that were previously not part of their feeding repertoire, as confirmed by discussions with local farmers noting their consumption of leguminous beans and peanuts. We also assume low levels of insectivory among the Caiquene-Cadique chimpanzees based on a previous feeding ecology study (Bessa *et al*., 2015). However, rates of insectivory may have increased and be reflected in the stable isotope data analysed in the current study.

Given the methods employed for the collection of hair samples, it is possible that we sampled a given community disproportionally by sampling the same individual on multiple occasions. The sampling protocol we employed attempted to reduce the likelihood of re-sampling the same individual by collecting chimpanzee hairs from nest clusters that had been built on the same day and therefore had been used by different individuals. However, only a small number of nests could be sampled on any given day due to the difficulty of safely climbing palm trees over 20 meters in height. During the study, the Caiquene-Cadique community was composed of at least 49 chimpanzees (M. Ramon, pers comm), with many pregnant or weaning females (Bessa *et al.*, 2015). It is possible that females were sampled more frequently, thus skewing our results. In humans, women have lower δ^{15}N values during gestation (Fuller *et al.*, 2004), and the same could be true for chimpanzees. Additionally, at the Tai National Forest female chimpanzees have significantly less access to animal protein compared to males (Boesch and Boesch-Achermann, 2000; Fahy *et al.*, 2013). However, females at the Tai Forest still had significantly higher Δ^{15}N values than the Caiquene-Cadique chimpanzees. Nonetheless, it is possible that a sex-specific behavioural difference is impacting stable isotope variation among community members.

Finally, it is important to highlight that the variation in Δ^{13}C and Δ^{15}N values across our four sites also means that all four sites differ substantially from the global average for CNP (see Fig. 5.2). This suggests that extra care must be taken in the interpretation of Δ^{13}C and Δ^{15}N values from a given site, even when these are based on samples collected from a relatively small geographical area. Our results show that pooling data from different communities, even those neighbouring each other and inhabiting similar environments, can produce a misleading ‘global’ isotope profile for the site.

Direct behavioural observation of the CNP chimpanzees would further our understanding of the stable isotope data we found. However, given how stable isotope values faithfully document feeding ecology, the variations found in these sympatric communities nonetheless highlight the plasticity of chimpanzee feeding ecology, and can point us towards small and nuanced differences in feeding strategies adopted by these four neighbouring communities. In turn, given that these differences are evident across the same habitat type and over a small geographic scale, they may potentially reflect group-specific, or cultural, phenomena. This research gives us a glimpse into how communities of *Pan* facing similar challenges across Africa might be adapting in different ways to the pressures of the Anthropocene (Hockings *et al.*, 2015; Kühl *et al.*, 2017), highlighting the importance of studying neighbouring communities rather than assuming that geographic proximity or environmental similarities will translate into the same behavioural adaptations. Studies like ours have the potential to be extremely valuable tools when developing site-specific conservation initiatives.

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5.9 Supplementary Material

Supplementary table 5.1. Plant sample list with date of collection, species name, site, indication of wild or cultivated plant, plant organ, sample weight, δ^{13}C and δ^{15}N values, and C/N ratio

Isotope ID	Project ID	Date of Collection	Site	Species Name	Wild/Cultivated	Plant Organ	Weight	d13C	d15N	%N	C/N Ratio
GBPS052	FRSCC001	03/03/2018	Caiquene-Cadique	*Adansonia digitata*	*cultivated*	fruit	2.97	-28.446	6.530	2.677	17.136
GBPS107	LSCC032	06/05/2018	Caiquene-Cadique	*Adansonia digitata*	*cultivated*	leaf	2.979	-29.361	6.196	1.253	34.879
GBPS152	FRSCB010	11/03/2018	Cambeque	*Adansonia digitata*	*cultivated*	fruit	2.75	-29.621	8.505	2.724	16.141
GBPS241	FRSL011	16/05/2018	Lautchande	*Adansonia digitata*	*cultivated*	fruit	2.961	-28.887	6.799	2.858	16.169
GBPS280	LSL027	16/05/2018	Lautchande	*Adansonia digitata*	*cultivated*	leaf	2.92	-30.760	5.722	1.318	31.858
GBPS399	LSM033	08/05/2018	Madina	*Adansonia digitata*	*cultivated*	leaf	2.717	-30.485	7.054	2.199	18.715
GBPS282	LSL029	29/05/2018	Lautchande	*amomum alboviolaceu*	*wild*	leaf	2.949	-33.160	0.106	2.164	21.410
GBPS103	LSCC029(a)	11/03/2018	Caiquene-Cadique	*Alchornea cordifolia*	*wild*	leaf	2.838	-29.719	1.387	4.527	10.757
GBPS044	FLSCC009	06/05/2018	Caiquene-Cadique	*nacardium occidental*	*cultivated*	flower	2.934	-30.254	4.434	1.935	24.742
GBPS076	LSCC002	27/02/2018	Caiquene-Cadique	*nacardium occidental*	*cultivated*	leaf	2.812	-31.044	4.366	1.762	28.041
GBPS113	NSCC001	06/05/2018	Caiquene-Cadique	*nacardium occidental*	*cultivated*	nut	2.795	-27.857	3.458	3.107	18.075
GBPS141	FLSCB002	11/03/2018	Cambeque	*nacardium occidental*	*cultivated*	flower	2.863	-31.816	2.647	1.759	26.971
GBPS189	LSCB027	11/03/2018	Cambeque	*nacardium occidental*	*cultivated*	leaf	2.68	-27.355	6.661	1.280	37.320
GBPS201	NSCB001	11/03/2018	Cambeque	*nacardium occidental*	*cultivated*	nut	2.674	-30.167	3.505	1.530	32.277
GBPS225	FLSL001	05/03/2018	Lautchande	*nacardium occidental*	*cultivated*	flower	2.929	-29.831	2.677	2.566	17.582
GBPS231	FRSL001	03/05/2018	Lautchande	*nacardium occidental*	*cultivated*	fruit	2.523	-27.461	3.333	1.768	23.483
GBPS270	LSL017	03/05/2018	Lautchande	*nacardium occidental*	*cultivated*	leaf	2.509	-30.797	5.473	1.678	29.409
GBPS340	FRSM007	05/03/2018	Madina	*nacardium occidental*	*cultivated*	fruit	2.905	-30.116	0.919	1.942	21.530
GBPS352	FRSM020	07/05/2018	Madina	*nacardium occidental*	*cultivated*	fruit	2.833	-28.643	2.841	1.287	32.549
GBPS396	LSM030	07/05/2018	Madina	*nacardium occidental*	*cultivated*	leaf	2.661	-31.293	2.613	1.925	25.124
GBPS415	NSM001	07/05/2018	Madina	*nacardium occidental*	*cultivated*	nut	2.577	-27.900	2.645	3.868	13.843
GBPS154	FRSCB012	11/03/2018	Cambeque	*Anisophyllea laurina*	*wild*	fruit	2.528	-29.059	4.160	1.189	38.051
GBPS191	LSCB029	11/03/2018	Cambeque	*Anisophyllea laurina*	*wild*	leaf	2.766	-29.596	4.909	1.826	23.087
GBPS365	FRSM034	31/05/2018	Madina	*Anisophyllea laurina*	*wild*	fruit	2.522	-29.972	-3.069	1.023	37.956
GBPS112	LSCC037	26/05/2018	Caiquene-Cadique	*Antiaris toxicaria*	*wild*	leaf	2.791	-26.555	6.313	2.698	16.468
GBPS400	LSM034	08/05/2018	Madina	*Antiaris toxicaria*	*wild*	leaf	2.771	-27.018	3.184	2.654	17.612
GBPS058	FRSCC007	07/03/2018	Caiquene-Cadique	*rtocarpus heterophyllu*	*cultivated*	fruit	2.74	-27.882	7.529	2.047	21.843
GBPS051	FLSCC017	23/05/2018	Caiquene-Cadique	*Avicennia germinans*	*wild*	flower	2.751	-27.255	3.445	1.930	25.265
GBPS084	LSCC010	23/05/2018	Caiquene-Cadique	*Avicennia germinans*	*wild*	leaf	2.833	-27.371	3.712	2.230	20.375
GBPS244	FRSL014	06/05/2018	Lautchande	*Borassus aethiopum*	*wild*	fruit	2.793	-30.791	2.066	0.494	84.000
GBPS062	FRSCC011	07/05/2018	Caiquene-Cadique	*Cajanus cajan*	*cultivated*	fruit	2.762	-27.871	3.496	2.966	14.164
GBPS104	LSCC029(b)	07/05/2018	Caiquene-Cadique	*Cajanus cajan*	*cultivated*	leaf	2.838	-29.719	1.387	4.527	10.757
GBPS351	FRSM019	07/05/2018	Madina	*Cajanus cajan*	*cultivated*	fruit	2.525	-24.444	1.601	3.701	10.991
GBPS407	LSM042	07/05/2018	Madina	*Cajanus cajan*	*cultivated*	leaf	2.631	-31.094	0.058	2.649	19.084
GBPS059	FRSCC008	07/03/2018	Caiquene-Cadique	*Carica papaya*	*cultivated*	fruit	2.938	-25.006	5.866	1.346	27.951
GBPS075	LSCC001	27/02/2018	Caiquene-Cadique	*Carica papaya*	*cultivated*	leaf	2.439	-26.129	2.562	5.433	7.545
GBPS156	FRSCB014	11/03/2018	Cambeque	*Carica papaya*	*cultivated*	fruit	2.754	-24.203	5.616	1.927	20.327
GBPS194	LSCB033	11/03/2018	Cambeque	*Carica papaya*	*cultivated*	leaf	2.736	-25.209	5.945	3.376	11.424
GBPS243	FRSL013	16/05/2018	Lautchande	*Carica papaya*	*cultivated*	fruit	2.661	-26.607	5.953	2.487	15.344
GBPS339	FRSM006	05/03/2018	Madina	*Carica papaya*	*cultivated*	fruit	2.811	-25.991	6.358	1.394	27.201
GBPS409	LSM044	12/05/2018	Madina	*Carica papaya*	*cultivated*	leaf	2.853	-25.805	2.252	4.587	9.376
GBPS097	LSCC023	10/03/2018	Caiquene-Cadique	*Ceiba pentandra*	*wild*	leaf	2.996	-30.398	9.703	2.152	20.486
GBPS150	FRSCB008	10/03/2018	Cambeque	*Ceiba pentandra*	*wild*	fruit	2.787	-27.203	3.037	1.696	26.928
GBPS181	LSCB019	10/03/2018	Cambeque	*Ceiba pentandra*	*wild*	leaf	3.198	-28.120	2.860	3.026	15.418
GBPS274	LSL021	05/05/2018	Lautchande	*Ceiba pentandra*	*wild*	leaf	2.725	-27.117	1.862	3.271	14.408
GBPS335	FRSM002	02/03/2018	Madina	*Ceiba pentandra*	*wild*	fruit	2.634	-26.590	5.179	3.424	12.353

GBPS386	LSM020	10/03/2018	Madina	*Ceiba pentandra*	*wild*	leaf	2.613	-33.206	0.211	1.631	25.664
GBPS036	FLSCC001	28/02/2018	Caiquene-Cadique	*Citrus aurantifolia*	*cultivated*	flower	2.393	-29.887	4.190	3.785	10.959
GBPS080	LSCC006	28/02/2018	Caiquene-Cadique	*Citrus aurantifolia*	*cultivated*	leaf	2.922	-30.069	3.871	2.949	15.503
GBPS155	FRSCB013	11/03/2018	Cambeque	*Citrus aurantifolia*	*cultivated*	fruit	2.699	-31.103	1.837	1.398	29.755
GBPS193	LSCB031	11/03/2018	Cambeque	*Citrus aurantifolia*	*cultivated*	leaf	2.774	-31.601	3.096	2.453	17.290
GBPS251	FRSL021	05/05/2018	Lautchande	*Citrus aurantifolia*	*cultivated*	fruit	2.428	-29.853	2.527	1.422	27.744
GBPS276	LSL023	05/05/2018	Lautchande	*Citrus aurantifolia*	*cultivated*	leaf	2.904	-31.321	2.009	1.719	25.487
GBPS338	FRSM005	06/03/2018	Madina	*Citrus aurantifolia*	*cultivated*	fruit	2.798	-27.097	6.219	2.135	19.133
GBPS370	LSM004	06/03/2018	Madina	*Citrus aurantifolia*	*cultivated*	leaf	2.504	-31.193	1.114	2.406	18.380
GBPS391	LSM025	11/03/2018	Madina	*Citrus aurantifolia*	*cultivated*	leaf	2.796	-29.014	7.282	2.495	17.733
GBPS405	LSM040	12/05/2018	Madina	*Citrus aurantifolia*	*cultivated*	leaf	2.927	-29.455	3.111	2.197	19.746
GBPS039	FLSCC004	05/03/2018	Caiquene-Cadique	*Citrus* limon	*cultivated*	flower	2.612	-30.243	9.516	3.520	12.591
GBPS056	FRSCC005	05/03/2018	Caiquene-Cadique	*Citrus* limon	*cultivated*	fruit	2.63	-28.855	7.539	1.859	22.197
GBPS072	FRSCC021	27/05/2018	Caiquene-Cadique	*Citrus sinensis*	*cultivated*	fruit	2.809	-27.071	7.268	1.642	26.720
GBPS092	LSCC018	06/03/2018	Caiquene-Cadique	*Citrus sinensis*	*cultivated*	leaf	2.89	-30.623	8.170	2.098	21.919
GBPS195	LSCB034	11/03/2018	Cambeque	*Citrus sinensis*	*cultivated*	leaf	2.94	-31.005	3.813	2.588	16.668
GBPS364	FRSM033	27/05/2018	Madina	*Citrus sinensis*	*cultivated*	fruit	2.789	-29.006	3.965	1.390	29.683
GBPS411	LSM046	27/05/2018	Madina	*Citrus sinensis*	*cultivated*	leaf	2.608	-28.015	6.697	2.621	16.771
GBPS090	LSCC016	05/03/2018	Caiquene-Cadique	*Citrus sp.*	*cultivated*	leaf	2.515	-29.755	6.628	4.309	10.860
GBPS188	LSCB026	11/03/2018	Cambeque	*Citrus* sp.	*cultivated*	leaf	2.729	-32.612	6.092	2.864	14.684
GBPS331	FLSM007	12/05/2018	Madina	*Citrus* sp.	*cultivated*	flower	2.878	-27.781	6.862	2.658	17.270
GBPS349	FRSM017	11/03/2018	Madina	*Citrus* sp.	*cultivated*	fruit	2.793	-29.140	6.597	1.170	35.995
GBPS158	FRSCB016	15/05/2018	Cambeque	*Detarium senegalense*	*wild*	fruit	2.574	-28.518	1.326	0.568	79.829
GBPS240	FRSL010	05/05/2018	Lautchande	*Detarium senegalense*	*wild*	fruit	2.5	-31.138	3.883	0.355	135.626
GBPS055	FRSCC004	04/03/2018	Caiquene-Cadique	*Dialium guineense*	*wild*	fruit	2.775	-27.074	2.157	1.143	40.838
GBPS081	LSCC007	03/03/2018	Caiquene-Cadique	*Dialium guineense*	*wild*	leaf	2.87	-33.291	1.710	1.975	23.327
GBPS145	FRSCB003	08/03/2018	Cambeque	*Dialium guineense*	*wild*	fruit	2.923	-28.511	0.584	1.198	39.770
GBPS177	LSCB015	08/03/2018	Cambeque	*Dialium guineense*	*wild*	leaf	2.659	-30.605	-1.226	1.359	34.202
GBPS256	LSL003	05/03/2018	Lautchande	*Dialium guineense*	*wild*	leaf	2.982	-27.970	3.898	2.851	15.711
GBPS341	FRSM009	06/03/2018	Madina	*Dialium guineense*	*wild*	fruit	2.837	-28.748	-1.760	0.912	48.764
GBPS374	LSM008	06/03/2018	Madina	*Dialium guineense*	*wild*	leaf	2.743	-35.549	-2.222	1.062	44.540
GBPS164	LSCB002	08/03/2018	Cambeque	*Diospyros heudelotii*	*wild*	leaf	2.35	-34.308	2.255	1.560	28.878
GBPS345	FRSM013	08/03/2018	Madina	*Diospyros heudelotii*	*wild*	fruit	2.932	-29.427	1.936	0.892	50.153
GBPS043	FLSCC008	05/05/2018	Caiquene-Cadique	*Elaeis guineensis*	*wild*	flower	2.551	-27.384	8.292	1.432	32.020
GBPS060	FRSCC009	07/03/2018	Caiquene-Cadique	*Elaeis guineensis*	*wild*	fruit	2.638	-28.938	6.314	0.794	81.698
GBPS095	LSCC021	07/03/2018	Caiquene-Cadique	*Elaeis guineensis*	*wild*	leaf	2.69	-31.094	4.811	2.443	18.674
GBPS151	FRSCB009	11/03/2018	Cambeque	*Elaeis guineensis*	*wild*	fruit	2.79	-27.730	0.353	1.502	27.287
GBPS180	LSCB018	10/03/2018	Cambeque	*Elaeis guineensis*	*wild*	leaf	2.689	-32.734	1.914	1.453	30.403
GBPS242	FRSL012	05/05/2018	Lautchande	*Elaeis guineensis*	*wild*	fruit	2.727	-29.511	-0.036	0.636	72.164
GBPS272	LSL019	05/05/2018	Lautchande	*Elaeis guineensis*	*wild*	leaf	2.744	-34.290	1.533	1.295	30.797
GBPS328	FLSM004	11/03/2018	Madina	*Elaeis guineensis*	*wild*	flower	2.652	-26.874	2.617	1.545	29.498
GBPS350	FRSM018	11/03/2018	Madina	*Elaeis guineensis*	*wild*	fruit	2.581	-27.591	2.046	0.753	87.462
GBPS393	LSM027	11/03/2018	Madina	*Elaeis guineensis*	*wild*	leaf	2.731	-28.897	0.039	1.503	27.520
GBPS233	FRSL003	06/03/2018	Lautchande	*Ficus aurea*	*wild*	fruit	2.601	-26.272	1.830	1.060	44.856
GBPS061	FRSCC010	08/03/2018	Caiquene-Cadique	*Ficus exasperata*	*wild*	fruit	2.547	-29.212	5.568	3.423	12.696
GBPS096	LSCC022	08/03/2018	Caiquene-Cadique	*Ficus exasperata*	*wild*	leaf	2.745	-28.512	5.104	2.169	17.821
GBPS185	LSCB023	10/03/2018	Cambeque	*Ficus exasperata*	*wild*	leaf	2.888	-30.330	2.545	1.833	20.295

GBPS239	FRSL009	05/05/2018	Lautchande	*Ficus exasperata*	*wild*	fruit	2.7	-28.986	3.272	4.056	10.312
GBPS275	LSL022	05/05/2018	Lautchande	*Ficus exasperata*	*wild*	leaf	2.917	-27.358	2.624	2.259	18.927
GBPS053	FRSCC002	04/03/2018	Caiquene-Cadique	*Ficus sp.*	*wild*	fruit	2.705	-30.016	2.113	1.475	32.642
GBPS146	FRSCB004	08/03/2018	Cambeque	*Ficus sp.*	*wild*	fruit	2.619	-29.709	1.421	1.101	41.309
GBPS148	FRSCB006	08/03/2018	Cambeque	*Ficus* sp.	*wild*	fruit	2.354	-27.694	0.746	1.649	25.860
GBPS261	LSL008	06/03/2018	Lautchande	*Ficus sp.*	*wild*	leaf	2.528	-31.118	1.661	1.002	45.506
GBPS342	FRSM010	06/03/2018	Madina	*Ficus sp.*	*wild*	fruit	2.855	-31.236	5.706	0.926	46.923
GBPS373	LSM007	06/03/2018	Madina	*Ficus sp.*	*wild*	leaf	2.465	-31.413	4.642	1.421	31.695
GBPS387	LSM021	10/03/2018	Madina	*Ficus sp.*	*wild*	leaf	2.507	-31.367	4.956	2.324	19.154
GBPS067	FRSCC016	27/03/2018	Caiquene-Cadique	*Ficus sur*	*wild*	fruit	2.607	-32.430	4.575	2.041	22.633
GBPS078	LSCC004	27/02/2018	Caiquene-Cadique	*Ficus sur*	*wild*	leaf	2.786	-33.136	4.675	1.857	20.377
GBPS183	LSCB021	10/03/2018	Cambeque	*Ficus sur*	*wild*	leaf	2.708	-27.918	3.394	2.596	17.353
GBPS238	FRSL008	05/05/2018	Lautchande	*Ficus sur*	*wild*	fruit	2.507		3.478	1.504	0.248
GBPS266	LSL013	06/03/2018	Lautchande	*Ficus sur*	*wild*	leaf	2.92	-30.014	2.958	2.080	18.810
GBPS332	FLSM008	13/05/2018	Madina	*Hibiscus sabdariffa*	*cultivated*	flower	2.702	-28.016	0.703	2.106	19.510
GBPS359	FRSM028	13/05/2018	Madina	*Hibiscus sabdariffa*	*cultivated*	fruit	2.812	-27.715	2.477	3.966	12.097
GBPS408	LSM043	13/05/2018	Madina	*Hibiscus sabdariffa*	*cultivated*	leaf	2.838	-27.570	3.310	4.341	8.888
GBPS071	FRSCC020	23/05/2018	Caiquene-Cadique	*Landolphia heudelotii*	*wild*	fruit	2.868	-29.715	2.226	0.966	46.409
GBPS083	LSCC009	03/03/2018	Caiquene-Cadique	*Landolphia heudelotii*	*wild*	leaf	2.825	-33.964	2.472	1.673	29.004
GBPS162	FRSCB020	15/05/2018	Cambeque	*Landolphia heudelotii*	*wild*	fruit	2.811	-27.475	-2.390	1.288	32.188
GBPS168	LSCB006	08/03/2018	Cambeque	*Landolphia heudelotii*	*wild*	leaf	2.618	-29.102	2.794	2.129	22.836
GBPS226	FLSL002	05/03/2018	Lautchande	*Landolphia heudelotii*	*wild*	flower	2.784	-30.567	2.454	2.293	20.581
GBPS236	FRSL006	06/03/2018	Lautchande	*Landolphia heudelotii*	*wild*	fruit	2.568	-29.657	1.079	0.809	58.220
GBPS249	FRSL019	29/05/2018	Lautchande	*Landolphia heudelotii*	*wild*	fruit	2.841	-29.094	3.541	0.737	63.233
GBPS258	LSL005	05/03/2018	Lautchande	*Landolphia heudelotii*	*wild*	leaf	2.954	-30.440	2.546	1.925	24.969
GBPS329	FLSM005	13/05/2018	Madina	*Landolphia heudelotii*	*wild*	flower	2.985	-28.306	3.115	2.017	23.428
GBPS380	LSM014	07/03/2018	Madina	*Landolphia heudelotii*	*wild*	leaf	2.842	-31.515	3.114	2.316	20.258
GBPS166	LSCB004	08/03/2018	Cambeque	*Landolphia hirsuta*	*wild*	leaf	2.435	-30.015	1.801	1.480	29.643
GBPS248	FRSL018	29/05/2018	Lautchande	*Landolphia hirsuta*	*wild*	fruit	2.901	-25.382	0.335	0.826	52.909
GBPS257	LSL004	05/03/2018	Lautchande	*Landolphia hirsuta*	*wild*	leaf	2.665	-29.908	3.497	1.906	23.523
GBPS163	LSCB001	08/03/2018	Cambeque	*Malacantha alnifolia*	*wild*	leaf	2.361	-33.596	-0.461	2.544	18.004
GBPS042	FLSCC007	13/03/2018	Caiquene-Cadique	*Mangifera indica*	*cultivated*	flower	2.897	-30.883	5.921	1.614	29.193
GBPS066	FRSCC015	13/03/2018	Caiquene-Cadique	*Mangifera indica*	*cultivated*	fruit	2.666	-32.485	2.859	1.219	35.435
GBPS106	LSCC031	13/03/2018	Caiquene-Cadique	*Mangifera indica*	*cultivated*	leaf	2.667	-30.624	4.348	1.280	36.219
GBPS157	FRSCB015	11/03/2018	Cambeque	*Mangifera indica*	*cultivated*	fruit	2.636	-32.139	0.465	1.571	27.799
GBPS190	LSCB028	11/03/2018	Cambeque	*Mangifera indica*	*cultivated*	leaf	2.799	-33.028	0.051	1.195	37.671
GBPS250	FRSL020	29/05/2018	Lautchande	*Mangifera indica*	*cultivated*	fruit	2.842	-26.691	1.195	0.749	53.892
GBPS252	FRSL022	29/05/2018	Lautchande	*Mangifera indica*	*cultivated*	fruit	2.761	-27.782	-0.078	0.455	88.802
GBPS285	LSL032	29/05/2018	Lautchande	*Mangifera indica*	*cultivated*	leaf	2.822	-30.766	-0.140	1.033	44.941
GBPS356	FRSM025	13/05/2018	Madina	*Mangifera indica*	*cultivated*	fruit	2.517	-30.157	2.724	0.554	73.720
GBPS357	FRSM026	12/05/2018	Madina	*Mangifera indica*	*cultivated*	fruit	2.836	-27.854	3.586	1.031	40.205
GBPS358	FRSM027	13/05/2018	Madina	*Mangifera indica*	*cultivated*	fruit	2.819	-28.813	1.224	0.752	54.041
GBPS406	LSM041	13/05/2018	Madina	*Mangifera indica*	*cultivated*	leaf	2.717	-32.170	1.961	1.074	43.253
GBPS165	LSCB003	08/03/2018	Cambeque	*Milicia regia*	*wild*	leaf	2.484	-28.888	2.438	3.224	13.572
GBPS254	LSL001	04/03/2018	Lautchande	*Milicia regia*	*wild*	leaf	2.924	-28.796	3.183	3.251	13.024
GBPS065	FRSCC014	12/03/2018	Caiquene-Cadique	*Parinari excelsa*	*wild*	fruit	3.016	-29.646	4.339	0.852	50.565
GBPS079	LSCC005	27/02/2018	Caiquene-Cadique	*Parinari excelsa*	*wild*	leaf	2.6	-36.383	2.506	1.770	26.442

GBPS187	LSCB025	10/03/2018	Cambeque	*Parinari excelsa*	*wild*	leaf	2.866	-30.956	2.833	2.214	20.744
GBPS227	FLSL003	06/03/2018	Lautchande	*Parinari excelsa*	*wild*	flower	3.108	-28.259	3.758	1.815	25.884
GBPS264	LSL011	06/03/2018	Lautchande	*Parinari excelsa*	*wild*	leaf	2.555	-36.112	2.981	1.972	22.518
GBPS367	LSM001	03/03/2018	Madina	*Parinari excelsa*	*wild*	leaf	2.489	-30.628	1.217	1.817	26.033
GBPS197	LSCB037	15/05/2018	Cambeque	*Parkia biglobosa*	*wild*	leaf	2.895	-29.949	3.892	1.484	31.838
GBPS278	LSL025	06/05/2018	Lautchande	*Parkia biglobosa*	*wild*	leaf	2.592	-26.078	3.006	2.951	15.814
GBPS037	FLSCC002	03/03/2018	Caiquene-Cadique	*Phoenix reclinata*	*wild*	flower	2.947	-30.272	2.110	2.475	18.609
GBPS069	FRSCC018	13/05/2018	Caiquene-Cadique	*Phoenix reclinata*	*wild*	fruit	2.698	-32.439	2.648	1.259	36.386
GBPS086	LSCC012	04/03/2018	Caiquene-Cadique	*Phoenix reclinata*	*wild*	leaf	2.524	-30.917	1.009	2.161	21.292
GBPS140	FLSCB001	08/03/2018	Cambeque	*Phoenix reclinata*	*wild*	flower	2.699	-26.517	2.499	1.048	42.727
GBPS174	LSCB012	08/03/2018	Cambeque	*Phoenix reclinata*	*wild*	leaf	2.317	-31.315	-0.176	1.275	36.195
GBPS237	FRSL007	03/05/2018	Lautchande	*Phoenix reclinata*	*wild*	fruit	2.771	-27.036	0.559	0.874	48.775
GBPS271	LSL018	03/05/2018	Lautchande	*Phoenix reclinata*	*wild*	leaf	2.609	-29.847	1.622	1.254	37.244
GBPS353	FRSM021	08/05/2018	Madina	*Phoenix reclinata*	*wild*	fruit	2.958	-24.952	2.770	1.330	33.262
GBPS397	LSM031	08/05/2018	Madina	*Phoenix reclinata*	*wild*	leaf	2.439	-31.978	0.607	1.343	33.492
GBPS153	FRSCB011	11/03/2018	Cambeque	*hyllanthus muellerianu*	*wild*	fruit	2.911	-28.491	1.031	1.026	42.158
GBPS232	FRSL002	06/03/2018	Lautchande	*hyllanthus muellerianu*	*wild*	fruit	2.989	-27.166	1.928	1.451	30.840
GBPS045	FLSCC011	07/05/2018	Caiquene-Cadique	*Psidium guajava*	*cultivated*	flower	2.627	-30.742	4.892	1.584	28.566
GBPS063	FRSCC012	07/05/2018	Caiquene-Cadique	*Psidium guajava*	*cultivated*	fruit	2.955	-28.942	6.830	1.643	28.178
GBPS093	LSCC019	07/03/2018	Caiquene-Cadique	*Psidium guajava*	*cultivated*	leaf	3.413	-31.106	5.238	1.759	26.880
GBPS108	LSCC033	07/05/2018	Caiquene-Cadique	*Psidium guajava*	*cultivated*	leaf	2.877	-30.866	4.415	1.851	26.623
GBPS192	LSCB030	11/03/2018	Cambeque	*Psidium guajava*	*cultivated*	leaf	2.859	-31.372	1.821	1.383	32.313
GBPS363	FRSM032	31/05/2018	Madina	*Psidium guajava*	*cultivated*	fruit	2.363	-28.051	0.944	0.876	49.572
GBPS412	LSM047	27/05/2018	Madina	*Psidium guajava*	*cultivated*	leaf	2.792	-27.395	3.521	1.907	23.869
GBPS143	FLSCB004	24/05/2018	Cambeque	*icinodendron heudelot*	*wild*	flower	2.764	-27.272	3.268	1.894	23.766
GBPS047	FLSCC013	09/05/2018	Caiquene-Cadique	*Saba senegalensis*	*wild*	flower	2.919	-29.143	5.944	2.301	21.585
GBPS082	LSCC008	03/03/2018	Caiquene-Cadique	*Saba senegalensis*	*wild*	leaf	2.877	-32.659	0.825	1.564	31.842
GBPS100	LSCC026	10/03/2018	Caiquene-Cadique	*Saba senegalensis*	*wild*	leaf	2.81	-29.979	4.943	2.026	24.460
GBPS142	FLSCB003	15/05/2018	Cambeque	*Saba senegalensis*	*wild*	flower	2.898	-27.698	2.492	2.056	23.953
GBPS160	FRSCB018	15/05/2018	Cambeque	*Saba senegalensis*	*wild*	fruit	2.429	-30.094	3.012	0.590	79.791
GBPS178	LSCB016	08/03/2018	Cambeque	*Saba senegalensis*	*wild*	leaf	2.363	-32.359	1.550	1.797	26.341
GBPS228	FLSL004	16/03/2018	Lautchande	*Saba senegalensis*	*wild*	flower	2.55	-26.764	5.058	2.113	22.999
GBPS229	FLSL005	29/05/2018	Lautchande	*Saba senegalensis*	*wild*	flower	2.876	-28.200	1.340	1.622	29.401
GBPS262	LSL009	06/03/2018	Lautchande	*Saba senegalensis*	*wild*	leaf	2.472	-32.228	1.612	2.374	20.319
GBPS361	FRSM030	20/05/2018	Madina	*Saba senegalensis*	*wild*	fruit	2.946	-30.283	1.448	0.482	97.194
GBPS404	LSM038	09/05/2018	Madina	*Saba senegalensis*	*wild*	leaf	2.647	-30.386	0.303	1.978	22.432
GBPS366	FRSM035	31/05/2018	Madina	*Saccharum officinarum*	*cultivated*	fruit	2.572	-12.091	1.925	2.125	19.595
GBPS414	LSM049	31/05/2018	Madina	*Saccharum officinarum*	*cultivated*	leaf	2.74	-13.824	2.344	0.732	58.939
GBPS159	FRSCB017	15/05/2018	Cambeque	*Salacia senegalensis*	*wild*	fruit	2.659	-31.740	1.862	1.271	36.336
GBPS230	FLSL006	29/05/2018	Lautchande	*Sarcocephalus latifolius*	*wild*	flower	2.874	-28.277	5.152	2.389	19.291
GBPS245	FRSL015	29/05/2018	Lautchande	*Sarcocephalus latifolius*	*wild*	fruit	2.626	-29.450	5.273	1.360	31.960
GBPS102	LSCC028	11/03/2018	Caiquene-Cadique	*Spondias mombin*	*wild*	leaf	2.923	-28.939	-0.830	2.838	16.416
GBPS184	LSCB022	10/03/2018	Cambeque	*Spondias mombin*	*wild*	leaf	2.626	-32.736	2.012	2.501	18.056
GBPS385	LSM019	10/03/2018	Madina	*Spondias mombin*	*wild*	leaf	2.811	-30.461	6.792	3.189	14.148
GBPS144	FRSCB002	08/03/2018	Cambeque	*Strombosia pustulata*	*wild*	fruit	2.498	-29.629	3.038	1.614	27.932
GBPS234	FRSL004	06/03/2018	Lautchande	*Strombosia pustulata*	*wild*	fruit	3.04	-31.727	3.364	3.131	13.928
GBPS235	FRSL005	06/03/2018	Lautchande	*bernaemontana africa*	*wild*	fruit	2.844	-29.954	5.288	1.617	27.974

GBPS077	LSCC003	27/02/2018	Caiquene-Cadique	*Treculia africana*	*wild*	leaf	2.756	-32.876	2.502	1.834	24.059
GBPS147	FRSCB005	08/03/2018	Cambeque	*Treculia africana*	*wild*	fruit	2.3	-26.640	4.355	1.042	44.008
GBPS173	LSCB011	08/03/2018	Cambeque	*Treculia africana*	*wild*	leaf	2.889	-33.137	1.140	1.622	27.274
GBPS247	FRSL017	29/05/2018	Lautchande	*Treculia africana*	*wild*	fruit	2.736	-29.895	2.697	2.548	17.511
GBPS255	LSL002	05/03/2018	Lautchande	*Treculia africana*	*wild*	leaf	2.425	-27.643	3.568	3.995	11.051
GBPS161	FRSCB019	15/05/2018	Cambeque	*Triclisia patens*	*wild*	fruit	2.49	-23.956	2.217	1.839	24.344
GBPS246	FRSL016	29/05/2018	Lautchande	*Triclisia patens*	*wild*	fruit	2.67	-25.758	2.039	1.436	30.482
GBPS149	FRSCB007	10/03/2018	Cambeque	unknown	wild	fruit	2.559	-31.192	2.013	1.561	27.083
GBPS171	LSCB009	08/03/2018	Cambeque	*Uvaria chamae*	*wild*	leaf	2.589	-30.438	-1.128	1.715	28.015
GBPS268	LSL015	06/03/2018	Lautchande	*Uvaria chamae*	*wild*	leaf	2.676	-31.568	1.869	2.247	18.356
GBPS376	LSM010	06/03/2018	Madina	*Uvaria chamae*	*wild*	leaf	2.915	-34.445	-0.042	2.470	18.391
GBPS394	LSM028	11/03/2018	Madina	*Uvaria chamae*	*wild*	leaf	2.698	-30.238	0.940	2.487	18.468
GBPS186	LSCB024	10/03/2018	Cambeque	*Vitex doniana*	*wild*	leaf	2.701	-30.739	2.508	1.495	30.502
GBPS263	LSL010	06/03/2018	Lautchande	*Vitex doniana*	*wild*	leaf	2.827	-27.066	3.155	1.752	25.631
GBPS377	LSM011	07/03/2018	Madina	*Vitex doniana*	*wild*	leaf	2.769	-29.865	-0.083	1.195	39.035

Supplementary table 5.2. Chimpanzee hair sample list with date of collection, site, number of hairs, method of collection, species, sample weight, $\delta^{13}C$ and $\delta^{15}N$ values, and C/N ratio.

Isotope ID	Project ID	Date of Collection	Site	Number of Hairs	Method of Capture	Species	Weight	d13C	d15N	%N	C/N Ratio
GBHS001	HSCB001	28/02/2018	Cambeque	1	nest	*Pan troglodytes*	482	-23.93	4.75632	14.164	3.093918662
GBHS002	HSCB002	18/03/2018	Cambeque	2	nest	*Pan troglodytes*	782	-24.601	6.55911	14.134	3.084725853
GBHS003	HSCB003	29/03/2018	Cambeque	2	other	*Pan troglodytes*	666	-24.254	4.11783	14.284	3.066519566
GBHS004	HSCB004	01/04/2018	Cambeque	1	nest	*Pan troglodytes*	650	-23.706	4.6255	14.192	3.065502679
GBHS005	HSCB005	01/04/2018	Cambeque	2	nest	*Pan troglodytes*	699	no data	no data	no data	no data
GBHS006	HSCB006	01/04/2018	Cambeque	7	other	*Pan troglodytes*	719	-23.763	4.434	13.122	3.281
GBHS007	HSCB007	06/04/2018	Cambeque	10	other	*Pan troglodytes*	926	-24.257	4.81679	13.215	3.267222183
GBHS008	HSCB008	22/04/2018	Cambeque	9	nest	*Pan troglodytes*	795	-23.726	4.69562	13.653	3.041285889
GBHS009	HSCB009	22/04/2018	Cambeque	9	nest	*Pan troglodytes*	721	-23.6	4.52001	13.636	3.073183798
GBHS010	HSCB010	22/04/2018	Cambeque	15	nest	*Pan troglodytes*	618	-23.687	4.2722	13.907	3.032341498
GBHS011	HSCB011	22/04/2018	Cambeque	6	nest	*Pan troglodytes*	768	-23.415	4.48388	13.428	3.078820783
GBHS012	HSCB012	23/04/2018	Cambeque	15	nest	*Pan troglodytes*	754	-23.865	4.53691	14.06	3.053915145
GBHS013	HSCB013	23/04/2018	Cambeque	15	nest	*Pan troglodytes*	657	-24.056	4.22973	7.9495	3.103381253
GBHS014	HSCB014	23/04/2018	Cambeque	8	nest	*Pan troglodytes*	766	-23.395	4.83728	14.346	3.050968988
GBHS015	HSCB015	26/04/2018	Cambeque	12	nest	*Pan troglodytes*	739	-23.849	4.29368	14.229	3.022353442
GBHS016	HSCB016	26/04/2018	Cambeque	10	nest	*Pan troglodytes*	716	-23.779	4.38288	12.669	3.021981383
GBHS017	HSCB017	26/04/2018	Cambeque	14	nest	*Pan troglodytes*	702	-23.361	4.3877	14.353	3.043716187
GBHS018	HSCB018	15/05/2018	Cambeque	13	nest	*Pan troglodytes*	769	-23.878	4.69198	11.137	3.135572603
GBHS019	HSCB019	15/05/2018	Cambeque	10	nest	*Pan troglodytes*	691	-24.177	4.72139	13.998	3.063067897
GBHS020	HSCB020	15/05/2018	Cambeque	15	nest	*Pan troglodytes*	795	-24.068	4.83261	14.003	3.057495553
GBHS021	HSCB021	16/05/2018	Cambeque	15	nest	*Pan troglodytes*	805	-24.075	4.67046	14.2	3.048386567
GBHS022	HSCB022	16/05/2018	Cambeque	15	nest	*Pan troglodytes*	729	-24.126	4.73879	9.6818	3.055262358
GBHS023	HSCB023	16/05/2018	Cambeque	15	nest	*Pan troglodytes*	776	-23.601	5.01204	13.853	3.084007291
GBHS024	HSCB024	16/05/2018	Cambeque	15	nest	*Pan troglodytes*	691	-24.09	4.46016	14.243	3.046
GBHS025	HSCB025	17/05/2018	Cambeque	15	nest	*Pan troglodytes*	716	-25.585	4.29858	14.080	3.070
GBHS026	HSCB026	17/05/2018	Cambeque	15	nest	*Pan troglodytes*	650	-24.233	4.54717	13.578	3.052142602
GBHS027	HSCB027	17/05/2018	Cambeque	14	nest	*Pan troglodytes*	826	-24.132	4.7241	13.03	3.122017984
GBHS028	HSCC003	02/03/2018	Caiquene-Cadique	1	fecal sample	*Pan troglodytes*	244	no data	no data	no data	no data
GBHS029	HSCC004	11/03/2018	Caiquene-Cadique	1	fecal sample	*Pan troglodytes*	696	-24.160	4.36906	14.25	3.007137895
GBHS030	HSCC005	07/04/2018	Caiquene-Cadique	5	other	*Pan troglodytes*	732	-25.180	4.51994	14.527	3.085434324
GBHS031	HSCC006	07/04/2018	Caiquene-Cadique	5	other	*Pan troglodytes*	804	-24.231	4.18721	14.705	3.028493807
GBHS032	HSCC007	07/04/2018	Caiquene-Cadique	5	nest	*Pan troglodytes*	788	-24.705	4.47765	14.429	3.098321554
GBHS033	HSCC008	07/04/2018	Caiquene-Cadique	5	other	*Pan troglodytes*	721	-24.545	4.09588	14.264	3.107889628
GBHS034	HSCC009	07/04/2018	Caiquene-Cadique	6	other	*Pan troglodytes*	741	-25.065	4.08237	14.158	3.092385276
GBHS035	HSCC010	07/04/2018	Caiquene-Cadique	6	other	*Pan troglodytes*	1177	-24.777	4.73557	13.598	3.111343676
GBHS036	HSCC011	11/04/2018	Caiquene-Cadique	7	other	*Pan troglodytes*	779	-24.58	4.33834	13.925	3.081325457
GBHS037	HSCC012	11/04/2018	Caiquene-Cadique	7	other	*Pan troglodytes*	724	-24.647	4.47933	13.874	3.091624016
GBHS038	HSCC013	17/04/2018	Caiquene-Cadique	18	nest	*Pan troglodytes*	790	-24.788	4.5715	12.856	3.037392574
GBHS039	HSCC014	17/04/2018	Caiquene-Cadique	10	nest	*Pan troglodytes*	869	-24.099	4.72811	14.382	3.006117542
GBHS040	HSCC015	02/05/2018	Caiquene-Cadique	16	other	*Pan troglodytes*	1,031	-24.713	4.006	14.312	3.068
GBHS041	HSCC016	02/05/2018	Caiquene-Cadique	15	other	*Pan troglodytes*	777	-25.053	4.404	14.193	3.153
GBHS042	HSCC017	02/05/2018	Caiquene-Cadique	9	nest	*Pan troglodytes*	765	-24.611	4.327	13.675	3.068
GBHS043	HSCC018	03/05/2018	Caiquene-Cadique	13	nest	*Pan troglodytes*	734	-24.342	3.886	12.862	3.059
GBHS044	HSCC019	23/05/2018	Caiquene-Cadique	12	nest	*Pan troglodytes*	719	-24.795	4.216	14.579	3.037
GBHS045	HSCC020	23/05/2018	Caiquene-Cadique	12	nest	*Pan troglodytes*	817	-24.267	4.274	14.543	3.034
GBHS046	HSCC021	24/05/2018	Caiquene-Cadique	11	nest	*Pan troglodytes*	744	-24.711	4.085	14.790	3.049

GBHS047	HSCC022	24/05/2018	Caiquene-Cadique	10	nest	*Pan troglodytes*	804	-24.982	4.141	14.262	3.069
GBHS048	HSCC023	24/05/2018	Caiquene-Cadique	10	nest	*Pan troglodytes*	934	-25.041	4.239	14.573	3.054
GBHS049	HSCC024	25/05/2018	Caiquene-Cadique	9	nest	*Pan troglodytes*	936	-24.390	3.920	14.503	3.058
GBHS050	HSCC025	25/05/2018	Caiquene-Cadique	1	nest	*Pan troglodytes*	463	-25.504	2.79923	2.344	2.84839502
GBHS051	HSCC026	25/05/2018	Caiquene-Cadique	9	nest	*Pan troglodytes*	744	-24.717	5.62966	0.891	2.800372279
GBHS052	HSCC027	25/05/2018	Caiquene-Cadique	6	nest	*Pan troglodytes*	729	-25.153	4.61615	2.872	2.908473067
GBHS053	HSCC028	31/05/2018	Caiquene-Cadique	6	nest	*Pan troglodytes*	787	-24.223	4.41257	4.863	2.959420859
GBHS054	HSCC029	31/05/2018	Caiquene-Cadique	7	nest	*Pan troglodytes*	782	-24.614	4.03291	3.722	3.198508409
GBHS055	HSCC030	31/05/2018	Caiquene-Cadique	7	nest	*Pan troglodytes*	714	-24.39	4.27243	6.641	2.991298698
GBHS056	HSCC031	03/06/2018	Caiquene-Cadique	14	nest	*Pan troglodytes*	707	-24.215	4.204	14.094	3.008
GBHS057	HSCC032	03/06/2018	Caiquene-Cadique	8	nest	*Pan troglodytes*	799	-24.426	4.010	14.227	3.082
GBHS058	HSCC033	03/06/2018	Caiquene-Cadique	2	nest	*Pan troglodytes*	875	-24.742	3.534	13.895	3.004
GBHS059	HSCC034	17/06/2018	Caiquene-Cadique	14	nest	*Pan troglodytes*	761	-24.688	3.710	13.929	3.121
GBHS060	HSCC035	17/06/2018	Caiquene-Cadique	10	nest	*Pan troglodytes*	972	-24.829	3.932	14.546	3.020
GBHS061	HSCC036	17/06/2018	Caiquene-Cadique	13	nest	*Pan troglodytes*	833	-24.677	3.854	12.386	2.950
GBHS062	HSCC037	17/06/2018	Caiquene-Cadique	3	nest	*Pan troglodytes*	708	-24.604	4.033	13.733	2.991
GBHS063	HSCC038	17/06/2018	Caiquene-Cadique	12	nest	*Pan troglodytes*	984	-24.301	3.8179	14.227	3.069275882
GBHS064	HSM001	23/03/2018	Madina	1	nest	*Pan troglodytes*	324	-23.924	3.99061	13.916	3.063541632
GBHS065	HSM002	22/03/2018	Madina	3	nest	*Pan troglodytes*	820	-24.123	4.45632	14.171	3.019716042
GBHS066	HSM003	22/03/2018	Madina	1	nest	*Pan troglodytes*	306	-22.599	4.12909	14.048	3.082358141
GBHS067	HSM004	22/03/2018	Madina	1	nest	*Pan troglodytes*	381	-23.897	3.55915	13.704	2.993738435
GBHS068	HSM005	12/03/2018	Madina	2	nest	*Pan troglodytes*	398	-24.521	3.96559	14.198	3.043673859
GBHS069	HSM006	22/03/2018	Madina	1	nest	*Pan troglodytes*	269	-22.999	3.96752	13.888	3.067620726
GBHS070	HSM007	22/03/2018	Madina	1	nest	*Pan troglodytes*	652	-23.975	4.62954	13.823	3.051699232
GBHS071	HSM008	22/03/2018	Madina	1	nest	*Pan troglodytes*	193	-23.988	4.35357	14.197	3.104147838
GBHS072	HSM009	24/03/2018	Madina	2	nest	*Pan troglodytes*	774	-24.158	4.35023	14.539	3.023423206
GBHS073	HSM010	24/03/2018	Madina	2	nest	*Pan troglodytes*	766	-23.716	4.62867	13.93	3.014462422
GBHS074	HSM011	24/03/2018	Madina	3	nest	*Pan troglodytes*	720	-24.187	3.93465	15.135	3.025619385
GBHS075	HSM012	14/04/2018	Madina	15	nest	*Pan troglodytes*	886	-24.291	4.57342	14.826	3.02087057
GBHS076	HSM014	15/04/2018	Madina	15	nest	*Pan troglodytes*	867	-23.846	4.63903	14.486	3.034416857
GBHS077	HSM015	15/04/2018	Madina	6	nest	*Pan troglodytes*	766	-24.251	4.58296	14.471	3.027621235
GBHS078	HSM016	16/04/2018	Madina	15	nest	*Pan troglodytes*	800	-24.05	4.40535	14.832	3.018487608
GBHS079	HSM017	16/04/2018	Madina	15	nest	*Pan troglodytes*	836	-24.287	3.9701	14.025	2.99798397
GBHS080	HSM018	16/04/2018	Madina	15	nest	*Pan troglodytes*	1156	-24.168	4.56418	14.51	3.06203306
GBHS081	HSM019	16/04/2018	Madina	11	nest	*Pan troglodytes*	856	-23.435	3.94086	14.871	3.009990764
GBHS082	HSM020	16/04/2018	Madina	15	nest	*Pan troglodytes*	998	-24.31	4.14545	14.346	3.113944661
GBHS083	HSM021	17/04/2018	Madina	4	nest	*Pan troglodytes*	511	-24.551	3.92332	14.469	3.0499741
GBHS084	HSM022	17/04/2018	Madina	10	nest	*Pan troglodytes*	876	-24.051	4.01501	14.079	3.071987486
GBHS085	HSM023	17/04/2018	Madina	2	nest	*Pan troglodytes*	760	-23.888	4.43597	14.166	3.012313739
GBHS086	HSM024	17/04/2018	Madina	8	nest	*Pan troglodytes*	735	-24.358	4.48891	14.374	3.035731292
GBHS087	HSM025	18/04/2018	Madina	2	nest	*Pan troglodytes*	730	-24.024	4.63771	14.008	3.108578303
GBHS088	HSM026	18/04/2018	Madina	7	nest	*Pan troglodytes*	927	-24.055	5.46046	14.119	3.06091817
GBHS089	HSM027	18/04/2018	Madina	15	nest	*Pan troglodytes*	1102	-24.274	5.34145	14.549	3.04679315
GBHS090	HSM028	21/04/2018	Madina	10	nest	*Pan troglodytes*	946	-24.04	5.06798	14.96	3.027342552
GBHS091	HSM029	21/04/2018	Madina	10	nest	*Pan troglodytes*	1060	-24.123	5.62706	14.917	3.032219152
GBHS092	HSM030	21/04/2018	Madina	6	nest	*Pan troglodytes*	736	-24.22	4.87092	14.68	3.035110434
GBHS093	HSM031	21/04/2018	Madina	6	nest	*Pan troglodytes*	720	-24.214	4.47018	15.063	2.993399297

GBHS094	HSL001	06/02/2018	Lautchande	5	nest	*Pan troglodytes*	1009	-24.102	4.77171	14.147	3.114973743
GBHS095	HSL002	06/01/2018	Lautchande	10	nest	*Pan troglodytes*	894	-24.354	4.53106	14.787	3.081241535
GBHS096	HSL003	06/02/2018	Lautchande	10	nest	*Pan troglodytes*	964	-24.495	4.74877	14.745	3.051745378
GBHS097	HSL004	15/03/2018	Lautchande	2	nest	*Pan troglodytes*	557	-24.429	3.73107	8.9348	3.013086687
GBHS098	HSL005	15/03/2018	Lautchande	1	nest	*Pan troglodytes*	347	-23.858	3.84527	23.016	3.011988745
GBHS099	HSL006	18/03/2018	Lautchande	4	nest	*Pan troglodytes*	1150	-24.137	5.08386	14.709	3.055986486
GBHS100	HSL007	18/03/2018	Lautchande	1	nest	*Pan troglodytes*	290	-24.554	4.593	13.342	3.036
GBHS101	HSL008	18/03/2018	Lautchande	2	nest	*Pan troglodytes*	1053	-23.163	4.684	14.083	3.182
GBHS102	HSL009	19/03/2018	Lautchande	4	nest	*Pan troglodytes*	814	-24.467	4.244	13.945	3.061
GBHS103	HSL010	23/03/2018	Lautchande	7	nest	*Pan troglodytes*	840	-24.529	4.462	14.966	3.013
GBHS104	HSL011	23/03/2018	Lautchande	8	other	*Pan troglodytes*	723	-24.971	4.213	14.241	3.138
GBHS105	HSL012	23/03/2018	Lautchande	5	other	*Pan troglodytes*	882	-24.546	4.392	14.735	3.050
GBHS106	HSL013	02/04/2018	Lautchande	3	other	*Pan troglodytes*	643	-24.605	4.033	14.473	3.057
GBHS107	HSL014	29/04/2018	Lautchande	8	nest	*Pan troglodytes*	919	-23.892	4.252	14.802	3.014
GBHS108	HSL016	29/04/2018	Lautchande	15	nest	*Pan troglodytes*	1462	-24.145	5.196	12.025	3.059
GBHS109	HSL017	29/04/2018	Lautchande	14	nest	*Pan troglodytes*	1000	-24.055	4.932	14.747	3.043
GBHS110	HSL018	02/05/2018	Lautchande	7	nest	*Pan troglodytes*	894	-23.904	4.121	14.49	3.062
GBHS111	HSL019	02/05/2018	Lautchande	7	nest	*Pan troglodytes*	914	-24.403	4.927	15.302	3.027
GBHS112	HSL020	02/05/2018	Lautchande	3	nest	*Pan troglodytes*	1268	-23.6	5.323	14.179	3.041
GBHS113	HSL021	02/05/2018	Lautchande	15	nest	*Pan troglodytes*	939	-24.664	4.02095	13.753	3.075781729
GBHS114	HSL022	03/05/2018	Lautchande	2	nest	*Pan troglodytes*	464	-24.225	3.8364	14.171	3.048080455
GBHS115	HSL023	05/05/2018	Lautchande	3	nest	*Pan troglodytes*	1107	-23.928	3.52057	13.81	3.015059579
GBHS116	HSL024	05/05/2018	Lautchande	6	nest	*Pan troglodytes*	867	-24.082	3.5472	14.261	3.022780016
GBHS117	HSL025	05/05/2018	Lautchande	6	nest	*Pan troglodytes*	1062	-24.499	3.82128	13.87	3.165968541
GBHS118	HSL026	05/05/2018	Lautchande	10	nest	*Pan troglodytes*	828	-24.864	3.57056	14.32	3.023238169
GBHS119	HSL027	06/05/2018	Lautchande	6	nest	*Pan troglodytes*	746	-24.355	3.79962	14.255	3.047585464
GBHS120	HSL028	06/05/2018	Lautchande	16	nest	*Pan troglodytes*	891	-23.998	3.61459	14.251	3.039146086
GBHS121	HSL029	06/05/2018	Lautchande	6	nest	*Pan troglodytes*	958	-24.351	3.68061	14.076	3.062600156
GBHS122	HSL030	06/05/2018	Lautchande	12	nest	*Pan troglodytes*	917	-24.035	3.80502	13.405	3.121059361
GBHS123	HSL031	02/06/2018	Lautchande	10	nest	*Pan troglodytes*	1097	-24.148	3.81847	14.027	3.065877054
GBHS124	HSL032	02/06/2018	Lautchande	10	nest	*Pan troglodytes*	967	-24.323	3.82189	14.065	3.064206191
GBHS125	HSL033	02/06/2018	Lautchande	16	nest	*Pan troglodytes*	804	-24.05	3.84714	13.991	3.060115086

CHAPTER 6

General Discussion

6. General discussion

In this thesis I investigate behavioural variation at a local, subspecies, and species level by studying, for two consecutive years, four neighbouring communities of western chimpanzees inhabiting the mosaic landscape of central Cantanhez National Park, Guinea-Bissau, and by comparing their behavioural repertoires with previously published data. By employing a combination of direct but mostly indirect methods of data collection I first produce a list of putative cultural behaviours for these communities (Chapter 2). Then I look closely at two specific types of behaviour, tool-assisted honey dipping (Chapter 3), one of the putatively cultural behaviours with clear evidence of inter-community variation, and buttress drumming (Chapter 4), a behaviour considered universal for the species. Finally, I finish by assessing dietary differences through stable isotope analysis (Chapter 5) and evaluate its applications in studies of behavioural variation in the wild.

6.1 Main results and contributions to the field

6.1.1 A multidisciplinary approach to the study of animal behavioural variation and culture in the wild

While animal behaviour research uses a variety of methods of data collection (Dawkins, 2007; Hughey et al., 2018; Bateson and Martin, 2021), the field of primatology has traditionally relied on habituation and long term daily following and observation of a target species, mostly focusing on one group or community at a time (Goodall, 1986; Matsuzawa et al., 2011; Nishida, 2011). Direct behavioural observation allows researchers to register their subjects' daily movements, interactions, and behaviours, and, at long-term research sites, compile a species' behavioural repertoire. These studies are undoubtedly valuable, giving us wonderfully detailed descriptions of wild populations and can revolutionise the way we think about animals as well as ourselves (e.g., Goodall, 1986). With the increasing number of animal cultural studies in the wild, it is clear that behavioural variation between animal groups is abundant, even at a local scale (e.g., Thornton et al., 2010; Luncz and Boesch, 2014; Koops et al., 2015). However, recent technological advances as well as concerns related to the habituation of certain species to human observers (Boyer-Ontl and Pruetz, 2014) have resulted in a shift in the way we investigate animal behaviour and its variation in the wild (Kühl et al., 2019).

In this thesis, I studied the behaviour and behavioural variation of four neighbouring chimpanzee communities, by employing a diverse set of traditional and new methodologies to maximise data collection and analysis, whilst all the time minimising risks to the study animals. All four study communities live in close proximity to humans and encounter them on a daily basis, during road crossings, crop feeding or even in the forest (Hockings and Sousa, 2012; Bessa et al., 2015; Vieira et al., 2019; Bersacola et al., 2021). Despite these habitual and largely non-violent human-chimpanzee interactions in CNP, chimpanzees still demonstrate some degree of fear towards humans and vice versa, a feeling that we should aim to preserve, given the increasingly frequent reports of chimpanzee attacks on children across Africa (see Hockings et al. 2010; McLennan and Hockings 2016). Therefore, in this study, habituation was never a goal or even a possibility. Instead, I started by first exploring the habitat and movements of these largely unknown chimpanzee communities. This information was collected during reconnaissance walks ("recces") performed in rotation across communities where direct (opportunistic encounters) and indirect (nest sites, faecal samples, feeding traces, prints, and abandoned tools) data of chimpanzee presence was collected, as well as data on the availability of resources (e.g., preferred fruits, nuts, honey). These "recces" also informed me on the best areas to set up the camera traps that ultimately produced a large amount of the behavioural data I analysed (Chapters 2 and 4). Using camera traps allowed me to collect data on behaviours that could have otherwise been missed without direct observation and daily follows, and also enabled a less biased sampling of individuals (e.g., including solitary males, female-only parties) than what sometimes accompanies direct observations.

However, due to the large amount of data collected from camera traps alone, I soon realised that its processing and analysis would be more challenging than previously imagined. An opportunity emerged to collaborate with a team of computer scientists, the Visual Geometry Group (University of Oxford), interested in developing a method for automated behavioural analysis. Together, we were able to create a system of automated drumming annotation that allowed for a much more efficient analysis of drumming videos (Appendix D, Chapter 4). For other behaviours however, video evidence was rare (e.g., for extractive honey dipping) but indirect evidence (i.e., discarded tools) was abundant (Chapter 3). By employing techniques borrowed from the field of primate archaeology, and by comparing the artefacts with previously published data, I was able to perform detailed analysis of the tools. Finally, the last set of data collected, came from a novel methodology in the field on animal culture – stable isotope analysis (Chapter 5). In collaboration with researchers from East Carolina University and the University of Colorado (USA) I was able to collect and analyse an unusually large set of plant and chimpanzee hair samples from the four studied communities. Given this multidisciplinary methodological approach I was able to, in a relatively short period of time, compile an extensive, albeit incomplete, list of behaviours (Chapter 2) and behavioural variants (Chapter 2, 3, 4, and 5), never before described for these communities. These results thus: 1) re-affirm the value of using a combination of direct and indirect methodologies to document the behaviour of unhabituated communities; 2) demonstrate the feasibility, practicality, and value of studying neighbouring communities; and 3) show the importance and potential of interdisciplinary collaborative work.

6.1.2 Patterns of inter-community variation in Cantanhez National Park

In Chapter 2 I identified 18 putative cultural traits that included some noteworthy novel behaviours for these communities (e.g., honey dipping). I also identified behaviours that do not leave noticeable material evidence behind and that are rare (e.g., raspberry vocalisation), seasonal (e.g., rain dance), or absent (e.g., salt water drinking) in other known chimpanzee communities (Nishida et al., 2010). Additionally, the fact that I chose to not restrict my data collection to behaviours that had previously been described for the species (Whiten et al., 1999; Kühl et al., 2019), led me to explore different habitat types such as the mangroves. Mangroves are an integral part of the Cantanhez landscape; therefore, it was not surprising to confirm that three of the four studied communities not only used these habitats but also utilised their resources (Chapters 2 and 3). My main goal in this chapter (2) was to not simply confirm the presence of behaviours, but more importantly, systematically assess inter-community variation within confirmed behaviours.

After identifying inter-community variation, I decided to focus on a specific group of behaviours – honey dipping with tools (Chapter 3). Honey consumption is a common behaviour among chimpanzees, and, unsurprisingly, I confirmed this behaviour in all studied communities. However, tool-assisted honey consumption is not common in western chimpanzees as they tend to use their hands (Boesch and Boesch, 1990; Lapuente et al., 2017). I found the first evidence that this behaviour occurs in Guinea-Bissau, with the identification of over 200 individual honey dipping tools in two of the four monitored communities. Moreover, I discovered evidence of variation between the two tool-using communities, differences in tools depending on the type of honey collected, and different tool functions. The tools encountered presented a high degree of complexity and could be divided into at least three functional types – exploratory probes, pounding and extractive tools (Boesch et al., 2009) that I hypothesise might be used in a specific sequence, working as a tool set (Boesch et al., 2009; Wilfried and Yamagiwa, 2014). The extractive tools were characterised by frayed/brush ends, a type of modification that has been described at other study sites, for fluid dipping (Sommer et al., 2012; Lapuente et al., 2017) or termite fishing where it is suggested to increase tool efficiency (Sanz and Morgan, 2007). Presently, I am unable to confirm if these modifications are intentional, as has been described for Comoé (Côte d'Ivoir) and the Goualougo Triangle (Republic of Congo) (Sanz and Morgan, 2007; Lapuente et al., 2017), or if they are simply a by-product of repetitive use (Takemoto et al., 2005). Nonetheless, I am confident that with further study effort this question will be soon answered. Finally, I describe the presence of possible multifunction tools, used both for pounding and extracting honey. This type of tool had never been described for western chimpanzees and is thought to represent a highly complex and sophisticated form of technology.

In contrast with the relative rarity among chimpanzees of the behaviours examined in Chapter 3, my next chapter (Chapter 4) looks at a species universal: buttress drumming. Already in 2004, McGrew, pointed out how surprising it was that so little attention was given to the study of chimpanzees' species-universal behaviours, but even since then only limited work has focused on how uniform they really are. Besides humans, chimpanzees have the largest cultural repertoire in the animal kingdom; nonetheless, when it comes to communication, despite the large repertoire of vocalisations, gestures, facial expressions and other signalling behaviours, the extent to which chimpanzees exhibit variation (individual, regional, or other) in communication is still unclear – in particular, there is little evidence of community-specific communication, unlike the extensive body of work showing population-specific dialects in birds and whales (Helweg et al., 2005; Garland et al., 2011; Williams and Lachlan, 2022). Given the rich data set I was able to collect on buttresses drumming, I set out to quantify the degree of variation exhibited in the behaviour and to examine which factors influenced such variation both within and across the four study communities. I focused on drumming duration as an easily quantifiable measure which has been shown to vary (Babiszewska et al., 2015). Firstly, I found evidence of inter-sex variation in drumming duration. This finding challenges previous claims that the drumming of males and females is indistinguishable to a listener (Boesch and Boesch-Achermann, 2000). Additionally, I argue that more attention should be given to female drumming (often overlooked in the literature): as my results show, female drumming frequently occurs in the presence of dependent offspring, representing a key opportunity for social learning. Secondly, my results support previous findings that the use of pant-hoot vocalisation in combination with drumming significantly increases drumming duration (Arcadi et al., 2004). Thirdly, I found evidence of inter-community variation in drumming duration. These results conform with one of the main functions of drumming, long distance communication, where community-specific-signatures in drumming can facilitate group gathering and cohesion. Finally, I found that the context (i.e., travel, feeding, agonistic display, and play) in which the drumming was performed significantly influenced its variation. When travelling, male drumming duration varied significantly between communities, but not in the context of agonistic display. I hypothesise that given the fission-fusion nature of chimpanzee communities, during travel, community specific information is more relevant to the listeners than it is during an agonistic display. During travel, being able to distinguish between friend or foe is essential for conflict avoidance and group cohesion. These findings suggest a high level of complexity in chimpanzee non-vocal communication which can be explored by focusing on inter-community variation in behavioural universals.

Given how little we still know about drumming it is hard to predict if rapid changes could occur due to changes in group structure, migration of individuals or even environmental disturbances. With the loss of large trees that chimpanzees rely on for drumming, or the construction of new roads that dissect chimpanzee habitats and impact the soundscape, one might expect chimpanzees to be forced to modify their communication behaviour in response. This is particularly the case if some of this communication is socially learnt, since we know that animal culture can be a source of adaptive behaviour that allows for rapid changes in group-specific behaviours, especially when environmental conditions suddenly and rapidly change.

In addition to communication, a key way in which individuals can quickly adapt to changing conditions is by changing or expanding their foraging habits in response to food scarcity or even to the introduction of new foods (e.g., human crops). Given the severity of human-induced change, these environment-driven behavioural adaptations might not last long, and we might miss them altogether. Traditional methods of studying feeding behaviour, such as direct observation or faecal analysis, are notoriously time consuming, especially if researchers aim to account for seasonality in the diet. However, stable isotope analysis of animal tissue, especially hair (which can be obtained from chimpanzee nests non-invasively), can provide us with an estimate of dietary patterns over several months (Chapter 5).

Given the highly anthropogenic nature of the areas inhabited by the four neighbouring communities, isotope analysis provides a useful approach that can point us towards specific feeding behaviours that had thus far not been detected in these communities. Despite all studied chimpanzees living in close geographic proximity and inhabiting similar environments with access to broadly the same food resources, I found evidence of significant dietary variation between all communities. It is unlikely that such inter-community variation is explained, solely, by resource (wild and cultivated) availability, and instead differences in behaviour provide the most likely explanation. For the purpose of this discussion, I will focus on the Caiquene-Cadique chimpanzee community, my biggest outlier. Chimpanzee hair samples from this community showed extremely low levels of $\delta^{15}N$, even when compared to other *Pan* isotope values. $\Delta^{15}N$ values are normally associated with trophic level; therefore, an initial interpretation might assume that this chimpanzee community does not hunt or feed on meat. However, molecular data from faecal samples has shown evidence that at least two species of mammals are eaten by Caiquene-Cadique chimpanzees (Hockings et al., 2021), although we do not know how frequently hunting occurs. Nonetheless, low levels of meat consumption are not the only possible explanation for these low $\delta^{15}N$ values; the consumption of certain foods like termites or legumes can also translate into lower $\delta^{15}N$ values. Termite feeding has never been confirmed at this site (or at any of the CNP study sites), despite the efforts of a previous study analysing faecal samples in Caiquene-Cadique (n=377 samples, Bessa et al., 2015) and the two years of data collection of the present study. Could it be that despite my best efforts I had missed such an important behaviour? Judging from reports of insectivory at other sites, and the ease with which insects are identified in faeces, I argue this is unlikely. However, it is possible that since the first feeding ecology study that took place in 2013 (Bessa et al., 2015), this behaviour has since been innovated, or re-emerged as a response to changes in wild food availability. Indeed, from discussions with local people, it became clear that there had been a recent shift in crop consumption in this chimpanzee community, and chimpanzees had recently started eating several species of legumes (e.g., beans and peanuts). This last piece of information is important, since it does show an apparent change in behaviour, with the possible implication that the same could be true for insectivory. Nonetheless, given the lack of current evidence, for now this explanation remains only a possibility. Overall, it is clear that there is enough variation in the feeding behaviour of these four communities for it to be reflected in the isotope values, demonstrating how isotope analysis can reveal inter-community variation at a small geographic scale. Even though at this time I can only speculate about what drives these differences, my results suggest that the study of stable isotopes is a valuable tool in the study of animal behaviour, steering researchers to investigate behaviours that might otherwise go unnoticed or unstudied. Further, they highlight the fact that animal behaviour is not static and should not be seen as such, especially when studying communities facing the constant pressure of the Anthropocene. My work thus underscores how the study of stable isotopes can prove to be an invaluable tool in assessing variation in behavioural adaptations employed by communities facing similar pressures.

Overall, my results demonstrate the value of studying chimpanzee communities for which there is little pre-existing behavioural or ecological information, partly in order to inform us of the behavioural repertoire of the species as a whole. In contrast to recent studies suggesting that cultural behaviours have on average 88% less chance of occurring in chimpanzee communities inhabiting highly anthropogenically impacted areas (Kuhl et al. 2019), my results shows that these communities are behaviourally rich. My work should encourage researchers to not restrict their attention to behaviours previously described for their study species (e.g., Whiten et al. 1999; Kuhl et al., 2019) and to further explore fine-scale differences within species' universals, especially in habitats where change happens quickly. Finally, during two years of fieldwork, this research identified behavioural variation across a relatively small geographical area. This shows that we must not presuppose that animals living in geographic proximity or in similar environments will all adapt to change in the same way – instead, particularly in the case of behaviours that are acquired at least in part by social learning, we may expect group-specific solutions to emerge and be maintained.

6.1.3 Implications for the field of animal culture

My study has contributed important new behavioural information to the continuously growing chimpanzee behavioural repertoire, challenged previous reports of low behavioural diversity in anthropogenic environments, highlighted the importance of studying neighbouring communities when researching animal culture, and shown that even species-universal behaviours should not be left out of cultural studies. It also re-affirms the feasibility of gathering detailed behavioural data from unhabituated communities, the importance of a multidisciplinary approach, and the practicality of doing so in multiple communities simultaneously. The exact combination of methods used in this study might not be applicable to the study of all species; however, my approach offers a valuable starting point to develop *in situ* studies of animal behaviour and culture. Importantly, my work also shows that behavioural research should not be constrained to a pre-selected list of behaviours (or even environments) already known for the species, and that we should take a step back from what we know such that we can approach new research sites, new study populations or new field seasons with fresh eyes.

6.2 Study limitations

Unfortunately, no work is devoid of limitations, especially when collecting data under challenging and difficult-to-control field conditions. Despite the volume of data collected we are far from having assembled a full picture of chimpanzee behavioural repertoires in these communities. This is particularly evident in Chapter 2, when trying to confirm the presence of universal behaviours in all four communities, for example; the longer a study lasts the more likely we are to confirm the presence of expected behaviours, but also uncover new unexpected ones. Confirming the *absence* of behaviours, on the other hand, can never be fully accomplished, even in long-term studies. By using mostly indirect methods of data collection, I sometimes lacked valuable direct observations that would have provided further context on specific patterns of behaviour. For example, in Chapter 3 observing tool manufacturing and use could have helped me answer the question of the intentionality of brush ends, or in Chapter 5, observing chimpanzee feeding behaviour could have helped me further understand the stable isotope data we found. Another limitation of this study is that by working with unhabituated communities it is hard to predict their movements within their home range. This was particularly noticeable in Chapter 4, where, despite similar study effort across sites, the number of videos collected varied substantially between communities. This was likely because for some of the communities I may have less accurately estimated the location of frequent drumming spots than for others. Additionally, working with unhabituated communities, especially with four simultaneously, meant that I was not able to identify most individuals (although this can still be the case even in studies with habituated communities). Individual identification would have allowed for further exploration of variation in drumming or tool use, for example.

All these limitations are an unavoidable consequence of the methodology used. However, I believe that the approach also has significant advantages: researchers should be seeking novel ways to restrict the harm we can unintentionally cause our study subjects (for example, through disease transmission; (Gilardi et al., 2015)), especially when they are Critically Endangered and hovering on the edge of extinction.

6.3 Future directions

My research has bolstered our understanding of chimpanzee behavioural variation, but has also generated many unanswered questions. I believe that some of these questions can be addressed by further analysis of the existing large data set, especially camera trap footage. Indeed, this data set is still expanding, as the camera traps continue to collect data beyond the time-frame of the current thesis.

The identification of individuals from camera trap footage is difficult and time consuming, but, given recent advances in the application of machine learning approaches to animal data, with time we are likely to be able to automate the process (see Schofield et al., 2019). This will provide us with much better resolution in the analysis of behavioural observations. Secondly, the collection and analysis of the drumming videos continues. Some of my camera traps have remained operational in CNP after the completion of my fieldwork, and I intend to use the additional data collected to explore, in more depth, aspects of drumming other than its duration. These will include specific drumming characteristics (e.g., number of beats, positioning within the pant hoot sequence, use of hand vs foot, handedness), behaviours surrounding drumming (e.g., pant hoot, leaf clipping, raspberry vocalisations), as well as individual identity. Additionally, I am currently part of another collaborative project with a group of biological physicists at ELTE University in Budapest, Hungary, with whom we are exploring further the automation of data collection from the drumming videos. Their efforts at developing a pipeline for pose estimation will enable me to expedite the collection of many of the behavioural variables listed above.

The use of mangroves and their importance for chimpanzees living in these fragmented habitats is also something I believe deserves further research. Why are these chimpanzees seeking salt? Could it be that there is a mineral/nutritional deficit that they are trying to address? Could it be a self-medication behaviour? Or, more simply, a “hedonistic” enjoyment of the flavour? Further remote data collection through camera traps will help us clarify which group members engage in these behaviours and when (i.e., patterns of variation related to age, sex, reproductive state, time of day or season). Such data, together with chemical analysis of the salt water, salty leaves, and perhaps chimpanzee faecal samples, will be necessary to explore these questions.

Additionally, the stable isotope analysis raised many interesting further questions. How can we explain such feeding variation between communities, especially the huge outlier that are Caiquene-Cadique’s $\delta^{15}N$ values? We need more targeted research on the feeding ecology of these communities, perhaps by combining further stable isotope analyses with DNA metabarcoding analyses of chimpanzee faecal samples, that would allow us to explore the possibility of insectivory, hunting frequency, the variety of crop feeding, and so much more.

Given the seemingly inevitable rate of habitat destruction that most species are currently facing, there has never been a more urgent need to develop new ways of protecting them. One of these ways is to understand how species, but more importantly how groups, communities, or populations, can and are adapting to changing conditions, as well as to ascertain how static or not their behavioural repertoires are. Understanding the dynamics of behavioural variation for specific communities can help us understand, for example, what key resources need protection, and help us identify important environmental corridors that allow not only continuous gene flow but also the flow of valuable cultural knowledge between communities. Additionally, understanding the human-animal dynamics at a specific site, and identifying where in their habitat, what type and how severe the different pressures are that animals are experiencing through human encroachment, is paramount in assessing the viability of a wild population. Finally, and perhaps most urgently, we must also consider the importance of working within a multidisciplinary team, and in close proximity to local authorities and stakeholders, to help develop targeted conservation strategies, to which findings like mine can and should contribute.

6.4 Conclusion

This study highlights the level of behavioural diversity we can find within a species, even at a small regional level, and the importance of diverting cultural primatology's attention from merely cataloguing the presence or absence of specific behaviours, and instead focussing our efforts in exploring fine-scale variation in shared behaviours. It also demonstrates not only the importance, but also the feasibility of simultaneously studying multiple neighbouring communities, without the need for habituation, with methodologies that can easily be used and/or adapted to the study of many different species. The advances made thus far on the techniques of data collection and analysis have not only changed the way we collect data but also the type and amount of data we can collect and analyse, which has major implications in the study of animal behaviour and establishment of new research sites. The rate of habitat destruction and climate change that wild species are currently facing means that there has never been a more relevant time to study how different animal communities behave and adapt to their changing environments in order to survive.

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APPENDIX A

Leprosy in wild chimpanzees

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Humans are considered as the main host for *Mycobacterium leprae*[1], the aetiological agent of leprosy, but spillover has occurred to other mammals that are now maintenance hosts, such as nine-banded armadillos and red squirrels[2,3]. Although naturally acquired leprosy has also been described in captive nonhuman primates[4–7], the exact origins of infection remain unclear. Here we describe leprosy-like lesions in two wild populations of western chimpanzees (*Pan troglodytes verus*) in Cantanhez National Park, Guinea-Bissau and Taï National Park, Côte d'Ivoire, West Africa. Longitudinal monitoring of both populations revealed the progression of disease symptoms compatible with advanced leprosy. Screening of faecal and necropsy samples confirmed the presence of *M. leprae* as the causative agent at each site and phylogenomic comparisons with other strains from humans and other animals show that the chimpanzee strains belong to different and rare genotypes (4N/O and 2F). These findings suggest that *M. leprae* may be circulating in more wild animals than suspected, either as a result of exposure to humans or other unknown environmental sources.

Leprosy is a neglected tropical disease caused by the bacterial pathogens *M. leprae* and the more recently discovered *Mycobacterium lepromatosis*[8,9]. In humans, the disease presents as a continuum of clinical manifestations with skin and nerve lesions of increasing severity, from the mildest tuberculoid form (or paucibacillary) to the most severe lepromatous type (or multibacillary)[10]. Symptoms develop after a long incubation period ranging from several months to 30 years, averaging 5 years in humans. As a result of sensory loss, leprosy can lead to permanent damage and severe deformity[11]. Although leprosy prevalence has markedly decreased over recent decades, approximately 210,000 new human cases are still reported every year, of which 2.3% are located in West Africa[12]. Transmission is thought to occur primarily between individuals with prolonged and close contact via aerosolized nasal secretions and entry through nasal or respiratory mucosae, but the exact mechanism remains unclear[13,14]. The role of other routes, such as skin-to-skin contact, is unknown.

Leprosy-causing bacteria were once thought to be obligate human pathogens[1]. However, they can circulate in other animal hosts in the wild, such as nine-banded armadillos (*Dasypus novemcinctus*) in the Americas and red squirrels (*Sciurus vulgaris*) in the UK[2,3]. Although initial infection was most probably incidental and of human origin, secondary animal hosts can subsequently represent a source of infection to humans[15–18]. In captivity, nonhuman primates, such as chimpanzees (*Pan troglodytes*)[4], sooty mangabeys (*Cercocebus atys*)[5,6] and cynomolgus macaques (*Macaca fascicularis*)[7], have been known to develop leprosy without any obvious infectious source. However, due to their captive status, it is unclear how they acquired *M. leprae* and whether these species can also contract leprosy in the wild.

Here, we report leprosy infections and their disease course in two wild populations of western chimpanzees (*P. troglodytes verus*) in Cantanhez National Park (CNP), Guinea-Bissau, and in Taï National Park (TNP), Côte d'Ivoire, using a combination of camera trap and veterinary monitoring (Extended Data Fig. 1a and Supplementary Notes 1 and 2). From analyses of faecal samples and postmortem tissues, we identified *M. leprae* as the causative agent of the lesions observed and determined

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the phylogenetic placement of the respective strains based on their complete genome sequences.

Chimpanzees at CNP are not habituated to human observers, precluding systematic behavioural observations. Longitudinal studies necessitate the use of camera traps, which we operated between 2015 and 2019. Of 624,194 data files (videos and photographs) obtained across 211 locations at CNP (Extended Data Fig. 1b, Extended Data Table 1 and Supplementary Table 1), 31,044 (5.0%) contained chimpanzees. The number of independent events (images separated by at least 60 min) totalled 4,336, and of these, 241 (5.6%) contained chimpanzees with severe leprosy-like lesions, including four clearly identifiable individuals (two adult females and two adult males) across three communities (Extended Data Fig. 2 and Supplementary Note 2). As with humans, paucibacillary cases in chimpanzees may be present but easily go undetected. Such minor manifestations of leprosy are not reported. All symptomatic chimpanzees showed hair loss and facial skin hypopigmentation, as well as plaques and nodules that covered different areas of their body (limbs, trunk and genitals), facial disfigurement and ulcerated and deformed hands (claw hand) and feet (Fig. 1a–c), consistent with a multibacillary form of the disease. Longitudinal observations showed progression of symptoms across time with certain manifestations similar to those described in humans (such as progressive deformation of the hands) (Extended Data Fig. 2 and Supplementary Videos 1–3). To confirm infection with *M. leprae*, we collected faecal samples and tested them with two nested polymerase chain reaction (PCR) assays targeting the *M. leprae*-specific repetitive element (RLEP) and 18 kDa antigen gene. One out of 208 DNA extracts from CNP was positive in both assays and a second was positive only in the more sensitive RLEP-PCR[19] (Extended Data Table 2, Supplementary Table 2 and Supplementary Note 3). Microsatellite analyses of the two positive samples confirmed that they originated from two distinct female individuals (Supplementary Note 4 and Supplementary Tables 3 and 4). Our results suggest that *M. leprae* is the most likely cause of a leprosy-like syndrome in chimpanzees from CNP.

At TNP, chimpanzees are habituated to the presence of researchers and have been followed daily since 1979. In addition, necropsy samples have been collected from all dead individuals recovered since 2000. In June 2018, researchers first noticed leprosy-like lesions on Woodstock, an adult male chimpanzee from one of the three habituated communities (south) (Extended Data Fig. 1c). The initial small nodules on the ears, lips and under the eye became more prominent and were followed by nodules on the eyebrows, eyelids, nostrils, ears, lips and face. The skin on facial nodules, hands, feet and testicles became hypopigmented and the loss and abnormal growth of nails was observed (Fig 1d–g, Extended Data Fig. 3 and Supplementary Videos 4 and 5). *Mycobacterium leprae* DNA was detected in all samples from June 2018 (Extended Data Table 2, Supplementary Table 2 and Supplementary Note 2). Here, continuous noninvasive detection of *M. leprae* was associated with the onset and evolution of a leprosy-like disease.

Retrospective PCR screening of all chimpanzee spleen samples (*n* = 38 individuals) from the TNP necropsy collection led to the identification of *M. leprae* DNA in two further individuals. An adult female from the same community named Zora, who had been killed by a leopard in 2009, tested positive in both PCR assays. The presence of *M. leprae* DNA was confirmed by PCR in various other organs (Extended Data Table 2). Retrospective analyses of photographs taken in the years before her death showed progressive skin hypopigmentation and nodule development since 2007 (Extended Data Fig. 3). Formalin-fixed skin samples (hands and feet) were prepared for histopathological examination using haematoxylin and eosin as well as Fite-Faraco stains. The skin presented typical signs of lepromatous leprosy characterized by a diffuse cutaneous cell infiltration in the dermis and the subcutis clearly separated from the basal layer of the epidermis (Extended Data Fig. 4a). We detected moderate numbers of acid-fast bacilli (single or in clumps) within histiocytes, indicative of *M. leprae* (Extended Data Fig. 4b). As antibodies against the *M. leprae*-specific antigen phenolic glycolipid-I (PGL-I) are a hallmark of *M. leprae* infection in humans[20], we also performed a PGL-I lateral flow rapid test[21] on a blood sample from this individual, which showed strong seropositivity (Extended Data Fig. 4c). Faecal samples collected in the years before Zora's death contained *M. leprae* DNA from 2002 onwards, implying at least 7 years of infection (Extended Data Table 2). In this case, disease manifestations, histopathological findings, serological and molecular data, as well as the overall course of the disease, all unambiguously point towards *M. leprae*-induced leprosy.

Fig. 1 | Clinical manifestations of leprosy in three chimpanzees at CNP, Guinea-Bissau and TNP, Côte d'Ivoire. **a**–**c**, Clinical signs of leprosy in two adult female chimpanzees in CNP (images extracted from camera traps). **a**, Rita has large hypopigmented nodules covering the entire body; disfigurement of the face, ears, hands and feet (ulcerated lesions and swelling). **b**, Rita has extensive plaques covering all limbs, with hair loss. **c**, Brinkos has large hypopigmented nodules covering the entire face, with extreme disfigurement of the face and ears, and ulcerated plaques on the arms and the nipples. **d**–**g**, Clinical signs of leprosy in an adult male chimpanzee, Woodstock, at TNP. **d**, Multiple hypopigmented nodules on the ears, brow ridges, eyelid margins, nostrils, lips and the area between the upper lip and the nose. **e**, Hypopigmentation and swelling of the hands with ulcerations and hair loss on the dorsal side of the joints. **f**, Claw hand with nail loss and abnormal overgrowth of fingernails. **g**, Scrotal reddening and ulceration with fresh blood.

To ascertain whether other individuals in the south community of TNP were infected at the time of Zora's death in 2009, cross-sectional screening of contact animals (*n* = 32) was performed by testing all available faecal samples (*n* = 176) collected in 2009 (Supplementary Table 2). Three other chimpanzees were PCR-positive in single samples, including Woodstock. Clinical symptoms of leprosy have not been observed in other individuals, despite daily monitoring of south community members for 20 years and of neighbouring communities for 40 years[22,23]. Considering that, over this period, 467 individuals have been observed, it seems that leprosy is a rare disease with low transmission levels in these chimpanzee communities.

To characterize the *M. leprae* strains causing leprosy in wild chimpanzees and to perform phylogenomic comparisons, we selected DNA extracts that were positive in both the RLEP and the less-sensitive 18-kDa PCR, which indicates relatively high levels of *M. leprae* DNA. For TNP, we selected individuals that were positive in multiple samples. Following targeted enrichment using hybridization capture, samples were subjected to Illumina sequencing. Sufficient *M. leprae* genome coverage

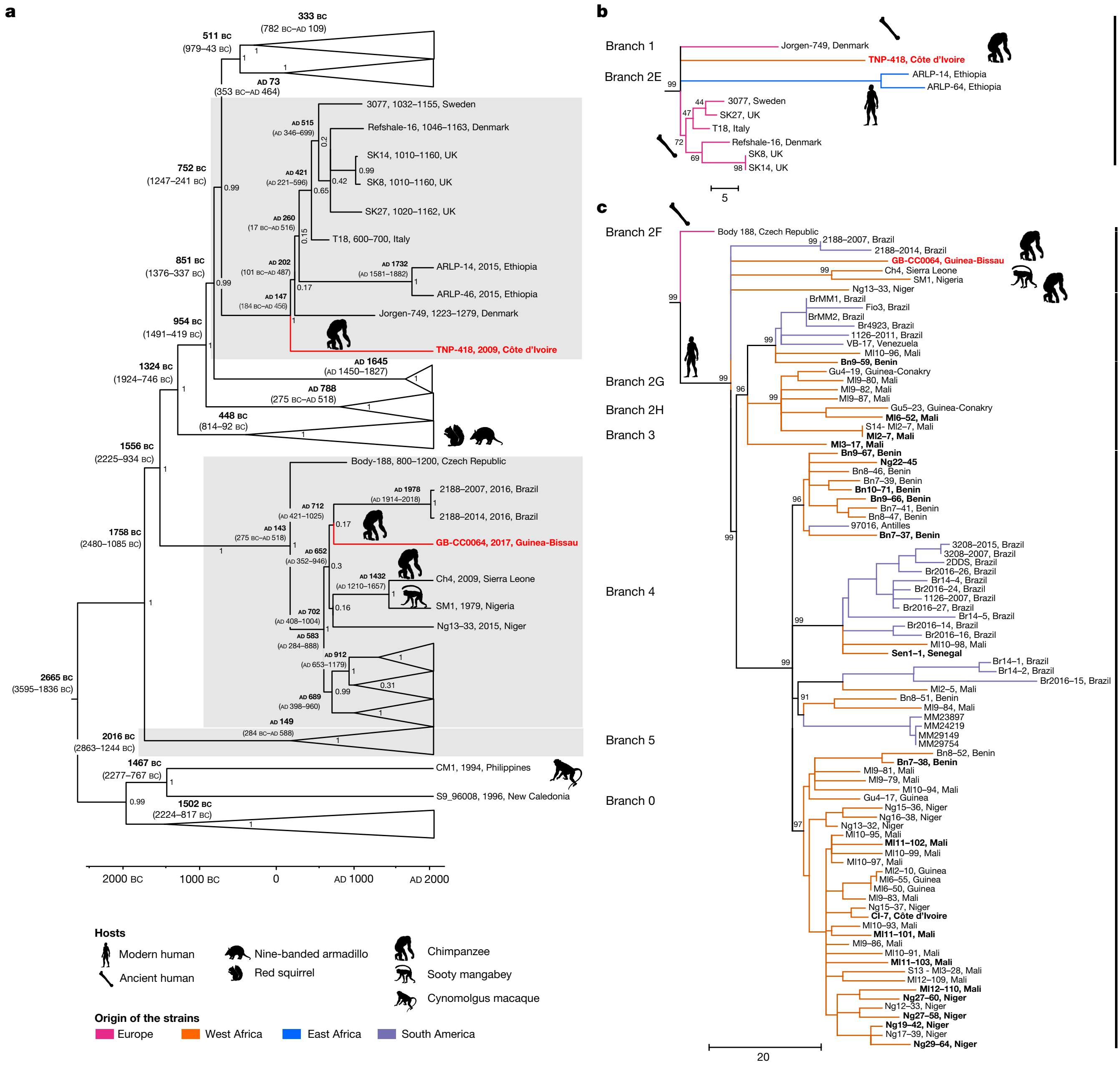

Fig. 2 | Phylogeny of *M. leprae* strains from human and animal hosts. **a**, Bayesian dated phylogenetic tree of 278 *M. leprae* genomes including the two new chimpanzee strains (in bold red). Hypermutated samples with mutations in the *nth* gene were excluded from the analysis. The tree is drawn to scale, with branch lengths representing years of age. Median estimates of node ages are shown in black above branches; 95% HPD intervals are shown in grey. Some *M. leprae* branches are collapsed to increase readability. **b**, Maximum parsimony tree of branch 2F. **c**, Maximum parsimony tree of the branch 4. The tree was initially constructed using 286 genomes (Supplementary Table 6), including 2 new chimpanzee strains (in bold red) and 21 new genomes from West Africa (in bold), 500 bootstrap replicates and *M. lepromatosis* as outgroup. Sites with missing data were partially deleted (80% genome coverage cutoff), resulting in 4,470 variable sites used for the tree calculation. Subtrees corresponding to branches were retrieved in MEGA7[65]. Corresponding genotypes are indicated on the side of each subtree. Samples are binned according to geographical origin as given in the legend. Scale bars (**b**, **c**), number of nucleotide substitutions. Animal silhouettes are available under Public Domain licence at PhyloPic (http://PhyloPic.org/).

was obtained for sample GB-CC064 (Guinea-Bissau) and for Zora (Côte d'Ivoire) with mean depth of 39.3× and 25.8×, respectively (Extended Data Table 2 and Supplementary Table 5). We generated 21 *M. leprae* genomes from human biopsies from five West African countries (Niger, Mali, Benin, Côte d'Ivoire and Senegal) and depth of coverage ranged from 4.7× to 170×. We assembled a dataset that included the genomes generated in this study and all previously available *M. leprae* genomes. Of the total 286 genomes, 64 originated from six West African countries (Extended Data Fig. 5 and Supplementary Note 5).

Bayesian and maximum-parsimony analyses (Extended Data Figs. 6 and 7) place the strain from Guinea-Bissau (GB-CC064) on branch 4, where it clusters outside the standard genotypes 4N, 4O and 4P, but within the so-called 4N/O genotype[24,25] (Fig. 2a, c). This 4N/O genotype is rare and only comprises five *M. leprae* strains; one strain (Ng13-33) from a patient in Niger, two strains (2188-2007 and 2188-2014) obtained from a single patient in Brazil (of 34 strains in Brazil)[26] and two strains from two captive nonhuman primates originating from West Africa (Ch4 and SM1)[25]. The branching order of these five strains and GB-CC064 was unresolved in our analyses, with a basal polytomy suggestive of star-like diversification within this genotype, and within the group comprising all genotype 4 strains (4N/O, 4N, 4P and 4O). Divergence from the most recent common ancestor for this group is estimated to have occurred in the sixth century AD (mean divergence time, 1,437 years ago, 95% highest posterior density (HPD) 1,132–1,736 years ago). The strain that infected Zora in Côte d'Ivoire, designated TNP-418, belongs to branch 2F, within which, the branching order was also mostly unresolved (Fig. 2a, b). The branch is currently composed of human strains from medieval Europe (*n* = 7) and modern Ethiopia (*n* = 2), and this genotype has thus far never been reported to our knowledge in West Africa. Bayesian analysis estimated a divergence time during the second century AD (mean of 1,873 years ago (95% HPD 1,564–2,204 years ago)), similar to previous predictions[27].

Samples from Woodstock did not yield enough Illumina reads to reconstruct full genomes for phylogenomic analysis. However, single-nucleotide polymorphisms (SNPs) recovered from the few available Illumina reads and Sanger sequences derived from PCR products allowed us to assign this second *M. leprae* strain from Côte d'Ivoire to the same genotype as TNP-418 (Supplementary Note 5). Overall, phylogenomic analyses show that *M. leprae* strains in chimpanzee populations at CNP and TNP are not closely related.

The finding of *M. leprae*-induced leprosy in wild chimpanzee populations raises the question of the origin(s) of these infections. *Mycobacterium leprae* is considered a human-adapted pathogen and previous cases of leprosy affecting wildlife were compatible with anthroponosis. Therefore, the prime hypothesis would be human-to-chimpanzee transmission. Potential routes of transmission include direct (such as skin-to-skin) contact and inhalation of respiratory droplets and/or fomites, with the assumption that, in all cases, prolonged and/or repeated exposure is required for transmission[11]. Chimpanzees at CNP are not habituated to humans and are not approached at distances that would allow for transmission via respiratory droplets. Although these chimpanzees inhabit an agroforest landscape and share access to natural and cultivated resources with humans[28], present-day human–chimpanzee direct contact is uncommon. The exact nature of historic human–chimpanzee interactions at CNP remains, however, unknown. For example, robust data on whether chimpanzees were kept as 'pets' or were hunted for meat are lacking. Long-term human–chimpanzee coexistence in this shared landscape makes humans the most probable source of chimpanzee infection. However, multiple individuals from several chimpanzee communities across CNP show symptomatic leprosy demonstrating that *M. leprae* is now probably transmitted between individuals within this population.

At TNP, the south chimpanzee community is distant from human settlements and agriculture. Human-to-animal transmission of pathogens has been shown at TNP[29,30] but involved respiratory pathogens (pneumoviruses and human coronavirus OC43) that transmit easily and do not require prolonged exposure. In addition, *M. leprae* is thought to be transmitted from symptomatic humans[31] and no cases of leprosy have been reported among researchers or local research assistants. Although a human source is impossible to rule out, low human contact coupled with the rarity of the *M. leprae* genotype detected in TNP chimpanzees among human populations in West Africa suggests that recent human-to-chimpanzee transmission is unlikely. This is supported by the absence of drug-resistant mutations (Supplementary Note 6). The relatively old age of the lineage leading to the chimpanzee strain at TNP nevertheless raises the possibility of an ancient human-to-chimpanzee transmission. However, the human population density 1,500–2,000 years ago was probably even lower than it is currently, making this unlikely. If such an ancient transmission had occurred and the bacterium had persisted for a long time in chimpanzees, it should have spread more broadly as observed in *M. leprae*-infected squirrels and armadillos[3,16,17]. Therefore, an ancient human-to-chimpanzee transmission is not the most plausible mechanism to explain the presence of *M. leprae* in chimpanzees at TNP.

These findings may be better explained by the presence of a nonhuman leprosy reservoir. As chimpanzees hunt frequently, transmission may originate from their mammalian prey[32]. Nonhuman primates are the most hunted prey at TNP[33] and are hunted at CNP (Supplementary Note 3). Chimpanzees also consume other mammalian prey such as ungulates. Notably, this scenario assumes that the animal host range of *M. leprae* is even broader than is currently known. Perhaps more intriguingly, an environmental source may be at the origin of chimpanzee infections. Other mycobacteria can survive in water, including *M. ulcerans* and other non-tuberculous mycobacteria[34,35], and molecular investigations have reported that *M. leprae* can survive in soil[36]. Experimental data also show that *M. leprae* multiplies in amoebae[37], arthropods[38] and ticks[39], which could contribute to the persistence of the bacteria in the environment. Testing these hypotheses will require thorough investigation of the distribution of *M. leprae* in wildlife and the environment and so shed light on the overall transmission pathways of the pathogen.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-03968-4.

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Methods

Study sites

Observational study and sample collections were performed at CNP in southern Guinea-Bissau and TNP in western Côte d'Ivoire (Extended Data Fig. 1a). CNP (1,067 km^2) comprises the Cubucaré peninsula in the sector of Bedanda, with the northeast of the park bordering the Republic of Guinea. The landscape at CNP consists of a mosaic of mainly mangroves, subhumid forest patches, savannah grassland and woodland, remnant forest strips dominated by palm groves as well as agriculture[40]. There are approximately 200 villages and settlements within the borders of the park, with an estimated human population of 24,000 individuals who comprise several ethnic groups[41]. Chimpanzees are not hunted for consumption within CNP due to local cultural beliefs and taboos[42] but are sometimes killed in retaliation for foraging on crops[43,44]. There is a minimum of 12 chimpanzee communities at CNP[41], all unhabituated to researchers, with approximately 35–60 individuals per community[45,46]. Numerous other wildlife taxa inhabit CNP, including six other nonhuman primate species[41,47].

The TNP (5,082 km^2) consists of an evergreen lowland rainforest and is the largest remaining primary forest fragment in West Africa. It is home to a wide range of mammals that include 11 different nonhuman primate species[48,49]. There are no settlements or agricultural areas inside the National Park. As of March 2021, the three habituated communities, north, south and east, comprised 22, 37 and 32 individuals, respectively, although community sizes have varied over time. Systematic health monitoring of these communities has been ongoing since 2000[23].

Longitudinal observations and health monitoring

At CNP, camera traps (Bushnell Trophy Cam models 119774, 119877 and 119875) were deployed at 211 locations, including across different habitat types (forest, mangrove-forest edge and orchards) within the home range of 8 of the 12 putative chimpanzee communities (Supplementary Table 1). Camera traps were set up over six data collection periods from 2015 to 2019 (Extended Data Table 1). Targeted camera traps were deployed to record and monitor chimpanzee behaviour and disease occurrence. To maximize the chances of recording specific behaviours and to identify leprosy-like symptoms in individuals, targeted camera traps were set up in locations that chimpanzees were known to use most often, sometimes in clusters, precluding uniform survey designs. Targeted camera traps were set up in video mode and were active 24 h per day. When triggered, targeted cameras recorded 10 to 60 s of video with a minimum interval of 0.6 s or 2 s, depending on the camera trap model. Furthermore, systematically placed camera traps were used to obtain measures of wildlife occurrence and habitat use across the heterogeneous landscape[41]. Systematic camera traps were deployed across central CNP, at a minimum distance of 1 km between sampling points, as well as within the home range of one chimpanzee community (Caiquene-Cadique) and were spaced at least 500 m from one another. The camera traps pointed towards animal paths (often chimpanzee paths), small human paths also used by wildlife and other areas presenting signs of animal activity. Systematic camera traps were set up to record three consecutive photographs when triggered. The GPS coordinates, habitat type, date, time and site description were recorded when setting up individual camera traps (targeted and systematic). Opportunistic observations of chimpanzees at CNP were made in 2013, during which chimpanzees were photographed and/or filmed using digital cameras.

Chimpanzees at TNP are fully habituated to human observers and all individuals in the habituated communities are individually identified. Behavioural and health monitoring of chimpanzees at TNP involves daily observation of habituated individuals by an interdisciplinary team comprising primatologists and veterinarians; investigations of wildlife mortality causes through necropsies on all animal carcasses found in the research area; and the collection of noninvasive samples such as faecal samples, laboratory investigations and the communication of the results to the park management for corrective and preventive measures[22]. Abnormalities in behaviour or clinical signs of disease are immediately reported and followed by detailed observation by the on-site veterinarian. To reduce the risk of transmission of human diseases to the chimpanzees, stringent hygiene measures have been put in place, including an initial 5-day quarantine for observers, keeping a distance of at least 7 m and obligatory wearing of masks, with only healthy observers allowed to work in the forest[50,51].

Faecal and necropsy sample collection

At CNP, chimpanzee faecal samples were collected between July 2017 and December 2018. The date and putative chimpanzee community were recorded for each faecal sample. As defecation was rarely observed and to prevent the collection of redundant samples from the same individual, we avoided multiple samples found under the same chimpanzee nest and paid special attention if multiple samples were found in proximity on trails[45,52,53]. All samples were collected with the aid of a wooden spatula and stored at ambient temperature in 15-ml tubes containing NAP buffer[54]. All samples were sent to the Robert Koch Institute for laboratory analysis. Even though chimpanzee faeces are easily distinguishable from those of other species and were found in areas where chimpanzees had recently been present with associated signs such as feeding remains or knuckle prints, we genetically confirmed the presence of chimpanzee DNA in faecal samples that tested positive in either of the *M. leprae* PCRs or the mammal PCR for diet analysis (Supplementary Note 3).

At TNP, the long-term health monitoring programme includes continuous collection of faecal and urine samples from known adult chimpanzees. Faeces are collected right after defecation, transferred to 2-ml cryotubes with the aid of a plastic spatula and frozen in liquid nitrogen the same day. A full necropsy is systematically performed on chimpanzees found dead by the on-site veterinarian. Necropsies follow a standardized biosafety protocol due to the occurrence of anthrax, Ebola and monkeypox in the area. This includes the use of full personal protective equipment and rigorous disinfection measures. Tissue samples of several internal organs are taken if the state of carcass decomposition allows. After collection, all samples are first stored in liquid nitrogen and subsequently shipped on dry ice to the Robert Koch Institute for analyses.

DNA extraction from faeces and necropsy samples

DNA extractions were performed at the Robert Koch Institute in a laboratory that has never been used for molecular *M. leprae* investigations. DNA was extracted from faecal and necropsy samples using the GeneMATRIX stool DNA purification kit (EURx) and the DNeasy Blood and Tissue kit (QIAGEN), respectively, following the manufacturers' instructions. Extracted DNA was then quantified using the Qubit dsDNA HS Assay kit (Thermo Fisher Scientific) and subsequently stored at −20 °C until further use.

Genetic identification of samples from infected chimpanzees at CNP

To determine whether faecal samples positive for *M. leprae* belonged to one or two individuals at CNP, we amplified chimpanzee DNA at 11 microsatellite loci and one sexing marker[55]. Owing to the small quantity of starting DNA, not all loci were amplified and in some cases the amplification quality was low, affecting our ability to confidently interpret allele peak profiles (for example, sample GB-CC064 failed to amplify for 5 out of the 11 loci) (Supplementary Note 4).

Molecular screening of *M. leprae* in faecal and necropsy samples

Mycobacterium leprae DNA was searched for using two nested PCR systems targeting the distinct but conserved repetitive element RLEP and the 18-kDa antigen gene as previously described (Extended Data Table 3). As 37 copies of RLEP are present in the *M. leprae* genome, this assay is considered to be more sensitive than 18 kDa, for which there is only a single copy. To prevent contamination at the laboratory at the Robert Koch Institute and to enable us to identify whether it occurs, we followed these procedures: (1) separate rooms were used for preparation of PCR master mixes and the addition of DNA in the primary PCR; (2) the addition of the primary PCR product in the nested PCR in another separate room; and (3) dUTPs were used for all PCRs instead of dNTPs. For both assays, primary PCRs were performed in 20-µl reactions: up to 200 ng of DNA was amplified using 1.25 U of high-fidelity Platinum Taq polymerase (Thermo Fisher Scientific), 10× PCR buffer, 200 µM dUTPs, 4 mM $MgCl_2$ and 200 nM of both forward and reverse primers. The thermal cycling conditions for the primary and nested PCRs were as follows: denaturation at 95 °C for 3 min, followed by 50 cycles of 95 °C for 30 s, 55 °C (18 kDa primers) or 58 °C (RLEP primers) for 30 s, and 72 °C for 1 min as well as an elongation step at 72 °C for 10 min. For nested PCRs, 2 µl of a 1:20 dilution of the primary PCR product was used as a template. Molecular-grade water was used as a template-free control. PCR products were visualized on a 1.5% agarose gel stained with GelRed (Biotium). Bands of the expected size were purified using the Purelink Gel extraction kit (Thermo Fisher Scientific). Both RLEP and 18-kDa nested PCR products are too short for direct Sanger sequencing. Therefore, fusion primers (primary PCR primers coupled with M13F and M13R primers) (Extended Data Table 3) were used for further amplification of the cleaned PCR products, applying the same conditions as in the primary PCR, but running only for 25 cycles. The resulting extended PCR products were then enzymatically cleaned using the ExoSAP-IT PCR Product Cleanup assay (Thermo Fisher Scientific) and Sanger sequenced using M13 primers. Resulting sequences were compared to publicly available nucleotide sequences using the Basic Local Alignment Search Tool (BLAST)[56].

Histopathology

To further confirm the infection, skin samples were sent to the German Primate Center in Göttingen, Germany for histopathological analyses. Samples were immersion-fixed in 10% neutral-buffered formalin, embedded in paraffin and stained with standard haematoxylin and eosin using the Varistain Gemini staining automat (Thermo Fisher Scientific). Samples were also stained with Fite-Faraco stain for the identification of acid-fast bacilli.

Serology

A whole-blood sample from Zora collected during the necropsy in 2009 was tested for the presence of the *M. leprae*-specific anti-PGL-I antibodies using a chromatographic immunoassay developed for use with human blood following the instructions provided by the test manufacturers with a 1:10 diluted whole-blood sample. This rapid lateral flow test was produced by R. Cho using the synthetic ND-O-BSA antigen with financial support of the NIH/NIAID Leprosy Research Materials contract AI-55262 at Colorado State University. Test results were interpreted at 5 and 10 min. Human serum from a patient with multibacillary leprosy donated by J. S. Spencer, Colorado State University, was used as a positive control. Whole blood collected during the necropsy of a chimpanzee (Olivia) at TNP who died of acute respiratory disease in 2009 was used as a negative control.

Library preparation, genome-wide capture and high-throughput sequencing for nonhuman primate samples

Selected *M. leprae*-positive faecal and necropsy samples (Supplementary Table 2) were converted into dual-indexed libraries using the NEBNext Ultra II DNA Library Prep kit (New England Biolabs)[57,58]. To reconstruct whole genomes, libraries were target-enriched for *M. leprae* DNA using in-solution hybridization capture with 80-nt RNA baits designed to cover the whole *M. leprae* genome (twofold tiling; design can be shared upon request to the corresponding author) and following the myBaits protocol as previously described[25]. Around 1.5 µg of each DNA library was captured in single or pooled reactions. Two rounds of 24-h hybridization capture were performed followed by a post-amplification step for each using the KAPA HiFi HotStart Library Amplification kit with 12 to 16 cycles to generate around 200 ng of enriched library per sample. Finally, enriched libraries were purified using the silica-based MinElute reaction cleanup kit (QIAGEN) followed by quantification with the KAPA library quantification kit (Roche). Libraries were then normalized and pooled across sequencing lanes on an Illumina NextSeq 500 for sequencing with a mid-output kit v.2 for 300 cycles (Illumina).

Sample collection, DNA extraction, library preparation, genome-wide capture and high-throughput sequencing of human specimens

Samples (skin biopsies or DNA extracts) from patients with leprosy from five West African countries who had a positive bacillary index (Niger ($n = 5$), Mali ($n = 8$), Benin ($n = 6$), Côte d'Ivoire ($n = 1$) and Senegal ($n = 1$)) were obtained from the respective National Leprosy Control Programmes in the framework of the leprosy drug-resistance surveillance programmes or from previous investigation[59].

DNA was extracted from skin biopsies using the total DNA extraction method as described previously[60]. DNA was quantified with a Qubit fluorometer using the Qubit dsDNA BR Assay kit (Thermo Fisher Scientific) before library preparation. DNA libraries were prepared using the KAPA Hyper Prep kit (Roche) as per the manufacturer's recommendation using KAPA Dual-Indexed Adapter (Roche) followed by in-solution capture enrichment with 80-nt RNA baits with 2× tiling density for 48 h at 65 °C as described previously[60]. Post-capture amplification was performed with seven cycles. Enriched libraries were purified using a 1× ratio of KAPA Pure beads (Roche) followed by quantification with the KAPA library quantification kit (Roche) and quality control of the fragment with the Agilent 2200 TapeStation (Agilent Technologies). Libraries were then normalized and pooled across sequencing lanes on an Illumina NextSeq 500 for sequencing with a high output kit v.2 for 75 cycles (Illumina).

Genomic data analysis

Raw reads were processed as described elsewhere[24]. Putative unique variants of GB-CC064 and TNP-418 strains were manually checked and visualized using the Integrative Genomics Viewer[61].

Genome-wide comparison and phylogenetic tree

SNPs of the two newly sequenced genomes from chimpanzees were compared to the 263 publicly available *M. leprae* genomes[25,60,62–64] (Supplementary Table 6) and 21 new genomes from West African countries (Supplementary Note 5). Phylogenetic analyses were performed using a concatenated SNP alignment (Supplementary Table 7). Maximum-parsimony trees were constructed in MEGA7[65] with the 286 genomes available (Supplementary Table 5) using 500 bootstrap replicates and *M. lepromatosis*[66] as outgroup. Sites with missing data were partially deleted (80% genome coverage cutoff), resulting in 4,470 variable sites used for the tree calculation.

Dating analysis

Dating analyses were performed using BEAST2 (v.2.5.2)[67] as described previously[24] with 278 genomes and an increased chain length from 50 to 100 million. In brief, concatenated SNPs for each sample were used for tip dating analysis (Supplementary Table 7). Hypermutated strains and highly mutated genes associated with drug resistance (in yellow, Supplementary Table 7) were omitted[24,60], manual curation of the maximum parsimony and BEAST input file was conducted at the positions described in Supplementary Table 9 for GB-CC064 and TNP-418. Sites with missing data as well as constant sites were included in the analysis, as previously described[24]. Only unambiguous constant sites (loci where the reference base was called in all samples) were included.

PCR genotyping of insufficiently covered *M. leprae* genomes from positive chimpanzees

The genome coverage for the strain infecting Woodstock was low. To be able to determine the genotype, we identified specific variants from the genome-wide comparison of TNP-418 (the strain infecting Zora, an individual from the same social group) with other strains from branch 2F (Supplementary Table 9). Variants were manually checked and visualized in the partially covered genome from the strain infecting Woodstock using IGV software (Supplementary Table 10). Two variants not covered by high-throughput sequencing data were also selected for specific PCR screening. Primers were designed using the Primer3 web tool (http://bioinfo.ut.ee/primer3-0.4.0/) based on Mycobrowser sequences[68] and are described in Extended Data Table 3. All PCR conditions were the same as in the *M. leprae* screening PCRs except for the primer sets and associated annealing temperatures.

Ethical oversight

For chimpanzees, all data were collected in accordance with Best Practice Disease and Monitoring Guidelines developed by the Section on Great Apes, IUCN SSC Primate Specialist Group (IUCN SSC PSG SGA). The collection of samples was noninvasive. All proposed data collection and analyses adhered strictly to ethics guidelines of the Association for the Study of Animal Behaviour (UK). Ethical approval for targeted leprosy camera trap surveys and faecal sample collection at CNP, Guinea-Bissau, was granted by the University of Exeter, UK. The Institute for Biodiversity and Protected Areas in Guinea-Bissau approved and collaborated directly on all aspects of this research. Ethical approval for the work by the Taï Chimpanzee Project at TNP was given by the Ethics Commission of the Max Planck Society. The Centre Suisse de Recherches Scientifiques en Côte d'Ivoire collaborates on the research at TNP.

For human participants, this study was carried out under the ethical consent of the World Health Organization Global Leprosy Program surveillance network. All human participants gave written informed consent in accordance with the Declaration of Helsinki.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Sequence data are available from the National Center for Biotechnology Information Sequence Read Archive, BioProject (PRJNA664360) and BioSample (16207289–16207321). BioSample codes for all samples used in this study are given in the Supplementary Data. Other relevant data are available in the Article and its Supplementary Information.

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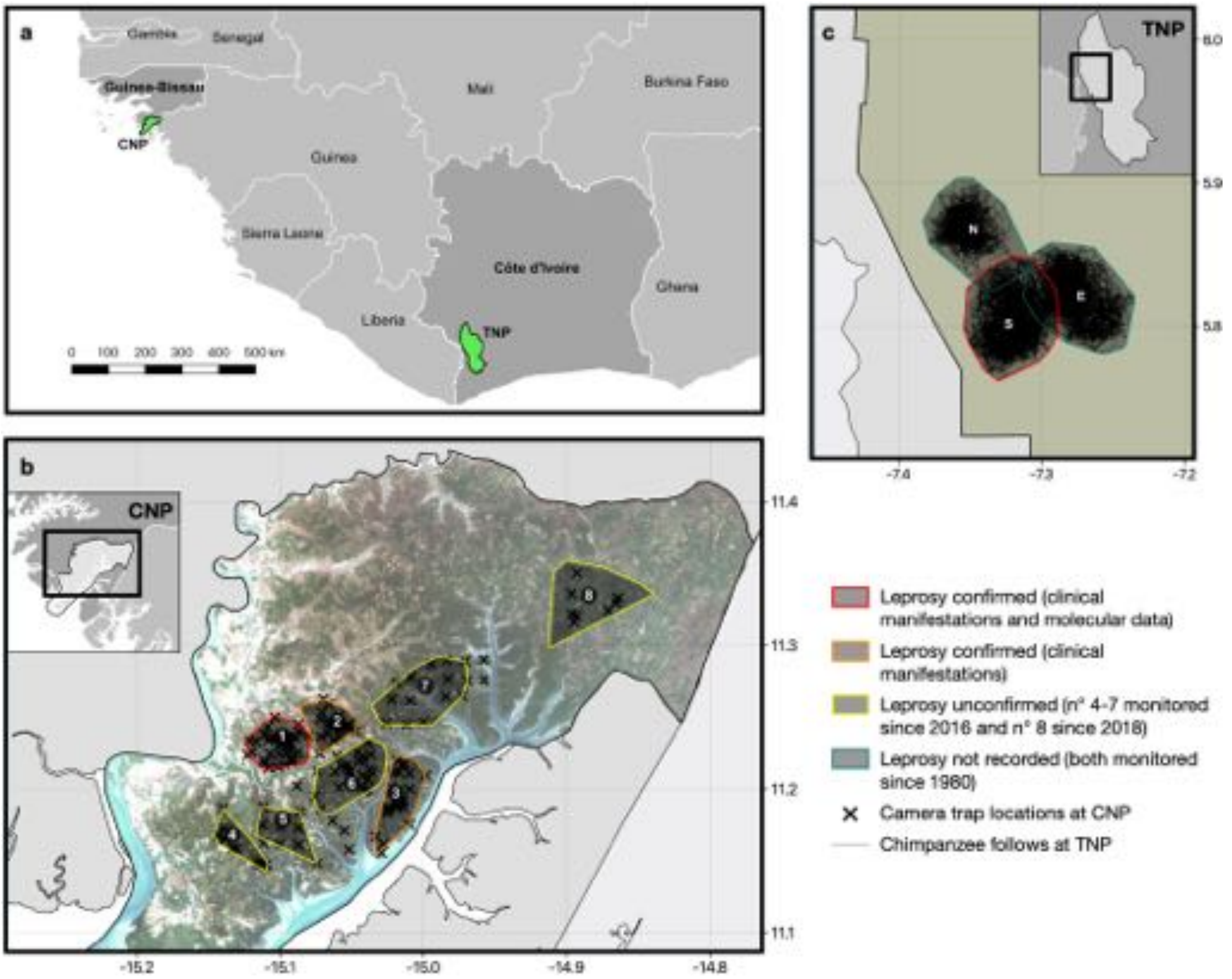
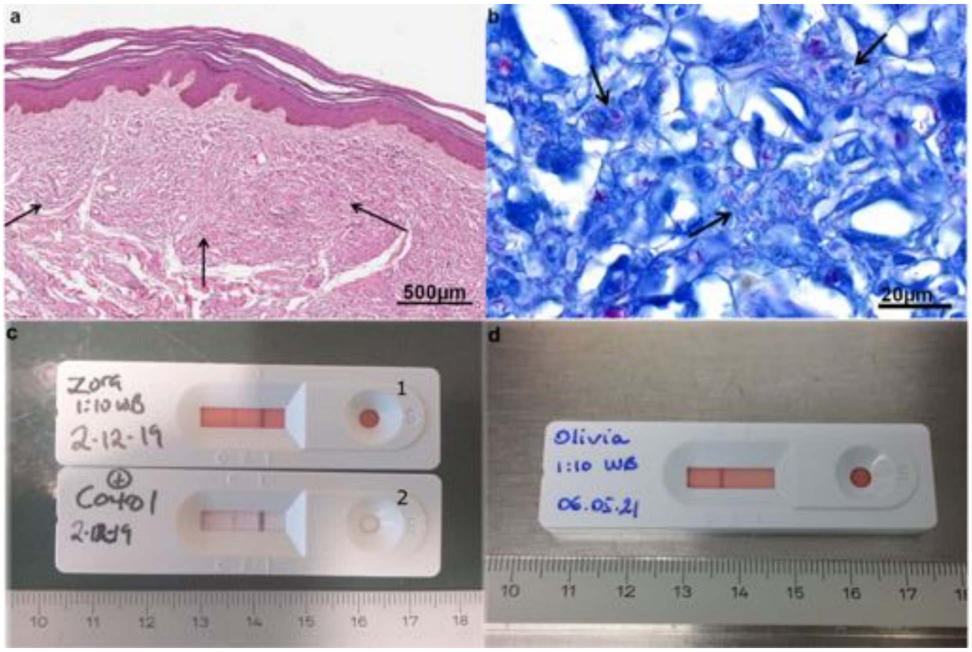


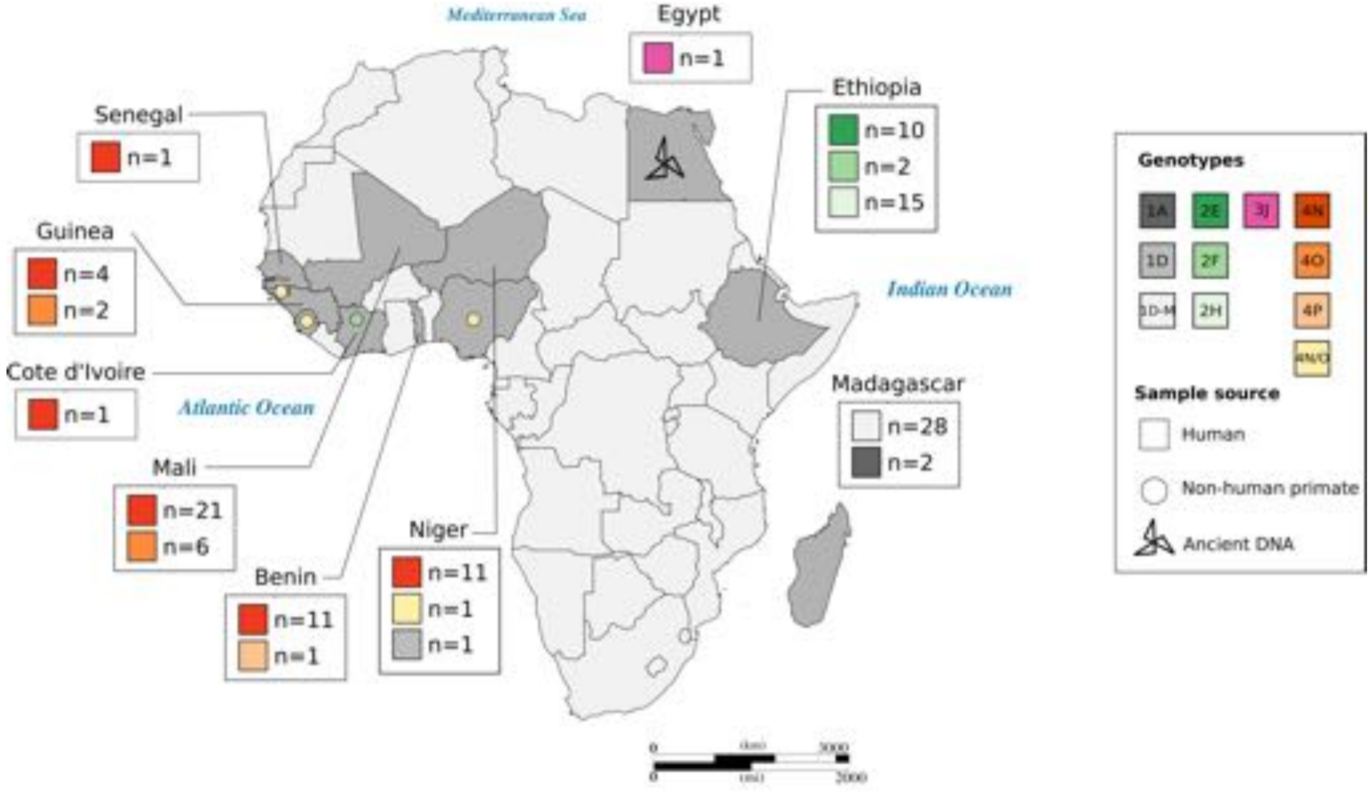
Extended Data Fig. 1 | **Maps of the chimpanzee study sites and chimpanzee communities. a**, Map of the CNP, Guinea-Bissau and the TNP, Côte d'Ivoire, West Africa. **b**, Location of the chimpanzee communities at CNP that were monitored between 2015 and 2019 (1, Caiquene-Cadique; 2, Lautchande; 3, Cambeque; 4, Cabante; 5, Canamine; 6, Madina; 7, Amindara; 8, Guiledje). Estimated home ranges of chimpanzee communities at CNP are shown by 100% minimum convex polygons of direct chimpanzee observations and indirect chimpanzee traces and nests during the study period. Red outline represents chimpanzee communities with at least one individual with clinical manifestations of leprosy, confirmed using molecular analysis; orange outline represents chimpanzee communities with at least one individual with clinical manifestations of leprosy; yellow colour represents monitored communities where clinical manifestations of leprosy have not been observed nor confirmed through molecular analysis. **c**, Location of the three habituated chimpanzee communities monitored at TNP (N, north; S, south; E, east). Estimated home ranges of chimpanzee communities at TNP are shown by 100% minimum convex polygons of direct chimpanzee follows from December 2013 to October 2016. Red outline represents the community with individuals with clinical manifestations of leprosy, confirmed using molecular analysis and serological tests; blue colour represents communities where leprosy has not been recorded.

Extended Data Fig. 2 | **Disease progression of leprosy in chimpanzees at CNP.** Adult female chimpanzee Rita over the course of 5 years (**a**–**d**) and disease manifestations in three additional adult chimpanzees (**e**–**g**). **a**, 2013/05 – Hypopigmentation of skin around the mouth and nose, small nodule on the lower lip and left ear (opportunistically recorded with a video camera before the start of longitudinal health monitoring with camera traps). **b**, 2015/12 – Large nodules between the upper lip and nose, with multiple small nodules on the eyelids, cheek, ears margins, lower lip and brow ridge. Small dry patches with hair loss on the wrists, knees and elbows. **c**, 2017/12 – Nodules increase in number, with apparent swelling and reddening, facial disfigurement and claw hand. Plaques appear on the wrist, knee and elbow joints, with an increase in hair thinning. **d**, 2018/05 – Face and ears completely covered by large nodules, with facial disfigurement and generalized hair loss on limbs and lower back. Nodule formation and swelling of fingers and toes, with disfigurement of hands and feet, and more severe claw hand. Some plaques on the body are ulcerated, and the individual has clear weight loss. **e**, Jimi (Lautchande in 2018/06) – First observation of lesions in 2015. The head is completely covered with multiple nodules of reddish colour, some of which are ulcerated. Ear margins are thickened. Hands and feet present nodules and plaques, and the scrotum is affected (not visible on picture). **f**, Baaba (Cambeque in 2017/08) – First observation of lesions in 2017. Multiple hypopigmented nodules on the brow ridge, cheek and upper and lower lips. Ears have thickened margins and nodules. There is hair thinning, with multiple small plaques present on the upper and lower limbs, back, abdomen and shoulders. **g**, Brinkos (Caiquene-Cadique in 2018/10) – First observation of lesions in 2015. Facial disfigurement, with the ulceration of nodules and a hanging lower lip. Hands and feet are ulcerated, and fingers are swollen. There are nodules on the nipples, and plaques covering the lower back, shoulders and arm are ulcerated, with hair loss.

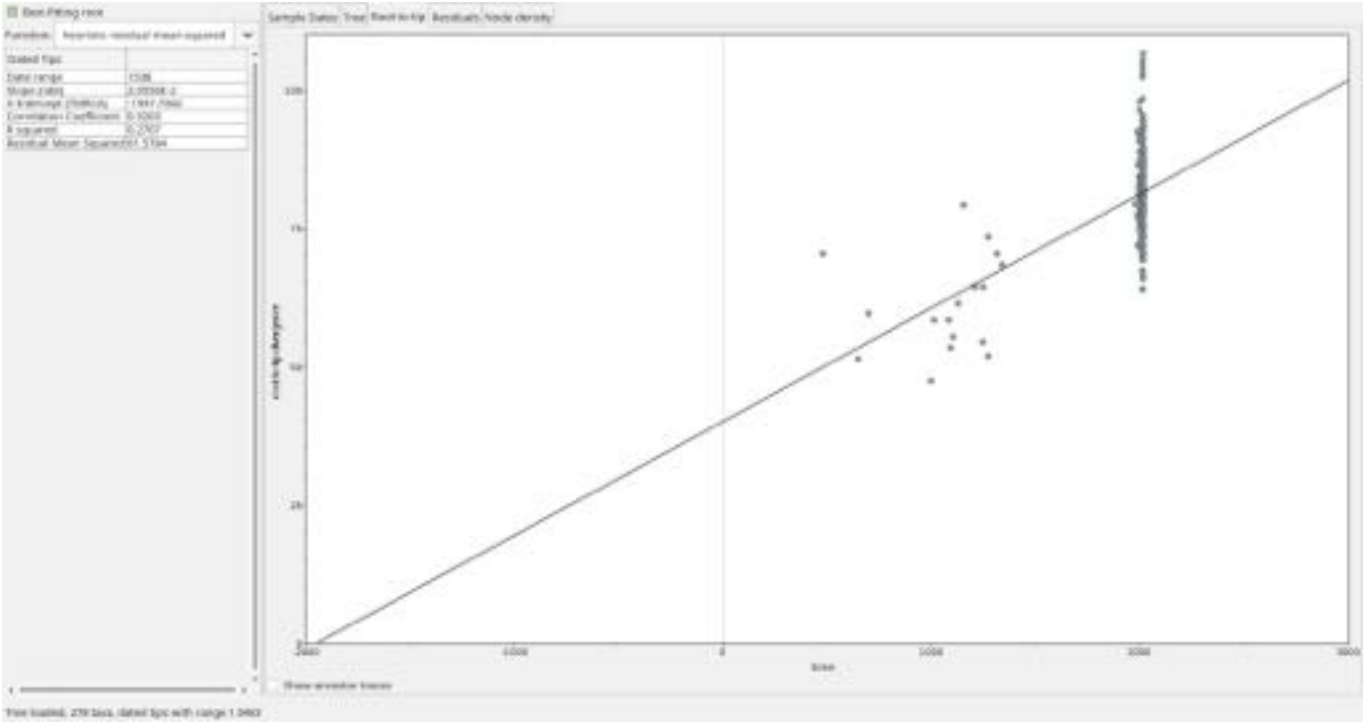
Extended Data Fig. 3 | Disease progression of leprosy in an adult male chimpanzee at TNP (Woodstock) over the course of 2 years (2018–2020) (a–i) and an adult female chimpanzee at TNP (Zora) over the course of 2008–2009 (j–m). **a**, 2017/01 – Woodstock before the appearance of clinical signs. **b**, 2018/06 – First hypopigmented nodules appear on the face (arrows), with swelling and hypopigmentation on both hands, and ulceration on the right hand. **c**, 2018/10 – Existing nodules increase in size and new smaller ones appear (arrows). Development of mucopurulent discharge from the left eye, and lower eyelid is turned outward. Hair loss and ulceration on dorsal part of right wrist and hand. **d**, 2019/04 – Most existing nodules increase in size and become pedunculated, and the nodule under the eye shrinks, and several new nodules appear (see arrows). Suspected start of nasal involvement, and right ear starts to become disfigured. Both hands are slightly swollen and hypopigmented, with the loss of nail plate on the fourth finger of the left hand, and the third and fifth fingers show early stage of abnormal nail overgrowth. **e**, 2019/10 – Facial lesions increase in size, and some become darkly pigmented. New lesions appear on the brow ridge, with nodules above the lips and between the lips, and the nose becomes pedunculated. The loss of nail plate, and nail bed becomes exposed on the first and second fingers of the left hand. **f**, 2020/04 – In general, facial nodules seem smaller than before, and the nodule under the left eye disappears. On the left hand, the nail of the fourth finger shows an advanced stage of abnormal nail overgrowth, and the third and fifth fingernails show early stage of abnormal nail overgrowth. **g**, 2020/07 – Facial nodules seem larger with many hypopigmented, and both ears are swollen and disfigured. Nasal involvement becomes apparent. Both hands are swollen and hypopigmented. Skin ulcerations present on the right hand, with possible claw hand on the left hand. **h**, 2019/04 – Slight hypopigmentation of scrotum. **i**, 2020/07 – Reddening and ulceration of scrotum; fresh blood observed. **j**, 2007/12 – Zora before the appearance of clinical signs of leprosy. **k**, 2008/01 – Appearance of nodules on the right ear and both eyebrow ridges. **l**, 2008/12 – Appearance of nodules on the left ear, and ulceration of the skin at the second, third and fourth proximal interphalangeal joint level of the right hand. **m**, 2009/04 – Nodular lesions on both ears and brow ridge seem aggravated, with nodular lesions on the lips, and above the mouth (four months before death).



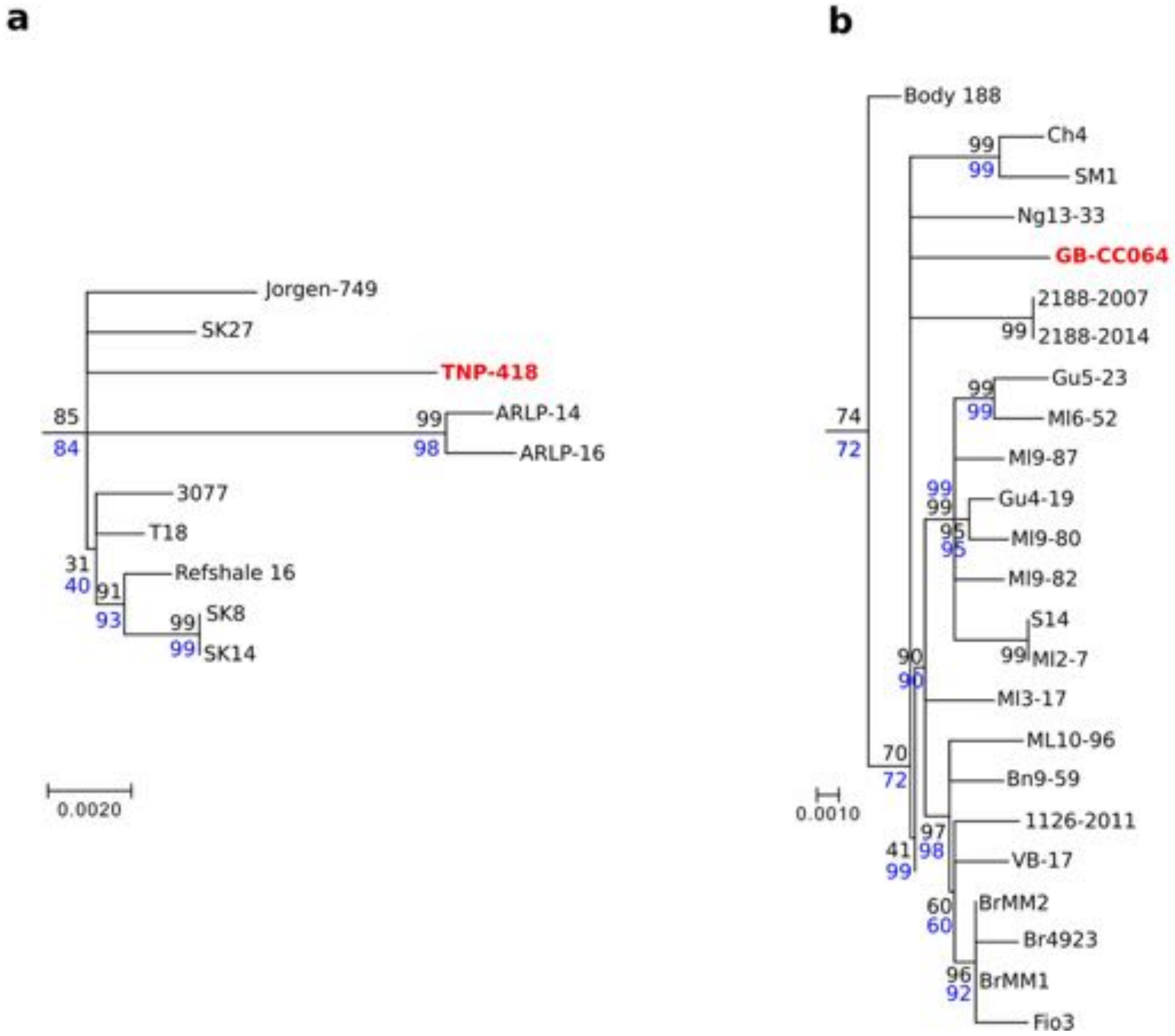
Extended Data Fig. 4 | Confirmation of leprosy infection in Zora through histopathology of skin sample and lateral flow test. a, Lepromatous leprosy, skin with diffuse histiocytic infiltrate in the dermis. The haematoxylin and eosin stain was conducted once; scale bar, 500 µm. **b**, Lepromatous leprosy, skin, acid-fast bacilli in histiocytes. The inflammatory infiltrate consists predominantly of histiocytes admixed with fewer lymphocytes. Histiocytes show foamy or vacuolated cytoplasm and containing bacteria surrounded by a clear zone. Fite-Faraco stain; scale bar, 20 µm. Fite-Faraco stain was conducted once and was controlled by a positive control slide containing mycobacteria. **c**, whole blood from Zora (1) and the positive control (2). **d**, whole blood from a chimpanzee at TNP (Olivia) not infected with *M. leprae*, used as negative control. C, control lane; T, test lane.



Extended Data Fig. 5 | Geographical distribution of *M. leprae* genotypes in Africa based on genome data. The genotype 2F has never been reported in West Africa and is the least identified in Ethiopia. The genotype 4N/O was only reported in one human sample from West Africa. Data included only *M. leprae* genomes (Supplementary Note 5 and Supplementary Table 7). The map was downloaded from https://www.amcharts.com/svg-maps/ under a free licence and modified for the current figure in Inkscape, an open source digital illustration software package (https://inkscape.org).

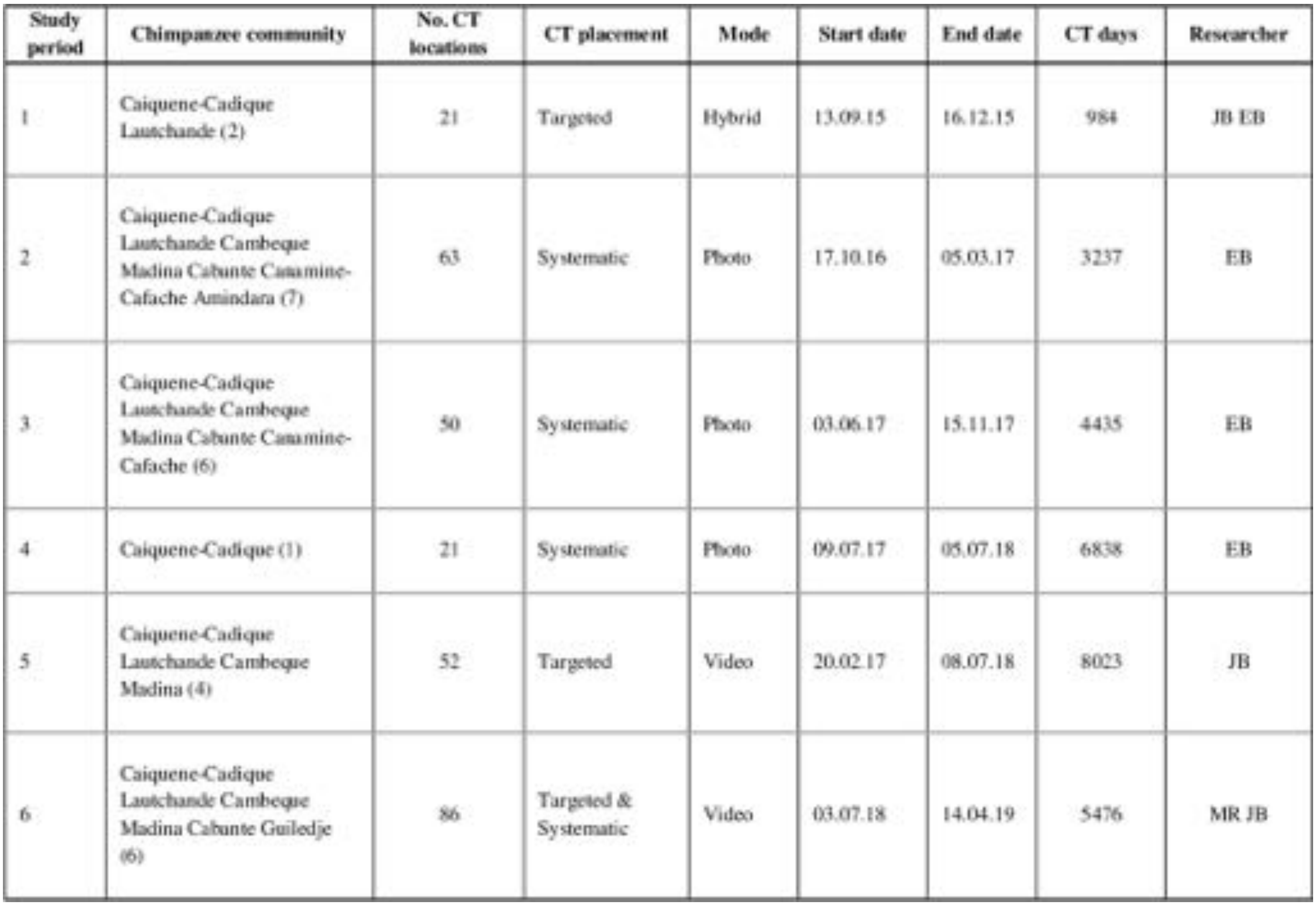


Extended Data Fig. 6 | Best-fitting root analysis using TempEst.



Extended Data Fig. 7 | Maximum Likelihood tree to confirm the topological placement of GB-CC064 and TNP-418. 286 genomes (Supplementary Table 6) were used, including the two new chimpanzee strains (in bold red), 500 bootstrap replicates (value in black with the Tamura Nei model and in blue for the general time model) and *M. lepromatosis* as outgroup. Sites with missing data were partially deleted (80% genome coverage cutoff). **a**, Maximum Likelihood tree of the branch 2F. **b**, Maximum Likelihood tree of the branch 4.

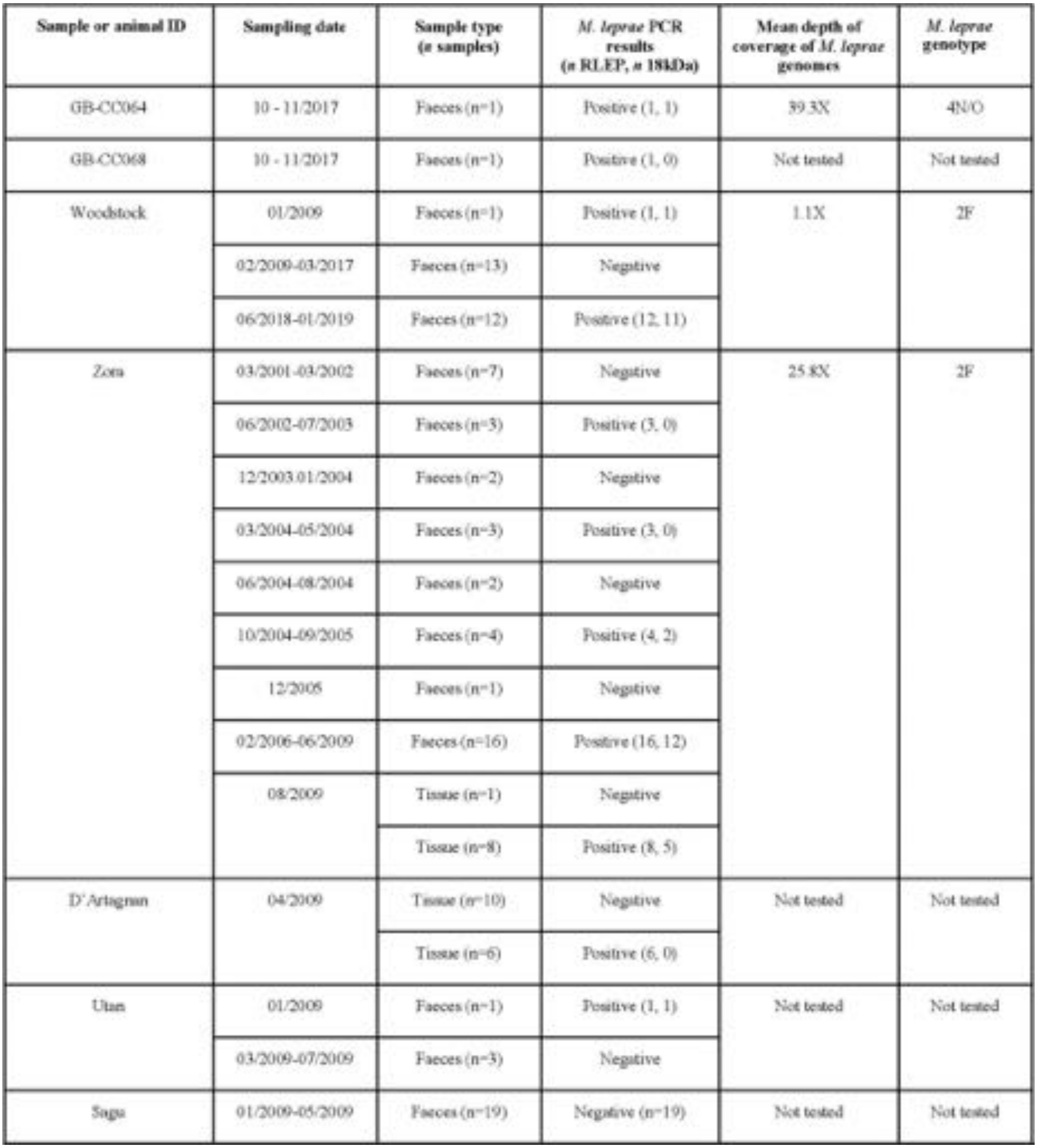
Extended Data Table 1 | The camera trap (CT) study periods with the focal chimpanzee community within Cantanhez National Park



Study period	Chimpanzee community	No. CT locations	CT placement	Mode	Start date	End date	CT days	Researcher
1	Caiquene-Cadique Lautchande (2)	21	Targeted	Hybrid	13.09.15	16.12.15	984	JB EB
2	Caiquene-Cadique Lautchande Cambeque Madina Cabunte Canamine-Cafache Amindara (7)	63	Systematic	Photo	17.10.16	05.03.17	3237	EB
3	Caiquene-Cadique Lautchande Cambeque Madina Cabunte Canamine-Cafache (6)	50	Systematic	Photo	03.06.17	15.11.17	4435	EB
4	Caiquene-Cadique (1)	21	Systematic	Photo	09.07.17	05.07.18	6838	EB
5	Caiquene-Cadique Lautchande Cambeque Madina (4)	52	Targeted	Video	20.02.17	08.07.18	8023	JB
6	Caiquene-Cadique Lautchande Cambeque Madina Cabunte Guiledje (6)	86	Targeted & Systematic	Video	03.07.18	14.04.19	5476	MR JB

The number of distinct CT locations for that study period is included (total number of CT locations = 211). Certain CT locations were used in more than one study period (Supplementary Table 1). For targeted CT placement if no chimpanzees were filmed for a certain period CTs were repositioned; hence not all cameras were working at the same time. The placement design of CTs was targeted or systematic. Targeted CTs were deployed to maximize detection of chimpanzees (such as chimpanzee drumming sites, fruiting trees and trails). Systematic CTs were placed following a survey design maximizing independence between CT sites and chimpanzee detection. The CT mode was either set to photograph or video or both (hybrid) and CTs were active for 24 h per day. The start and end dates of each study period are included as well as the number of CT days. CT days are the sum of number of days for each active CT after removing days when cameras were inactive due to malfunctioning, batteries running out, trees falling in front of the CT or theft (total CT days = 28,993). The researcher initials are included (J.B., Joana Bessa; E.B., Elena Bersacola; M.R., Marina Ramon).

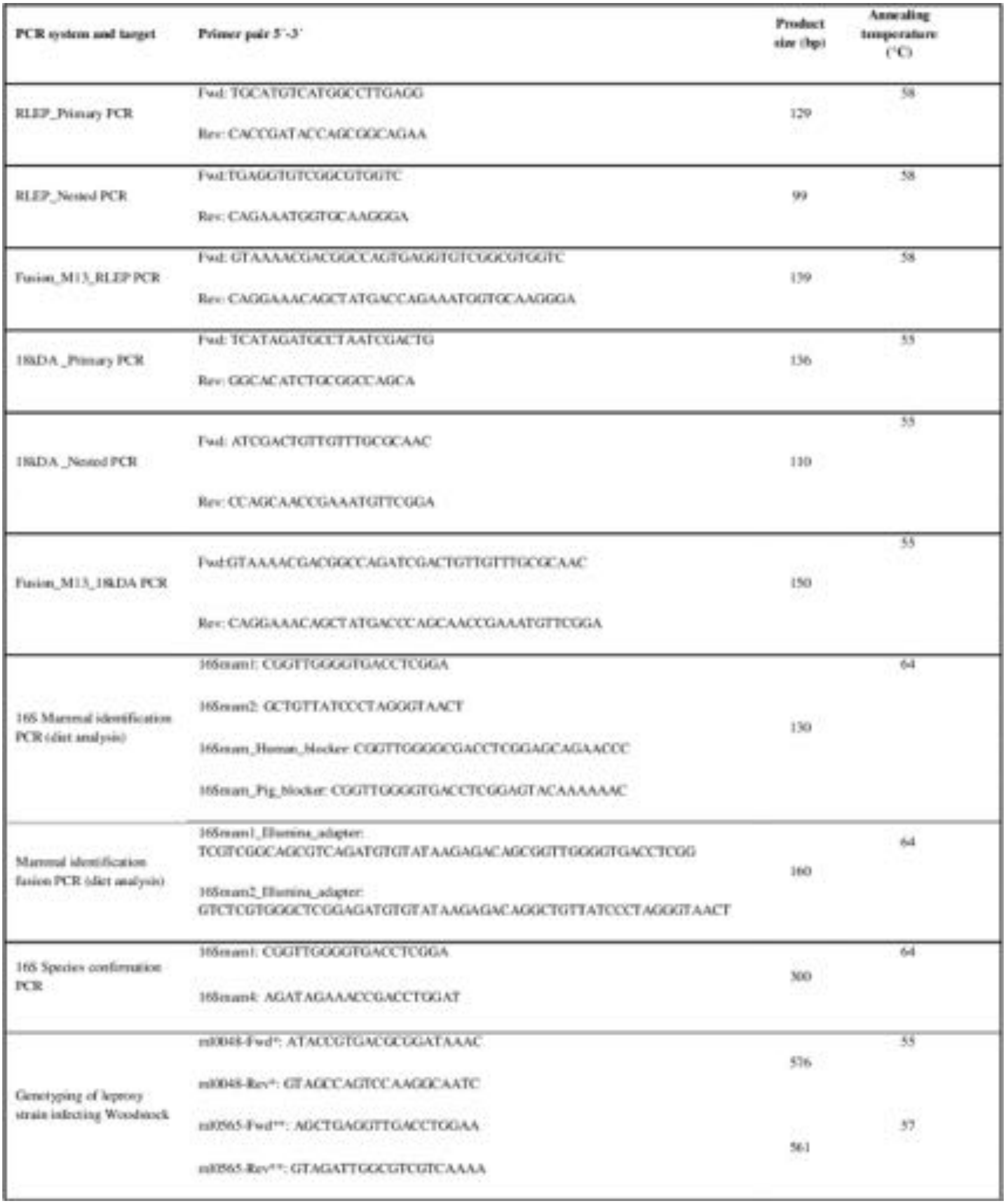
Extended Data Table 2 | Samples (CNP) and animals (TNP) that tested positive for *M. leprae* DNA



Sample or animal ID	Sampling date	Sample type (*n* samples)	*M. leprae* PCR results (*n* RLEP, *n* 18kDa)	Mean depth of coverage of *M. leprae* genomes	*M. leprae* genotype
GB-CC064	10 - 11/2017	Faeces (n=1)	Positive (1, 1)	39.3X	4N/O
GB-CC068	10 - 11/2017	Faeces (n=1)	Positive (1, 0)	Not tested	Not tested
Woodstock	01/2009	Faeces (n=1)	Positive (1, 1)	1.1X	2F
	02/2009-03/2017	Faeces (n=13)	Negative		
	06/2018-01/2019	Faeces (n=12)	Positive (12, 11)		
Zora	03/2001-03/2002	Faeces (n=7)	Negative	25.8X	2F
	06/2002-07/2003	Faeces (n=3)	Positive (3, 0)		
	12/2003.01/2004	Faeces (n=2)	Negative		
	03/2004-05/2004	Faeces (n=3)	Positive (3, 0)		
	06/2004-08/2004	Faeces (n=2)	Negative		
	10/2004-09/2005	Faeces (n=4)	Positive (4, 2)		
	12/2005	Faeces (n=1)	Negative		
	02/2006-06/2009	Faeces (n=16)	Positive (16, 12)		
	08/2009	Tissue (n=1)	Negative		
		Tissue (n=8)	Positive (8, 5)		
D'Artagnan	04/2009	Tissue (n=10)	Negative	Not tested	Not tested
		Tissue (n=6)	Positive (6, 0)		
Utan	01/2009	Faeces (n=1)	Positive (1, 1)	Not tested	Not tested
	03/2009-07/2009	Faeces (n=3)	Negative		
Sugu	01/2009-05/2009	Faeces (n=19)	Negative (n=19)	Not tested	Not tested

Samples are considered as PCR-positive if they were positive either in the RLEP or in the RLEP and the 18-kDa assay (see Supplementary Table 2 for additional information).

Extended Data Table 3 | Primers used for the identification of *M. leprae* in chimpanzee tissues and faeces, diet analysis, the genotyping of *M. leprae* strains and confirmation of chimpanzee origin of the samples



PCR system and target	Primer pair 5'-3'	Product size (bp)	Annealing temperature (°C)
RLEP_Primary PCR	Fwd: TGCATGTCATGGCCTTGAGG Rev: CACCGATACCAGCGGCAGAA	129	58
RLEP_Nested PCR	Fwd:TGAGGTGTCGGCGTGGTC Rev: CAGAAATGGTGCAAGGGA	99	58
Fusion_M13_RLEP PCR	Fwd: GTAAAACGACGGCCAGTGAGGTGTCGGCGTGGTC Rev: CAGGAAACAGCTATGACCAGAAATGGTGCAAGGGA	139	58
18kDA _Primary PCR	Fwd: TCATAGATGCCTAATCGACTG Rev: GGCACATCTGCGGCCAGCA	136	55
18kDA _Nested PCR	Fwd: ATCGACTGTTGTTTGCGCAAC Rev: CCAGCAACCGAAATGTTCGGA	110	55
Fusion_M13_18kDA PCR	Fwd:GTAAAACGACGGCCAGATCGACTGTTGTTTGCGCAAC Rev: CAGGAAACAGCTATGACCCAGCAACCGAAATGTTCGGA	150	55
16S Mammal identification PCR (diet analysis)	16Smam1: CGGTTGGGGTGACCTCGGA 16Smam2: GCTGTTATCCCTAGGGTAACT 16Smam_Human_blocker: CGGTTGGGGCGACCTCGGAGCAGAACCC 16Smam_Pig_blocker: CGGTTGGGGTGACCTCGGAGTACAAAAAAC	130	64
Mammal identification fusion PCR (diet analysis)	16Smam1_Illumina_adapter: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGGTTGGGGTGACCTCGG 16Smam2_Illumina_adapter: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGTTATCCCTAGGGTAACT	160	64
16S Species confirmation PCR	16Smam1: CGGTTGGGGTGACCTCGGA 16Smam4: AGATAGAAACCGACCTGGAT	300	64
Genotyping of leprosy strain infecting Woodstock	ml0048-Fwd*: ATACCGTGACGCGGATAAAC ml0048-Rev*: GTAGCCAGTCCAAGGCAATC	576	55
	ml0565-Fwd**: AGCTGAGGTTGACCTGGAA ml0565-Rev**: GTAGATTGGCGTCGTCAAAA	561	57

Fwd, forward; Rev, reverse. *Mutation C1193T in *ml0048* (genome position 60123); a T is found in TNP-418 and TNP-566. **Mutation C319T in *ml0565* (genome position 683097); a T is found in TNP-418 and TNP-566.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used
Data analysis	All raw reads were adapter- and quality-trimmed with Trimmomatic v0.35. The quality settings were “SLIDINGWINDOW:5:15 MINLEN:40”. Paired-end (PE) data were additionally processed with SeqPrep (https://github.com/jstjohn/SeqPrep) to merge overlapping pairs. Preprocessed reads were mapped onto the M. leprae TN reference genome (GenBank AL450380.1) with Bowtie2 v2.2.5. SNP calling was done using VarScan v2.3.9. To avoid false-positive SNP calls the following cutoffs were applied: minimum overall coverage of five non-duplicated reads, minimum of three non-duplicated reads supporting the SNP, mapping quality score >8, base quality score >15, and a SNP frequency above 80%. InDel calling was done using Platypus v0.8.1 followed by manual curation. We used the Integrative Genomics Viewer v 2.8.13 and Basic Local Alignment Search Tool (BLAST) v 2.11.0+. Dating analyses were done using BEAST2 v2.5.2.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sequence data are available from the NCBI Sequence Read Archive (SRA) Bioproject PRJNA664360 Biosamples SAM16207289-16207321. Biosample codes for all samples used in this study are given in the Supplementary Data. Other relevant data supporting the findings of the study are available in this published article and its Supplementary Information files.

Field-specific reporting

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Study description – We report on leprosy-like lesions in two wild populations of western chimpanzees in the Cantanhez National Park (CNP), Guinea-Bissau, and the Taï National Park (TNP), Côte d'Ivoire, West Africa. We screen chimpanzee faecal and necropsy samples for the presence of M. leprae and conduct phylogenomic comparisons with other strains from humans and other animals.
Research sample	The research sample is represented by two populations of wild chimpanzees (Pan troglodytes verus) in CNP and TNP. We conducted this study in these two populations in response to leprosy-like lesions observed during behavioural monitoring. We did not discriminate between age and sex classes, instead we collected data on as many individuals as possible for analysis of leprosy symptoms. Analyses in this paper focus on symptomatic individuals. These two chimpanzee populations include male and female individuals and age estimates range from newborn to adult (~40 years of age). There are a minimum of 12 chimpanzee communities at CNP, all unhabituated to researchers, with approximately 35-60 individuals per community (age and sex composition of all communities unknown). At one community (Caiquene-Cadique), we estimate at least 48 individuals, including 16 adult females, 13 adult males, 3 subadults and 16 immatures (juveniles and infants). At TNP, the three human-habituated chimpanzee communities include a total of 91 individually recognised chimpanzees.
Sampling strategy	We performed non-invasive sampling through the collection of faeces from symptomatic and asymptomatic chimpanzees at CNP and TNP. In CNP, where chimpanzees are not habituated to human observers, this is performed by collecting faecal material found under chimpanzee nests or in proximity to chimpanzee signs (e.g. food remains or knuckle prints). At the time of faecal collection, the identity of the chimpanzee was not known. At CNP, camera traps were deployed at 211 locations including across different habitat types within the home range of eight of the 12 putative chimpanzee communities. Targeted camera traps were deployed to record and monitor chimpanzee behaviour and disease occurrence. Systematic camera traps were deployed across central CNP at a minimum distance of 1km between sampling points. At TNP chimpanzees are followed by researchers on a daily basis and faeces are collected right after observing defecation. In both cases, faeces are collected with the aid of a plastic or wooden spatula and placed in 2ml or 15ml tubes dry or with RNAlater. For this study we analysed all available faecal samples from individuals which displayed clinical signs of leprosy and optimal sample sizes could not be determined beforehand. For TNP we included only samples from the South community since leprosy was observed only in members of this chimpanzee community. Necropsies on dead chimpanzees were performed by trained veterinarians at TNP as part of the health monitoring program. For this study, we tested all available chimpanzee necropsy samples in our collection.
Data collection	Data collection was performed by local field assistants, researchers and veterinarians working at CNP and TNP. At CNP, clinical data on unhabituated chimpanzees were collected using camera traps and faecal samples were collected with the aid of a wooden spatula and stored at ambient temperature in 15ml tubes containing NAP buffer. At TNP, data were collected by research assistants both on paper sheets and using the Cybertracker app, and by veterinarians who documented via pictures and videos. At TNP, the long-term health monitoring program includes continuous collection of faecal and urine samples from known adult chimpanzees. Faeces are transferred in 2ml cryotubes with the aid of a plastic spatula and frozen in liquid nitrogen. A full necropsy is systematically performed on chimpanzees found dead by the on-site veterinarian. Tissue samples of several internal organs are taken if the state of carcass decomposition allows.
Timing and spatial scale	Camera traps were set up over six data collection periods ranging from 2015 to 2019 across CNP (1067 km2). There were six study periods in total: (1) 13.09.15-16.12.15 (984 camera trap (CT) days, targeted CT placement, 2 communities); (2) 17.10.16-05.03.17 (3237 CT days, systematic, 7 communities); (3) 03.06.17-15.11.17 (4435 CT days, systematic, 6 communities); (4) 09.07.17-05.07.18 (6838 CT days, systematic, 1 community); (5) 20.02.17-08.07.18 (8023 CT days, targeted, 4 communities); (6) 03.07.18-14.04.19 (5476 CT days, targeted and systematic, 6 communities). Data collection was stopped once we had obtained sufficient camera trap footage to determine leprosy presence across chimpanzee communities. Since 2020, the Cantanhez Chimpanzee Project has continued monitoring the health of this population. At TNP sample collection for the project started in 1994 and has been routinely carried out ever since. Over 25 years we have accumulated a collection of chimpanzee faecal and urine samples and necropsy samples from all wildlife found dead in the area. For this study, we tested samples collected between 1998 and 2019.
Data exclusions	No specifica data were excluded from the study.
Reproducibility	To confirm our results of leprosy infection we used two PCR systems in parallel and tested several samples for each individual/community. Positives were then further confirmed via next generation sequencing. For this purpose several individual libraries were generated to confirm M. leprae DNA presence in the samples.
Randomization	Randomization is not relevant for this type of study, which is based on investigating infectious causes of illness in wildlife. To maximize our chances of pathogen detection we sampled all individuals, whenever possible.

Blinding	Not applicable to this study since this is a study on a naturally occurring disease in wild animals.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Guinea-Bissau (36,125 km2), West Africa, lies within the Guinean forest-savannah mosaics, a biodiverse ecoregion buffering the Guinean moist forests in the south and the West Sudanian savannah in the north. The climate in Guinea-Bissau is characterized by a rainy season from mid-May to the end of October and a long dry season from November to mid-May. Cantanhez NP (N11° 14.287′ W15° 02.281′) comprises the Cubucaré peninsula in the Tombali Region bordering Guinea-Conakry. The landscape in Cantanhez NP consists of a mosaic of coastal sub-humid forest patches, mangroves, savannah grassland, woodland and agriculture including mostly cashew orchards, shifting cultivation fields and mangrove swamp rice fields. Approximately 24,000 people across 200 villages and settlements are present inside the park. The TNP (5,082 km 2), located in the south-west of Ivory Coast bordering Liberia (N5° 38 56 W7° 05 43), consists of an evergreen lowland rainforest and is the largest remaining primary forest fragment in West Africa. It is home to a wide range of mammals that include 11 different nonhuman primate species. There are no settlements or agricultural areas inside the National Park. The climate in TNP is characterized by a rainy season from March/April to the end of October and a dry season from November to February/March.
Location	Tai National Park, Ivory Coast and Cantanhez National Park, Guinea Bissau
Access and import/export	Research conducted at CNP is authorised by the Institute for Biodiversty and Protected Areas (IBAP) in Guinea-Bissau, who are partners and co-authors on this research. All research at TNP is conducted under the umbrella of a collaboration with Ivorian partners and health authorities. Samples are routinely exported to Germany for diagnostic purposes following international guidelines and prior official authorization through CITES permits, where necessary. CITES permits for importing necropsy samples from Ivory Coast are regulary issued to the RKI. The most recent ones were issued on March 30th 2021 under the number DE-E-05895/20 and DE-E-05896/20.
Disturbance	All activities conducted for this study were carried out as part of the Cantanhez Chimpanzee Project and the Tai Chimpanzee Project. All samples and observations collected are done with the minimum disturbance to wildlife and the environment. At CNP, camera traps are used to collect data and cause minimum disturbance to chimpanzees. Faecal samples are collected when animals are no longer at the site. At TNP, a minimum distance of 7 meters is maintained from chimpanzees and samples are collected after the animals have moved away. Only non-invasive samples such as faeces and urine are collected.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study	
☒	☐	Antibodies
☒	☐	Eukaryotic cell lines
☒	☐	Palaeontology
☐	☒	Animals and other organisms
☐	☒	Human research participants
☒	☐	Clinical data

Methods

n/a	Involved in the study	
☒	☐	ChIP-seq
☒	☐	Flow cytometry
☒	☐	MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	This study did not involve laboratory animals.
Wild animals	At CNP, chimpanzees are not habituated to human observers and all data are collected remotely using camera traps. The age and sex distribution of chimpanzees within this population have not be calculated (as this requires accurately identifying all individuals). At one community (Caiquene-Cadique), we estimate at least 48 individuals, including 16 adult females, 13 adult males, 3 subadults and 16 immatures (juveniles and infants). At TNP, wild chimpanzee communities have been habituated by researchers since 1979. A team of field assistants and researchers follow the animals on a daily basis from a 7-meter distance, recording behavioural data and collecting faeces and urine samples whenever possible. In normal situations, each assistant or researcher has one focal individual per day to collect data and samples from. In disease outbreak situations, monitoring efforts are reinforced and sampling is attempted from all symptomatic and asymptomatic individuals. These populations include male and female individuals and estimation of age range is from newborn to adult (~40 years of age). As of March 2021, there are 91 individuals (40 males and 51 females), including 43 adults (14 males and 29 females), 5 adolescents (4 males, and 1 female), 19 juveniles (6 males and 13 females), and 24 infants (16 males and 8 females).
Field-collected samples	At CNP, chimpanzee faecal samples are collected by visiting chimpanzee nesting and feeding sites. Faecal samples were stored at room temperature in 15ml tubes containing NAP buffer, and shipped to Robert Koch Institute in Germany. At TNP, samples are

	collected upon defecation or urination of the chimpanzees and stored in 2ml cryotubes. The research camps of the Tai Chimpanzee Project are equipped with liquid nitrogen tanks for storage of samples. Samples are then transported to Abidjan for temporary storage at the Centre Suisse de Recherches Scientifiques and subsequently shipped to RKI on dry ice whenever someone is traveling. Since these samples were collected from wild living animals, no other parameter needs to be specified (e.g. housing or photoperiod).
Ethics oversight	All data were collected in accordance with Best Practise Disease and Monitoring Guidelines of the Great Ape Section of IUCN Primate Specialist Group. The collection of samples was strictly non-invasive. All proposed data collection and analyses adhered strictly to ethics guidelines of the Association for the Study of Animal Behaviour (UK). Ethical approval for targeted leprosy camera trap surveys and faecal sample collection at CNP, Guinea-Bissau, was granted by the University of Exeter, UK. The Institute for Biodiversity and Protected Areas (IBAP) in Guinea-Bissau approved and collaborated directly on all aspects of this research. Ethical approval for the work at Tai Chimpanzee Project was given by the Ethics Commission of the Max Planck Society. The Centre Suisse de Recherches Scientifiques en Côte d'Ivoire collaborates on the research at TNP.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	M. leprae strains were collected from skin samples of newly diagnosed patients with positive bacillary index. These were obtained from the respective National Leprosy Control Programs in the framework of the leprosy drug resistance surveillance programs. Among the 21 patients included retrospectively in this study, seven were female and 13 were male (one unknown), ranging from 18 to 80 years in age. They originated from Mali (n=8), Benin (n=6), Niger (n=5), Côte d'Ivoire (n=1) and Senegal (n=1).
Recruitment	Patients were not recruited for this study. Inform consent were collected by the respective National Leprosy Control Programs during diagnosis to allow the use of the M. leprae strain genetic informations.
Ethics oversight	This study was carried out under the ethical consent of the WHO Global Leprosy Program surveillance network. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

APPENDIX B

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Research

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Inter-community behavioural variation confirmed through indirect methods in four neighbouring chimpanzee communities in Cantanhez NP, Guinea-Bissau

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Culture, while long viewed as exclusively human, has now been demonstrated across diverse taxa and contexts. However, most animal culture data are constrained to well-studied, habituated groups. This is the case for chimpanzees, arguably the most 'cultural' non-human species. While much progress has been made charting wild chimpanzees' cultural repertoire, large gaps remain in our knowledge of the majority of the continent's chimpanzees. Furthermore, few studies have compared neighbouring communities, despite such comparisons providing the strongest evidence for culture, and few have studied communities living in anthropogenic habitats although their culture is in imminent danger of disappearing. Here we combine direct, indirect and remote methods, including camera traps, to study, over 2 years, four unhabituated neighbouring chimpanzee communities inhabiting human-impacted habitats in Cantanhez NP, Guinea-Bissau. From traces collected during 1089 km of reconnaissance walks and 4197 videos from 56 camera trap locations, we identified 18 putative cultural traits. These included some noteworthy novel behaviours for these communities, and behaviours possibly new to the species. We created preliminary behavioural profiles for each community, and found inter-community differences spanning tool use, communication, and social behaviour, demonstrating the importance of comparing

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[†]These authors contributed equally to this work.

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5849139.

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neighbouring communities and of studying previously neglected communities including those inhabiting anthropogenic landscapes.

1. Introduction

Animal culture, defined as patterns of behaviour that are group-typical and transmitted, at least in part, through social learning [1], is argued to be a source of adaptive behaviour: individuals can more readily discover the solution to a problem if they attend to or copy the behaviour of other individuals facing the same problem, allowing them to better exploit their natural and social environment. Furthermore, cultural traditions can influence evolutionary rates and trajectories by homogenizing behaviours within a population and allowing rapid changes in a population's behavioural repertoire [3]. Overall, the study of animal culture can inform researchers of different ways animals adapt to their environment (e.g. foraging techniques, migratory patterns, communication of information), and might be a valuable tool when planning conservation strategies [2,4–6]. The first evidence for animal culture was described over 70 years ago where the transmission of new behaviour (milk-bottle opening) by titmice (*Parus* sp.) was tracked across the United Kingdom [7]. Soon after, novel foraging behaviours that spread through kin networks were found in Japanese macaques (*Macaca fuscata*) (e.g. sweet potato washing) [8], and regional birdsong dialects were discovered in white-crowned sparrows (*Zonotrichia leucophrys)* [9]. Since then, evidence of animal culture has been identified in numerous taxa, from fish to meerkats, cetaceans and apes (e.g. [10–14]), both in the wild and in captivity.

Multiple approaches to studying animal culture have been proposed. Among these, the one most widely used in the wild to date is the ethnographic method [15] or 'method of exclusion' [16] that identifies culture by ruling out possible ecological or genetic explanations for inter-group behavioural variation documented. A paradigmatic example of this method was the first large-scale chimpanzee cross-population study that extracted 39 candidate behavioural traits from six habituated chimpanzee communities in West and East Africa [17]. These included habitual or customary behaviours that could not be explained by ecological or genetic differences between populations, hence by exclusion were considered cultural variants. Some experimental methods rely on a similar rationale: for example, translocation experiments, where individuals are moved from one population to another or whole populations are exchanged between sites [10,18], seek to establish whether existing behavioural variation is more likely to be traditional than due to genetic or environmental influences. Nonetheless, many argue that methods of exclusion, if rigorously applied, might erroneously reject cases of animal culture (e.g. [2,15,19]), and in fact, ecology, genetics and social learning are inexorably interlinked and can all influence, to some degree, behavioural variation [19,20]. For more direct demonstrations of cultural processes, some researchers have employed elegant field experiments where new resources or information are artificially introduced into a wild community to observe, in real time, their diffusion through social learning (e.g. [21,22]). Nonetheless, although these studies allow us to confirm that new behaviours can spread through groups through social learning, they do not inform us about the nature and spread of naturally occurring behavioural variation [11]. Statistical approaches try to tackle this problem: for example, network-based diffusion analysis (NBDA) has been used to study the transmission of newly invented behaviours in chimpanzees [23], humpback whales [14] and bottlenose dolphins [24]. While powerful when the right data are available, one limitation of the NBDA approach is that it requires long-term data collection on the same population, as well as some degree of luck in witnessing the natural emergence and transmission of a novel behaviour.

While large-scale ethnographic studies have given us valuable insights into species-level variation in putatively cultural behaviours [17,25], it has been suggested that more compelling evidence for culture might come from studying the same subspecies (e.g. [12,19]). For example, while all four subspecies of *Pan troglodytes*, but not all known communities, engage in army ant (*Dorylus* spp.) dipping, there is variation in the technology employed even among different communities of the same subspecies [26]. Some of this variation can be linked to the aggressiveness of the ant species exploited, but in some cases, such an ecological account does not fully explain the variation found [5,27]. Therefore, many have recently suggested that the study of neighbouring communities might be the most informative approach to studying animal culture in the wild (e.g. [11,12,28,29]): comparing communities where groups broadly face the same ecological constraints and individuals migrate between communities, make ecological or genetic explanations for behavioural variation less likely or important compared to a cultural explanation. Illustrating this approach, Luncz *et al.* [12] compared the selection of wooden and stone hammers for coula (*Coula edulis*) nut-cracking in three habituated neighbouring chimpanzee communities in Tai National Park, Ivory Coast. Even though these neighbouring communities inhabit the same forest habitat with minimal ecological variation between their home ranges, the study showed that there was marked inter-community variation in hammer size and raw material preferences [12]. Similarly, Thornton *et al.* [11] have shown that neighbouring groups of meerkats show consistent differences in their time of emergence from their sleeping burrows, despite overlaps in burrow use and extensive gene flow between groups. In sum, it is clear that different methods have their pros and cons and their feasibility is dependent on species, environment, financial and ethical constraints, among others [6], but many of the examples here described emphasize the need for more comparative studies of behavioural variation at a local scale, both in terms of the presence/absence of specific behaviours across communities and their detailed descriptions that may reveal subtle variation in form.

In chimpanzees, arguably the most 'cultural' among non-human species, much effort has focused on building a comprehensive catalogue of the species' cultural repertoire [17,25]. Yet, while much of the species' range has been studied [5,30–35], new behaviours and behavioural variants keep being identified, suggesting that gaps still remain to be filled. At the same time, increasing human disturbance, and consequent fragmentation, degradation and change of habitats and available resources, are suggested to have the potential to bring about both the loss of existing behavioural variants [5] and their modification as an adaptive response to environmental changes [4,32,36]. This highlights the importance of surveying communities inhabiting anthropogenically impacted areas. Specifically, little is known about the westernmost populations of the species' distribution. This means that not only are we lacking detailed information about potential regional cultural variation but we may have a limited time in which to chart it: behaviours may go extinct, and we may lose our ability to document how chimpanzees respond to life in the Anthropocene [37].

The present study aims to fill these gaps by being the first to explore cultural variation between neighbouring chimpanzee communities inhabiting Cantanhez National Park (CNP), Guinea-Bissau. CNP is thought to be home to approximately 10–12 chimpanzee communities [38] inhabiting a mosaic of habitats. Bessa *et al.* [39] studied the feeding ecology of the chimpanzee community of Caiquene-Cadique in CNP for nine consecutive months, where apart from the use of leaf sponges, a universal chimpanzee behavioural trait, no other type of tool use was confirmed (unpublished data). A recent study, however, has found evidence of honey-dipping tools in other CNP communities [40]; Bessa *et al.* [39] also suggest, based on indirect data (i.e. traces such as accumulation of snail shells, use-wear marks in wooden anvils and bite marks in soft tissue of discarded snails), the possibility of the Caiquene-Cadique chimpanzees cracking giant African snails (*Achatina achatina)* against wooden anvils and eating them (a behaviour first described in a recent study of Bili-Uéré's (DRC) chimpanzees, though direct evidence is yet to be found [41]). Additionally, a recently discovered behaviour—accumulative stone throwing [34,42]—has also been confirmed in Boé National Park, Guinea-Bissau. Overall, preliminary work indicates the presence of a potentially unique cultural profile for the poorly studied Guinea-Bissau chimpanzees.

We collected behavioural data on four unhabituated neighbouring chimpanzee communities in central CNP. Due to the communities' close proximity to local people, we chose not to habituate chimpanzees, therefore any direct observation was merely opportunistic, and the majority of data collection relied on a combination of indirect methods and camera traps. As such, our aim was also to demonstrate the feasibility of using this combination of methods to achieve our three primary goals: (1) to document new behaviours for the studied communities, expanding our to-date limited knowledge of their behavioural repertoire; (2) to identify behavioural variation among communities; and (3) to contribute to continental-level comparisons in the ever-growing chimpanzee behavioural repertoire. Our indirect data collection methods (see below) were particularly suitable for behaviours that leave trace evidence (extractive tool use, resource consumption), while the camera trap data were our principal source of information for behaviours that are more ephemeral/do not leave traces (communication and display, social behaviour). We structure our results according to these categories.

2. Methods

2.1. Study site

CNP is located in the Tombali region of south-west Guinea-Bissau. CNP has an area of 1057 km^2 and is a mosaic of settlements, agricultural fields, sub-humid forest, secondary forest, palm groves, mangrove and

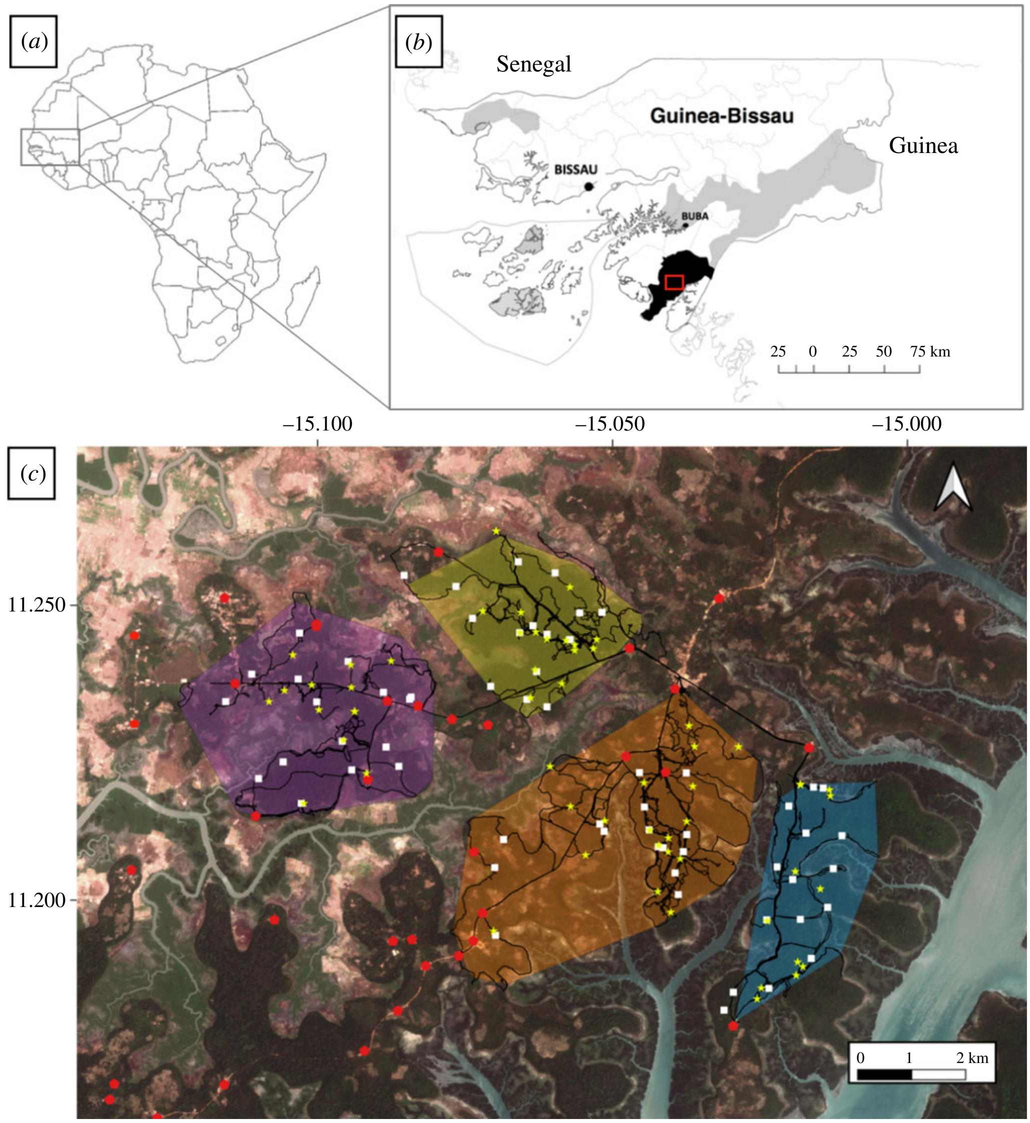

Figure 1. Locations of research sites and study communities' core home ranges. (*a*) Map of Africa showing the location of Guinea-Bissau. (*b*) Map of Guinea-Bissau showing the locations of CNP (black) and other protected areas (light grey). (*c*) Aerial image showing the locations of the four study sites in CNP. The core home ranges of the four chimpanzee communities, minimum bounding polygons estimated from direct and indirect chimpanzee data points, are illustrated in different colours: [CC] Caiquene-Cadique (purple), [LA] Lautchandé (yellow), [MA] Madina (orange) and [CB] Cambeque (blue); the minimum bounding polygon for the core home range of this chimpanzee community provides only a rough estimate given the paucity of direct and indirect data points available. Black lines indicate recces walked, red pentagons correspond to locations of villages and other human settlements, white squares correspond to 50 × 50 plots, and yellow stars correspond to camera trap locations. Sentinel-2 imagery was downloaded from the Sentinel Hub, Sinergise Ltd (https://www.sentinel-hub.com/). All maps were created using QGIS version 3.12 (https://www.qgis.org).

savannah. Recent work has revealed that a large percentage of forest cover remains across the habitat matrix, but these forest areas are highly fragmented and interspersed with cultivated land. Areas cleared for agriculture often have remnant forest edges that together with fallow land and orchards can work as corridors for some wildlife [38] (figure 1). Data collection took place in four neighbouring unhabituated chimpanzee communities at CNP: Caiquene-Cadique, Lautchandé, Madina and Cambeque, over the course of 23 consecutive months (February 2017–December 2018). We identified different communities based on previous research, including genetic studies, local knowledge and the presence of natural and anthropogenic barriers (e.g. villages, roads, rivers and estuary branches that form small peninsulas) [38,39,43–46]. The size of each community's ranging area was estimated using minimum convex polygons: Caiquene-Cadique 14.8 km^2, Madina 19.0 km^2, Cambeque 7.1 km^2 and Lautchandé 8.4 km^2. Additionally, previous work had estimated the minimum community size in

Caiquene-Cadique as 49 individuals (Ramon, personal communication) and Madina as 48 individuals [44]; for Lautchandé or Cambeque we did not possess sufficient information to estimate community sizes.

2.2. Data collection

As the chimpanzees of CNP are unhabituated to researchers, a combination of direct, indirect and remote data collection methods was employed. To ensure that data collection was systematic and comparable to that of other study sites across Africa, methods were adapted from the Pan African Programme guidelines [5,47] and are explained in detail below. Study efforts across the four sites are summarized in table 1.

2.2.1. Indirect data

Reconnaissance walks ('recces', which followed given compass bearings while walking the path of least resistance [47]) were conducted at each study site by following chimpanzee paths and forest trails that covered as many different habitat types as possible. This method was chosen over systematic transects in order to minimize disturbance to an already highly fragmented habitat and to avoid opening up new trails for hunters. Recces were walked in rotation between communities, from February to July 2017 and from February to July 2018 spanning across the second half of the dry season and the first half of the wet season (see table 1 for number of recces and total distances covered per community). During recces, data on direct encounters (i.e. group size, composition, location and behaviours) and indirect signs of chimpanzee presence and behaviour, including faeces, feeding traces, nests, tool-use sites and foot and knuckle prints, were collected. These data were used to estimate chimpanzee core ranging areas and to find the best locations to set up camera traps. Any plant and animal species associated with chimpanzee behaviour (e.g. feeding traces; tools used in extractive foraging) that were not identified *in situ* were collected for later identification by local people or, when necessary, by consulting the relevant literature (see [48,49]). Every behaviour that was identified in one community was added to a list of candidate behaviours for inter-community variation, and efforts were made to confirm the presence of each in all of the other communities.

2.2.2. Remote data

Camera traps (Bushnell Trophy Cam HD Aggressor No-glow) were set up at 56 different locations in CNP (see table 1 for a more detailed breakdown). Camera traps were motion triggered and recorded 1-minute videos, and were set up in places habitually used by chimpanzees and where (1) tools had been found and/or tool use behaviour was expected to take place (e.g. stingless bee hives; natural water sources) and (2) where there was evidence of habitual presence of chimpanzees and where other behaviours of interest could occur (e.g. trees with large buttress roots with clear signs of wear). To maximize the chances of capturing behaviours of interest, some cameras were moved during the study to account for seasonal changes in chimpanzee ranging patterns, and to capture previously undocumented behaviours.

2.2.3. Resource availability data

During recces, data on the presence of specific resources were recorded *ad libitum*: nut-bearing trees, movable stones, beehives, termite and army ant nests and trails and giant African snails. These resources were selected since they are associated with specific putative cultural behaviours, mostly tool use, in other studied communities. Additionally, fifteen 50 × 50 m plots were established at random locations in each of the four communities' ranging areas to assess the presence/absence of the same resources. When movable stones were found they were tested for hardness and sturdiness by hitting them twice with significant force against a wooden or stone substrate; if the stone did not fragment it was considered potential tool raw material [47].

2.3. Data analysis

We compiled a list of behaviours present based on the data collected (including camera trap footage) and categorized observations as either species-typical behaviours (i.e. present universally across all habituated chimpanzee populations), or putatively cultural (i.e. present at some or all of our study sites but not across the whole species range, or present at at least one, but not all of our study sites). The categorization was done based on previously published data on chimpanzee culture and behavioural variation (following [5,17]). Definitions for each behaviour are given in table 2 (adapted

Table 1. Summary of study effort at each of the study sites, in Cantanhez National Park in Guinea-Bissau. All relevant resources were confirmed in all studied communities apart from *Melipona* sp., only confirmed in Cambeque and Madina. Information is provided on number of recces and kilometres (km) walked; camera trap (CT) number, operational period (first day and last day active) and total number of days active and functional; total number of videos recorded that contained chimpanzees; estimated ranging area; indirect data points collected; number of confirmed behaviours; behaviours and their categorization (C, presence confirmed through indirect data; C*, presence confirmed through direct evidence (video or observation); U, unconfirmed) at the four study sites in CNP. Additionally, the total number of indirect data (I) and camera trap videos (V) of each behaviour is given for each community after the behaviour categorization (I,V).

		chimpanzee community			
		Caiquene-Cadique	Cambeque	Lautchandé	Madina
recces	no. of recces	48	49	48	50
	distance walked (km)	230.8	260.4	236.6	361.5
camera trap deployments	no. of CTs	12	11	15	18
	first CT day	05/04/2017	07/03/2017	06/05/2017	20/02/2017
	last CT day	08/12/2018	22/11/2018	10/12/2018	04/12/2018
	total CT days	2367	2391	2109	2828
	total no. chimpanzee videos	2254	828	468	647
data	estimated ranging areas (km^2)	14.8	7.1	8.4	19.0
	no. of indirect traces	538	230	230	749
	no. of confirmed behaviours	17	14	9	18

(*Continued.*)

Table 1. (*Continued.*)

		chimpanzee community			
		Caiquene-Cadique	Cambeque	Lautchandé	Madina
behaviour	fluid-dip	U (0,0)	U (0,0)	C (1,0)	U (0,0)
	honey-dip	U (0,0)	U (0,0)	U (0,0)	C (2,0)
	honey-dip large stingless bees	U (0,0)	C* (63,2)	U (0,0)	C (1,0)
	honey-dip small stingless bees	U (0,0)	C (38,0)	U (0,0)	C* (100,8)
	leaf-sponge	C (6,0)	C (6,0)	C (5,0)	C* (4,3)
	honey-feed, no tools	C* (10,4)	C (3,0)	C (5,0)	C (2,0)
	mangrove-eat	C* (6,7)	C (2,0)	U (0,0)	C (7,0)
	saltwater-drink	C* (0,4)	U (0,0)	U (0,0)	C* (0,4)
	aimed-throw	C* (0,4)	U (0,0)	U (0,0)	U (0,0)
	branch-drag	U (0,0)	U (0,0)	U (0,0)	C* (0,1)
	branch-shake	C* (0,10)	C* (0,2)	C* (0,2)	C* (0,8)
	buttress-drum	C* (15,624)	C*(8,110)	C* (11,141)	C* (32,171)
	ground-slap	C* (0,3)	C* (0,2)	U (0,0)	C* (0,1)
	ground-slap knuckles	C* (0,1)	C* (0,1)	U (0,0)	C* (0,1)
	leaf-clip, fingers	C* (0,33)	C* (0,1)	C* (0,3)	C* (0,4)
	leaf-clip, mouth	C* (0,34)	C* (0,4)	C* (0,7)	C* (0,19)
	leaf-drag	C* (0,11)	U (0,0)	C* (0,2)	U (0,0)
	leaf-pull, finger	C* (0,10)	C* (0,1)	U (0,0)	C* (0,5)
	leaf-pull, mouth	C* (0,2)	C* (0,1)	C* (0,1)	C* (0,3)
	rain-dance	C* (0,13)	U (0,0)	U (0,0)	U (0,0)
	raspberry	C* (0,3)	C* (0,1)	U (0,0)	C* (0,14)
	food-share	C* (0,7)	U (0,0)	U (0,0)	C* (0,13)

Table 2. Definition of behaviours recorded in the present study that show potential variation among study sites (adapted from Whiten *et al*. [17] and Nishida *et al*. [50]).

behaviour	definition
extractive tool use	
fluid-dip	manufacturing a probe from a twig to extract fluid.
honey-dip	manufacturing a probe from a twig, to extract honeybee (*Apis mellifera*) honey from nest.
honey-dip large stingless bee forest	manufacturing a probe from a twig, to extract large stingless bee honey (*Melipona* sp.) from nest, generally found in open secondary forest.
honey-dip small stingless bee mangrove	manufacturing a probe from a twig, to extract small stingless bee honey (*Meliplebeia* sp.) from nest, generally found in mangroves.
leaf-sponge	bundling leaves/vegetation, chewing or folding, to collect water and squeeze into the mouth.
resource consumption, feeding and habitat use	
Honey-feed, no tools	feeding on bee honey without a tool, employing snatch and run approach.
mangrove-eat	collecting salty leaves of *Avicennia germinans* (found exclusively in mangrove areas) from tree, either ingesting or chewing and spitting out (wadge).
saltwater-drink	drinking mangrove salt water that collects in puddles.
communication and display	
aimed-throw	aiming and throwing of object.
branch-drag	dragging a large branch as part of a display.
branch-shake	shaking of branch, producing a conspicuous sound, prior to a buttress-drumming display.
buttress-drum	beating/drumming with hands or feet on buttress or trunk of a tree, normally preceded by pant-hoot vocalization.
ground-slap	striking substrate with open hands/feet or alternate hands/feet during display, sometimes followed by pant hoot vocalization.
ground-slap, knuckles	as above, but substrate is struck with the knuckles instead of open hands.
leaf-clip, fingers	ripping apart of one or more, normally dried, leaves from the ground using the thumb and index fingers, one by one, producing a conspicuous and distinctive ripping sound. Typically precedes buttress-drumming display.
leaf-clip, mouth	as above, but clipping is performed with the mouth. Typically precedes buttress-drumming display.

(*Continued.*)

Table 2. (*Continued.*)

behaviour	definition
leaf-drag	walking forward fast quadrupedally with head down and shoulders hunched, while pushing dry leaves with hands and feet, producing distinctive sound. Sometimes performed before and/or after buttress drumming.
leaf-pull, fingers	pulling of leaves, one by one, from a shrub or a twig, with index finger and thumb. Typically precedes buttress-drumming display.
leaf-pull, mouth	as above, but pulling is performed with the mouth. Typically precedes buttress-drumming display.
rain-dance	performing vigorous charging displays at the start of heavy rain. May include slow as well as rapid charges, and may involve a variety of display patterns (e.g. ground slap, branch drag, branch shake, throw).
raspberry	producing a spluttering sound by pressing air and saliva through lips.
social behaviours	
food-share	feeding by two or more individuals simultaneously on an item of food obtained by one of the individuals. Theft is excluded.

from [5,17,50]). Given that these are previously unstudied and unhabituated communities, data were insufficient to categorize behaviours as customary (occurs in all or most members of at least one age–sex class) or habitual (not customary but seen repeatedly in several individuals), as defined in [25]. Therefore, the following categories were used to describe our knowledge of the prevalence of each behaviour: Confirmed (C)—the behaviour was clearly identified in the community; Unconfirmed (U)—the behaviour was not yet recorded in the community, but this may be explained by insufficient observation opportunities.

3. Results

During the study period a total of 1089 km were walked over 195 recces, and camera traps were set up in 56 locations, for a combined total of 9695 days, yielding a total of 4197 videos of chimpanzees (see electronic supplementary material, table S1). During recces, 1747 indirect chimpanzee traces were recorded, including 204 extractive stick tools. For detailed information on study effort and data collected in each community, see table 1.

Our surveys of resource availability revealed the following results. At all sites the availability of three species of nut-producing trees (*Elaeis guineensis, Detarium senegalense* and *Parinari excelsa*) was confirmed. Termites (*Macrotermes* sp. and *Cubitermes* sp.) were present at all study sites, as were army ants (*Dorylus* sp.). At other chimpanzee research sites, these resources are exploited with the use of tools (i.e. nut-cracking and termite and ant fishing/dipping) (see [25]). Nonetheless, no evidence of tool-assisted nut-cracking or insectivory was confirmed for CNP; additionally, a previous study that analysed hundreds of faecal samples in Caiquene-Cadique found no macroscopic evidence of insectivory [39]. Movable stones were present in all CNP communities but infrequently encountered. The stones found were fragile and broke easily, and no evidence of their use was found. Giant African snails (*Achatina achatina*) were present in all CNP communities' home range, nonetheless no conclusive evidence (e.g. direct observation) of the exploitation of snails was found. Honeybees (*Apis mellifera*) were present at all five study sites and a species of stingless bee (*Meliplebeia* sp.) was confirmed for all four CNP sites. A second species of stingless bee (*Meliponula* sp.) was only confirmed at Cambeque and Madina. Tool-use evidence as well as trace evidence of discarded honeycombs with teeth marks confirmed that each CNP community fed on at least one of the different types of honey (see [40]).

A total of 22 behaviours of interest were identified during the study period; figure 2 and table 1 show their distribution across the study communities, and figure 3 charts how their cumulative number varied with study effort (number of months of data collection). Four of these behaviours—Leaf-sponge, Buttress-drum, Branch-drag and Branch-shake—are universal for the species [25], while the remaining 18 behaviours are putatively cultural. Four of the putatively cultural behaviours (Leaf-clipping with mouth and with fingers, Leaf-pulling with fingers, and Honey consumption without the use of tools) appear to be universal for our four CNP study sites. Five other behaviours were only confirmed at one of the study sites: these included Rain-dance (Caiquene-Cadique), Aimed-throw (Caiquene-Cadique), Branch-drag (Madina), Honey-dip (Madina), Fluid-dip (Lautchandé). The site with the greatest number of behaviours confirmed was Madina (18), while Lautchandé had the fewest (9). The following sections describe variation in different behavioural domains across the four sites in more detail.

3.1. Extractive tool use

A total of 204 dipping tools and 16 leaf sponges were found across the CNP study sites. The pattern of tool recovery exhibited some marked variation among the four neighbouring communities. No evidence of extractive dipping tools to access fluid (including honey) was found in Caiquene-Cadique, while the extraction of different honey types with tools appeared to be frequent in Cambeque (100 tools) and Madina (103 tools) (table 1). Video evidence of extractive tool use was recorded in Madina and Cambeque.

3.2. Feeding and mangrove resource use

The use of mangroves and their resources (saltwater and salty leaves) was confirmed in three of the CNP communities. Chimpanzees at Caiquene-Cadique and Madina were seen Saltwater-drinking, and they, as well as Cambeque chimpanzees, were confirmed to chew on the salty leaves of black mangrove trees, *Avicennia germinans* (Mangrove-eat). No evidence of mangrove use was found at Lautchandé even

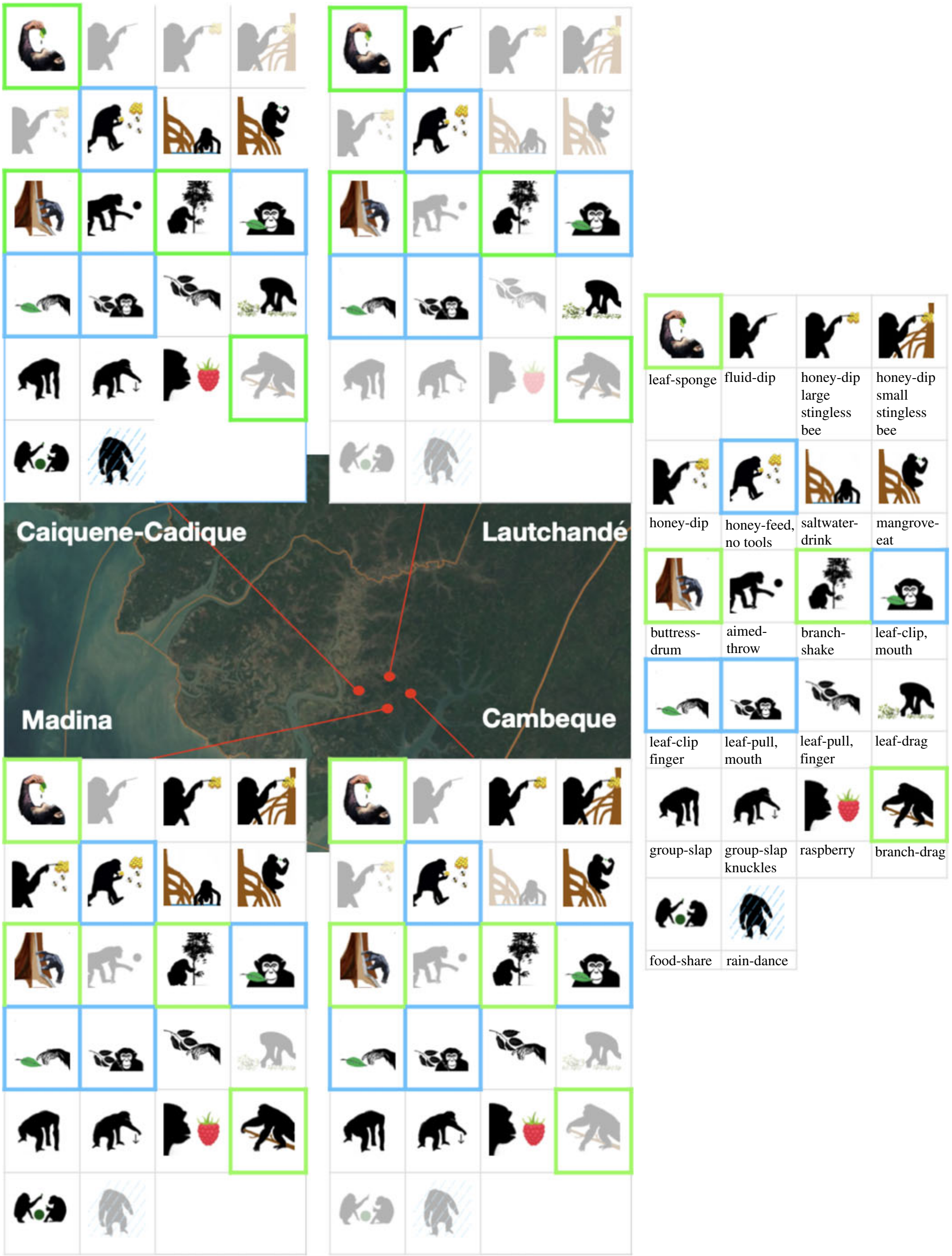

Figure 2. Distribution of behaviours across the four study sites (Caiquene-Cadique, Lautchandé, Madina and Cambeque). Coloured icons represent confirmed behaviours (C), and faded icons represent behaviours for which presence is still unconfirmed (U). See figure key for correspondence of icons to specific behaviours, described in detail in table 2. Green squares represent known species universals, and blue squares represent universals for the CNP study sites. The Sentinel-2 imagery was downloaded from the Sentinel Hub, Sinergise Ltd (https://www.sentinel-hub.com/). All maps were created using QGIS version 3.10.5 (https://www.qgis.org).

though mangroves are present within the chimpanzees' known ranging area. Camera trap evidence of Mangrove-use was recorded for Madina and Caiquene-Cadique.

3.3. Communication and display

Leaf-clipping, a type of non-extractive tool use, was observed in all four CNP communities. All Leaf-clipping was confirmed through camera trapping and performed in association with Buttress-drumming.

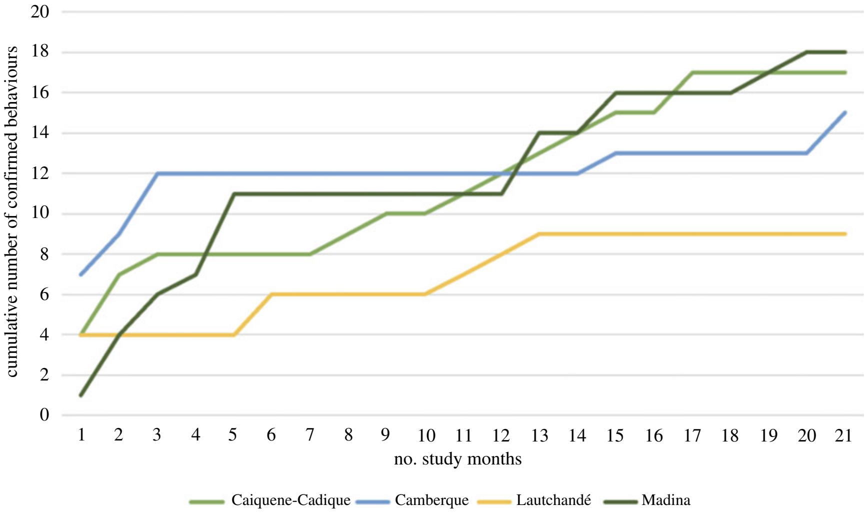

Figure 3. Cumulative number of confirmed behaviours found in each study community over the study period.

Leaf-pulling in association with drumming was also recorded in all CNP communities. Raspberry vocalization was captured on camera in three of the CNP communities, but not at Lautchandé. Additionally, in Caiquene-Cadique male chimpanzees were caught on camera throwing the giant fruit of *Treculia africana* during drumming displays.

3.4. Social behaviours

Fruit-sharing of large *T. africana* fruits was recorded on camera in Caiquene-Cadique and in Madina, between a mother and her dependent offspring as well as between adults. Rain-dance display was only confirmed at Caiquene-Cadique.

4. Discussion

An essential component of identifying cultural variation in non-human species is documenting behavioural variation across populations. However, studying behaviour and its variation in groups of unhabituated wild animals is a notoriously difficult and long process (e.g. [41]). Verifying the presence of specific behaviours and identifying inter-community variation are hampered by the patchy nature of available data, where the length of study and the methods employed have implications on the amount and nature of data that can be collected. Behaviours that leave behind material evidence, specific artefacts or modifications in the environment (e.g. feeding traces, tools, constructions) are easier to detect, while behaviours that leave no trace evidence (e.g. social or communicative behaviours) and/or are infrequent or rare in nature are impossible to document without direct *in situ* or remote observation. It is also evident that the longer a study runs the more researchers can learn about a population's behavioural repertoire, not simply because data accumulate over time but also because these studies may end up habituating or semi-habituating the individuals to researchers. Nonetheless, despite the constraints of working with unhabituated populations, the present study was able to compile, through a combination of direct and indirect methods, and remote monitoring, a list of behaviours and potential behavioural variations in four neighbouring chimpanzee communities in Guinea-Bissau. Furthermore, we were able to identify some noteworthy novel behaviours for these communities, as well as behaviours that are possibly new to the species as a whole, thus filling gaps in our knowledge of the chimpanzee behavioural repertoire at a continental scale.

We identified 22 distinct behaviours, some of which had previously been described as universal at a species level, such as Buttress-drumming and Leaf-sponging [17], and, therefore, will not be considered candidates for cultural behaviours. Even though we did not find all the universal behaviours in all the study communities we cautiously assume that this was an artefact of sampling time and methodology and that with increased study effort these will, in time, be identified for all communities. Several other behaviours appear universal at a local level in CNP such as Leaf-clipping or Honey-feeding (without tools), but when put into context at a subspecies or species level, are good candidates for cultural behaviour. This is either because the behaviour is known to be absent at least at some other study sites (e.g. Honey-feeding), the behaviour is exhibited in different contexts (e.g. Leaf-clipping), or there is as yet no evidence of the behaviour existing elsewhere (e.g. Mangrove-eat), suggesting variation across the species' range. Given our limitations (in terms of both data collection methods and study duration) it is not possible to conclusively confirm the *absence* of most of the studied behaviours, but considering the encounter rates of certain behaviours some putative variation seems likely. Perhaps the most striking case in point is Honey-dipping. While we confirmed the presence of at least one type of stingless bee (*Meliplebeia* sp.) in all four CNP communities' ranges and the presence of honeybee (*Apis mellifera*) at all four study sites, evidence of dipping for honey was only found in two of the four CNP communities. Although we cannot conclude with certainty that dipping for honey is absent in Caiquene-Cadique and Lautchandé—and indeed the picture may change as research efforts continue—it is likely that it occurs with at least greater frequency in the communities of Cambeque and Madina (for further discussion see [40]). We chose to separate honey dipping into three types depending on the type of honey exploited (stinging bee, small stingless bee and large stingless bee honeys) since a previous study found significant differences in the number and types of tools used to extract these different types of honey [40]. We succeeded in confirming not only extractive tool use, but also non-extractive tool use. This type of behaviour does not produce recognizable artefacts and can only be documented through observation. Yet, despite working with unhabituated chimpanzees, we were able to remotely observe this behaviour through the use of camera traps. Leaf-clipping/pulling behaviours were confirmed in all CNP communities, and even though their function is as of yet unknown, they appear to play a role in Buttress-drumming displays. Hence, they could be considered examples of communicative or social tool use [51]. Additionally, there appears to be variation in Leaf-clipping and Leaf-pulling behaviours, and through further analyses we will be able to characterize these variations in more detail (manuscript in preparation). For example, in some cases a single leaf is carefully clipped, in others, several leaves are clipped simultaneously; on some occassions both varieties of leaf-clipping happen simultaneously (i.e. with mouth and hand) or leaf clipping and pulling are combined during the same event. Interestingly, such variation contrasts with what has been described at Tai Forest, the only other community known to Leaf-clip prior to drumming: there, according to Boesch [52], the behaviour is highly stereotyped among all the individuals of the group, unlike the within-community variation we observed. Also associated with buttress drumming we identified a Raspberry vocalization that is commonly heard in captivity and has been described as species atypical behaviour [53]. This behaviour has only been described in the wild once, in the Ngogo community (Uganda), but in the context of grooming [54].

One group of behaviours, which occurred exclusively in the mangrove habitat, is of particular interest. To our knowledge the use of mangroves has only been described in Loango (Gabon) [55], but see [56] for another potential example, although this study did not report whether chimpanzees used the mangroves within their habitat. Mangroves are an integral part of the Cantanhez landscape and evidence of chimpanzees utilizing resources within was confirmed for three of the four study sites. At the fourth, Lautchandé, we were not able to confirm any mangrove-related behaviours despite the presence of mangroves in the community's habitat. It may be the case that with the increase of study effort this will change; however, similarly to honey dipping, it is possible that different communities rely more on this habitat type than others. In the sense that mangrove-related behaviours are so clearly dependent on the environment, they can be compared to cave use by chimpanzees at other sites in West Africa (e.g. Fongoli in Senegal [31]). Ecology can easily explain the absence of the behaviour in many communities, yet it can still vary between communities where ecological characteristics alone cannot account for such variation, and, as such, cave use was considered a cultural behaviour by Kühl and colleagues [5]. This affirms the value of incorporating ecology and habitat in studies of behavioural variation, and illustrates why it is fruitful to explore new areas and habitat types when studying chimpanzee behaviour. In a similar vein, our findings regarding some less common social behaviours, such as Food-sharing and Rain-dance, detected in at least one of the studied communities, demonstrate the importance not only of study duration but also of diversification in the range of possible behaviours that researchers should look for in unhabituated communities.

It is worth noting that despite the study effort being similar across the four sites (table 1), the number of videos and indirect traces collected varied substantially between communities. This may in part have been due to the fact that at the beginning of our study we had a better baseline knowledge of the movements of the Caiquene-Cadique community than any of the other three communities, and hence were able to record a much larger number of chimpanzee videos at Caiquene-Cadique. However, this would not explain why in the first month of the study we in fact confirmed many more behaviours in

Cambeque than in the other communities (figure 3). Additionally, the differences in ranging areas (with chimpanzees at Madina and Caiquene-Cadique estimated to range over larger areas than those at Cambeque and Lautchandé) could also help account for some of the disparity in number of indirect traces found: larger ranging areas may correspond to larger population sizes, which, in turn, may produce larger numbers of traces. It is likely that with an increase in study effort better parity in data volume across the four communities would be achieved, which may further clarify how much of the observed intercommunity variation found is due to behavioural differences.

Notwithstanding, in 2 years of research, we were able to compile an extensive list of wild chimpanzee behaviours never before described for CNP, for Guinea-Bissau, or (in the case of some behaviours) for the rest of Africa. We concede that this list is likely to be far from comprehensive since it is clear that longevity of study and close, direct observation of individuals are key factors that influence the size of the behavioural repertoire assembled for each chimpanzee community, as is evident through the increase in the number of confirmed behaviours over time (figure 3). The recent study by Kühl *et al.* [5] comparing the presence/absence of 31 behaviours among 144 chimpanzee communities (46 previously largely unstudied communities along with information from published literature on an additional 106 communities) concluded that these behaviours had on average 88% less chance of occurring in chimpanzee communities inhabiting highly anthropogenically impacted areas compared to low impacted areas [5]. This would thus seem to discourage the study of communities that, like ours, inhabit fragmented forest mosaics [57]. However, our results show that chimpanzee communities that inhabit human-impacted environments demonstrate rich behavioural repertoires. In CNP we were able to confirm 9 of the 65 behaviours described by Whiten *et al.* [25] and 7 of the 31 behaviours described by Kühl *et al.* [5]. These numbers might seem low, but given that the original lists only include, by definition, behaviours that show variation across communities, it is in fact expected that we will only encounter some of these behaviours in newly studied communities. Examining the data presented by Kühl *et al.* [5], even communities that are fully habituated and have been studied for over 40 years, only show a subset of the 31 behaviours analysed (e.g. 18 for Bossou, Guinea and 17 for Gombe, Tanzania).

Thus, our research shows that studying the behaviour of neighbouring primate communities inhabiting human-impacted areas can be a useful source of information in studies of animal culture, and re-affirms the value of using a combination of direct and indirect methodologies to document the behaviour of unhabituated communities. The fact that over the course of our relatively short study we identified behaviours which do not leave noticeable material evidence behind and that are rare, seasonal, or absent in other known chimpanzee communities, provides further justification for the validity of this approach. Additionally, and contrary to some previous studies, our approach was not constrained to a pre-selected list of behaviours. This allowed us to, for example, explore behaviours specific to unusual habitat types, such as mangroves, and identify previously unknown behaviours that we would have missed otherwise. At the same time, it is important to remember that behavioural repertoires are not static and should not be seen as such: behaviours can disappear [58], resurface after years of absence [59], change [32] or be, as far as we know, newly invented [23]. In fact, the ever-changing conditions that chimpanzees inhabiting disturbed habitats face might translate into a need to rapidly adapt through changes in established behaviours or through innovation [4]. This means that while some behavioural variants might disappear [5], given the flexible nature of chimpanzee behaviour others might arise anew and be passed down to the next generation via social learning [4]. Furthermore, human–chimpanzee dynamics are different in areas where local people have prolonged sympatry or exposure to wildlife and more gradually encroach into wildlife habitats, than those where human encroachment is more rapid and chimpanzees are killed for food or as 'pests' [4,60]. Thus, chimpanzees that for generations have been facing human disturbances and are tolerated by local people, such as our study communities, might display a rich behavioural repertoire that has allowed them not to become dependent on specific foods or habitat types (e.g. primary forest) to survive, and therefore might be better equipped for a continued existence in human-disturbed areas. In a related vein, it is increasingly evident that in addition to comprehensive and up-to-date information on the species' and subspecies' status, distribution and population trends, genetic and cultural diversity are also important to guide effective conservation activities [4]. Our research helps build baselines for chimpanzee cultural diversity in CNP and for the species as a whole which has the potential—if done carefully [61]—to be integrated into existing, evidence-based conservation frameworks.

Ethics. This research was reviewed and approved by the Instituto da Biodiversidade e das Áreas Protegidas (IBAP) in Guinea-Bissau. All research involving wild chimpanzees was non-invasive and purely observational, and strictly adhered to ethics guidelines detailed by the Association for the Study of Animal Behaviour (United Kingdom).

Data accessibility. All camera trap data used for this research have been made accessible in the form of an Excel file uploaded as electronic supplementary information—electronic supplementary material, table S2.

The data are provided in electronic supplementary material [62].

Authors' contributions. J.B.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, visualization, writing—original draft, writing—review and editing; D.B.: conceptualization, funding acquisition, investigation, methodology, resources, supervision, writing—review and editing; K.H.: conceptualization, funding acquisition, investigation, methodology, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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APPENDIX C

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ORIGINAL RESEARCH
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First Evidence of Chimpanzee Extractive Tool Use in Cantanhez, Guinea-Bissau: Cross-Community Variation in Honey Dipping

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Wild chimpanzee tool use is highly diverse and, in many cases, exhibits cultural variation: tool-use behaviours and techniques differ between communities and are passed down generations through social learning. Honey dipping – the use of sticks or leaves to extract honey from hives – has been identified across the whole species' range. Nonetheless, there seems to be marked variation in honey dipping at a species level, with most descriptions originating from central Africa, and involving the use of complex tool sets, or even multifunctional tools. In West Africa, while honey consumption is common, in most cases tools are not used. We document, for the first time, the use of honey dipping tools in unhabituated chimpanzee (*Pan troglodytes verus*) communities at Cantanhez National Park (CNP), Guinea-Bissau. Over a 23-month period we employed a combination of direct (camera traps, n = 1944 camera trap days) and indirect (1000km of reconnaissance walks, collection of abandoned tools) methods to study four neighbouring communities in central CNP. Fluid dipping tools were found in three of the four communities; here we analyse 204 individual stick tools from the 70 tool-use ateliers found. In addition to documenting individual tool dimensions and raw materials, we adopt methods from primate archaeology to describe the typology of different tools based on use-wear patterns. We describe differences in tools used for different honey types, between communities, and tools and tool kits that show an unexpected degree of complexity. Our data also suggest the use of tool sets, i.e., tools with different functions used sequentially toward the same goal; as well as possible multifunction tools (pounding and dipping), never before described for western chimpanzees. Our study fills gaps in our knowledge of the wild chimpanzee cultural repertoire and highlights how chimpanzee tool manufacture and use can vary even at local scales.

Keywords: animal culture, behavioural variation, dipping, honey, tool use, West Africa

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INTRODUCTION

Apart from humans, chimpanzees show the greatest diversity of tool use in the animal kingdom, making and using a variety of complex tools as part of their daily lives (McGrew, 2004). In addition, different communities exhibit different tool-use repertoires (Goodall, 1986; Whiten et al., 1999; Boesch and Boesch-Achermann, 2000; McGrew, 2004; Matsuzawa et al., 2011;

Pruetz et al., 2015) – a phenomenon commonly ascribed to cultural variation. West African chimpanzees, for example, are known to crack nuts using wooden or stone hammers, whereas this behaviour is entirely absent from East Africa despite the presence of the necessary resources and raw materials within East-African chimpanzees' ranges (Boesch and Boesch-Achermann, 2000; Matsuzawa et al., 2011). How such regional differences emerge and are maintained are key questions in understanding the spread of cultural traits, and, given chimpanzees' close evolutionary proximity to humans, are also relevant to understanding the origins of hominin technology and culture. Several different methods to identify culture have been suggested, most prominently those applying the "method of exclusion" (e.g., Whiten et al., 1999) and those positing a tri-dimensional approach to traditions (Fragaszy and Perry, 2003). The former proposes the explicit exclusion of ecological or genetic factors as drivers of inter-population behavioural variation within a species (Wrangham et al., 1994; Whiten et al., 1999), whereas the latter places the strongest emphasis on demonstrating social learning, without which a behaviour cannot be considered cultural (Fragaszy and Perry, 2003). Subsequent work argued that ecology, genetics and social learning are in fact inexorably interlinked and can all influence, to some degree, behavioural variation (Laland and Janik, 2006; Koops et al., 2013), thus leading to a useful convergence between the two main frameworks.

The past two decades have seen further refinement of these methodologies in the study of wild primates. First, while early works on chimpanzee culture conducted comparisons at a species-wide scale (Whiten et al., 1999, 2001; Schöning et al., 2008), it has been suggested that more compelling evidence for culture in chimpanzees might come from the study of behavioural variation within the same subspecies (e.g., Laland and Janik, 2006; Luncz et al., 2012). In particular, comparisons of neighbouring communities, where habitat types are similar and individuals broadly face the same ecological constraints and migrate between communities, make ecological or genetic explanations for behavioural differences less likely compared to a cultural explanation (i.e., one based on local innovation and subsequent diffusion through social learning). Illustrating this approach, Luncz et al. (2012) compared the selection of wooden and stone hammers for coula (*Coula edulis)* nut-cracking in three neighbouring chimpanzee communities in Taï National Park (Ivory Coast). Even though these neighbouring communities inhabit the same forest habitat and ecological variation is minimal, the study showed that there was still marked variation in hammer size and raw material preferences between communities (Luncz et al., 2012). This confirms that studying nuanced differences in details of the *same* behaviour between communities that exist in close proximity can yield tantalising evidence for subtle behavioural variation. Second, direct evidence for social learning being involved in the maintenance of specific behaviours has become available through observations of natural immigration and the emergence of subsequent conformity (Luncz and Boesch, 2014). Furthermore, novel social-network-based analyses have also confirmed the socially mediated diffusion of a newly invented behavioural variant in wild chimpanzees (Hobaiter et al., 2014). Taken together, these studies elegantly bridge the gap between the method of exclusion and the tri-dimensional approach to the study of animal traditions.

Honey dipping behaviour – chimpanzees' use of sticks or leaves to extract honey from hives – has been identified across the whole species' range (Boesch and Boesch, 1990; Tutin et al., 1995; Ohashi, 2006; Fowler and Sommer, 2007; Sanz and Morgan, 2009; McLennan, 2011). However, it is from Central Africa that most descriptions of honey dipping of arboreal and terrestrial honey from different stinging and stingless beehives seem to originate. Central African chimpanzees (*Pan troglodytes troglodytes*) use complex tool sets, sometimes composed of up to five tools with different functions used in sequence to gain access to and extract honey, and have even been described using multifunctional tools, i.e., tools where a single object can have different functions (Bermejo and Illera, 1999; Sanz and Morgan, 2007; Boesch et al., 2009). In East Africa, the use of stick tool sets to access honey by chimpanzees is rare. However, chimpanzees at Bulindi in Uganda use tool sets, including both digging sticks and more slender sticks to probe the stingless bees' narrow underground entry tubes (McLennan, 2011; McLennan et al., 2019). In West Africa, honey consumption also occurs frequently, but in many cases no tools are used to extract the honey (Boesch and Boesch, 1990). This might be because this subspecies of chimpanzee (*Pan troglodytes verus*) feeds more frequently on the honey of stinging bees (*Apis* sp.) whose painful sting does not allow individuals to spend long enough near hives to use tools, meaning they must instead adopt other approaches (such as using hands only; Boesch and Boesch, 1990). Nonetheless, in the Ivory Coast the chimpanzees inhabiting Comoé National Park have been described to frequently use dipping stick tools not only to access honey but also water. These chimpanzees were even observed modifying their tools by chewing on their end to create a 'brush tip' prior to use (Lapuente et al., 2017). Variations in honey dipping thus appear to exist between subspecies and within the same subspecies, hence both genetic and environmental explanations might be at play. Hence, it is of particular interest to study this behaviour between neighbouring communities inhabiting similar habitats, where variation due to the latter two influences is expected to be minimal. Furthermore, we still lack a complete picture of the full chimpanzee cultural repertoire, despite long-term study across much of the species' range, and the addition of new sites of unhabituated chimpanzee communities identifying new behaviours and behavioural variants (e.g., Sanz et al., 2004; Pruetz and Bertolani, 2007; Gruber et al., 2015; Hockings et al., 2015; Kühl et al., 2016; IUCN, 2020). Specifically, no long-term studies have yet reached the westernmost populations of the species' distribution. Recent work in Cantanhez National Park (CNP), Guinea-Bissau, has started to fill this gap (Sousa et al., 2011; Hockings and Sousa, 2012; Bessa et al., 2015; Vieira et al., 2019; Hockings et al., 2020). For example, a 9-month study at CNP found, through the analysis of faecal samples and other indirect data, that these chimpanzees fed on wild bee honey with some degree of frequency, however no tools were ever found (Bessa et al., 2015). In more recent surveys, however, evidence of dipping tools began to emerge (E. Bersacola, personal communication), confirming the presence of the behaviour at CNP.

In the present study, we employ a combination of direct and indirect methods to systematically survey CNP for honey-dipping tools, and compare four neighbouring chimpanzee communities unhabituated to the presence of researchers. Specifically, we aim to (1) identify the presence of honey dipping tools in the four communities' home ranges, (2) compare the characteristics of tools used to exploit different honey sources, and (3) compare the characteristics of tools across those communities that use them.

METHODS

Study Site

Cantanhez National Park (CNP, N11°14.287′ W15° 02.281′) is located in the Tombali region of south-west Guinea-Bissau. CNP is a mosaic of settlements, cropland, sub-humid forest, secondary forest, mangrove, and savannah (Catarino and Palminha, 2014). There are two marked seasons in Guinea-Bissau: dry season (November to mid-May) and rainy season (mid-May to October). During 2017, annual rainfall was 2351 mm with an average temperature of 26.3°C (15.6°C min to 38.6°C max). It is estimated that there are 10-12 chimpanzee communities in CNP as a whole (Bersacola, 2019). In the forested areas of central-southern CNP, genetic, behavioural and ecological research support the presence of seven different chimpanzee communities (Hockings and Sousa, 2013; Sá, 2013; Bessa et al., 2015; Bersacola, 2019; Vieira et al., 2019; Hockings et al., 2020); these include the four studied communities: Caiquene-Cadique, Lautchandé, Madina and Cambeque. Due to the unhabituated nature of these communities, at present little is known about their community sizes and compositions; nonetheless, previous works estimate that the communities' range between 35-60 individuals (Bessa et al., 2015; Vieira et al., 2019).

Data Collection

Data collection took place over the course of 23 consecutive months (February 2017 - December 2018). Since the main aim of this study was to assess the presence of and potential inter-group variation in honey-dipping behaviour in neighbouring chimpanzee communities, where none of the studied communities were habituated to researchers, a combination of direct and indirect methods of data collection were employed. A total of 187 reconnaissance walks ("recces") were walked, covering just over 1,000 km. Since several neighbouring communities were being studied, five consecutive recces in each were initially performed to assess preliminary core ranging area and habitat composition. After obtaining this information, recces were walked 6 days a week. These were performed in rotation across communities, accumulating information that would help maximise data collection at each, while also ensuring that all communities were sampled equally across different months/seasons. All data were collected by JB who was accompanied by two field assistants at all times. Camera traps were set up by JB during recces and checked every two-weeks by JB or one of the trained field assistants. **Supplementary Table 1** presents a summary of the cumulative study effort in each of the communities.

Resource Availability

Data on resource availability were collected during recces at each study site by following chimpanzee paths and forest trails that covered as many different habitat types as possible. This method was chosen over systematic transects in order to minimise disturbance to an already highly fragmented habitat, and to avoid opening up new trails for hunters. Honey availability was assessed during recces *ad libitum*: every time a hive of honey bees or stingless bees was encountered, a GPS point of its location was taken, the habitat was classified [dry forest, riparian forest, woodland, palm grove, mangroves, fallows, croplands, savannah woodland and grassland (Catarino et al., 2020)], and hive type (e.g., arboreal, subterranean) and bee species (local name and scientific name when possible) were recorded. Honey is an important subsistence resource for the local human communities, therefore, hives were usually easily located by one of the field assistants. A careful visual search was conducted to locate honey bee hives, as well as the small tubular entrances of stingless bee hives. Honey bee hives were also located through the sound of the swarm. Initially, local honey harvesters were contacted for information about the potential location of hives; other than hives that were impossible to deplete, most of the locations identified in this way were already depleted (or destroyed), or were likely to become so in the near future.

Indirect Data Collection: Home Ranges, Evidence of Honey Consumption, and Tools

Chimpanzee ranging areas were estimated using minimum bounding polygons from direct chimpanzee encounters and camera trap data, as well as indirect (e.g., nests, feeding traces, faecal samples, abandoned tools) data points, continuously collected during reconnaissance walks from February 2017 to July 2018. Additionally, the highly fragmented nature of the chimpanzees' habitat, with human settlements, roads, cultivated areas as well as many mangrove estuaries acting as natural and artificial boundaries, was helpful when estimating the home ranges. Data points that were collected in areas where there might be overlap between communities were excluded.

Hives and their surroundings were inspected for evidence of honey consumption by chimpanzees: detached wax from the hive's entrance, honeycomb traces, tools discarded by the hive, detached fresh green leaves (debris from tool manufacture), freshly snapped branches, or any other chimpanzee signs (e.g., prints, faecal or feeding traces).

When a tool use atelier – a location where tools were used to extract a resource, and were then left behind – was encountered, we recorded its exact location by GPS, photographed the site with the tools *in situ,* and photographed the individual tools. We registered the bee species associated with the tool use atelier, the species of tree in which the hive was located, whether it was an arboreal or terrestrial hive, and its distance from the ground (using a tape measure or a rangefinder depending on its height). Only sticks that showed clear signs of modification, such as stripped bark, lateral branches removed, frayed or blunt ends, or signs of honey or wax at one or both extremities, were considered tools (e.g., Hernandez-Aguilar et al., 2007; Lapuente et al., 2017). We then collected each tool, gave it a label, recorded its species, and, when possible, we measured its distance from the source by refitting (i.e., finding the presence of a scar left in a plant as a result of the chimpanzees harvesting the raw material) the tool to its original source (methods adapted from Koops et al., 2015; Pascual-Garrido, 2018). If more than one tool was found by the same hive we grouped the tools into age categories depending on colour, pliability and degree of decay (new – still green and pliable; recent – browning in colour and less pliable; old – dry appearance, no pliability/fragile, with possible signs of decay; adapted from Pascual-Garrido, 2018). Collected tools were photographed, measured (length; mid, proximal and distal diameter; length of fray), and all modifications were recorded (fragmented/detached from substrate; percentage of bark left; stripped ends; attachments removed; signs of use on extremities; bite marks; and presence of honey/wax). Additionally, we recorded use-wear patterns on the extremities, categorised into three types: brushed/frayed (significantly frayed with long and separated wood fibres), blunt/mashed (minimal to no rounding with significant fringing and lateral/backward bending of terminal wood fibres) and fragmented (broken end with sharp edges) (adapted from Heaton and Pickering, 2006; Boesch et al., 2009). In order to record whether the same hive was repeatedly exploited, the tool use sites were revisited every week, unless it could be confirmed that the hive had been depleted and the bees had abandoned it.

Remote Data Collection: Camera Trap Sampling

Since the chimpanzee communities were unhabituated to researchers' presence, the opportunities to observe them directly were few. However, eight camera traps (Bushnell Trophy Cam HD Aggressor No-glow) provided observational data in the form of video footage. The camera traps were set up in chosen locations, on video mode, and programmed to film for 1 min when triggered by movement. These camera traps were set up in places where tools had been found and the resource (honey) had not been totally depleted (i.e., was likely to be revisited) and/or tool use behaviour was likely to take place due to the presence of beehives. To maximise the chances of capturing behaviours of interest on video, some of the cameras were moved during the study period, for example if bees had abandoned a particular hive, if water and/or salt had disabled a camera in a mangrove location, or if the chimpanzees were known to be utilising new travel routes due to seasonal changes in their habitat. 12 camera traps (three in Caiquene-Cadique, three in Cambeque, two Lautchandé and four in Madina) were operating for a cumulative total of 1923 days (399 days in Caiquene-Cadique, 648 days in Cambeque, 363 in Lautchandé and 513 in Madina), from February 2017 to December 2018 (see **Supplementary Table 1**).

Data Analysis

All statistical tests were performed in R (version 1.1.463), using t-tests and Chi-square tests. Given that data were based on indirect evidence, we assumed that each event was an independent event (but see section "DISCUSSION"). Data were also tested for normal distribution using the Shapiro-Wilk's method, and for homogeneity of variance using Bartlett's test. Two-sample t-tests and Welch's two-sample t-tests were used when comparing datasets with equal and unequal variance, respectively.

Our principal comparisons of interest focused on the dimensions (e.g., length) and characteristics (e.g., modifications) of tools used to collect different types of honey, and of tools used to collect the same type of honey but by different communities. Given the nature of the data collected, where most hives had less than five tools found associated with them, we were not able to use a generalized linear model. Instead, we compared tool dimensions and characteristics using t-tests. For descriptive characteristics we employed Chi-square tests.

RESULTS

Based on the 4293 direct chimpanzee encounters and camera trap data, as well as 1796 indirect data collected during reconnaissance walks, the four communities' ranging areas surveyed in the present study were estimated using minimum convex polygons: Caiquene-Cadique 14.8 km^2, Madina 19.0 km^2, Cambeque 7.1 km^2, and Lautchandé 8.4 km^2 (**Figure 1**).

Resource Availability

All beehives were arboreal, at varying distances from the ground (15–350 cm), and could normally only be accessed by a very small opening covered with dry hard wax (see **Figures 2A,C** for examples). No subterranean hives were found. Three types of bees and respective hives were identified: one species of stinging honey bee (local name bagueira, scientific name *Apis mellifera,* hereafter BH) and two species of stingless or sweat bees, large stingless bees (local name bagueira mudo, scientific name *Meliponula* sp., hereafter BM) and small stingless bees (local name mosca mel, *Meliplebeia* sp., hereafter MM). Hive characteristics varied between and within bee species. *Apis mellifera* were normally found inside live trees with varying entry sizes; *Meliplebeia* sp. were always found in live trees with very small entries and only a single entrance tube (see **Figure 2A**); and *Meliponula* sp. were found both in dead and live trees, with generally large entries in dead trees (see **Figure 2B**) and generally small entries in live trees (see **Figures 2C,E**).

Of all bee species, the hives of MM were most commonly encountered during recces (N = 80). They were present in all study communities but in different numbers and at different densities: 40 in Cambeque, 22 in Madina, 17 in Caiquene-Cadique and one in Lautchandé. The next most common hive was that of BH (N = 23): 10 in Madina, seven in Caiquene-Cadique, four in Cambeque, and two in Lautchandé. BM (N = 7) was only encountered six times in Cambeque and once in Madina. **Supplementary Table 2** presents these densities standardized against home range size and km of recces walked.

Hives were found across different habitats. MM was almost exclusively found in the mangrove area (97.5%) with the remainder in woodland, BM mostly in woodland (71.4%) with

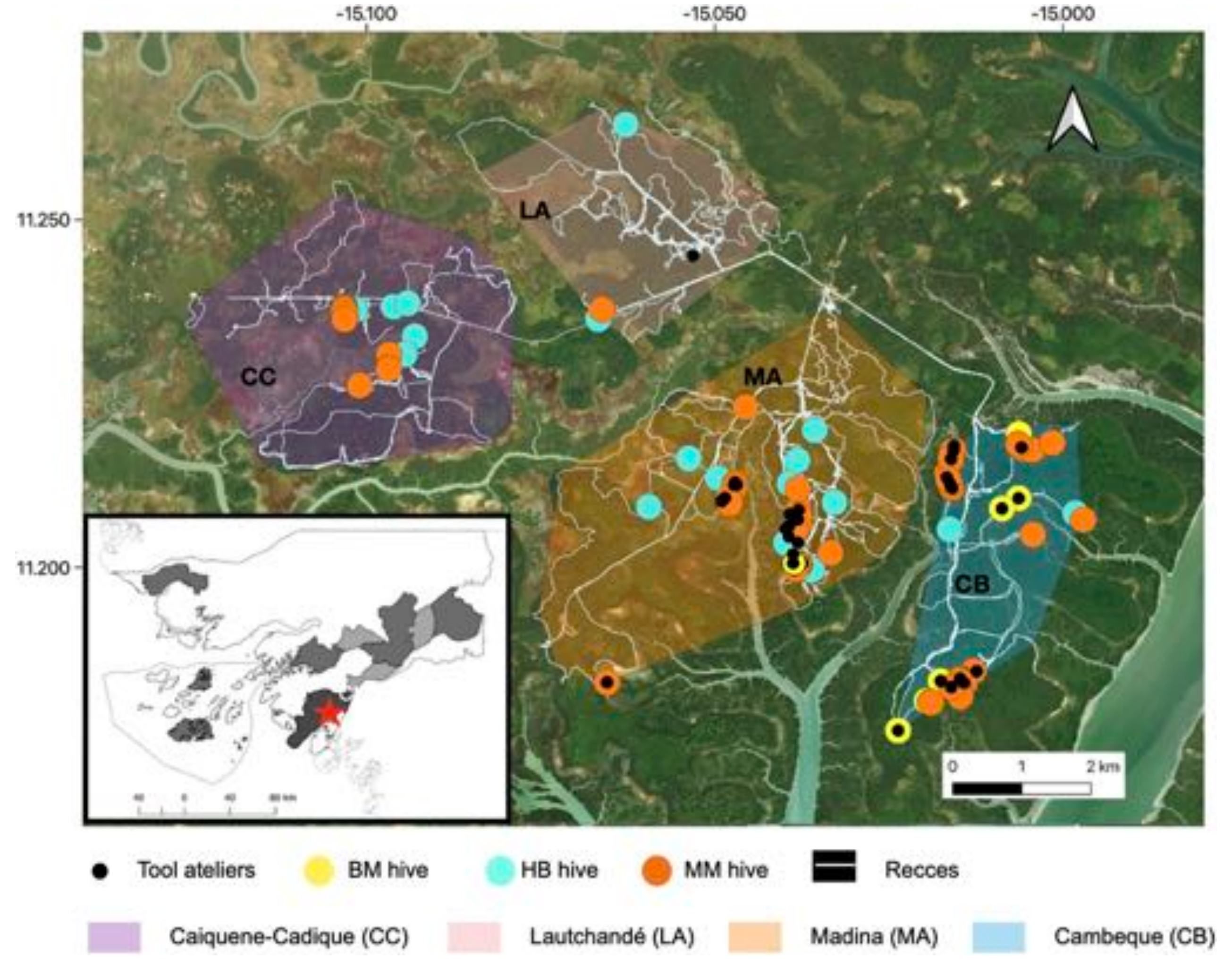

FIGURE 1 | Map of the study site showing each of the study communities' estimated core home range. CNP is marked by a red start, Caiquene-Cadique is shown in purple, Lautchandé in pink, Madina in orange and Cambeque in blue. Ranging areas during the study period were estimated using minimum bounding polygon from 4293 direct (chimpanzee encounters and camera trap data) and 1796 indirect (nests, feeding traces and faecal samples) data points, collected during reconnaissance walks from February 2017 to July 2018.

the remainder in the mangrove area and cropland. Most BH was found in dry forest (60.9%), followed by mangrove and cropland.

Indirect Evidence of Honey Consumption Without Tools

We found indirect traces for the consumption of BH, without tools, in all four study communities. These traces consisted of honeycomb or wax that was discarded after it had been exploited. They presented distinct tooth marks or were left behind in the form of wadges.

Tools Collected

In total we collected 204 individual stick tools from 70 tool use ateliers (see **Figures 2B,D** for examples). Stick tools were found in three of the four study communities' home ranges, although at different frequencies. 50% of all tools were found in Madina (*N* = 103) and 49% in Cambeque (*N* = 100), with only a single tool found in Lautchandé, and none in Caiquene-Cadique. These tools were associated with four types of fluid extraction: MM, BM, and BH honey, and, on a single occasion, an unidentified fluid (UF). Tools were mainly recovered from mangrove areas (67%), followed by closed secondary forest (30%), open secondary forest (2%) and an agricultural field (1%). In Madina we found tools associated with the extraction of MM (97%), BH (1.9%), and BM (1%), while in Cambeque tools were only associated with the extraction of BM (62%) and MM (38%). In Lautchandé the single tool found was associated with UF. By grouping tools found by the same hive into age classes as well as revisiting hives that were not depleted after the first raiding event we were able to confirm their repeated exploitation, with the use of tools, on separate occasions. One BM hive in Cambeque was successfully raided twice and on two other occasions chimpanzees attempted to exploit it without success

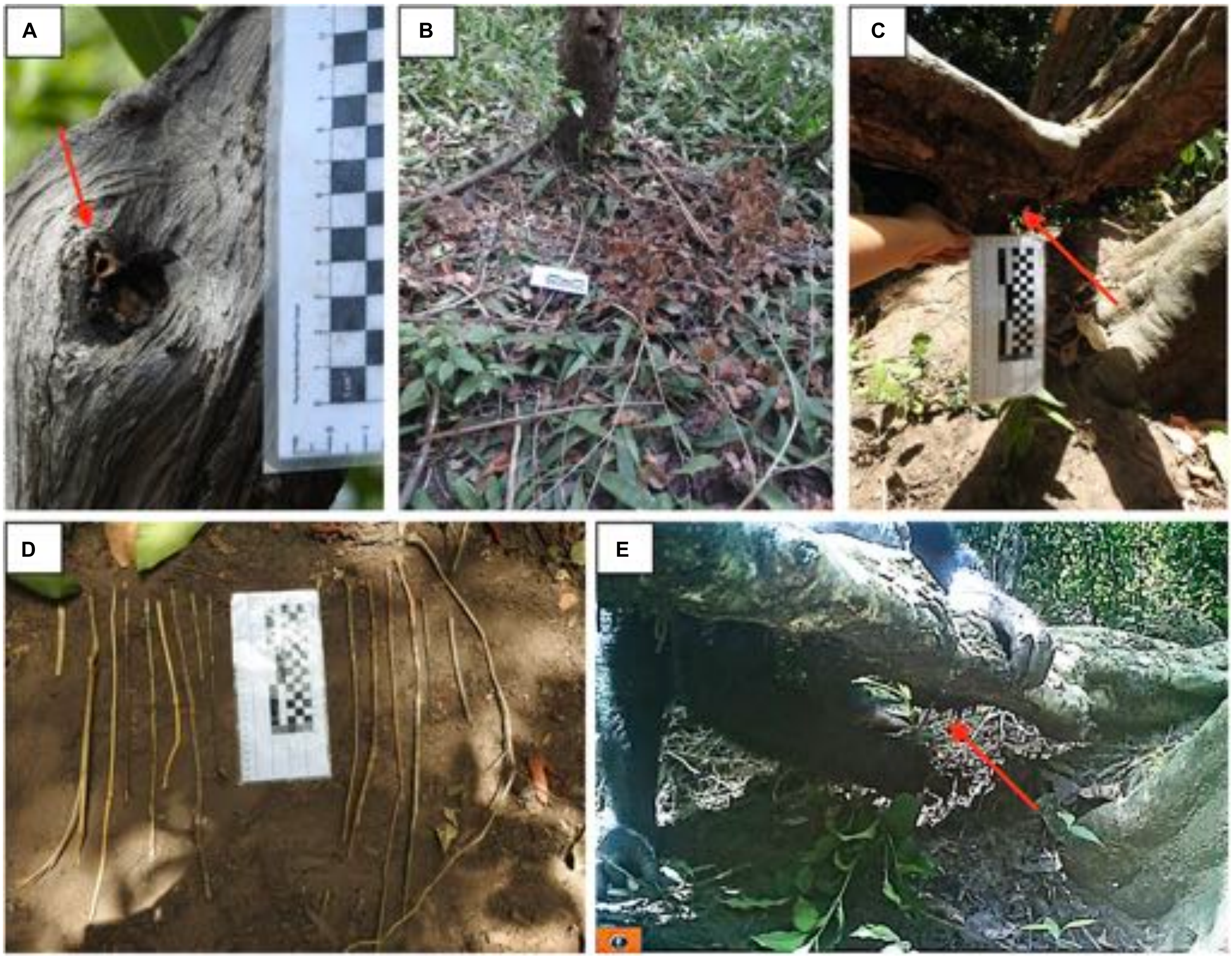

FIGURE 2 | Beehives and tools used to extract honey by chimpanzees in CNP. **(A)** A small stingless bee (Meliplebeia sp.; MM) hive located in the mangrove area at Madina. The arrow points to the entrance tube of the stingless bees' hive; the hive's entrance covered by wax has been exposed, tools were found next to the hive. **(B)**. A large stingless bee (*Meliponula* sp.; BM) hive exploited by chimpanzees in secondary open forest at Cambeque, with tools *in situ* **(C)**. A large stingless bee (*Meliponula* sp.; BM) hive located in secondary forest at Cambeque. The arrow points to the entrance tube of the stingless bees' hive; the hive's entrance covered by wax has been exposed. **(D)** Tools found by hive **(C)** with two different estimated ages of use – one-day-old (new) on the left, approx. 1-week-old (recent) on the right. **(E)** Screenshot from camera trap footage showing a juvenile chimpanzee inserting a manufactured tool into the hive shown in **(C)** in Cambeque secondary open forest (tool indicated by red arrow) (see **Supplementary Material 3**. Observation 2).

(recorded on video). At least seven MM hives were exploited more than once by chimpanzees in Madina.

Table 1 presents descriptive statistics for all tools and tool characteristics. Overall, the mean number of tools found per atelier was 3.05 (± 2.75). Mean tool length was 40.79 (± 15.39) cm and mean mid diameter 6.46 (± 15.3) mm. 90.7% of all tools had four to six modifications. Tools were found with distinct wear patterns – brushed/frayed, blunt/mashed and fragmented (see **Figures 3A–C** for examples of each type of wear pattern).

112 (54.9%) of 204 tools were found to have at least one frayed end and 106 (52%) tools had at least one blunt end. 14.7% ($n = 30$) of tools had two different wear patterns at opposite extremities, suggesting possible multifunction for those tools (**Figure 3E**). Sticks that had both ends fragmented or without a clear wear pattern (i.e., absence of brush or blunt end) were only considered tools if they had traces of honey on at least one of the extremities (n = 15, 7.4% tools). Honey residue was found on 132 tool extremities: on 66 (51.2%) brush tips, 36 (35.6%) blunt ends, and 30 (26.5%) fragmented ends (**Figure 3D**).

Of the tools with at least one frayed end, 63.4% ($n = 71$) had a frayed proximal end and the mean fray length was 26.31 (± 17.46) mm. Of the tools with at least one blunt end 71.7% ($n = 76$) had blunt distal ends. 62.4% ($n = 128$) of all tools had signs of wear at only one extremity, of these 56.3% ($n = 72$) had proximal wear patterns. 109 (53.4%) tools presented signs of honey/wax/insects and 20 (9.8%) had distinct bite marks.

Tools were made out of fresh twigs from tree species typically found no further than 5 m from the hive (mean ± SD = 0.436 ± 1.026), with most being sourced from the same tree where the hive was located. In the mangroves

TABLE 1 | Summary of tools and tool characteristics as a function of type of fluid exploited.

Type of fluid	Number of tool ateliers	Number of tools	Mean number of tools per atelier	Tool characteristics						
				Mean length (cm)	Mean diameter (mm)	Mean number of modifications	Mean length of fray (mm)	Only 1 end used	Both ends used	Possible multifunction tools
MM	59	138	2.53 ± 1.79 [1-8]	40.4 ± 12.1 [13.4-78.8]	6.2 ± 1.5 [3.3-11.2]	4.87 ± 0.94 [2-6]	22.4 ± 11.0 [3.4-65.5]	98 (71.1%)	40 (28.9%)	20
BM	9	64	7.86 ± 4.71 [1-14]	42.58 ± 20.8 [6.8-133]	6.9 ± 2.6 [3.2-15.1]	4.9 ± 0.98 [2-6]	35.6 ± 25.7 [4.9-113.0]	30 (46.8%)	23 (35.9%)	10
BH	1	2	2	34.15 ± 14.8 [23.7-44.6]	5.0 ± 2.0 [3.6-6.4]	6	18.0 ± 10.3 [4.3-27.8]	0	2	0
UF	1	1	1	32.8	10.4	6	33.5	0	1	0
TOTAL	70	204	3.08 ± 2.75 [1-14]	40.79 ± 15.39 [6.8-133]	6.46 ± 15.3 [6.8-133]	4.9 ± 0.95 [2-6]	26.31 ± 17.46 [3.4-118.0]	128	76	30

MM, Meliplebeia sp, small stingless bee; BM, Meliponula sp., large stingless bee; BH, Apis mellifera, honey bee; UF, Unknown fluid.

one species was used exclusively as raw material, the mangrove tree (*Avicennia germinans*). Similarly, in cropland only one species was used, the orange tree (*Citrus sinensis*). In dry forest, several different species were chosen as raw material, including *Strombosia pustulata, Sarcocephalus latifolius, Vitex doniana, Dialium guineense, Ceiba pentandra, Antiaris toxicaria, Trichilia monodelpha and Albizia ferruginea.*

Comparison of Tools by Site and by Resource Exploited

Table 1 further breaks down tools and tool characteristics by the type of fluid exploited. Of the four sources of fluid exploited, MM was associated with the highest number of tools and tool use ateliers encountered (138 and 59, respectively), followed by BM with 64 tools and 9 ateliers, BH with two tools and one atelier, and UF with a single tool recovered.

Given the low frequency of tools recovered in association with BH and UF these will not be used in subsequent comparisons. Additionally, the single tool found in Madina associated with BM extraction was not included in the comparisons. Given the structure of the dataset that remains, in which only one chimpanzee community repeatedly exploited more than one type of fluid, and only one type of fluid repeatedly exploited by more than one community, we first focus on potential differences in tool use associated with two different resources frequently consumed at one particular site, then compare tool use at two different sites associated with a single resource.

Comparison of Tools Found in Cambeque for the Extraction of BM and MM

Cambeque was the only site at which more than one type of honey (BM and MM) was frequently exploited with the use of tools (**Table 2**). The mean number of tools found per atelier was significantly higher for BM (7.88 ± 4.36) compared to MM (2.47 ± 1.99) (Welch's two-sample t-test: $t = 3.33$, $df = 8.6$, $p = 0.009$) (**Figure 4A**). Mean tool length was similar for MM (43.88 ± 13.10 cm) and BM (40.93 ± 15.47 cm), confirmed by a non-significant test result (Welch's two-sample t-test: $t = 0.48$, $df = 97.9$, $p = 0.633$) (**Figure 4B**). The mean mid diameter of BM tools was higher than that of MM tools but this difference was also not significant (6.88 ± 1.9 mm and 6.25 ± 1.31 mm, respectively; Welch's two-sample t-test: $t = 1.52$, $df = 95.2$, $p = 0.131$). MM and BM tools had a similar number of modifications: on average 4.84 and 4.90 respectively. The percentage of bark left after modification was significantly lower in BM (60.47 ± 33.94%) than MM (79.45 ± 23.30%) tools (Welch's two-sample t-test: $t = 3.31$, $df = 96.6$, $p = 0.001$) (**Figure 4C**).

Fifty one percent ($n = 19$) of MM tools and 49% ($n = 31$) of BM tools had at least one frayed end. Blunt ends were present in 47% ($n = 18$) and 46% ($n = 29$) of MM and BM tools, respectively. 17% of all BM tools and 3.2% of MM tools had fragmented ends without any further signs of wear (i.e., no blunt or frayed ends). There was a significant difference when comparing tools that had signs of wear on both ends: this was the case for 13% ($n = 5$) of MM tools and 35% ($n = 22$) of BM tools (Chi-square test: $X^2{}_2 = 11.34$, $p = 0.001$). Of these tools 45% ($n = 10$) BM and 40% ($n = 2$) MM tools had different wear types at the two tool ends, i.e., they were potential multifunction tools. Finally, the average length of fray on frayed ends was significantly longer on BM tools (35.44 ± 25.48 mm) than MM tools (25.16 ± 11.91 mm) (Welch's two-sample t-test: $t = 2{,}09$, $df = 55.5$, $p = 0.041$).

Comparison of Tools Used to Exploit MM in Cambeque and Madina

MM honey was the overall most frequently exploited resource with the use of stick tools; however, no evidence was found in Caiquene-Cadique and Lautchandé despite the presence of MM hives at those two sites. We therefore compare MM tool characteristics between the communities of Cambeque and Madina only (**Table 2**).

In Madina we found 43 ateliers with a total of 100 tools, while in Cambeque 16 ateliers provided 38 tools. The mean number of tools found per atelier was similar between the two communities, with 2.47 and 2.40 tools in Cambeque and Madina, respectively (two-sample t-test: $t = 0.04$, $df = 51$, $p = 0.970$) (**Figure 4D**). Mean tool length was significantly higher in Cambeque (43.9 ± 13.1 cm) compared to Madina (39.1 ± 11.53 cm) (two-sample t-test: $t = 2.12$, $df = 136$,

FIGURE 3 | Examples of tools found abandoned in tool use ateliers. **(A)** Brush tips; **(B)** blunt tool ends; **(C)** fragmented tool ends; **(D)** tool ends with honey residue (indicated by the red arrows); **(E)** potential multifunction tools with fray/brush ends and blunt/mash ends at opposite tool ends.

p = 0.036) (**Figure 4E**), but there was no significant difference in mean mid diameter (two-sample t-test: t = 0.18, df = 136, p = 0.850). The percentage of bark left after modification was not significantly different between communities (two-sample t-test: t = 0.68, df = 136, p = 0.496) (**Figure 4F**). All tools found in both communities were sourced from the same tree species of mangrove (*Avicennia germinans*).

In Cambeque 51% (n = 19) and in Madina 58% (n = 58) of all tools had at least one frayed end. Blunt ends were present in 47% (n = 18) and 56% (n = 56) of all tools in Cambeque

TABLE 2 | Summary of tools and tool characteristics as a function of chimpanzee community.

Community	Number of tool ateliers	Number of tools	Mean number of tools per site	Mean length (cm)	Mean diameter (mm)	Mean number of modifications	Average length of fray (mm)	Only 1 end used	Both ends used	Possible multifunction tools
Cambeque MM	16	38	2.47 ± 1.99 [1-8]	43.9 ± 13.1 [21.2-78.8]	6.3 ± 1.3 [3.3-15.1]	4.84 [3-6]	25.2 ± 11.9 [8-46.2]	32 (84.2%)	5 (13.1%)	2
Cambeque BM	8	63	7.88 ± 4.36 [1-14]	40.93 ± 15.47 [6.8-133]	6.88 ± 1.9 [3.2-15.1]	4.9 ± 0.96 [2-6]	35.4 ± 25.5 [4.9-113.0]	30 (47.6%)	22 (34.9%)	10
Madina MM	43	100	2.40 ± 1.53 [1-6]	39.1 ± 11.51 [13.4-71.5]	6.2 ± 1.5 [3.3-11.2]	4.89 [2-6]	21.8 ± 10.5 [3.4-65.5]	66 (66%)	32 (32%)	18

Shown, are tools associated with the exploitation of small stingless bee honey (MM), for the two communities (Cambeque and Madina) as well as large stingless bee honey (BM), for one community (Cambeque).

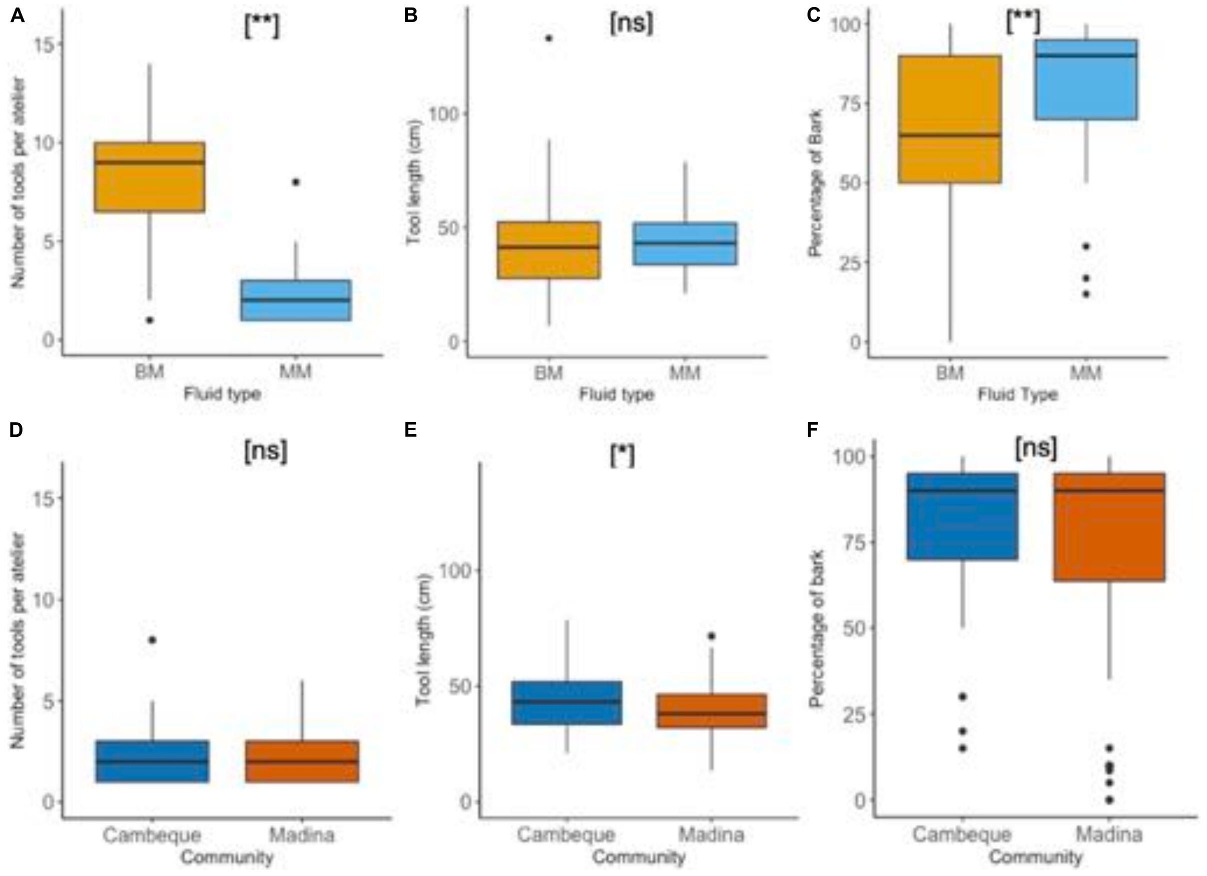

FIGURE 4 | Honey dipping tool characteristics. Tools per atelier, tool length (cm) and percentage of bark remaining, compared between fluid types BM and MM at Cambeque **(A–C)** and between two neighbouring communities, Cambeque and Madina, exploiting MM **(D–F)**. Boxplots indicate upper and lower quartile; thick horizontal lines represent median. Maximum and minimum data range indicated by whiskers; black dots show individual outliers. *P*-values are indicated by [ns] – $p > 0.05$; [*] – $p \leq 0.05$; [**] – $p \leq 0.01$.

and Madina respectively. 5.4% of tools in Cambeque and 4.0% Madina had no signs of wear at either extremity. Tools with signs of wear at both ends were significantly more frequent in Madina (n = 32, 32%) compared to Cambeque (n = 5, 15.8%) (Chi-square test: $X^2{}_2 = 4.495$, $p = 0.034$); of these 56% and 40% tools had different wear patterns at the two ends for Madina and Cambeque, respectively. The average length of fray on frayed ends was longer in Cambeque (25.16 ± 11.91mm) than Madina (21.81 ± 10.54mm), but the difference was not significant (two-sample t-test: $t = 1.20$, $df = 82$, $p = 0.233$).

Remote Camera Trap Observations of Tool Use and Honey Extraction

Camera traps provided footage of chimpanzees extracting or attempting to extract MM, BM, and HB. In total, 6977 videos were captured, of which 386 (5.5%) were of chimpanzees. Of the 386 chimpanzee videos, 12 (3.1%) videos provided evidence of tool use to extract MM in Madina and BM in Cambeque (**Figure 2E** and **Supplementary Video 1**). These 12 videos corresponded to seven independent events (where an event started when an individual approached a hive and finished when the individual left and did not return into the camera's view). See **Supplementary Material 3** where we describe each tool-use event.

Additionally, camera traps captured chimpanzees on three separate occasions in Caiquene-Cadique raiding a natural HB hive, by inserting their hands deep inside a tree trunk. As soon as these chimpanzees removed their hands from the active hive, swarms of bees emerged, and the chimpanzees ran off holding honeycomb. After the hive was abandoned by the bees (no more bees could be seen or heard in the camera trap footage) one individual inspected it using the same method but was seen leaving calmly and empty handed. All individuals were adult males. In Madina a natural HB hive that had been exploited the previous day by humans was checked by an adult female chimpanzee. She looked inside and inserted her hand into the tree trunk, but no honey was extracted.

DISCUSSION

Our study examined the use of dipping tools to access different types of honey in four neighbouring chimpanzee communities in central CNP, Guinea-Bissau. This is the first evidence of honey dipping tool use by chimpanzees in Guinea Bissau. Dipping tools were found in three of the four studied communities, but only in two of these (Madina and Cambeque) were we able to positively identify the tools as honey dipping tools. These results are puzzling since honey is present in the home ranges of all four studied communities, and a previous study on the feeding ecology of CNP chimpanzees, based on indirect data collection and faecal sample analyses, have shown that honey is an important part of the diet at Caiquene-Cadique (Bessa et al., 2015), one of the communities where dipping tools were not found. This discrepancy might be due to a number of methodological, ecological or behavioural factors.

Firstly, these communities are unhabituated to human researchers – we therefore had to rely on a combination of indirect methods and camera traps to study their tool use behaviour. Thus, it is possible that tool use related to honey extraction is present at Caiquene-Cadique as well, but we simply failed to find evidence for it. In a similar vein, we were also unable to attribute tools found to specific individuals, and as such, one or a few individuals could have been responsible for the manufacturing of a large proportion of tools found at a given site (note, however, that our video footage, albeit limited, supports the idea that within a given community there are indeed different individuals making and using tools). Most of our knowledge on wild chimpanzee behaviour comes from communities that can be followed daily and their behaviour studied directly (e.g., Goodall, 1986; Boesch and Boesch-Achermann, 2000; Matsuzawa et al., 2011; McLennan et al., 2019). Hence, when studying unhabituated communities, much of the subjects' behavioural repertoire remains inaccessible to the researcher. Nonetheless, studies on these communities are beginning to gain traction, partly because of a reduction in efforts to habituate new communities given that so many already live in fragmented habitats and in close proximity to humans, where the loss of fear of humans could be counter-productive to conservation and welfare. It is also worth noting that the number of communities already habituated to researchers is extremely low compared to the species' total population size. In West Africa, for example, only five chimpanzee communities are fully habituated to researchers (one at Bossou, Guinea, Matsuzawa et al., 2011; three in the Taï Forest, Ivory Coast, Boesch and Boesch-Achermann, 2000; and one in Fongoli, Senegal, Pruetz and Bertolani, 2007) representing a total of approximately 200 individuals out of an estimated 52,811 (CI 17,577-96,564) chimpanzees in the region (Heinicke et al., 2019). As such, our current knowledge of chimpanzee behaviour and behavioural variation - that is biased towards those communities that can be followed - may represent only a small fraction of the full picture. On the other hand, despite the limitations of studying unhabituated communities, studies have already successfully discovered new behaviours and behavioural variation through a combination of suitable methods that did not rely on habituation (e.g. Kühl et al., 2019). Furthermore, in our research, we employed the same methodology and level of effort in each of the four studied communities, suggesting that the variation we found is unlikely to be a function of differential research effort or observational bias.

Another possible explanation for the differences we found may be rooted in differential resource availability. Honey was recorded in all the studied communities' home ranges; however, in Lautchandé honey was not encountered frequently and chimpanzees did not seem to utilize the mangrove areas where stingless bee honey is commonly found. This might explain the low incidence of tool use (with only a single tool found) at Lautchandé. Nonetheless, honey was encountered frequently in Caiquene-Cadique, where the chimpanzees often use mangroves to access different parts of their home range and pass many stingless bee hives along their routes. This suggests that occasions for honey extraction are plentiful at Caiquene-Cadique, hence lack of opportunity cannot solely explain the complete absence of tools there.

A third, related explanation for the apparent lack of tool use for honey-extraction in Caiquene-Cadique might be that chimpanzees feed on honey from stinging honey bees (*Apis mellifera*) more frequently, either from natural or artificial hives (Bessa et al., 2015). To exploit this particular resource, chimpanzees have been observed employing a different approach that is quicker than tool use and therefore less likely to subject them to painful stings from the bees: they perform rapid hit-and-run raids on the hives.

Lastly, the differences in the numbers of tools recovered at the four sites might be due to variation in material culture between communities. Many studies have shown that different chimpanzee communities exhibit different behaviours, including the use of tools, even without obvious ecological or genetic determinants on these behaviours (Whiten et al., 1999;

Luncz et al., 2012). This has been described as cultural variation, i.e., the emergence and maintenance of behavioural variants through local innovation and subsequent social learning. While the validity of a genetics-ecology-culture trichotomy has rightly been called into question (Laland and Hoppitt, 2003; Laland et al., 2009; Koops et al., 2013, 2014), evidence of behavioural variation even across neighbouring chimpanzee communities that share migrants and that inhabit very similar habitats has provided some of the most convincing evidence so far of cultural processes at work (Luncz et al., 2012; Koops et al., 2015; Pascual-Garrido, 2019). Nonetheless, it is also important to note that even in cases where ecological or genetic differences exist, intergroup variation in behaviour may still be attributable to culture, as long as the given behaviour is acquired at least in part by social learning. In fact, if different communities exhibit the same behavioural pattern, but these behaviours are socially learnt, they still qualify as cultural. Although we cannot state with certainty that honey dipping tools are absent in Caiquene-Cadique and Lautchandé, from the evidence gathered in this study their use certainly appears less frequent than that of Cambeque and Madina chimpanzees. Due to the spatial proximity of these four communities, and the resulting exchange of migrants and similarities in ecology and resource availability in at least three of the studied communities, we may therefore hypothesize that these differences in honey exploitation are, at least in part, cultural in nature. Nonetheless, direct evidence of social learning, as emphasised particularly strongly by the tridimensional model of animal traditions (Fragaszy and Perry, 2003), would be necessary for this hypothesis to stand. Collecting such evidence – which typically requires extended periods of direct observation – was beyond the scope of our current study.

At a more fine-grained level, when comparing tools used to exploit different types of stingless bee honey, some further notable differences were found. BM tool ateliers tended to have significantly more tools associated with each honey extraction event than did MM or BH tool ateliers, tools where both the proximal and distal ends were used were more frequently found in BM ateliers, and, when ends were frayed, the fray length tended to be significantly longer for BM tools. These differences might be due to the type of habitat where the different bee species' hives are normally found. MM are mostly found in mangrove areas, an open landscape where shelter is limited and where humans frequently pass. Given that BM is normally found in the forest where the likelihood of encountering humans is much lower, chimpanzees might have the opportunity to spend longer periods of time exploiting them. The hives' location and structure might be related to these differences as well. Most MM hives found were located high up in live trees and had extremely small entrances, while some BM nests were found in dead or fragile tree trunks which meant that the small hive opening could be enlarged more easily, or the trunk could be fragmented. A larger opening will allow increased access to honey and therefore more time spent at the site. This may in turn translate to more repetitions of using the same tool (thus making the fray longer), until it has to be substituted by using the other tool end (leading to more tools with both the distal and proximal ends used) and/or by manufacturing a new tool (leaving behind a higher number of tools at the site).

Additional differences were also found when comparing the tools used for MM extraction in Madina and Cambeque. In Cambeque, tools were significantly longer than in Madina, and in Madina there were significantly more tools found with both ends showing signs of use or modifications. Importantly, at both sites tools were used to exploit the same resource, the raw material used was the same (*Avicennia germinans*), and the hives were exclusively arboreal, located in live trees with small entryways. Hence, the differences in tools cannot easily be explained through environmental differences, and a genetic explanation is unlikely given the documented gene flow between communities (Sá, 2013). Therefore, again a cultural explanation is likely, this time pertaining to subtle differences in the characteristics of tool manufacture and use between the Cambeque and Madina communities.

When analysing the CNP chimpanzees' dipping tool kit as a whole, some important patterns were found. Many of the tools collected had one or both ends frayed. Frayed/brush ends have been described in many other chimpanzee communities for fluid dipping (Stanford et al., 2000; Fowler and Sommer, 2007; Boesch et al., 2009; Sommer et al., 2012; Lapuente et al., 2017) or termite fishing (e.g., Sanz and Morgan, 2007). In Comoé National Park (Ivory Coast) chimpanzees have been seen biting the ends of tools to loosen the fibers creating a brush (Lapuente et al., 2017), and at Goualougo (Republic of Congo) a similar manufacture process to create brushed ends for termite fishing tools is associated with increased termite harvest (Sanz and Morgan, 2007). However, in other cases brushed ends have been linked to the fibre structure of the raw material that, when broken, might naturally form a brush (Takemoto et al., 2005). In the case of CNP we do not know if the fray is a simple by-product of use or if it is specifically added by chimpanzees prior to use. Notably, we found all three types of wear (brush, blunt and fragmented) on tools made out of multiple different species of raw material. Furthermore, all tools used to exploit MM were made out of the same raw material but exhibited all three types of wear. Taken together, these observations are more supportive of a pattern of production rather than fraying being merely a by-product of the fibre structure. Interestingly, the human communities that live alongside the CNP chimpanzees also exploit the same types of honey opportunistically: when they encounter a stingless bee hive they will often enlarge the opening of the hive, for easier access to the honey, and chimpanzee tools are sometimes encountered by these trees (JB, personal observation). Human traces, however, are clearly distinct from those of chimpanzees given that they present clean cuts made by a knife or machete on the end opposite to the brush, all side branches are sliced off, and the bark is peeled off using the same cutting tool, while the hole of the hive will also present signs of having been enlarged by a machete. As such, the likelihood that we misclassified human tools as chimpanzee tools is very low. Nonetheless, these observations raise important questions about how human activities such as honey harvesting and traditional apiculture might impact chimpanzee tool use (see Hockings et al., 2015 for similar research), and should be the focus of future research.

As tools with different wear patterns were found associated with the same hives, including subsets that were the same approximate age (see, for example, **Figure 2D**), it is possible they were all used during the same honey extraction episode, in a potential sequence. This suggests the use of tool sets by chimpanzees at CNP. When comparing the patterns of wear encountered in CNP with other published data it is possible that the CNP tool set has at least three types of tools. The first are exploratory probes, where very little modification is present. On some occasions (as confirmed by camera trap footage) chimpanzees simply procure a small twig, remove some side branches with leaves, and use it in a delicate motion to test if there is any honey present in the hive to collect. The second are pounding tools, where one or both tool ends present blunt or mashed ends, suggesting a pounding motion, possibly to break or separate the hard wax in the hive. Finally, the third type represent extraction tools – these have brush ends that are either a by-product of use or a deliberate modification (see Boesch et al., 2009). Given the scarcity of video evidence to date, we can only speculate that these tools serve these specific functions and that they may have been used in sequence, nonetheless the fact that tools that were made of the same raw material presented such distinct wear patterns gives us some degree of confidence that they were used for different functions. Additionally, comparing our indirect data to direct evidence collected in central Africa, where tool sets of up to five different tool types are used in sequence [e.g., Loango NP, Gabon (Boesch et al., 2009); Moukalaba-Doudou NP, Gabon (Wilfried and Yamagiwa, 2014)], strengthens our hypothesis that a similar sequence of use could be present in CNP.

Finally, we describe another characteristic of the CNP honey-dipping tool kit. In a few cases, tools showed different types of wear patterns at opposite ends, one frayed and one blunt. This suggests that these tools may have had more than one function, i.e., they were multifunctional tools, used both for pounding and for extraction. Such tools have been described for honey extraction in central Africa (at Goualougo, by Sanz and Morgan, 2009 and at Loango, by Boesch et al., 2009). Multifunctional tools were once thought to be unique to humans, manifestations of highly sophisticated and complex technology. While we now know them to also be present within the wild chimpanzee tool kit, they have never until now been described for West African chimpanzees. Indeed, the use of tools to collect honey within Western chimpanzees has been regarded as less common than among Central African chimpanzees (Boesch and Boesch, 1990; Bermejo and Illera, 1999; Ohashi, 2006; Lapuente et al., 2017). It is important to note that given the lack of direct evidence of tool manufacture, we cannot completely discount the possibility that different wear patterns on the same tool resulted from an individual re-using another individual's tool for a different function.

Our study illustrates the importance of research on previously unstudied chimpanzee communities, including chimpanzees inhabiting human-impacted areas. They allow us to fill some gaps in our knowledge of the chimpanzee behavioural repertoire, revealing interesting new behaviours, and adding to the list of habitat types that we now know to be exploited by wild chimpanzees. CNP chimpanzees' use of the mangrove habitat is one such example (note that previous, albeit rare, reports of mangroves being part of chimpanzee home ranges did not describe their use by the resident communities – e.g., Loango chimpanzees in Head et al., 2019). Over 67% of the tools we recovered were found in mangroves, which suggests that this habitat type may have great significance for chimpanzees inhabiting the westernmost limit of the species' distribution. Our results also suggest potential cultural variation between neighbouring communities, and, the fact that our evidence was gathered through a combination of indirect and remote methods confirms that multifaceted methodologies are able to provide meaningful data even when studying populations where habituation is not possible or appropriate. It is clear, like in any other study of wild animal behaviour, that the longer the study continues the more we will learn about the lives and behaviour of these chimpanzees. It is, therefore, imperative that studies like ours continue long term, not only informing us about chimpanzee behaviour but also about chimpanzee behavioural variation and behavioural plasticity that might aid future conservation strategies, for example by identifying key chimpanzee resources, adaptations to anthropogenic changes, or even cryptic behaviours in response to added land pressures.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This research was reviewed and approved by the Instituto da Biodiversidade e das Áreas Protegidas (IBAP) in Guinea-Bissau. All research involving wild chimpanzees was non-invasive and purely observational, and strictly adhered to ethics guidelines detailed by the Association for the Study of Animal Behaviour (United Kingdom).

AUTHOR CONTRIBUTIONS

JB, KH, and DB conceived the ideas and designed the methodology. JB collected and analysed the data. JB led the writing of the manuscript, all authors contributed critically to the drafts and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.625303/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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APPENDIX D

ORGANISMAL BIOLOGY

Automated audiovisual behavior recognition in wild primates

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Large video datasets of wild animal behavior are crucial to produce longitudinal research and accelerate conservation efforts; however, large-scale behavior analyses continue to be severely constrained by time and resources. We present a deep convolutional neural network approach and fully automated pipeline to detect and track two audiovisually distinctive actions in wild chimpanzees: buttress drumming and nut cracking. Using camera trap and direct video recordings, we train action recognition models using audio and visual signatures of both behaviors, attaining high average precision (buttress drumming: 0.87 and nut cracking: 0.85), and demonstrate the potential for behavioral analysis using the automatically parsed video. Our approach produces the first automated audiovisual action recognition of wild primate behavior, setting a milestone for exploiting large datasets in ethology and conservation.

INTRODUCTION

The field of ethology seeks to understand animal behavior from both mechanistic and functional perspectives and to identify the various genetic, developmental, ecological, and social drivers of behavioral variation in the wild (*1*). It is increasingly becoming a data-rich science: Technological advances in data collection, including biologgers, camera traps, and audio recorders, now allow us to capture animal behavior in an unprecedented level of detail (*2*). In particular, large data archives including both audio and visual information have immense potential to measure individual- and population-level variation as well as ontogenetic and cultural changes in behavior that may span large temporal and spatial scales. However, this potential often goes untapped: The training and human effort required to process large volumes of video data continue to limit the scale and depth at which behavior can be analyzed. Automating the measurement of behavior can transform ethological research, open up large-scale video archives for detailed interrogation, and be a powerful tool to monitor and protect threatened species in the wild. With rapid advances in deep learning, the novel field of computational ethology is quickly emerging at the intersection of computer science, engineering, and biology, using computer vision algorithms to process large volumes of data (*3*).

The aim of this paper is to automate animal behavior recognition in wild footage. Deep learning–based behavior recognition has thus far been shown in constrained laboratory settings (*4*, *5*) or using still images (*6*) and has yet to be effectively demonstrated on unconstrained video footage recorded in the wild. Measuring animal behavior from wild footage presents substantial challenges—often, behaviors are hard to detect, obscured by motion blur, occlusion, vegetation, poor resolution, or lighting. If successful, then the tools would enable exploration of a multitude of research questions in ethology and conservation. Increasingly, research is revealing fine-scale variation between individuals and populations of wild animals (*7*); however, capturing this variation is often laborious and not feasible on the large scale through manual annotation. Automated approaches allow us to examine in more detail the variation, through cross comparison of animal groups in a wide variety of contexts. Detailed time series data of individual behavior enables integration of time depth perspectives into field research to more comprehensively reconstruct how behavior develops across the life span (ontogenetically) as well as examine how other processes such as social transmission, demography, and ecology interact to drive behavior change over time (*8*). These detailed behavioral data are also a crucial component of conservation research: They enable us to investigate how anthropogenic pressures such as climate change and habitat fragmentation disrupt animal behavior (*9*) (migratory patterns, foraging, reproduction, etc.) and to develop novel behavioral metrics to monitor the risks to and viability of threatened populations (*10*, *11*). Here, we demonstrate the potential of such an approach by developing a system for the automated classification of two distinct wild chimpanzee behaviors with idiosyncratic audiovisual features: nut cracking and buttress drumming. We also analyze pilot data of sex and age differences in percussive behaviors (nut cracking and drumming) from longitudinal archive and camera trap datasets. Chimpanzees are an ideal species for testing behavioral recognition; owing to their large fission-fusion societies, complex sociality, and behavioral flexibility, they exhibit exceptionally rich behavioral repertoires (*12*). Our target behaviors, nut cracking and buttress drumming, differ in their function—extractive tool use versus long-distance communication, respectively—but both involve

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percussive actions that produce distinctive sounds, i.e., the pounding of a hammer stone against a nut balanced on an anvil stone and the pounding of hands or feet against large buttress roots. Whereas nut cracking is limited to some West African and Cameroon chimpanzees (*Pan troglodytes verus* and *Pan troglodytes ellioti*), buttress drumming is a universal behavior across all chimpanzee communities (*12*).

In relation to previous works using deep learning, individual reidentification has been a critical first step toward full automation (*13*, *14*), but this alone cannot capture the full complexity of behaviors that animals perform in the wild across space and time. Existing methods have used deep learning for markerless pose estimation to track the movement of animal body parts (*15*), but pose estimation models perform poorly at recognizing actions using posture and limb movements alone (*16*). Other approaches have used single-image analysis to identify basic activities of wild animals using tagged information from camera traps, but these fail to capture the dynamic sequences of behavior required for detailed analysis (*17*). Recent advances in human action recognition in the field of computer vision have used three-dimensional (3D) convolutional neural networks (CNNs) (*18*), which incorporate spatiotemporal information across video frames (*19*), but thus far have only been applied to animal species to produce broad behavioral classification limited to the visual domain (*20*).

Given that both behaviors have strong audio and visual signatures, we recognize actions using both audio and visual streams. Our automatic framework consists of two stages: (i) body detection and tracking of individuals through the video (localization in space and time) and (ii) audiovisual action recognition (Fig. 1 and movie S1). Audio allows us to determine temporal segments where the nut cracking and buttress drumming occur ("scene level") but does not pinpoint the individual responsible. By visually detecting and tracking all chimpanzees that appear in the video, frame by frame, we are able to determine the spatial position of each individual present. The next stage of our framework uses both the scene level audio and the visual content of each track to specify which individual is performing the behavior ("individual level"). Both stages in our pipeline use a deep CNN model (see Materials and Methods).

The audio stream can also be used to provide a preview mechanism to filter out behavioral sequences for human annotators to label (*21*), substantially reducing the time required to collect annotations. This is achieved using an audio-only action recognition model (which operates at the scene level) and can identify "proposals" or short video sequences where the action is likely to occur. A human annotator then only verifies whether the sequence contains the action or not. This allows us to efficiently create a labeled action recognition dataset that can be used to train the second stage of our automatic pipeline.

Our method is able to identify where fine-grained movements such as striking and drumming are occurring in time and space automatically. It consists of a deep CNN model, which predicts actions using audio only, visual only, and both audio and visual modalities together. We demonstrate the use of our pipeline on two different data sources: For nut cracking, we use part of a longitudinal video archive recorded by human-operated camcorders at an "outdoor laboratory" in Bossou, Guinea (*14*, *22*); while for buttress drumming, data were collected between 2017 and 2019 by 25 motion triggered cameras in Cantanhez National Park, Guinea-Bissau (*23*). Last, we also demonstrate possible next steps in behavioral analysis enabled by the automatically parsed video. This approach represents the first automated audiovisual action recognition of species in the wild.

RESULTS

For nut cracking, we apply our pipeline to 40.2 hours of video containing 2448 nut cracking sequences (see Materials and Methods for definition of a sequence), resulting in a total of 24,700 individual body tracks (linked detections through video frames of the same

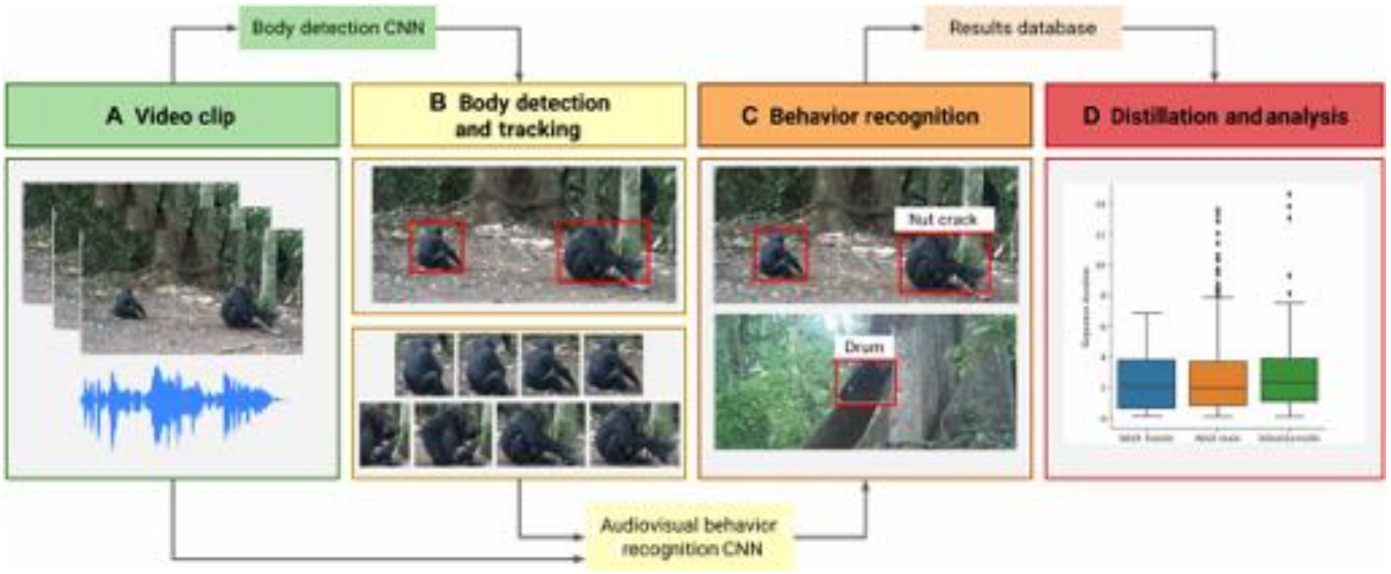

Fig. 1. Fully unified pipeline for wild chimpanzee behavior recognition and analysis from raw video footage. The pipeline consists of the following stages: (**A**) Frames and audio are extracted from raw video. (**B**) Body detection is performed over the video frames using a deep CNN single-shot detector (SSD) model, and the detections are tracked using a Siamese tracker. (**C**) The body tracks are classified (e.g., is this individual cracking nuts?) using the audio data and spatiotemporal visual information for the track by a deep CNN audiovisual behavior model. The system only requires the raw video as input and produces labeled body tracks and metadata as temporal and spatial information. This automated system can be used to perform large-scale analysis (**D**) of behavior. Photo credit: Kyoto University, Primate Research Institute.

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individual; Fig. 1C and fig. S1B). The training set for our model consists of data taken from three years (2004, 2008, and 2012), while we test the performance of our model entirely on data from a different year (2013) to demonstrate generalizability over time. Our audio-only nut cracking recognition CNN model obtains high average precision (85%; Table 1) at the scene level. Results at a scene level only detect time periods where nut cracking is being performed in the video, but they do not isolate the nut cracker, given that multiple individuals may be nut-cracking in the video at the same time (Fig. 2). We also predict results at an individual level, identifying whether a particular individual is nut-cracking or not. Our chimpanzee body detector achieved an average precision of 92% (fig. S1), and our nut cracking recognition model performed well on different poses and lighting conditions typical of videos recorded in the wild (Fig. 2 and movie S1), achieving an overall average precision of 77% at an individual level (Table 1 and Fig. 3).

For buttress drumming, 10.8 hours of camera trap footage are analyzed, resulting in a total of 1251 drumming sequences. We trained our model on data from two chimpanzee communities (Cabante and Caiquene-Cadique) and evaluated our model on manually labeled held-out test data from a third community (Lautchandé). Data from an additional community (Cambeque) are included in the analysis. Our drumming recognition CNN model achieved 87% average precision at a scene level (using audio only) and 86% average precision at an individual level (Table 1 and Fig. 3).

To demonstrate the potential applications of this framework, we used the output of our automatic pipeline to further characterize nut cracking and buttress drumming behaviors. For nut cracking, we trained a visual classifier to identify eating events: This model followed the protocols of the visual-only drumming and nut cracking classifiers and sought to identify instances when food was passed from hand to mouth (an indication of successful nut cracking). Given that an individual typically eats in conjunction with nut cracking, our audio prescreening narrowed down the search space, allowing us to efficiently label 896 body tracks of individuals consuming nuts. This enabled us to analyze, as a function of age/sex class, the average time spent nut cracking per eating event (a proxy for the number of nuts successfully cracked and consumed) (Fig. 4). For buttress drumming, we automatically detect the first and last beats of each drumming bout to precisely measure drumming bout length as a function of age/sex class, allowing us to map the distribution of drumming events throughout the day (Fig. 5; details of the automatic beat detection method are found in the "Analysis" section in Materials and Methods).

Table 1. Recognition results for both nut cracking and buttress drumming. We provide a baseline (random), which shows the chance performance of a random classifier. Bold indicates the highest performing method for the task.

Task	Method	Average precision	
		Nut cracking	Buttress drumming
I. Scene level	Random	0.09	0.11
	Audio	**0.85**	**0.87**
II. Individual level	Random	0.12	0.13
	Audio	0.30	0.81
	Visual	0.76	0.64
	Audiovisual	**0.77**	**0.86**

For Bossou chimpanzees, nut cracking bouts were predominantly performed by adult males (n = 4665 bouts) followed by adult females (n = 5485 bouts) and juveniles (n = 2134 bouts), while infants (n = 1) were not observed nut-cracking. The mean time spent nut-cracking and the proportion of time spent nut-cracking differed between age/sex groups. Adult males spent a greater proportion of their time nut-cracking than adult females (males, mean ± SD = 9.21 ± 9.49%; and females, mean ± SD = 7.97 ± 9.19%), while juveniles required longer nut cracking sequences per nut consumed than adult males and females (males, mean ± SD =16.8 ± 6.46 s; females, mean ± SD =15.7 ± 10.41 s; and juveniles/infants, mean ± SD = 43.4 ± 39.0 s) (Fig. 4C and table S3), confirming previous reports on the ontogeny of nut cracking (*24*). This suggests that adult males consumed the greatest number of nuts.

For buttress drumming, we analyzed 992 drumming bouts; the majority of bouts were performed by adult males (n = 845), confirming previous observations that this is a predominantly male activity (*25*), and occurred throughout the day, following a bimodal distribution with peaks in the morning and in the afternoon (Fig. 5B). When analyzing bout duration, adult males had, on average, shorter bouts (mean ± SD = 2.21 ± 1.80 s) than immature individuals (mean ± SD = 2.72 ± 2.39 s) and adult females (mean ± SD = 2.75 ± 1.79 s). There is a marked variation within each age/sex group, especially in adult males (min = 0.21 s and max = 19.48 s). In addition, drumming context (travel, feeding, and agonistic display) was analyzed for both adult males and adult females. In both groups drumming, during "travel" was the most common (509 bouts for males and 34 bouts for females), followed by "agonistic display" (225 bouts for males and 25 bouts for females), and lastly "feeding" (111 bouts for males and nine bouts for females). The proportion of drumming events for different contexts was approximately equal for both adult males and females (fig. S3). All drumming performed by immature individuals was done in a "play" context. Drumming bout duration varied between contexts in both adult males and adult females as well as between sexes. Feeding drumming bouts were, on average, shorter (males, mean ± SD = 1.74 ± 1.13 s; and females, mean ± SD = 2.17 ± 1.09 s) than agonistic display (males, mean ± SD = 2.31 ± 2.61 s; and females, mean ± SD = 2.78 ± 1.78 s) and travel drumming bouts (males, mean ± SD = 2.27 ± 1.35 s; and females, mean ± SD = 2.89 ± 1.97 s).

DISCUSSION

Overall, our model demonstrates the efficacy of using deep neural network architectures for a biological application: the automated recognition of percussive behaviors in a wild primate. Unlike older, rule-based automation methods, our method is entirely based on deep learning and is data-driven. It also improves on previous single-frame methods by being video-based: It uses 3D convolutions in time (*18*) to reason about temporal information, which is important for action detections, and exploits the multimodality of video to use the audio and visual streams jointly to classify behaviors.

Often, it is challenging to curate datasets large enough to train action recognition models without sifting through a significant amount of footage (20 and 4.1% of footage yielded our behaviors of interest for the nut cracking and buttress drumming data, respectively). A key aspect of our approach is the use of audio as a prescreening

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Fig. 2. Behavior recognition results demonstrate the CNN model's robustness to variations in pose, lighting, scale, and speed of action. Example of correctly labeled body tracks from unseen and unheard videos (nut cracking and drumming for the top two and bottom two rows, respectively). Middle two rows: Multiple individuals nut-cracking and buttress-drumming showing variations in lighting, pose, background, and number of chimpanzees. Photo credit: Kyoto University, Primate Research Institute; The Cantanhez Chimpanzee Project.

mechanism, which substantially cuts down the large search space of video for annotation.

Furthermore, we do not constrain the video data in any way, as is done commonly for deep learning methods applied to primate recognition and analysis by aligning individual detections or selecting for age, resolution, or lighting (*26*). Instead, we are able to perform the task "in the wild" and ensure an end-to-end pipeline that will work on raw video with minimum preprocessing (Fig. 2). We also demonstrate that our method is applicable to both long-term targeted field video recordings (including in a field experimental setting) and to

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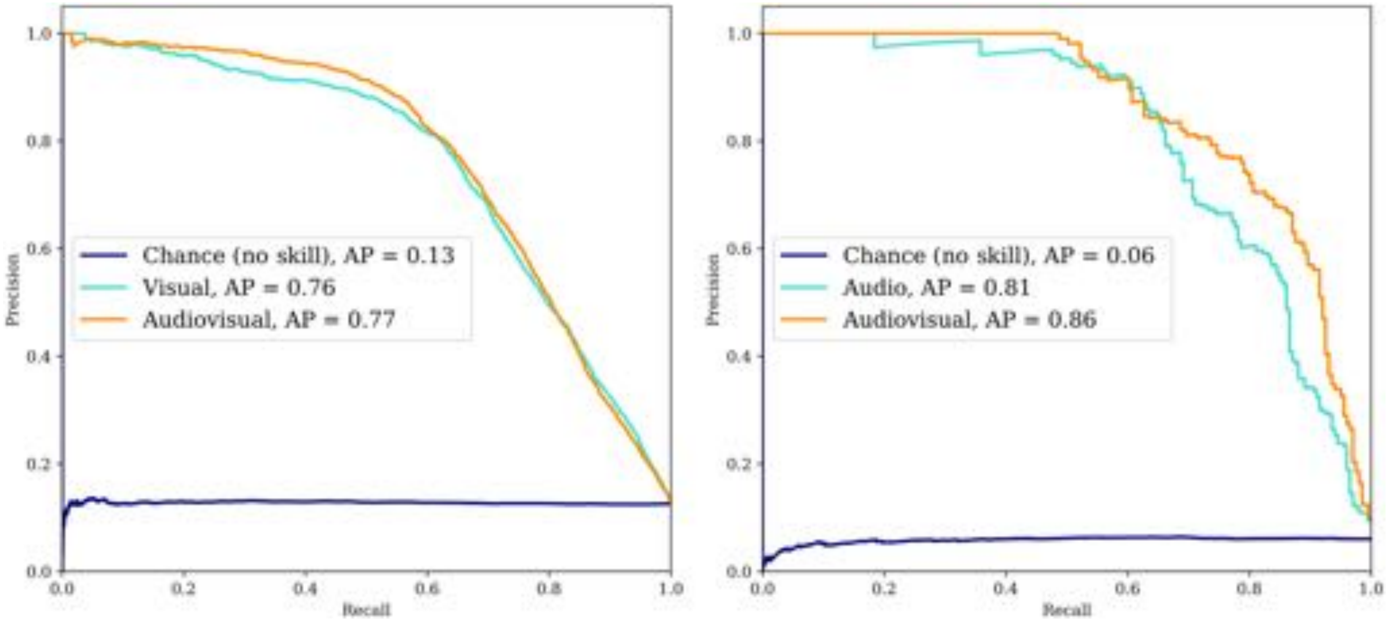

Fig. 3. Performance of the audio, visual, and audiovisual models for individual-level behavior classification. The curves for nut-cracking (**left**) and buttress-drumming (**right**) demonstrate that audiovisual outperforms single-modality methods. Instances where the behavior is either visually or audibly occluded can be compensated by using the other modality (AP: Average Precision).

remote monitoring camera trap datasets, demonstrating its usefulness across different data collection protocols.

The pipeline can be applied to data where only audio or only visual information is available (e.g., camera trap recordings where the behavior occurs off-screen, video recordings with noisy or corrupted audio, or microphone-only recordings). The benefits of our audio method are that it is not affected by visual distractors such as lighting, pose, size, and occlusion and is also computationally cheaper to run. Certain actions (such as buttress drumming) are also more discriminative in the audio space than the visual space (Table 1) and hence require less training data. Audio also allows greater coverage, by detecting actions beyond the field of view of the camera. Our visual-only method, on the other hand, provides the added benefit of allowing localization at an individual level, predicting which individuals are performing particular actions. This is a key advantage for potential future applications in, for example, the monitoring of individual behavior and welfare, both in the wild and in captive settings. Our audiovisual model combines the benefits of both modalities. For drumming, we demonstrate that our model works well even on camera trap data from locations unseen by our model during training (which therefore might contain different tree species) and communities of chimpanzees.

Our model's performance demonstrates the effectiveness of using multimodal deep learning for behavioral recognition of individual animals in longitudinal video archives and camera trap datasets in the wild. Using a novel combination of data collection methods (automated classifiers and manual annotations) and video datasets (archival footage and camera traps), we validate our approach by reproducing known findings on the ontogeny of nut cracking from the existing literature (*24*) and go further to gain preliminary insights into drumming behavior in unhabituated communities as well as revealing potential sex and age differences in different contexts (previously neglected in published work). Ultimately, the integration of computer vision and ethology using automated behavior recognition can aid behavioral research and conservation, moving beyond inferences of social structure and demographics that can be inferred using individual identification [e.g., (*14*)] to capturing the full complexity and dynamics of social interactions and behaviors. Typically, the time and resources required for manual data collection of multiple behaviors (either through in situ observation or retrospective video coding) prohibits analysis of large scale datasets. Adopting automated behavioral recognition is scalable, increasing the speed, quantity, and detail of data that can be collected and analyzed. Once classifiers have been trained, such work can move beyond broad classification of general behavioral states (eating, resting, etc.) to include fine-grained analysis at multiple layers/dimensions of behavior (*27*)—for example, using pose estimation to quantify postural kinematics or detect the number and order of elements in a behavioral sequence (e.g., nut cracking strikes) or investigating temporal co-occurrences between the behavior of individuals in the same group. We also envisage that our method could have a large impact in conservation science. Anthropogenic pressures are increasingly affecting animal behavior, with habitat fragmentation and population loss posing an imminent threat to "cultural species" through the erosion of behavioral diversity (*28*). Automating the measurement of behavioral diversity and activity budgets could be crucial for developing more sophisticated metrics to monitor the health and stability of wild populations (*10*).

There are some limitations to our study, notably that the audio preview step is limited to actions that contain a distinctive sound (such as percussion). Nonetheless, none of the pipeline steps are specific to primate behavior, and the method can be readily applied to other animal species and behaviors. Furthermore, behaviors that are audio distinctive exist in multiple domains, and we envisage possible applications for our pipeline in, for example, marine and terrestrial animal communication (vocalizations), movement (wing

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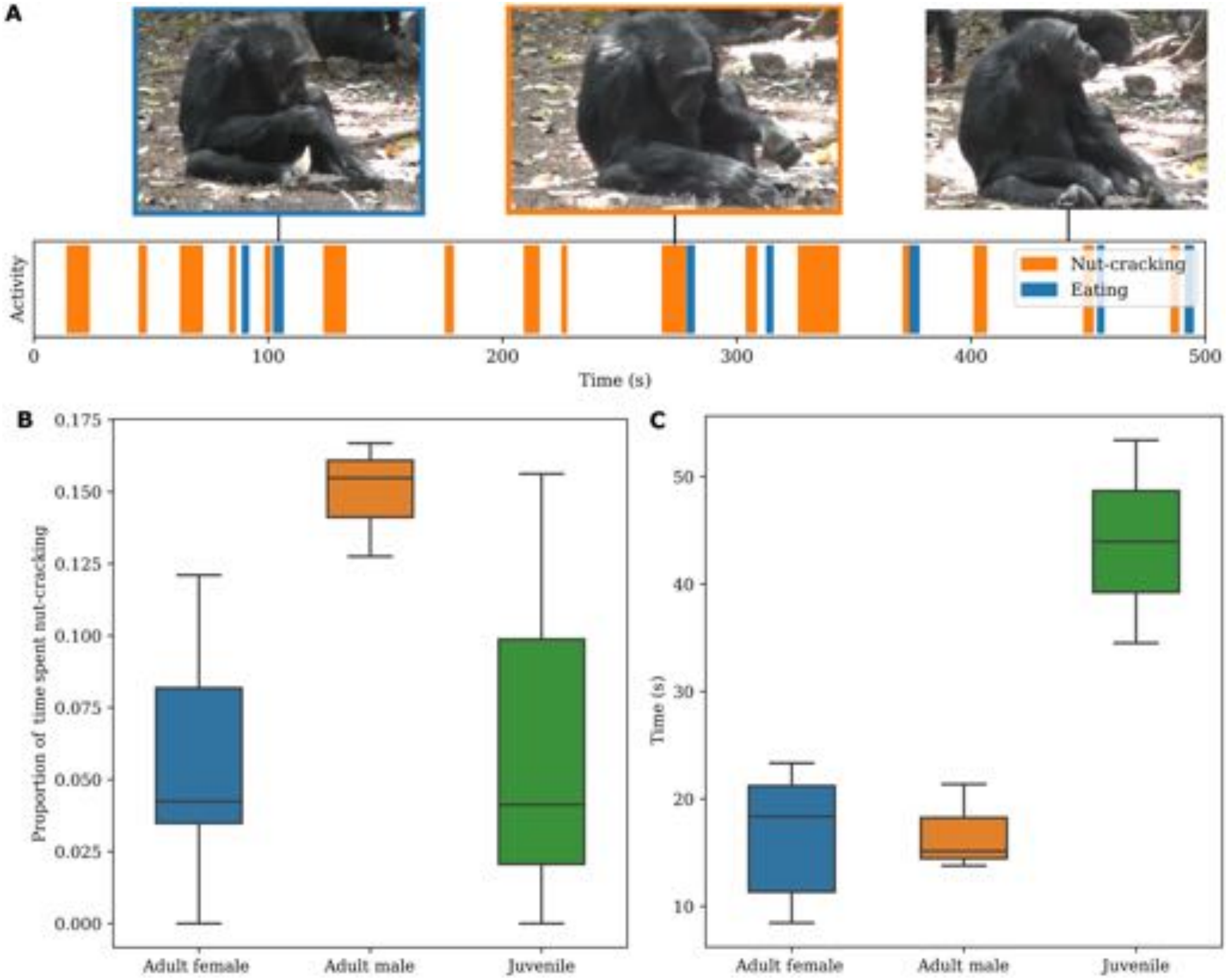

Fig. 4. Nut cracking analysis. (**A**) An example activity sequence following a single individual over the course of a video. The blank white spaces are any activities that are not nut cracking or eating. Note that eating typically follows nut cracking events. (**B**) Proportion of time spent nut cracking as a fraction of total time visible. (**C**) Average time spent nut cracking per eating event. Computed by dividing the cumulative time spent nut-cracking over the total number of eating events as a function of age and sex. Photo credit: Kyoto University, Primate Research Institute.

flapping and stepping), self-maintenance (scratching), aggression (hitting, slapping, and screaming), and foraging (tearing, smashing, and chewing). These analyses could be performed on data not only from remote sensors but also from animal-borne audio-only biologgers. Another limitation concerns the fact that, for individual-level recognition, our method is heavily reliant on the performance of the body detector: Individuals that are not detected or tracked cannot have their behavior classified. For example, the detector often fails to detect infants on their mother's backs, although for our present analyses, this poses no problem, because young chimpanzees do not nut-crack or buttress-drum while being carried. For behaviors that specifically require a visual classifier (such as successful nut cracking being identified through the hand-to-mouth motion of eating), visual occlusion or motion blur poses challenges. However, we note that the body detector has far fewer missed individual detections than other methods that are reliant on face detection (*14*, *22*). Future directions to improve our pipeline include adopting active learning, which minimizes annotator effort by automatically selecting informative samples from a pool of unannotated data for a human to annotate to retrain the network (*29*). In addition, self-supervised learning enables label-free pretraining, initializing the model in such a way that reduces the annotation requirement for training (*30*).

Our pipeline provides a critical first step in large-volume automated behavioral coding and represents a breakthrough in measuring behavior. It will permit detailed intraindividual, interindividual, and cross-site comparisons, automated collection of activity budgets, and longitudinal studies of behavior at individual and population levels, enabling detailed investigation into ontogeny, cultural evolution, and the persistence/decline of behavioral variation over time and how these relate to environmental change. It has transformative potential to science, setting a milestone for exploiting large datasets in ethology and conservation.

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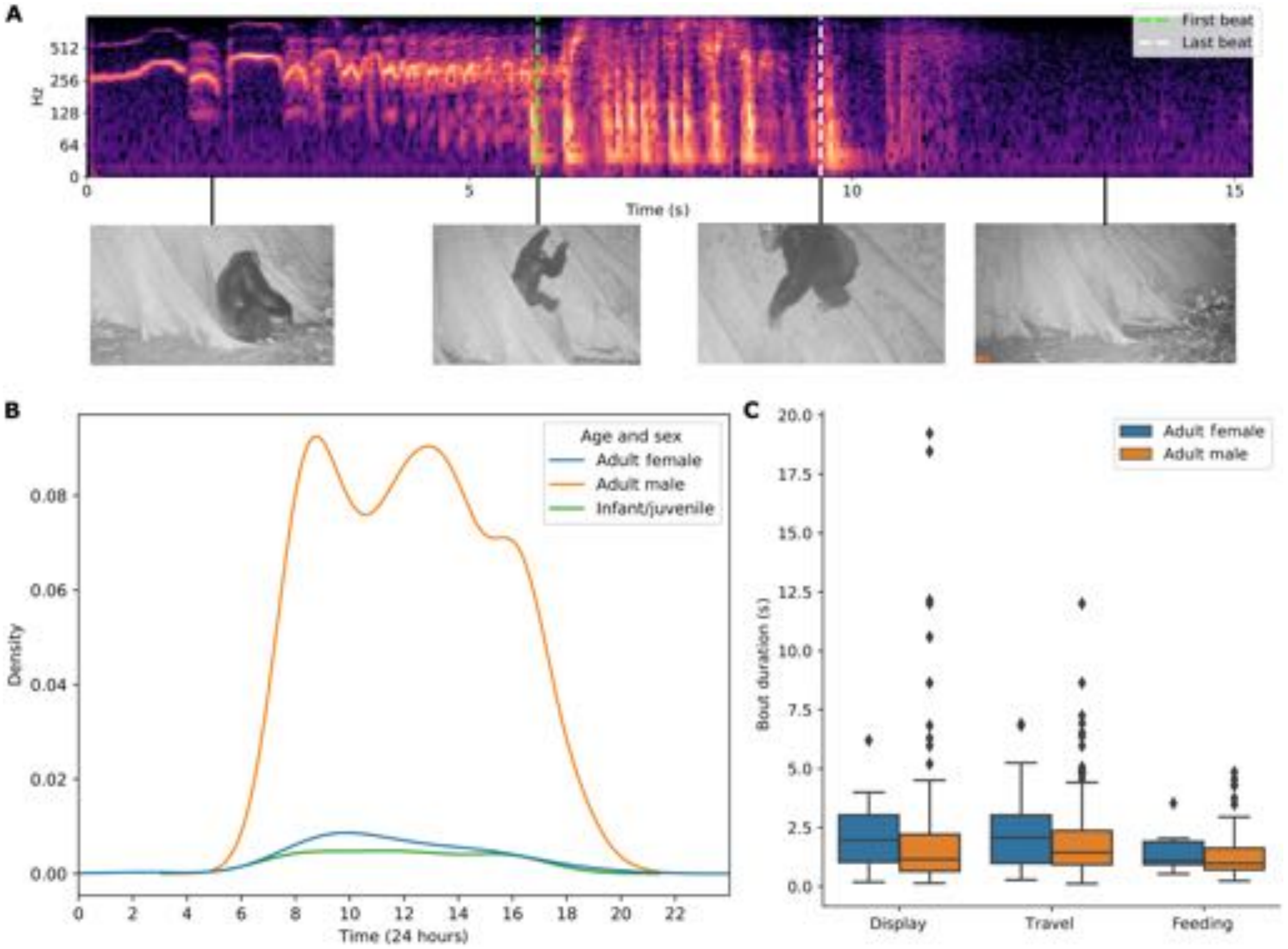

Fig. 5. Buttress drumming analysis. (**A**) Spectrogram showing a detected drumming bout delineated by the first and last beats, with video frames visualized. (**B**) Kernel density estimation plot showing the diel distribution of buttress drumming bouts, based on hh:mm:ss data captured by camera traps. (**C**) Duration (in seconds) of buttress drumming bouts by context (agonistic display, travel, and feeding) and by age/sex (adult females and adult males). Photo credit: The Cantanhez Chimpanzee Project.

MATERIALS AND METHODS

Video archive

Description of actions

Nut cracking has been described as the most complex tool-use behavior in wild chimpanzees, with the nut cracker typically combining three objects (*31*, *32*). It involves placing a hard-shelled nut on an anvil and then using a hammer to pound the nut until the edible kernel is exposed—sometimes one or two wedges are used to stabilize the anvil. We defined nut cracking "sequences" as beginning when the hammer is raised before the initial strike of a nut and ending when the hammer makes contact with the nut or anvil for the final time before the nut is consumed or abandoned or the camera is moved away. Sequences often included multiple strikes for a single nut.

Buttress drumming is a universal and frequent behavior across all chimpanzee communities, but there is much left to understand about its functions and potential cross-community variation. Drumming occurs when a chimpanzee slaps or stamps rhythmically on the buttress of a tree, often accompanied by a distinct vocalization called a pant hoot. Multiple functions of drumming have been proposed, including long-distance communication (*25*, *33*) and intimidation accompanying agonistic displays (*34*). Distinctive individual drumming patterns and pant hoot vocalizations are thought to act as signals that coordinate group movement and distribution when traveling, as well as containing information about the individual's identity (*35*). These distinct drumming patterns have been described for both males and females (*36*), but male chimpanzees appear to drum more frequently when traveling (*35*). We defined buttress drumming sequences as beginning when the first beat was detected and ending with the last beat; any behavior, such as pant hoots, occurring immediately before the first beat or immediately after the last beat were not included. Beats were detected visually, when at least one hand or foot was in contact with the buttress, and auditorily, when the distinct beat sound was heard.

Structure of the data

Nut cracking at Bossou, Guinea. Data used were collected in the Bossou forest, southeastern Guinea, West Africa, a long-term chimpanzee field site established by Kyoto University in 1976 (*14*). Bossou is home to an outdoor laboratory: A natural forest clearing (7 m by 20 m)

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located in the core of the Bossou chimpanzees' home range (07°39′N and 008°30′W) where raw materials for tool use—stones and nuts—are provisioned, and the same group has been recorded since 1988 (*14*, *22*). The use of standardized video recording over many field seasons has led to the accumulation of more than 30 years of video data, providing unique opportunities to analyze chimpanzee behavior over multiple generations. In total, we analyzed 43.1 hours of video footage.

Buttress drumming in Cantanhez National Park, Guinea-Bissau. Data used were collected by camera traps ($n = 25$) deployed in the home ranges of four different communities (Caiquene-Cadique, Lautchandé, Cambeque, and Cabante) in Cantanhez National Park, Southern Guinea-Bissau, West Africa (11°14′17.2″N and 15°02′16.9″W) between February 2017 and December 2018. Chimpanzees in Cantanhez National Park inhabit an agroforest landscape and are not habituated to researchers. The camera traps were set up in areas that chimpanzees' frequented and pointed to trees with large buttress roots with clear signs of wear from chimpanzee buttress drumming. Some cameras were moved during the study period to account for seasonal changes in chimpanzee ranging patterns and when a new area of interest was located. Cameras were motion sensitive and were set to record 1-min video clips when triggered. Approximately 41,000 video clips were collected over the study period, of which 4745 contained footage of chimpanzees, spanning a total of 47.2 hours of video footage.

Dataset splits: Training, testing, and analysis. We divide the dataset into different sections. Part of the data is manually annotated by human annotators, which provides data for training and testing our automated framework (described in the "Methods" section). The remaining data are unlabeled by humans. Our framework is applied to these unlabeled data automatically (this stage is referred to as inference) for analysis (described in the "Analysis" section). Dataset statistics are provided in table S1.

Methods

Our pipeline for the detection of audio-discriminative percussive behaviors consists of the following two stages: (i) chimpanzee detection and tracking and (ii) audiovisual action recognition (Fig. 1).

To efficiently collect annotations for the second stage (ii) audiovisual action recognition, we also use an additional "audio preview stage" (described below in the "Audio action recognition" section) only when collecting training data. This optional audio preview stage uses audio only to determine temporal segments where the behaviors (nut cracking and drumming) occur at a scene level. This markedly reduces the total search space of the video, allowing for efficient annotations, used to train a model on both scene-level audio and the visual content of each track to determine which individual is carrying out the behavior.

With this trained model, our pipeline can then be applied directly to previously unseen videos without any human input. At this point, we do not require the audio preview stage and only use (i) detection and tracking and (ii) audiovisual action recognition.

All stages in the method are implemented using deep CNNs. For audio previewing, we train a CNN on the spectrogram image of the audio. For detection, we use a single-shot detector (SSD) object category detector (*37*) to detect individuals. The detections for an individual are then grouped across frames (time) using a pretrained tracker. The final audiovisual action recognition stage involves a spatiotemporal CNN for the visual features and a spectrogram CNN for the audio. The training data were obtained by using the VGG Image Annotator (VIA) annotation tool (*38*). We provide a detailed description for each stage of the pipeline in the following sections and then describe how the analysis is carried out given the detected behaviors.

Audio action recognition

With the audio data alone, our framework is able to classify actions at the scene level. The nut cracking and buttress drumming audio classifier achieved 85 and 87% average precision, respectively, on unseen test data (Table 1).

Network architecture. For the audio model, we use a 2D CNN (ResNet-18), pretrained on VGGSound (*39*). The output is passed through two linear layers and then a final predictive layer with two neurons and a softmax activation function, resulting in a binary classifier for each target action.

Inputs. We use short-term magnitude spectrograms as input to a ResNet-18 model. All audio is first converted to single-channel, 16-bit streams at a 16-kHz sampling rate for consistency. Spectrograms are then generated in a sliding window fashion using a hamming window with a width of 32 ms and a hop of 10 ms, with a 512-point fast Fourier transform. This gives spectrograms a size of 257 × 201 for 3 s of audio. The resulting spectrogram is integrated into 64 mel-spaced frequency bins with a minimum frequency of 125 Hz and a maximum frequency of 7.5 kHz, and the magnitude of each bin is log-transformed. This gives log mel spectrogram patches of 64 × 201 bins, used as input to the CNN.

Augmentations. Temporal jittering of 0.5 s is used as well as augmentation to positive samples by randomly adding background audio samples (audio that does not contain nut cracking and buttress drumming).

Training. Binary cross-entropy is used as the training objective, along with an Adam optimizer with a learning rate of 5×10^{-3}.

Audio preview for manual annotation. Videos in the wild (including from camera traps) contain a lot of dead footage, where the actions of interest may be captured rarely. Manually searching through all this footage is a labor-intensive task. Hence, we use an inexpensive and computationally efficient prescreening method to automatically sift through many hours of footage, proposing short videos that contain the action and discarding the rest. This is done using the audio alone, because our actions of interest are all percussive and make a distinct sound.

The audio model is applied using a sliding window of size 3 s, with a stride of 0.5 s over the raw video footage. This produces a probability score $P(\text{action})$ of the action of interest being present within each temporal window. We then use the most confident 7% of windows (using the probability score as the confidence) for discrete video labeling, resulting in 2418 discrete, 3-s long video proposals to be annotated. The more expensive body detection and tracking is performed only on these "audio proposals." The body tracks are visualized on the proposals, allowing the annotators to label each actor in the proposal with a binary label denoting whether or not they are performing the action. Given that the drumming video footage is already segmented into short clips and annotated, the audio preview step was not required for the buttress drumming data at training time, so it was only used for nut cracking here.

At inference time, the audio preview can be used as a filtering step first before the full framework, providing computation savings. Because audio is much cheaper computationally than the full framework (detection, tracking, and audiovisual classification), this can be useful in resource-constrained environments such as running

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the framework on the camera traps themselves. Because this work was not constrained in terms of compute, we did not use the audio preview step at inference. For buttress drumming, the trade-off is minimal; a computation saving of 64% still captures 97% of drumming events. For nut cracking, the trade-off is greater; a computation saving of 64% captures 70% of nut cracking events. As there are many off-screen nut cracking events, the sound of nut cracking is not definitively on-screen.

Visual detection and tracking

A prerequisite for our method of automated detection of primate behavior is the detection and tracking of the target animal, producing spatiotemporal tracks following individuals through time. Deep learning has proved to be highly successful at object detection and tracking, and previous works describe the protocol and results of this applied to footage of wild animals (*13*, *14*, *40*, *41*).

In more detail, we follow the same protocol as in (*13*), which involves fine-tuning an SSD object detector (*37*) on bounding box annotations of chimpanzee bodies. Because the two datasets contain very different sources of footage, including camera traps for drumming and direct longitudinal recordings for nut cracking (the former containing night vision, varied lighting, and out of focus blur; with the latter having higher quality video but consisting of close-ups as well as medium shots), we separately fine-tune the two object detectors, one for each dataset.

For the nut cracking dataset, we fine-tune on 16,000 bounding box annotations across 5513 video frames. For the buttress drumming dataset, we fine-tune on 2200 bounding box annotations across 2137 video frames. All video frames were sampled every 10 s.

Tracking. The object tracker used to link the resulting detections through time is a pretrained Siamese network. Pairs of detections in consecutive frames with a Jaccard overlap greater than 0.5 are given as input to the network. Detection pairs with a similarity score greater than 0.5 are deemed to be from the same track.

Evaluation for the detectors. Evaluation is performed on a held-out test set using the standard protocol outlined in (*42*). The precision-recall curve is computed from a method's ranked output. Recall is defined as the proportion of all positive examples above a given rank, while precision is the proportion of all examples above that rank, which are from the positive class. For the purpose of our task, high recall is more important than high precision (i.e., false positives are less dangerous than false negatives) to ensure that no chimpanzees are missed. The Bossou and Cantanhez detectors achieved average precision scores of 0.92 and 0.91 on their respective test sets. Precision-recall curves for both detectors are shown in fig. S1A.

Programming implementation details. The detector was implemented using the machine learning library PyTorch and trained on two Titan X Graphical Processing Units (GPUs) for 20 epochs (where 1 epoch consists of an entire pass through the training set) using a batch size of 32 and two subbatches. Flip, zoom, path, and distort augmentation was used during preprocessing with a zoom factor of 4. The ratio of negatives to positives while training was 3, and the overlap threshold was 0.5. The detector was trained without batch normalization. The tracker was also implemented in PyTorch.

Audio-visual action recognition

Network architecture. For the visual stream, we use a 3D ResNet-18, with 3D convolutions (*30*). The output is passed through two linear layers and then a final predictive layer, with two neurons and a softmax activation function. For the audiovisual fusion model, 512 dimensional embeddings from the ResNet backbone in each stream are concatenated and then passed to the final predictive layer, with two neurons and a softmax activation function.

Inputs. For the audio stream, the preprocessing is identical to the "Audio action recognition" stage. Video frames are sampled at 25 frames per second, and all detections are resized to 128 × 128—we feed in 40 frames over 2.5 s, with three red-green-blue channels each, sampled randomly during training and uniformly during inference. This gives final inputs of size 40 × 128 × 128 × 3.

Augmentations. Standard augmentation techniques are applied to the visual inputs: color jittering, random cropping, and horizontal flipping. For the audio, we repeat the augmentations in the "Audio action recognition" section.

Training. All models are trained with a binary cross-entropy loss. In this stage, we use the annotations obtained from the "Audio action recognition" stage of the pipeline to train the model.

Evaluation. Evaluation for the action recognition models is performed on a held-out test set, the statistics of which are supplied in Table 1. The audiovisual fusion model performed the best at the individual level for both nut cracking and buttress drumming (77 and 86%, respectively), demonstrating its robustness across domains and actions and demonstrating its efficacy over audio or vision alone.

The models are evaluated on their precision recall at either the scene level or individual level. For the scene level, we evaluate the audio-only model with a stride of 0.5 s and a forgiveness collar of 0.5 s. For the individual level, we evaluate the audio, visual, and audiovisual models with a stride of 0.5 s per track and a forgiveness collar of 0.5 s.

Implementation details. The networks for action recognition were trained on four Titan X GPUs for 20 epochs using a batch size of 16. We trained both models end to end via stochastic gradient descent with momentum (0.9) weight decay (5×10^{-4}) and a logarithmically decaying learning rate (initialized to 10^{-2} and decaying to 10^{-8}). The visual stream is initialized with weights from (*30*), and the audio model is initialized with weights pretrained on VGG-Sound (*39*).

Action-specific implementation details

Nut cracking analysis: Success detection. To further analyze nut cracking behaviors, we additionally measure another action: passing food from hand to mouth, which is an indication of successful nut cracking. Here, the shell has been successfully cracked and the individual passes the kernel to their mouth using their hand; henceforth, this action is referred to as "eating." Because this behavior has a strong visual signature, we train a visual classifier to determine this. This model follows the protocol of the visual-only drumming and nut cracking classifiers. The training labels for eating events were gathered from the audio preview proposals, totaling 896 tracklets of individuals eating. While the audio preview searches for nut cracking, eating is often found shortly after successful nut cracking events, so the short audio proposals often contain this action as well. Furthermore, individuals often nut-crack together, resulting in multiple individuals in a video proposal. Training the model on data from 2004 and 2008 results in 89% accuracy in classifying eating on unseen tracks from 2012.

Buttress drumming duration analysis. We investigate the duration of drumming bouts by determining the start and the end beat in a drumming bout using audio-based beat detection. Beat detection is performed in an automated fashion by using low-pass filtering

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and onset detection to the audio signal of the drumming bout. The audio sequence is first low pass–filtered using a Butterworth filter with a cutoff frequency of 800 Hz. Onset detection is then performed on the filtered audio waveform. We use the onset detection method provided by the Librosa Python toolbox. The hyperparameters were chosen to achieve the best beat counting accuracy on 30 drumming bouts hand-labeled with the number of beats. During evaluation, we apply a forgiveness collar of 0.25 s on either side of the drumming event boundaries to be more lenient toward imprecise boundary annotation. From the beat detections, we define the duration of a drumming bouts to be the interval between the first and last beats. This beat detection method predicts drumming duration with a mean and median error of 0.205 and 0.131 s, respectively.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abi4883

View/request a protocol for this paper from *Bio-protocol*.

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