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A Comparison of *Eulemur* Social Systems and Vocal Communication During the Mating Season: Implications for the Speciation and Conservation of Blue-Eyed Black Lemurs and Black Lemurs

Caitlin Logan Eschmann

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Life Science

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ABSTRACT

Within the last ten years, two closely-related, parapatric species of lemurs, the blue-eyed black lemur (*Eulemur flavifrons*) and the black lemur (*E. macaco*), were classified as distinct taxa. Despite this, morphologically intermediate forms have been reported from an area of potential overlap in the two species' distributions. If hybridisation between *E. flavifrons* and *E. macaco* is, or was, ongoing in this region, pre-mating barriers reinforcing reproductive isolation between the two species may be incomplete. No published studies compare comprehensively their behavioural ecology. Therefore, the overall aims of this study were to identify an area of contact between the two species and to illuminate the role of species-specific behaviours as potential pre-mating isolation mechanisms. The social systems and vocal communication of three distinct populations of *E. flavifrons* (*Ef-1*, *Ef-2*, and *Ef-3*) and three distinct populations of *E. macaco* (*Em-1*, *Em-2*, and *Em-3*) were examined over three mating seasons (May-July 2015-2017). Each population, which could contain multiple groups, was selected to be representative of a unique allopatric (*Ef-1*, *Em-1*, and *Em-3*) or parapatric location (*Ef-2*, *Ef-3*, and *Em-2*) within the two taxa's geographic distributions. In addition, the two species' potential contact zone was surveyed. Although *E. flavifrons* was observed outside of its established range during this time, I was unable to conclusively determine whether the two species currently overlap. The comparisons of the social organisation, social structure, and mating systems of *E. flavifrons* populations and *E. macaco* populations did not reveal clear interspecific differences. Instead, one *E. macaco* population (*Em-1*) was found to differ from the others consistently. Preliminary findings suggest that social system plasticity in these species may be partially related to local ecological conditions. The comparison of the vocal repertoires and acoustic parameters of specific calls also revealed no clear interspecific differences. The lack of evidence supporting *E. flavifrons* and *E. macaco* social system and acoustic communication divergence suggests that it is unlikely that these characters serve as mate recognition mechanisms between the two species or prevent their interbreeding in natural settings. Based on these findings, possible modes of speciation for *E. flavifrons* and *E. macaco* are evaluated.

DEDICATION

In memory of Felicia Ruperti
for without you, none of this would have transpired

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ………………………………………………………………

DATE: …………………………………………………………………

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ACRONYMS AND ABBREVIATIONS

AEECL: Association Européenne pour l'Etude et la Conservation des Lémuriens

AOO: area of occupancy

BLC: brown lemur complex

BSC: biological species concept

CSC: cohesion species concept

DFA: discriminant function analysis

EcSC: ecological species concept

Ef-1: Ankarafa Forest population of *E. flavifrons*, observed in 2015

Ef-2: Ambodimanga population of *E. flavifrons*, observed in 2016

Ef-3: Angodrahely population of *E. flavifrons*, observed in 2017

Em-1: Andranomatavy population of *E. macaco*, observed in 2016

Em-2: Mahadera population of *E. macaco*, observed in 2016

Em-3: Lokobe National Park population of *E. macaco*, observed in 2017

EOO: extent of occurrence

ESC: evolutionary species concept

F0: fundamental frequency

F1: first formant

F2: second formant

F3: third formant

F4: fourth formant

FFD: fission-fusion dynamics

GKPA: Galoko-Kolobinono Protected Area (GKPA),

GLC: general lineage concept

GSC: genotypic species concept

IUCN: International Union for Conservation of Nature

LD1: first linear discriminant

LD2: second linear discriminant

LMM: linear mixed-effect model

MANOVA: multivariate analysis of variance

MBG: Missouri Botanical Gardens

MEF/T: Ministère de l’Environnement et des Forêts/ et du Tourisme

MEM: Ministère de l’Energie et des Mines

MNP: Madagascar National Parks

MSR: Manongarivo Special Reserve

PAI: pairwise affinity index

PAR: perimeter-area ratio

PhSC: phenetic species concept

PSC: phylogenetic species concept

RT: relationship tenor

SD: standard deviation

SIRNAP: Sahamalaza-Iles Radama National Park

SMRS: specific mate recognition system

TLG: train of long grunts

TNR: Tsaratanana Nature Reserve

Chapter 1

Introduction

1.1 Species concepts

Species have been designated as the basic units of classification across all disciplines of biology (Hull, 1977) and, as such, their delimitation remains important in understanding the process of speciation (i.e. how species originate and evolve over time; Chandler & Gromko, 1989). Despite this significance, consensus on how to identify and delimit a species remains a lingering issue among biologists (Sites Jr. & Marshall, 2003; Sites Jr. & Marshall, 2004; Balakrishnan, 2005; Hausdorf, 2011; Pavlinov, 2013). This is partially due to the fact that it can be difficult to distinguish between taxonomic differences that contributed to the speciation process and those that occurred after the fact (Templeton, 1981). Because of this, there are currently more than 26 contemporary alternative concepts, of which many offer conflicting and discordant definitions of what constitutes a species, as well as underscore different evolutionary processes and patterns operating between taxa (for review see Mayden, 1997; Wheeler & Meier, 2000; Hey, 2001; Sites Jr. & Marshall, 2004; Hausdorf, 2011).

The majority of these concepts can be boiled down to their major mechanisms for recognizing species (de Queiroz, 2005). The biological species concept (BSC) requires individuals to breed together and focuses on reproductive isolation of populations to identify taxa (Mayr, 1942; Lambert & Spencer, 1995). Closely related to the BSC, is the concept of a specific mate recognition system (SMRS), which emphasises shared signal-reception mechanisms, whether behavioural or gametic, that have evolved to identify potential mates (Paterson, 1980). The ecological species concept (EcSC) identifies species based on shared niches and resources (Andersson, 1990; Nosil & Harmon, 2009). The evolutionary species concept (ESC) views species as separate lineages with distinct historical and evolutionary paths (Simpson, 1951; Wiley, 1978). The cohesion species concept (CSC) defines species based on demographic or genetic unity (Templeton, 1989). The genotypic species concept (GSC) requires species to form genetic clusters with no intermediate forms (Mallet, 1995). The phenetic species concept (PhSC) relies on similarities in morphology or charaters (Sokal & Crovello, 1970), while the phylogenetic species concept (PSC) identifies species based on a common ancestor with shared traits (Donoghue, 1985; Hennig, 1999).

In addition, de Queiroz (1998, 2005, 2007) argues that all species concepts share an underlying and unifying idea that species are metapopulation lineages in the process of evolving independently. Disagreements arise because of the different secondary criteria (e.g. ecological niches, similarities in morphology, etc.) used in each of the alternative species concepts. Instead, each criterion should be considered as cumulative lines of evidence in identifying species boundaries. Each can arise at different points over time, and they do not have to occur in a systematic order (de Queiroz, 1998). By adopting a general lineage concept (GLC) of species, the use of multiple criteria, especially when they are in concordance, can provide strong evidence for recent divergence in populations (de Queiroz, 2007). A number of species have been identified and described using a more integrative approach, including plants (*Humulus lupulus*: Reeves & Richards, 2011), invertebrates (*Amblyomma parvum*: Nava et al., 2016; Oxynoidae: Berriman et al., 2018), reptiles (*Pseudogekko* spp.: Siler et al., 2016), amphibians (*Rhinella* spp.: Murphy et al., 2017), birds (Campbell et al., 2016), and mammals (*Eulemur* spp.: Markolf et al., 2013; *Pteronotus* spp.: Pavan & Marroig, 2016).

The absence of an agreed species concept among biologists is problematic as different theoretical concepts can lead to dissenting conclusions regarding the number of specific species/subspecies (Apagow et al., 2004) and/or the size and geographic range of a particular taxon (de Queiroz, 2007). In fact, Agapow & colleagues (2004) found that the use of the PSC definition yielded 49% more species than the BSC definition. This highlights how different definitions can impact measures of species richness and species abundance, which in turn affects the creation of management plans and the establishment of areas of significant conservation need (Myers et al., 2000; Agapow et al., 2004; Balakrishnan, 2005). Given that nearly 30% of the world's assessed species are facing possible extinction, including 14% of birds, 25% of mammals, and 40% of amphibians (IUCN, 2019), an agreed upon definition to accurately delimit species is undoubtedly a conservation priority (Mace, 2004).

It has been argued that from a conservation perspective, when reproductive isolation is used as a criterion to delimit species, "minimum harm and maximum potential benefits in terms of fitness and adaptive evolutionary potential" are possible (Frankham et al., 2012). This is especially the case for populations that are highly fragmented and where isolation may result in inbreeding depression (reduced fitness of offspring from related mates due to decreased genetic variation/increased homozygosity) and/or outbreeding depression (mating between distantly-related individuals resulting in offspring with reduced fitness that are maladapted to parental environments). In essence, species should be defined in such a way that minimises outbreeding depression as much as possible, while simultaneously maximising opportunities to mate that reduce inbreeding depression (Frankham et al., 2011, 2012).

1.2 Modes of speciation

Modes of speciation can be classified based on whether the barriers to gene flow have geographic, environmental, or genetic origin, although these are not necessarily mutually exclusive (Coyne & Orr, 2004). Allopatric speciation, which is also referred to as geographic speciation, occurs when physical barriers isolate two populations and prevent gene exchange. Geographic barriers can be bodies of water, topographical features (e.g. mountain ranges), and/or harmful habitats. Over time these populations diverge genetically, resulting in the formation of separate, allopatric species. If the physical barrier is eventually removed and secondary contact is established between the two species, reproductive isolation will prevent their interbreeding. This form of speciation is thought to be one of the most prevalent modes through which new mammal taxa originate (Coyne & Orr, 2004; Singh, 2012). With parapatric speciation, adjacent populations diverge, gene exchange becomes limited, and eventually the two populations become reproductively isolated. This mode of speciation usually is the result of sexual selection as individuals venture into new habitats along an environmental cline, although there is no geographic separation (Bush, 1975; Coyne & Orr, 2004). With sympatric speciation, assortative mating and reproductive barriers develop within a single population that originally mated randomly, leading to two distinct populations. As opposed to parapatric speciation, reproductive isolation occurs before populations expand to new environments (Bush, 1975; Coyne & Orr, 2004; Singh, 2012). Lastly, allele substitutions and rearrangements, cytoplasmic incompatibility, and chromosomal changes may all contribute to genetic incompatibility of parapatric and/or sympatric populations, leading to their speciation (Coyne & Orr, 1998; Wu & Ting, 2004).

Both parapatric and sympatric speciation require the formation of intrinsic isolation mechanisms to limit the exchange of genes, as opposed to extrinsic mechanisms such as physical separation (Mayr, 1970; Templeton, 1989; Coyne & Orr, 2004). Isolation mechanisms that act to prevent breeding are known as pre-mating barriers and can take three different forms—temporal, ecological, and/or ethological. Temporal isolation is when the reproductive periods of populations occur at different times. If populations use different habitats to mate or rely on different resources and do not come in contact, ecological isolation may occur (Rice, 1987). Ethological isolation occurs as a result of different social or sexual behaviours that reduce the mating opportunities of two populations (Mayr, 1970; Templeton, 1989; Coyne & Orr, 2004).

Post-mating prezygotic isolation mechanisms include mechanical or gametic incompatibility. With the former, physical differences in genitalia will prevent the transfer of sperm to females. With the latter, although sperm and egg come in contact, fertilisation will not take place (Mayr, 1970; Templeton, 1989; Coyne & Orr, 2004). If mating and the formation of hybrid offspring do occur, postzygotic barriers may result in F1 hybrids with reduced viability and/or sterility, or an F2 generation that experiences hybrid breakdown and a reduction of fitness. In areas of sympatry especially, these post-mating isolation mechanisms are hypothesised to aid in the natural selection against hybrid offspring and can serve to reinforce assortative mating at the pre-mating level (Mayr, 1970; Templeton, 1989; Naisbit et al., 2001; Coyne & Orr, 2004).

1.3 The phenomenon of character displacement

The speciation phenomenon of character displacement has been suggested as a way for closely-related, congeners to coexist (Brown & Wilson, 1956). According to this process, in areas of contact, characters will diverge, and differences will be accentuated. The selection for and evolution of divergent traits can therefore limit interactions and competition and strengthen isolation barriers between two sympatric taxa. Conversely, in locations where the taxa do not overlap, characters will converge, and differences will appear minimalized, sometimes to the point where certain characters are indistinguishable. Therefore, character displacement results in divergent traits between two interacting species, as well within the allopatric and sympatric populations of each individual species (Brown & Wilson, 1956).

Two forms of character displacement are possible. Ecological character displacement acts on traits that are associated with resource use to allow coexisting populations to exploit novel niches, thereby limiting costly resource competition (Brown & Wilson, 1956). This form of character displacement is hypothesised to have contributed to the adaptive radiation of sympatric, congeneric species (Schluter, 2000) including birds (*Geospiza* spp.: Grant & Grant, 2006), mammals (*Neotoma* spp.: Scurtliff et al., 2013; primates: Houle, 1997), and fish (*Gasterosteus* spp.: Schluter & McPhail, 1992). For example, while significant differences in jaw morphology have allowed sympatric populations of salamander species (*Plethodon* spp.) to differentiate the types and size of prey that they consume, corresponding jaw differences were not found in allopatric populations (Adams & Rohlf, 2000). Similarly, Davies & colleagues (2007) reviewed sister species of carnivores and found a negative relationship between range overlap and teeth shape, with parapatric and sympatric taxa have more different dentition than allopatric taxa. They hypothesised that the differing dental morphologies of coexisting species indicated consumption of unique food sources, which would ultimately serve to reduce competition (Davies et al., 2007).

The second form of displacement, reproductive character displacement, serves to strengthen reproductive barriers by selecting for traits that limit costly sexual interactions (Brown & Wilson, 1956; Pfennig & Pfennig, 2009). This type of character displacement can influence the process of sexual selection by impacting the traits involved in sexual signalling, mate competition, or mate choice (Pfennig & Pfennig, 2009). Therefore, the divergence of behaviours involved in mate recognition of co-occurring congeners serves to reinforce mechanisms of reproductive isolation (Shaw & Parsons, 2002). Divergence of signals involved in species recognition and mate attraction is well documented (Templeton, 1979; Paterson, 1980; Shaw & Parsons, 2002; Vasey & Tattersall, 2002; Coyne & Orr, 2004). In areas of sympatry as compared to allopatry, female frogs (*Hyla* spp.) have shown acoustic preference for mates with more pronounced vocalisation differences than co-occurring heterospecifics (Höbel & Gerhardt, 2003), while male fish (*Poecilia latipinnai)* preference for specific behavioural traits of conspecific females was found to be stronger when coexisting with a closely-related taxa than when not (Gabor & Ryan, 2001). Divergence of morphological characters to reinforce reproductive isolation has been found in Cercopithecini primates. The distinct facial features of sympatric congeners are hypothesised to have evolved to be more dissimilar to each other than to allopatric species, thus aiding in overlapping taxa's specific mate recognition systems (Allen et al., 2014). These examples highlight the role diverging reproductive characters play in mate recognition and acquisition, and in maintaining reproductive isolation of coexisting congeners (Bush, 1975; Kaneshiro, 1980; Paterson, 1980; Templeton, 1981; Hendry et al., 2007).

1.4 The role of hybrid zones

If strong pre-mating reproductive isolation barriers are not maintained between closely-related species that are in contact, interbreeding could result in hybrid offspring. Hybridisation, which increases the gene flow between two taxa, may lead to the loss of genetic integrity and species-specific behaviours, the loss of one or both parental taxa at the local level, the formation of a stable zone of introgression (gene flow) between the two taxa, or the formation of new species (referred to as hybrid speciation; Allendorf et al., 2001; Wolf et al., 2001; Seehausen, 2004; Arnold & Meyer, 2006; Shurtliff, 2013). Hybrid zones therefore have been the focus of evolutionary biologists (Barton & Hewitt, 1985; Harrison, 1990; Hewitt, 2001) as they attempt to connect observations from areas of contact with patterns in acquired and inherited characters, mate recognition systems, species concepts, and the evolution of distinct taxa (Harrison, 1993; Shaw & Parsons, 2002; Pastorini et al., 2009; Shurtliff, 2013). Additionally, contact zones between closely-related species can have important implications for the conservation management of those taxa, including identifying appropriate IUCN Red List statuses and CITES legislation and deciding how to allocate funds accordingly (Lehman & Wright, 2000; Mace 2004; Zinner et al., 2011).

Although originally hypothesised to be uncommon, natural hybridisation has been found in at least 25% of plant species and 10% of animals (Mallot, 2005), including invertebrates (Bullini & Nascetti, 1990; Sakamoto & Yago, 2017), fishes (Hubbs, 1955; Scribner et al., 2000), amphibians (Sanderson et al., 1992), reptiles (Sovic et al., 2016), birds (den Hartog et al., 2007; Sattler et al., 2007), and mammals (Shurtliff, 2013). While every hybrid zone is under a unique set of selective pressures, several cumulative key patterns have emerged. Hybrid zones tend to have a narrower geographic distribution than either of the parental taxa (Barton & Hewitt, 1989; Evans et al., 2001). Changes in characters typically occur over a gradient, or cline, and many of these variations have genomic links to the parental population (Barton & Hewitt, 1989).

The fitness of hybrid offspring can have important consequences in shaping the overall dynamics of the hybrid zone, as well as influencing the stability of the parental populations. Many hybrid zones are thought to be tension zones where random dispersal of parental forms and selection against hybrids interact to maintain a cline of characters (Barton & Hewitt, 1989). In such instances, F1 hybrids may have a reduced fitness in comparison to the parental taxa, showing signs of outbreeding depression (Wolf et al., 2001). Often these offspring are sterile, as with certain *Chorthippus* spp. (Hewit et al., 1987) and *Eulemur* spp. hybrids *(*Djlelati et al., 1997), and/or non-viable, as with certain *Eulemur* spp. (Wyner et al., 1999) and *Rana* spp. hybrids (Abt & Reyer, 1993). Inferior hybrid fitness can limit the long-term gene flow and result in reduced population growth rates to the point where parental populations are not being sufficiently replaced (i.e., demographic swamping; Wolf et al., 2001).

Conversely, genetic swamping is a possibility if hybrid offspring have superior fitness to the parental forms. In such cases, hybrids may be able to supplant one or both of the parental taxa, which can be particularly dangerous to highly threatened species (Wolf et al., 2001; Todesco et al., 2016). For example, the introduction of a non-native species of damselfish (*Abudefduf vaigiensis*) to Hawaii has resulted in hybridisation with the native taxa (*A. abdominalis*); this native species is now facing local extinction (Coleman et al., 2014). Similarly, the introduction of the common mallard duck (*Anas platyrhynchos*), and its resulting introgression and hybridisation with local taxa, has resulted in outbreeding depression and genetic mixing. Such events have been credited with the near collapse of several species of rare ducks around the world (for review see Rhymer, 2006).

Despite these concerns, hybridisation should not always be viewed through a negative lens, as it also has the potential to lead to the formation of new species. Hybrid speciation has been difficult to identify, especially in animals, in part due to the difficulty in discerning between cryptic species. But with advent of new genetic techniques, the role of hybridisation in the diversification of mammalian species is slowly being elucidated (Mallet, 2005, 2007; Shurtliff, 2013). One of the first taxa to be identified through the use of mitochondrial DNA markers as originating from the hybridisation of two separate species was the Japanese mouse (*Mus musculus molossinus*); it is likely that colonisation events with European (*M. m. musculus*) and Asiatic species (*M. m. castaneus*) resulted in this hybrid form (Yonekawa et al., 1988). More recently, Amaral & colleagues (2014) presented genetic and morphometric data that supported the formation of a new species of dolphin, distinct from either parental taxon (*Stenella* spp.), and suggested that female sexual selection for conspecific behaviours may have driven this process. These emerging studies help to highlight the potential role that hybrid speciation may play in the adaptive radiation and diversification of mammal taxa (Seehausen, 2004; Mallet, 2007).

1.5 Hybridisation in primates

In primates, two modes of hybridisation have been suggested to occur naturally in the wild: secondary contact of allotaxa and hybridisation of sympatric species. Allotaxa are parapatric populations of closely-related species that have diverged and evolved independently in some capacity (Grubb, 1999). If the populations come back into contact after a period of isolation, interbreeding may occur if sufficient barriers have not yet evolved (Zinner et al., 2011). This type of secondary contact often occurs along geographic boundaries, such as river headwaters or the transition between two different phytogeographic regions (Groves, 1993). In comparison to this form of parapatric hybridisation, the natural hybridisation of distinct, sympatric species is hypothesised to occur rarely. Changes in local environmental conditions, such as increased forest edge and habitat fragmentation, increased predation, and/or disease, may make locating conspecific mates difficult. If individuals come into contact only with heterospecifics during this time, interbreeding may occur. In such instances, the formation of a stable hybrid zone is unlikely though (Detwiler et al., 2005).

Primate hybrid zones are well-documented (*Alouatta* spp.: Aguiar et al., 2007, 2008; Agostini et al., 2008; Kelaita & Cortés-Ortiz, 2013; *Cercopithecus* spp.: Detwiler et al., 2005; de Jong & Butynski, 2010; *Macaca* spp.: Bernsteil, 1966; Bynum et al., 1997; Evans et al., 2001; *Papio* spp.: Dunbar & Dunbar, 1974; Alberts & Altman, 2001; Bergman & Beehner, 2003), with recent interest in the Malagasy strepsirrhines (*Eulemur* spp.: Djlelati et al., 1997; Lehman & Wright, 2000; Pastorini et al., 2000; Johnson, 2002; Wyner et al., 2002; Delmore et al., 2011, 2013; Johnson et al., 2016; *Microcebus* spp.: Gligor et al., 2009; Hapke et al., 2011; *Varecia* spp.: Vasey & Tattersall, 2002). The natural hybridisation of baboons (*Papio* spp.) remains one of the best-documented examples among primates (Alberts & Altmann, 2001; for review see Detwiler et al., 2005). The well-researched hybrid zone between *P. hamadryas anubis* and *P. h. hamadryas* occurs in the Awash National Park in Ethiopia (Bergman & Beehner, 2003). There these two baboon species come in contact along an ecological transition zone between dry thornscrub and moister savanna regions. Both taxa have distinct social organisations and mating systems, ranging from the male-bonded, multi-level society comprised of one-male units of *P. h. hamadryas* (Kummer, 1968) to the female-bonded, multimale-multifemale society of *P. h. anubis* (Packer, 1979b). Male competition for oestrus females is intense in *P. h. anubis* (Packer, 1979b), while male and female *P. h. hamadryas* form long-term bonds (Kummer, 1968). In the Awash hybrid zone, it has been hypothesised that the reproductive success of males is tied to the predominant type of male-female bonding present in the group (Bergman & Beehner, 2003). That is to say, if a group is comprised primarily of *P. h. anubis*, male competition is a favourable mating strategy, while in predominantly *P. h. hamadryas* groups, female selection tends to favour the formation of stable male-female relationships. In evenly mixed groups, which exhibit phenotypically intermediate individuals and aspects of both species' social systems (Berman & Beehner, 2004), intermediate male mating behaviour is more successful, suggesting that sexual selection does not necessarily act against hybrids (Bergman & Beehner, 2003).

Several hybrid zones also have been described for species of howler monkeys throughout the Neotropics, including *Alouatta caraya* x *A. guariba* and *A. pigra* x *A. palliata* (for review see Cortés-Ortiz et al., 2015). Hybrids have been identified based on morphological (Aguiar et al., 2007; Agostini et al., 2008; Kelaita & Cortés-Ortiz, 2013), acoustic (da Cunha et al., 2014; Kitchen et al., 2015, 2017), and genetic indicators (Cortés-Ortiz et al., 2007). It is hypothesised that chromosomal differences may lead to hybrid incompatibility between certain species, which could explain the lack of viable F1 males and the maintenance of hybrid tension zones (*A. pigra* and *A. palliate*; Cortés-Ortiz et al., 2007, 2015; Steinberg et al., 2008). There is tentative support for the formation of these hybrid zones as the result of recent forest fragmentation and habitat disturbances (Agostini et al., 2008; Dias et al., 2013; Cortés-Ortiz et al., 2015). Despite the frequency of hybridisation reports, much research is still needed to understand the mechanisms involved in howler monkey hybridisation and the selective pressures acting for or against hybrid individuals (Cortés-Ortiz et al., 2015).

Several *Macaca* species have also been documented hybridising throughout their range. A hybrid zone in Sulawesi between *M. maura* and *M. tonkeana* appears to be maintained through a combination of selection against hybrids, which have a presumed lower fitness, and dispersal of parental taxa into the contact zone (Evans et al., 2001). Similarly, the hybridisation of *M. fascicularis* (formally *M. irus*) x *M. nemestrina* in Malaysia, as determined by mixed-species groups with morphologically intermediate individuals, was attributed to anthropogenic disturbances. The local population of *M. nemestrina* had been nearly extirpated by hunting, forcing remaining individuals to look outside of conspecifics for potential mates (Bernsteil, 1966). To better understand the evolution of *Macaca* lineages, Tosi & colleagues (2003) analysed the maternal, paternal, and biparental molecular markers of 27 taxa. Among other findings, they hypothesised that *M. arctoides* is the result of a hybridisation event during the Pleistocene between *M. fascicularis* and *M. sinica* (then proto-*M. fascicularis* and proto-*M. assamensis*/*thibetana*). This merging of ancient *Macaca* lineages has been credited with the formation of new hybrid species (Tosi et al., 2003).

1.6 Madagascar and lemurs

Madagascar, the fourth largest island in the world, reaches 1,600 km in length and covers an area of 590,000 km^2. Separated from mainland Africa approximately 160-180 million years ago and India 90 million years ago (de Wit, 2010), Madagascar is an ideal micro-continent to study the diversification of primate species due to its time in isolation (Martin, 1972; Myers et al., 2000; Mittermeier et al., 2010, 2011). Boasting 111 primate species and subspecies that span five lemur families, Madagascar is the only country in the world that can claim 100% endemism of its primates (Mittermeier et al, 2010; Schwitzer et al., 2014a), contributing to its classification as a biodiversity hotspot region (Myers et al., 2000; Mittermeier et al., 2011). Unfortunately, the rapid increase in human population and resulting forest fragmentation and habitat loss has created increasingly threatened populations (Schwitzer et al., 2014a; Estrada et al., 2017). Of the known species, at least 95% are either Critically Endangered, Endangered, or Vulnerable (Schwitzer et al., 2014a). It is therefore of little surprise that lemurs represent some of the most endangered primates in the world (Schwitzer et al., 2017).

Due to advances is genetic analyses that elevated many subspecies to species and detected numerous cryptic nocturnal species, there have been 46 newly classified taxa of lemurs since 2003 despite not having discovered many new populations (Tattersall, 2007). The diversification and widespread distribution of lemurs across Madagascar has been attributed to the country's seven unique climatic and phytogeographic regions (Humbert, 1955; Martin, 1972; Tattersall & Sussman, 1975), the prevalence of river barriers (Pastorini et al., 2003), the elevation of river headwaters (Goodman & Ganzhorn, 2004), the direction of drainage systems (Ganzhorn et al., 2006), the isolation of watersheds (Wilmé et al., 2006), and the partitioning of ecological niches (Martin, 1972; Blair et al., 2013).

1.7 Genus *Eulemur*

In 1988, Simons & Rumpler separated five 'true' lemur species from the genus *Lemur* and designated them as *Eulemur* based on cytogenetic, anatomical, and behavioural data. This partition included the black lemur (*Eulemur macaco*), the polytypic brown lemurs (*E. fulvus*, with six or seven subspecies), the red-bellied lemur (*E. rubriventer*), the mongoose lemur (*E. mongoz*), and the crowned lemur (*E. coronatus*). In 2001, Groves elevated all *E. fulvus* subspecies to separate species based on distinct morphological and craniodental features. There are now six taxa within the brown lemur complex (BLC), which include *E. fulvus*, *E. sanfordi, E. albifrons*, *E. cinereiceps* (previously *E. albocollaris;* Johnson & Wyner, 2000; Johnson et al., 2008), *E. rufus*, and *E. collaris* (Wyner et al., 1999). Although the validity of *E. mayottensis*, which is found on the Comoros island of Mayotte, as a separate taxon within the BLC has been suggested (Schlegel, 1866 as cited in Tattersall, 1977), current consensus is that it represents an introduced population of *E. fulvus* (Pastorini et al., 2000, 2003; Mittermeier et al., 2008). Fausser & colleagues (2000) were the first to recognize two distinct subspecies of black lemurs, *E. macaco macaco* and *E. m. flavifrons*. In 2008, *E. flavifrons* (blue-eyed black lemur) was elevated to full species status based on a combination of morphological differences (Mittermeier et al., 2008) and genomic differences consistent with those found within the BLC (Pastorini et al., 2000). Further genomic analyses revealed that the two species diverged only relatively recently, approximately 160 thousand years ago (Meyer et al., 2015).

Eulemur species are found in all of Madagascar’s forest types, except for the spiny forests in the south (Ossi & Kamilar, 2006; Mittermeier et al., 2010; Blair et al., 2013). These lemurs all show some degree of sexual dichromatism (Pastorini et al., 2000). There have been many reports of hybridisation between the different species with fertile offspring sometimes resulting, both in the wild and captivity (Petter, 1969; Rabarivola et al., 1991; Djlelati et al., 1997; Lehman & Wright, 2000; Pastorini et al., 2000; Johnson, 2002; Wyner et al., 2002; Jekielek, 2003; Pastorini et al., 2009; Delmore et al., 2011, 2013; Johnson et al., 2016). Such findings suggest that the reproductive isolation mechanisms and pre-mating barriers between *Eulemur* congeners may not be fully established (Pastorini et al., 2009).

1.8 *Eulemur flavifrons* and *E. macaco*

Arguably one of the most threatened lemur species is the blue-eyed black lemur (*Eulemur flavifrons*). Little was known about *E. flavifrons* until the early 1980s when the species was rediscovered (Koenders et al., 1985). These lemurs are susceptible to extensive human-induced disturbances including hunting, trapping, logging, pet trade, and slash-and-burn agricultural practices (Andrianjakarivelo, 2004; Schwitzer et al., 2006; Seiler et al., 2010; Andriaholinirina et al., 2014a). These threats have resulted in a species decline of more than 80% in the past 24 years, leading to their classification as Critically Endangered by the IUCN Red List in 1996 (Andriaholinirina et al., 2014a) and inclusion as a Top 25 Most Endangered Primate 2008-2014 (Mittermeier et al., 2009, 2012; Schwitzer et al., 2014b). A recent population viability analysis concluded that *E. flavifrons* will be extirpated in the wild within the next 100 years if nothing is done to counteract levels of habitat destruction (Volampeno et al., 2015). This outlook is especially worrisome as the distribution of *E. flavifrons* is thought to be limited to less than 2,700 km^2 of forest largely restricted to the Sahamalaza Peninsula of Madagascar; only a small and understudied population extends northeast off the peninsula (Koenders et al., 1985; Randriatahina & Rabarivola, 2004; Schwitzer et al., 2006; Mittermeier et al., 2010; Volampeno et al., 2010; Andriaholinirina et al., 2014a; Randriatahina et al., 2014).

In comparison, the black lemur (*Eulemur macaco*), which is a closely-related sister species of *E. flavifrons*, is currently listed as Vulnerable by the IUCN Red List due to a fragmented and declining geographic range and continued hunting practices in northwest Madagascar (Mittermeier et al., 2010; Andriaholinirina et al., 2014b). In addition, the islands of Nosy Be and Nosy Komba, located off the northwest coast of Madagascar, also contain populations of *E. macaco*. Collectively, their 11,740 km^2 range covers two national parks, a special reserve and a strict nature reserve (Birkinshaw et al., 2000; Goodman & Schütz, 2000; Schwitzer and Lork, 2004; Mittermeier et al., 2010; Rakotoarinivo et al., 2011), providing the species with considerably more protection than *E. flavifrons*.

The geographic range of *E. flavifrons* is bounded by the Maeverano River to the south, the Sandrakota River and Manongarivo Special Reserve to the east, and the Mozambique Channel to the west (Koenders et al., 1985; Petter & Andriatsarafara, 1987; Randriatahina & Rabarivola, 2004; Schwitzer & Lork, 2004; Andriaholinirina et al., 2014a; Figure 1.1). The Andranomalaza River, which runs east-west, is the presumed northern geographic barrier for *E. flavifrons* and the southern geographic barrier for *E. macaco* (Koenders et al., 1985; Andriaholinirina et al., 2014a, 2014b). The range of *E. macaco* is further bounded by the Mahavay River in the north and the Tsaratanana Massif to the east (Andriaholinirina et al., 2014b). As the Andranomalaza River tapers off in the east, the two species' distributions are thought to overlap in a wide contact zone near the Manongarivo Special Reserve. It is here that the hybridisation of *E. flavifrons* with *E. macaco* has been reported, based largely on morphological data (Meyers et al., 1989; Rabarivola et al., 1991; Goodman & Schütz, 2000; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004; Schwitzer & Lork, 2004).

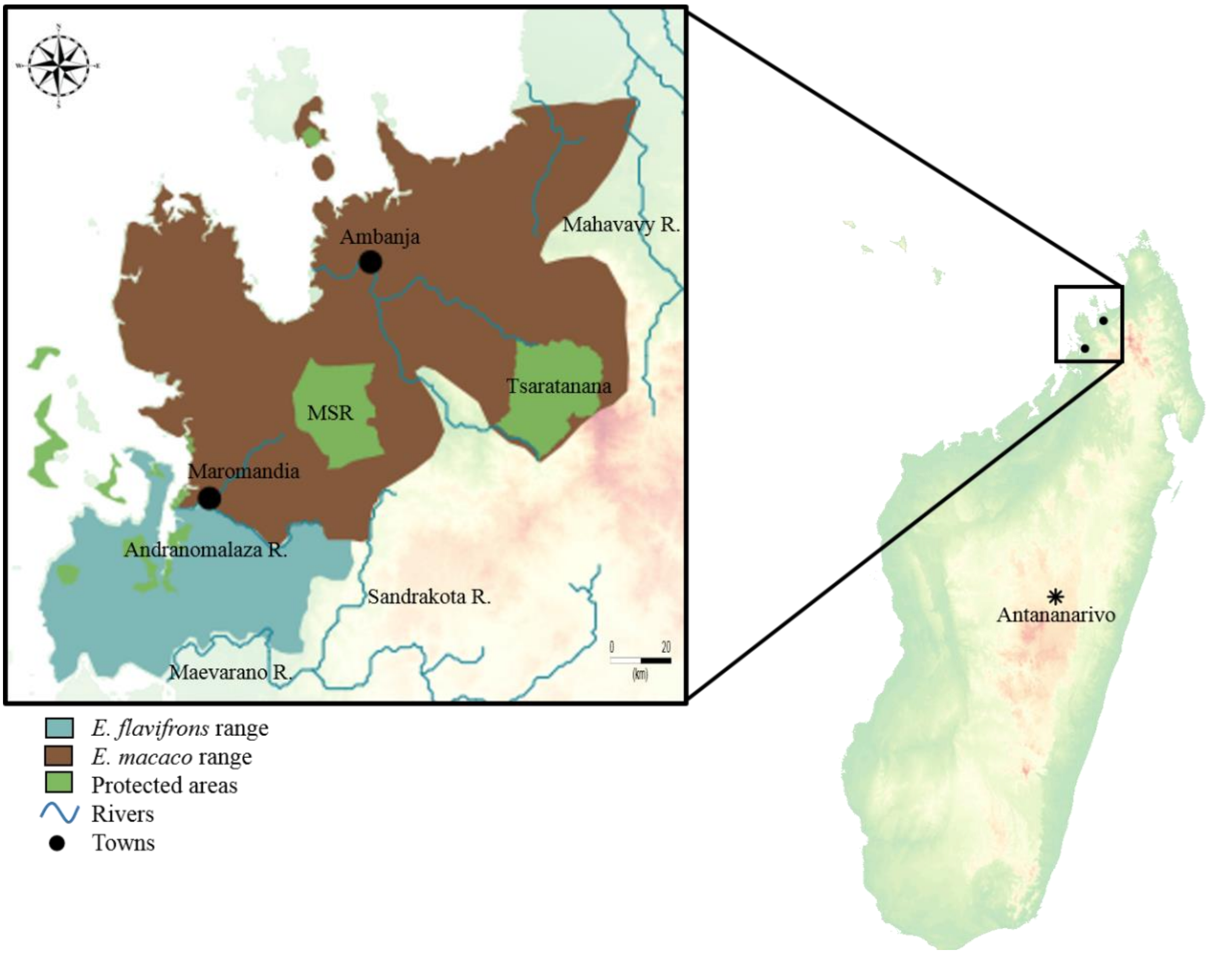

Figure 1.1 Map of Extent of Occurrences for *Eulemur* spp. in northwest Madagascar, courtesy of the IUCN.

Morphologically very similar, both species are 90-110 cm long (Terranova & Coffman, 1997; Mittermeier et al., 2010) with a mean body mass of 1.79 kg for *E. flavifrons* and 2.47 kg for *E. macaco* (Terranova & Coffman, 1997). *E. flavifrons* and *E. macaco* are sexually dichromatic—males are black and females a golden tan. Although similar in the aforementioned features, the two species differ most distinctly in eye colour and the presence/absence of ear ruffs. *E. flavifrons* has blue to blue-grey eyes and no ruffs, whereas *E. macaco* has yellow-orange eyes and exhibits ear ruffs (Mittermeier et al., 2010). See Appendix A for a more detailed breakdown of the morphological differences between the two species.

In the wild, *E. flavifrons* and *E. macaco* groups are composed of 2-15 males and females (Colquhoun, 1993; Randriatahina & Rabarivola, 2004; Bayart & Simmen, 2005; Randriatahina et al., 2014). Both species typically exhibit female dominant societies (Digby & Kahlenberg, 2002; Bayart & Simmen, 2005; Digby & Stevens, 2007). *E. flavifrons* and *E. macaco* are seasonal breeders; the mating season has been observed beginning as early as April and lasting as late as mid-June (Bayart & Simmen, 2005; Volampeno et al., 2011a, 2011b). During this time, increased agonistic encounters have been observed, with males competing for access to reproductive females (Digby, 1999; Bayart & Simmen, 2005). The two species are cathemeral, with activity levels fluctuating depending on ambient moon light (Colquhoun, 1998a; Schwitzer et al., 2007a), seasonality (Colquhoun, 1993; Colquhoun, 1998a), and forest composition (Schwitzer et al., 2007a). Their diet, which is also highly seasonal, is composed primarily of fruit and supplemented with leaves, flowers, seeds, arthropods, sap, soil, and fungi (Koenders, 1989; Colquhoun, 1993; Birkinshaw, 1999, 2001, 2003; Simmen et al., 2007; Van den Abbeele, 2014). One captive study (Gosset et al., 2001) detailed the full vocal repertoire of *E. macaco*, and one comprehensive study assessed repertoire differences between multiple *Eulemur* species (Gamba et al., 2015). The grunts of *E. flavifrons* and *E. macaco* differ significantly (Gamba & Giacoma, 2008).

1.9 Project goals and thesis structure

Despite their recent classification as separate species based on genetic differences (Fausser et al., 2000; Mittermeier et al., 2008), few studies have compared the behavioural ecology of the two taxa, which could provide extended support for this taxonomic distinction. The absence of further study is surprising given the withering long-term viability of *E. flavifrons* (Volampeno et al., 2015) and the reports of hybridisation with *E. macaco* (Meyers et al., 1989; Rabarivola et al., 1991; Goodman & Schütz, 2000; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004; Schwitzer & Lork, 2004). Therefore, the overall aims of this study are (1) to provide additional lines of evidence that either support or refute the classification of *E. flavifrons* and *E. macaco* as separate species and (2) to illuminate the role of species-specific behaviours as potential pre-mating isolation barriers between the two taxa. The following are the overarching questions that this study endeavours to answer:

- Do *E. flavifrons* and *E. macaco* range distributions currently overlap in a contact zone, and if so, what is the effect on the behaviours and vocalisations of those populations? Is there any evidence of hybridisation?
- Are there intersexual, interspecific, and/or intraspecific variations in characters? Could any of these potentially serve as reproductive isolation mechanisms?
- How do these data contribute to what is known regarding the speciation of the two taxa?

To address these questions, two key components of mate recognition systems were chosen for investigation, social systems and vocal communication. Originally, comparisons were to be made between allopatric, parapatric, and sympatric populations of both species. As such, in Chapter 2, I first investigate whether or not the ranges of *E. flavifrons* and *E. macaco* do in fact overlap in a zone of contact. In collaboration with Jen Tinsman, a doctoral student at Columbia University, the presence of *Eulemur* in Manongarivo Special Reserve is discussed. This first data chapter is in press in *Oryx*. Although we both contributed equally to writing the manuscript, Tinsman is responsible for the literature review and the updated Extent of Occurrence (EOO) and Area of Occupancy (AOO) polygons. In Chapter 3, I characterise the social organisation of *E. flavifrons* and *E. macaco,* identify patterns of intersexual and intrasexual interactions and associations, and discuss similarities and/or differences between the two taxa. Specifically, group composition and cohesion, the strengths of dyadic associations, and the rates of affiliative and agonistic interactions are explored. The mating systems of *E. flavifrons* and *E. macaco* are described in Chapter 4. To do this sexual behaviours and mating tactics of four populations are explored, with comparisons made within and between the two species. Chapter 5 investigates the acoustic communication of *E. flavifrons* and *E. macaco* and contributes newly characterised calls to their vocal repertoires. Overall conclusions are drawn in Chapter 6.

1.10 Study locations and populations

Data were collected from a combination of seven different *E. flavifrons* and *E. macaco* locations throughout the two species' ranges (Figure 1.2). Each location was comprised of multiple groups of lemurs, collectively referred to as a population. The lack of habituation and clear morphological markers made reliable identification of groups and/or individuals very difficult. Because of this, data in this study are presented at the population level only unless otherwise noted.

These populations exist along a bioclimatic and phytogeographic cline in northwest Madagascar (Cornet, 1974; Moat & Smith, 2007). Study sites were found in tropical regions characterised by humid forests (Sambirano domain), in increasingly drier regions with sub-humid forests (found in the upper

Western domain), or in areas of transition between the two ecotones. The Sambirano domain covers an area of approximately 420,000 ha and includes the islands of Nosy Be and Nosy Komba; the Ampasindava Peninsula; and the Tsaratanana, Manongarivo, and Galoko-Kalobinono Massifs (Humbert, 1955). This domain is characterised as being structurally similar to the wet evergreen region in the east, while also having a unique combination of locally endemic species (Koechlin et al., 1974). In comparison, the Western domain runs along much of the western coast of Madagascar. The composition of dry, deciduous vegetation varies throughout locations, depending on soil type and moisture content (Tattersall & Sussman, 1975).

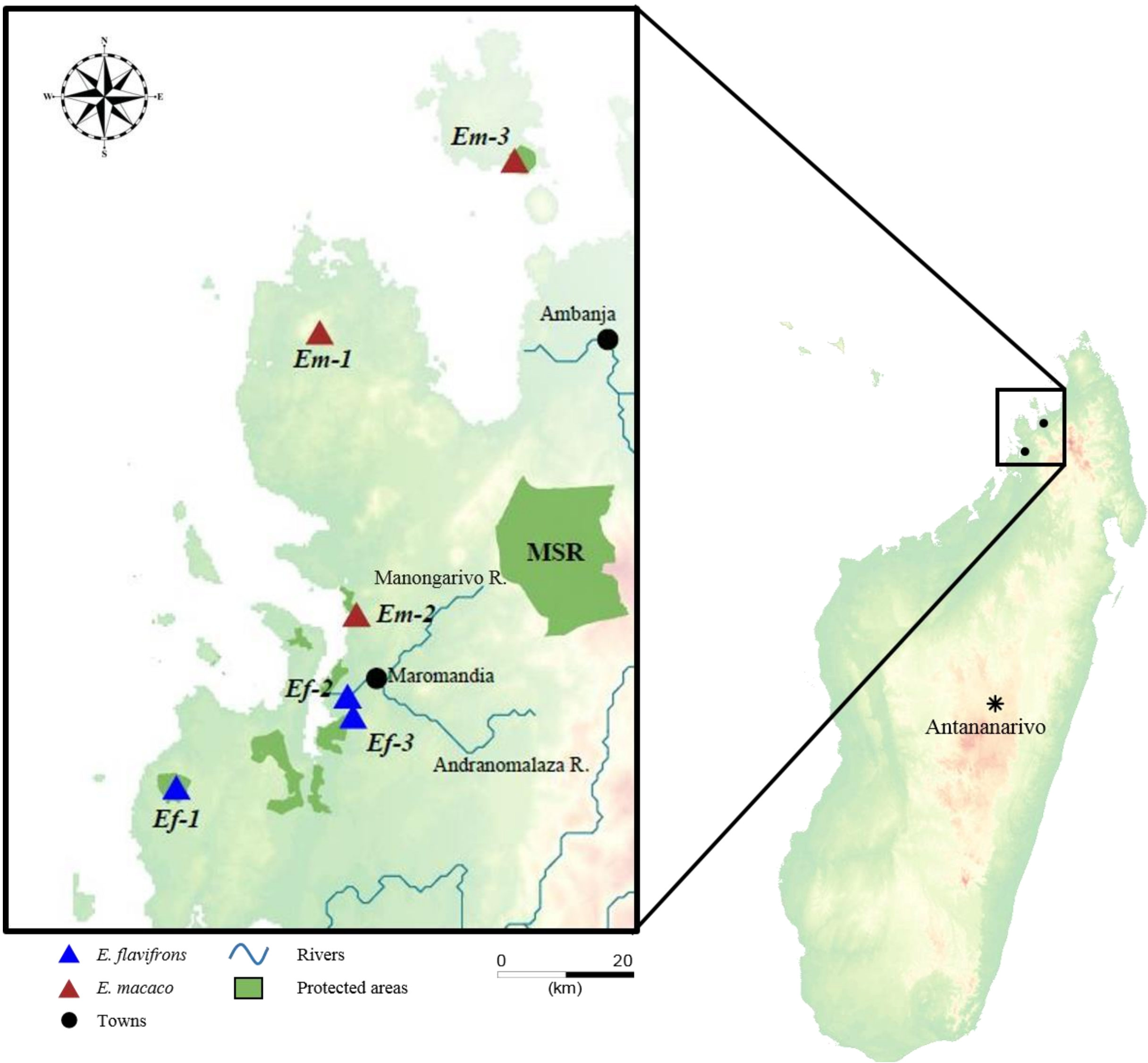

Figure 1.2 Locations of the seven study sites where data were located over three field seasons (April-July 2015-2017) in northwest Madagascar. *Ef-1*: Ankarafa Forest, 2015; *Ef-2*: Ambodimanga, 2016; *Ef-3*: Angodrahely, 2017; *Em-1*: Andranomatavy, 2016; *Em-2*: Mahadera, 2016; and *Em-3*: Lokobe, 2017; and MSR: Manongarivo Special Reserve, 2015 & 2016.

1.10.1 Ankarafa Forest (*Ef-1* population)

The Sahamalaza Peninsula is located in the Western domain that is part of the Sofia region of Madagascar. This area was first recognised as an UNESCO Biosphere Reserve in 2001 and then later as a National Park in 2007 (Sahamalaza-Iles Radama National Park, SIRNP; Schwitzer et al., 2007b). SIRNP is comprised of 59,000 ha of forest and marine coastal areas (Fausser et al., 2000; Randriatahina & Roeder, 2013), which are bounded by the Mozambique Channel to the west, the Sahamalaza Bay to the east, and the Loza River to the south (Volampeno, 2009). SIRNP lies in a transition zone between the drier, deciduous region to the southwest and the more northern and humid Sambirano region. As such, the remaining semi-humid forest on the peninsula is a unique combination of plants from both neighbouring ecoregions, as well as locally endemic species (Birkinshaw, 2004; Volampeno, 2009). Strongly seasonal, the Sahamalaza Peninsula experiences a rainy season from November to April and dry season from May to October (Volampeno, 2009). There are strong trade winds for half of the year (Volampeno et al., 2013).

Located within SIRNP is the Ankarafa Forest (14°22’64.2”S and 47°45’31.5”E). At approximately 185 ha in size (Seiler et al., 2014), this area represents the largest remaining forest fragment on the peninsula. Ankarafa Forest is comprised of six parcels that are a mixture of old growth and regenerated vegetation, surrounded by low-diversity grasslands (Volampeno, 2009; Seiler, 2012; Seiler et al., 2014; Mandl, 2017; Mandl et al., in press). The forest is characterised by both native and exotic species, including significant patches of mango (*Mangifera indica*) and bamboo (family Poaceae, Volampeno et al., 2013). A measurement of abiotic factors has determined that up to 70% of Ankarafa is comprised of forest edge (Mandl, 2017), with all parcels exhibiting some degree of edge effect (Schwitzer et al., 2007a, 2007b; Seiler, 2012). Tree composition in the core was found to be taller, larger, and more diverse than edge vegetation suggesting that the inner forest may be of better quality (Volampeno et al., 2013).

Ankarafa Forest harbours many endemic reptiles and amphibians (Penny et al., 2017), as well as four species of lemurs: the Sahamalaza sportive lemur (*Lepilemur sahamalaza*), the northern giant mouse lemur (*Mirza zaza*), the fat-tailed dwarf lemur (*Cheirogaleus medius*), and the blue-eyed black lemur (*E. flavifrons*; Seiler et al., 2010). The aye-aye (*Daubentonia madagascariensis*) has been sighted on the peninsula (C. Schwitzer, pers. comm.), although it has yet to be confirmed in Ankarafa.

The Association Européenne pour l’Etude et la Conservation des Lémuriens (AEECL) has managed a research station in Ankarafa since 2001. Despite its presence, there are continued anthropogenic disturbances from subsistence farming, tree-felling, bushfires, hunting, and livestock grazing (Ruperti, 2007; Schwitzer et al., 2007b; Seiler et al., 2010, 2012). In 2018, uncontrolled fires were seen burning inside the forest (S. Solofondranohatra, pers. comm.). Much of AEECL’s research and conservation focus has been on *E. flavifrons*. The Ankarafa lemurs remain the largest (Schwitzer et al., 2006) and most well studied population of this taxa. The population density in Ankarafa is estimated to be 1 individual/ha of forest (Volampeno et al., 2010). Habituated in 2004, lemurs were collared and studied extensively until 2009 (Volampeno, 2009). Despite the initial momentum, there have been few recent long-term studies of these lemurs, with most of the previous research occurring between October and January (Mainiero, 2014; Van den Abbeele, 2014; Prodger, 2015). I collected social system and acoustic data from this location in 2015.

1.10.2 Andranomatavy (*Em-1* population)

The Ampasindava Peninsula, found in the Diana region of the island, is located between 13°49’40.0” and 13°40’60.0”S and 47°58’40.0” and 48°58’30”E. The peninsula is characterised by humid and subhumid Sambirano forests and mangroves, mean rainfall over 2,200 mm, and pronounced wet and dry seasons (Humbert, 1955; Tahinarivony, 2014; Razafimandimby, 2017). The 145,000 ha peninsula has been reported to have lost approximately 40,000 ha of forest between 1991 and 2013 (Tahinarivony, 2014). During this time there was a reduction in primary forest (old growth) and an increase in secondary forest (new growth), presumably due to logging and *tavy*, a slash-and-burn rice cultivation technique (Tahinarivony, 2014). In 2015, under the management of Missouri Botanical Gardens (MBG), 7,648 ha of core forest and the surrounding 84,142 ha buffer zone (including areas of occupancy, sustainable use, and development) were classified as protected (MEEMF, 2015).

The four remaining massifs on the peninsula are no longer connected (Tahinarivony, 2014). The largest massif, Andranomatavy (13°40’26.4”S and 47°59’35.1”E), is 2,543 ha of humid, evergreen, core forest. The canopy is dense and continuous and reaches up to 30 m high (MBG, 2015). While floral diversity remains high, Andranomatavy is characterised by the dominance of *Canarium* spp. and *Dypsis* spp. (see Tahinarivony, 2014 for a detailed description of vegetation). Eight lemur species are found here (*Avahi unicolor*, *Daubentonia madagascariensis*, *E. macaco, Hapalemur occidentalis, Lepilemur mittermeieri, Microcebus sambiranensis*, *Mirza zaza*, and *Phaner parienti*), as well 26 species of endemic reptiles and amphibians, and 69 species of birds (MBG, 2015). Although there are no *E. macaco* behavioural or ecological studies published from here, the density of the species is reported to be 0.043 individuals/ha (MBG, 2015). I collected social system and acoustic data from Andranomatavy in 2016.

1.10.3 Ambodimanga (*Ef-2* population), Mahadera (*Em-2* population), and Angodrahely (*Ef-3* population)

Ambodimanga, Mahadera, and Angodrahely are three small villages surrounding the town of Maromandia (14°12’11.9”S and 48°04’54.1”E) in the Sofia Region of northwest Madagascar. Maromandia sits in between the juncture and the estuary of the Andranomalaza and Manongarivo Rivers. The Andranomalaza River serves as the purported geographical boundary between *E. flavifrons* and *E. macaco* (Koenders et al., 1985; Andriaholinirina et al., 2014a, 2014b). In 2016 the mayor of Maromandia confirmed that groups of both species could be found on their respective side of the river less than 50 years ago. Given that these taxa can live up to an estimated 30 years in captivity (Leigh & Terranova, 1998), the two species could have been in visual and auditory contact in as few as three generations ago. In fact, a 2010 survey of the area surrounding Maromandia identified several forest fragments still containing small groups of *E. flavifrons* and *E. macaco*, each on their respective sides of the Andranomalaza River (Dumoulin, 2011).

In 2016, Ambodimanga and Mahadera were chosen as field sites for this study, representing locations of close contact between the two species. Behavioural and acoustic recordings were to be collected in 2017, but due to extenuating and dangerous circumstances, these sites were no longer viable options for the final field season. As a result, the nearby town of Angodrahely and the island of Nosy Be were selected for data collection in 2017.

The village of Ambodimanga (14°14’21.2”S and 48°2’15.4”E) is located approximately 5 km south of Maromandia and 700 m from the Andranomalaza River. The remaining vegetation fragment is approximately 2.4 ha and is extremely degraded (Razafindramoana, 2015). Rice fields, the National Road 6, and houses surround the fragment. Local villagers rely heavily on the resources of the forest, resulting in continual disruptions. The local association Taratra monitors the *E. flavifrons* population, although this remains debatable. In 2016, collection of vocalizations began but was forced to end early due to unforeseen safety concerns. Although I had planned to return to this location again in 2017 to finish data collection, the lemurs could not be located during the month of May. After over a week of searching the small fragment and the surrounding areas, data collection was moved to nearby Angodrahely. The group was spotted two months later (J. van Mildert, pers. comm.), which was outside the scope of this study. No other species of lemur have been observed in this location.

Angodrahely (14°16’18.4”S and 48°2’47.4”E), which is located a further 7 km south of Ambodimanga, is neither under the jurisdiction of MNP nor under the control of the local authority ruling Maromandia. Instead, it is 44 ha of privately-owned land comprised of degraded primary and secondary vegetation. This fragment is home to *E. flavifrons*, *Microcebus* spp., and *Lepilemur sahamalaza*. To my knowledge, no previous research has been conducted in Angodrahely. In 2017, I located lemurs and collected social system and acoustic data from Angodrahely.

The village of Mahadera (14°6’44.0”S and 48°2’59.1”E), which is 11 km north of Maromandia, is comprised of approximately 11 ha of highly fragmented vegetation. *E. macaco* are found in this location, which is to the north of the Andranomalaza River. Lemurs here live in extreme proximity to villagers and undoubtedly experience considerable noise pollution as a result. The vegetation here is protected by MNP and residents also abide by local *fady*, or taboo, that considers *E. macaco* sacred. A further 2 km north is the village of Kapany, which supports a large number of provisioned *E. macaco*. It seems unlikely that connecting forest corridors remain.

1.10.4 Lokobe National Park (*Em-3* population)

Lokobe National Park is found between 13°22’14.8” and 13°25’30.1”S and 48°18’6.9” and 48°21’34.2”E in the southeast corner of the island of Nosy Be. With a climate that is hot and humid, the mean annual rainfall exceeds 2,000 mm and the mean temperature is around 26° C. Lokobe is both a marine (122 ha) and terrestrial (740 ha) protected area managed by Madagascar National Parks (MNP) since 1996. It is comprised of a combination of mangrove and Sambirano vegetation that reaches 430 m above sea level. *Dypsis* spp. are the park’s flagship tree species. The low altitude forest here is easily accessible and therefore has been exposed to more anthropogenic threats than the inner core forest (MNP, 2013).

There are three species of lemurs found on the island—*E. macaco*, *Lepilemur tymerlachsonorum*, and *Microcebus mamiratra* (Randriatahina & Volampeno, 2013). Despite the fact that there has been little long-term research conducted on *E. macaco* in Lokobe since the 1990s (Koenders, 1989; Rabarivola et al., 1996; Andrews & Birkinshaw, 1998; Birkinshaw & Colquhoun, 1998; Birkinshaw, 1999, 2001), lemurs there are habituated to human presence due to high levels of ecotourism and the numerous peripheral villages (Randriatahina & Volampeno, 2013). In 2011, park officials conducted density surveys determining that there are approximately 0.4 individuals/ha of forest (MNP, 2013).

The Nosy Be *E. macaco* were found to be smaller (mean adult mass 1.78 kg; Junge & Louis, 2007) and more genetically dissimilar to *E. macaco* inhabiting the nearby islands of Nosy Komba and Nosy Ambato and the mainland, than the latter three locations were to each other. It is has been hypothesised that the Nosy Komba and Nosy Ambato lemurs were introduced from the mainland more recently (Rabarivola et al., 1996), while the Nosy Be lemurs developed in isolation from the mainland around 8,000 years ago (Battistini, 1960 cited by Rabarivola et al., 1996).

1.10.5 Manongarivo Special Reserve

Found in the Diana region, the 32,700 ha Manongarivo Special Reserve (MSR) was established in 1956 (MEF & MNP, 2010). The reserve is managed by MNP, although deforestation remains a significant concern at the lower elevations (Rakotondrainibe & Quansah, 1994). The headwaters of several key northern rivers originate in MSR, including the Andranomalaza, Manongarivo, and Sambirano. Reaching elevations over 1,800 m, the Sambirano forest can be extremely steep and difficult to navigate (Rakotondrainibe & Quansah, 1994). Because of this characteristic the reserve remains an area of species richness, including 70 taxa of reptiles and amphibians, 103 species of birds, and at least 10 taxa of lemurs, two of which are confirmed *Eulemur* (*E. macaco* and *E. fulvus*; MNP, 2015). *E. macaco* and *E. flavifrons* hybrids have been reported from this location (Meyers et al., 1989; Rabarivola et al., 1991; Goodman & Schütz 2000; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004), although comprehensive support for this is lacking.

1.11 Data collection effort

Data were collected with the help of Malagasy graduate students and volunteer field assistants. Prior to the start of the field season, all researchers received training in the appropriate methods to collect behavioural and acoustic data. A minimum inter-rater (principal investigator vs. volunteer/student) reliability score of 90% was required for each researcher to begin collecting data.

1.12 Ethics approval

This research received institutional approval from the University of Bristol, as well as in-country approval from the Ministère de l'Environnement et des Forêts (MEF) and MNP. Permits included:
No.50/15/MEEF/SG/DGF/DCB.SAP/SCB, No.286/15MEEMF/SG/DGF/DAPT/SCBT,
No.55/16/MEEMF/SG/DGFDAPT/SCBT, No.76/16/ MEEMF/SG/DGFDAPT/SCBT,
No.64/17/MEEF/SG/DGF/DSAP/SCB.

1.13 Note on lemur genomics

The analysis of genomic information is the most accurate way to determine the relatedness of individuals and populations. This type of research has been used extensively to identify new species and subspecies across a wide range of animals (Herbert et al., 2004; Clare et al., 2007; Fouquet et al., 2007; Mutanen et al., 2013), including primates (Yoder et al., 2000; Groeneveld et al., 2009), and is particularly relevant to the recent boom in new lemur taxa (Pastorini et al., 2000, 2001; Craul et al., 2007; Rasoloarison et al., 2013). Throughout the three field seasons of this project, faecal samples of observed individuals were collected whenever possible. These samples are currently being analysed in collaboration with J. Tinsman. Although it was initially intended that genomic results would confirm or refute hybridisation between *E. flavifrons* and *E. macaco* in areas of purported contact, and thereby bolster findings from this study, results have not been obtained in time to be included here.

Chapter 2

Range and Conservation Updates for the Critically Endangered Blue-Eyed Black Lemur (*Eulemur flavifrons*) and the Vulnerable Black Lemur (*E. macaco*)

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ABSTRACT

The Critically Endangered blue-eyed black lemur *Eulemur flavifrons* of north-western Madagascar is one of the most threatened primates. The majority of research and conservation efforts for the species have been restricted to the Sahamalaza Peninsula but there are unstudied and unprotected populations further inland. The dearth of information regarding the transition between *E. flavifrons* and its parapatric sister species, the Vulnerable black lemur *Eulemur macaco*, and the possibility of a hybrid population complicates conservation planning for both species. We surveyed 29 forest fragments across both species' ranges to investigate the boundary between the taxa, whether hybrids persist, and the threats to lemurs in the region. We found *E. flavifrons* in six fragments and *E. macaco* in seventeen. We never observed *E. flavifrons* and *E. macaco* in the same location and we found no conclusive evidence of hybrids. Three fragments in which *E. flavifrons* was present were north of the Andranomalaza River, which had been previously considered the barrier between the two species. Based on these observations and a literature review, we provide updated ranges, increasing the Extent of Occurrence (EOO) of *E. flavifrons* by 28.7% and reducing the EOO of *E. macaco* by 44.5%. We also evaluate the capacity of protected areas to conserve these lemurs. We recommend additional surveys and the implementation of an education programme in this region to help conserve both species.

2.1 INTRODUCTION

Madagascar, a biodiversity hotspot (Myers et al., 2000), is home to >100 endemic species of lemurs, accounting for >20% of global primate diversity; however, 94% of lemur species are threatened by hunting and deforestation (Schwitzer et al., 2014a). One of the species most affected is the Critically Endangered blue-eyed black lemur (*Eulemur flavifrons*), which is subject to poaching and habitat loss as a result of slash-and-burn rice cultivation (*tavy*), logging, and livestock rearing (Andrianjakarivelo, 2004; Seiler et al., 2010; Andriaholinirina et al., 2014a). The population of blue-eyed black lemurs declined by >80% during 1990–2014 (Andriaholinirina et al., 2014a).

Estimates of *E. flavifrons* numbers have focused mainly on the protected population in the Ankarafa Forest of Sahamalaza–Iles Radama National Park, where there are estimated to be 60–130 individuals/km^2 (Schwitzer et al., 2006; Volampeno et al., 2010). Surveys of the isolated fragments where *E. flavifrons* occurs further inland found much lower densities, with a mean of 24 individuals/km^2 (Adrianjakarivelo, 2004). Based on these surveys there are only an estimated 2,780–6,950 individuals remaining (Schwitzer et al., 2006). A population viability analysis concluded that the Ankarafa population, which is the largest remaining, could be extirpated by 2026 (Volampeno et al., 2015). The vulnerability of *E. flavifrons* is partially attributable to its unique habitat; the species occurs only in the transitional, subtropical forest between Madagascar's western dry deciduous forests and the humid evergreen rainforests of the east (Schwitzer et al., 2007b). The plant, amphibian, reptile, and mammal communities of the Sahamalaza Peninsula include endemic species that occur nowhere else on the island (Birkinshaw, 2004; Schwitzer et al., 2006; Penny, et al. 2017).

Although not confined to the Sahamalaza Peninsula, *E. flavifrons* has one of the smallest geographical ranges of the genus *Eulemur* (Volampeno et al., 2010). Its estimated Extent of Occurrence (EOO; i.e. the smallest, continuous area encompassing all known or projected occurrences of a species; IUCN, 2001) is <2,700 km^2 (Andriaholinirina et al., 2014a). Its Area of Occupancy (AOO; i.e. the area of suitable habitat that is actually occupied within a species' EOO; IUCN, 2001) must be even smaller, although it had not been estimated previously.

The EOO of *E. flavifrons* is bounded by the Mozambique Channel to the west and the Maevarano River to the south (Koenders et al., 1985; Petter & Andriatsarafara, 1987; Randriatahina & Rabarivola, 2004; Schwitzer & Lork, 2004; Andriaholinirina et al., 2014a; Figure 2.1). It extends east to the Sandrakota River and Manongarivo Special Reserve, which comprises 32,000 ha of protected Sambirano rainforest (MEF & MNP, 2010). Previous studies have identified the Andranomalaza River, also called the Maitsomalaza in the local Sakalava dialect, as the boundary between *E. flavifrons* and its parapatric sister species, the black lemur (*Eulemur macaco*, Koenders et al., 1985; Andriaholinirina et al., 2014a). However, there is conflicting evidence regarding whether the lemurs between the Andranomalaza River and the more northern Manongarivo River are hybrids, intermediate-appearing forms on a phenotypic cline, or typical members of either species (Meyers et al., 1989; Rabarivola et al., 1991; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004).

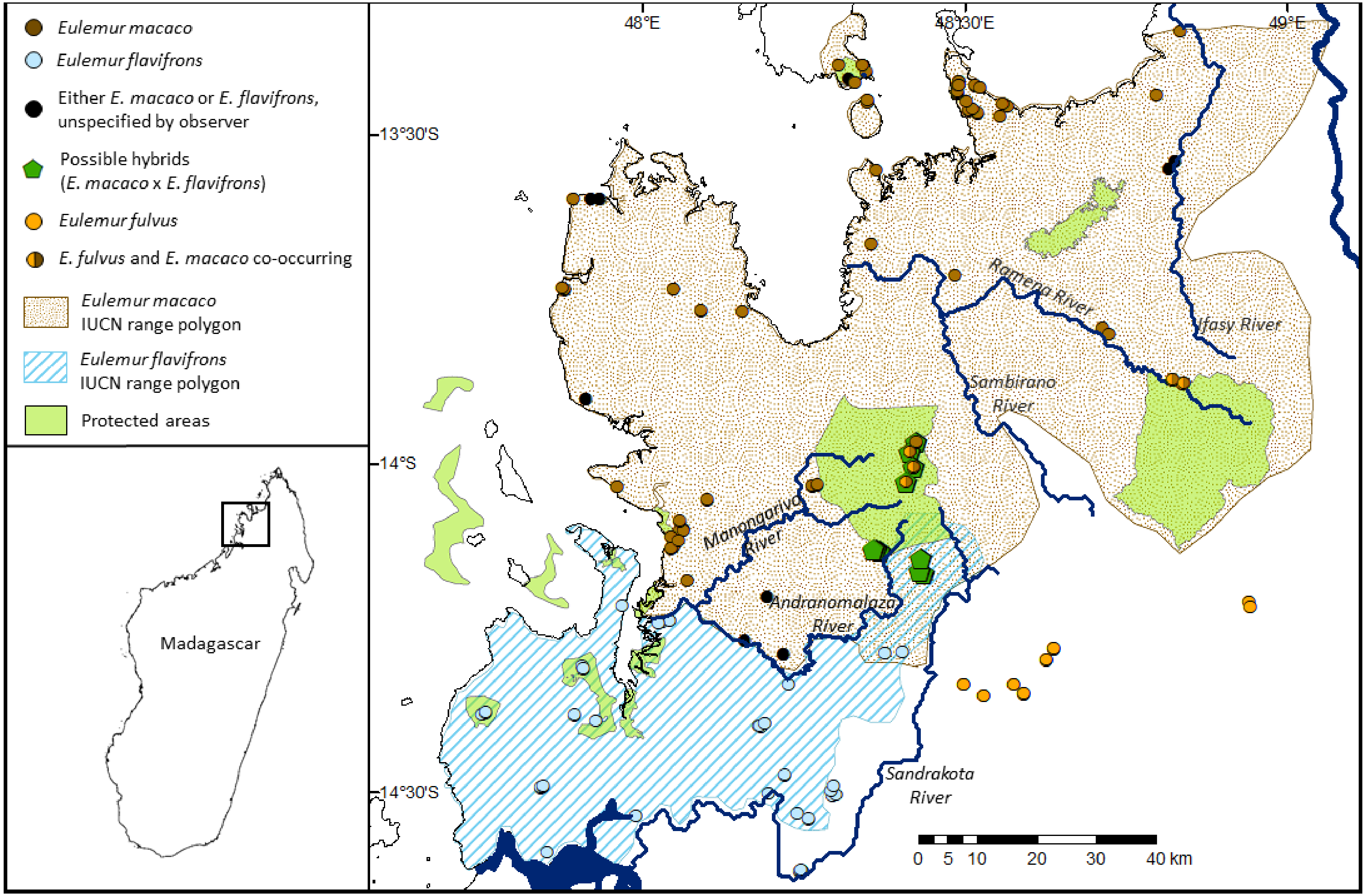

Figure 2.1 Previous understanding of Extent of Occurrences for *Eulemur* spp. courtesy of the IUCN. Points represent all known occurrence records for *E. flavifrons* and *E. macaco*, putative hybrids, and for *E. fulvus* in the area. Rivers are labelled.

The first report of phenotypic variation was by Meyers et al. (1989), who observed two distinct groups of lemurs unlike typical *E. flavifrons* or *E. macaco*. The first group was at Beraty, and individuals had light brown eyes and short ruffs of hair around their ears, characteristics that are intermediate between *E. flavifrons* and *E. macaco*. The second group was at Ambodivoahangy, and individuals had darker eyes and redder coats than is typical of Sahamalaza *E. flavifrons*. However, when Andrianjakarivelo (2004) visited Ambodivoahangy he found animals that 'greatly resembled' *E. flavifrons*. Goodman & Schütz (2000) surveyed the eastern slopes of Manongarivo Special Reserve, north of Ambodivoahangy, and identified groups containing both *E. macaco* and 'hybrid' individuals but did not detail their criteria for these distinctions. Their assessment was complicated by the presence of *E. fulvus* in that area, which may be perceived as having a reddish coat (Goodman & Schütz, 2000).

Updated assessments of the lemurs in this region are necessary to establish effective conservation initiatives for these two species (Rakotonirina et al., 2011, 2014). They could also improve estimates of the species' ranges, especially considering the area has not been assessed since 2004 (Schwitzer et al., 2014a). We investigated the presence and phenotypes of *Eulemur* species from Sahamalaza–Iles Radama National Park to Manongarivo Special Reserve, and the threats to their survival, to (1) establish the continued existence of *E. flavifrons* outside protected habitat, (2) locate the purported contact zone between *E. flavifrons* and *E. macaco*, and (3) understand anthropogenic pressures in and around the protected areas in this region. We provide updated EOOs and new AOOs for both species, and report on the status of the lemurs between the Manongarivo and Andranomalaza Rivers.

2.2 METHODS

We conducted surveys during June–September and November–December 2015, June 2016, and April–June 2017. To evaluate possible barriers between *E. flavifrons* and *E. macaco* we worked eastwards from the coast near Maromandia (14°12′11.9″S, 48°04′54.1″E) to the north-eastern slopes of Manongarivo Special Reserve (14°00′42.8″S, 48°22′47.3″E), focusing our efforts around the Andranomalaza and Manongarivo rivers. We visited eight sites within and 10 outside the Reserve (Figure 2.2) and 11 other sites throughout the region to record threats to lemur survival and to observe typical members of both species (Figure 2.2 and Figure 2.3). We searched for lemurs for up to seven days at each site, calling the site an absence if we could not find lemurs after a week of diurnal surveys led by a local person who worked in the forest. We walked the interior of each fragment, relying on paths when possible, until we heard lemur vocalisations or movements. We recorded global positioning system (GPS) coordinates for all observed *Eulemur* spp. and noted key morphological features (eye colour, presence of ruffs) to distinguish between *E. flavifrons* and *E. macaco*. Evidence of *tavy*, livestock incursions, traps, and hunting were also noted.

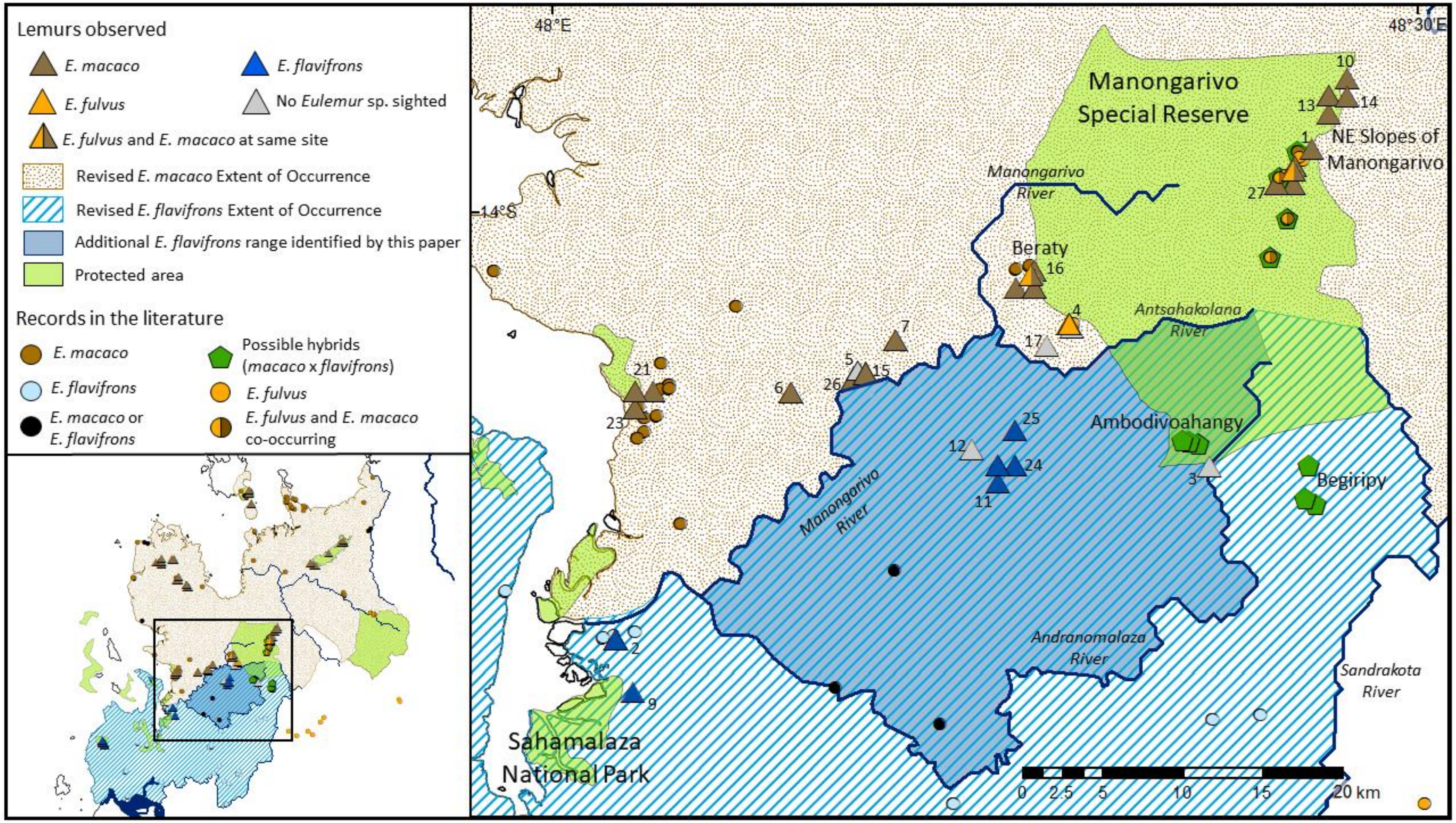

Figure 2.2 Updated Extent of Occurrences for *Eulemur flavifrons* and *E. macaco* based on new field work (triangles) and in previous published studies (circles. Numbers correspond to those describing forest fragments in Table 2.1. Manongarivo Special Reserve (MSR), Sahamalaza Iles-Radama National Park (SIRNP), and rivers are labelled.

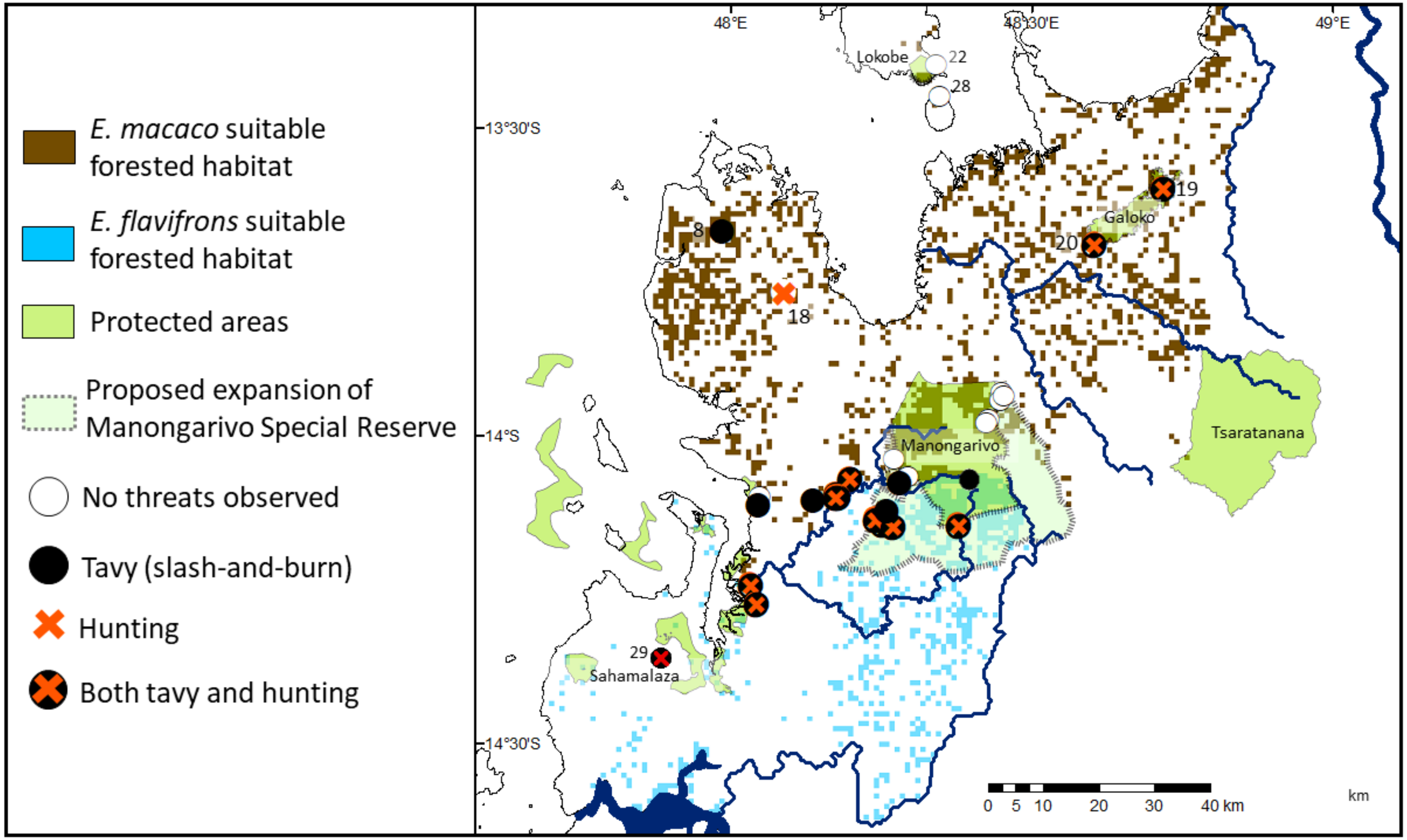

Figure 2.3 Map of remaining, suitable habitat for *Eulemur flavifrons* and *E. macaco* and of threats observed throughout the study area. Numbers correspond to those describing forest fragments listed in Table 2.1. Manongarivo Special Reserve (MSR), Sahamalaza Iles-Radama National Park (SIRNP), Tsaratanana Nature Reserve (TNR), the Galoko-Kolobinono Protected Area (GKPA), and Lokobe Reserve (LR) are labelled.

In addition to field surveys we conducted a thorough review of the literature for occurrence records of *E. flavifrons* and *E. macaco*. We searched four online databases (ReBioMa, Manis, VertNet and GBIF) for *Eulemur* spp. records. All relevant articles published in *Lemur News*, *Madagascar Conservation and Development*, *Primate Conservation*, and *Malagasy Nature* were scanned visually for GPS coordinates. We also conducted several searches in Google Scholar using combinations of the following terms: *Eulemur macaco*, *Eulemur flavifrons*, GPS, occurrence, coordinates, and range. These articles, databases, and our field efforts yielded 119 unique records for *E. flavifrons* and 182 for *E. macaco*. New EOOs were determined by comparing these records to the river catchments in this region. River data were downloaded from WWF's HydroSHEDS project (Lehner et al., 2008).

To approximate AOOs for these species we used these occurrence records to construct ecological niche models. Points were thinned to no closer than 2.5 km apart, to reduce spatial autocorrelation (Kramer-Schadt et al., 2013). Environmental data related to arboreal life history were downloaded from WorldClim, CliMond, SoilGrids, WorldGrids, NASA EOSDIS, and CIRCAD. Only variables with relatively low correlation to each other ($|R^2| < 0.85$; mean $|R^2| = 0.38$) were included in analyses to reduce model overfitting (Dormann et al., 2013; Pearson et al., 2014). Niche models were constructed with *MaxEnt 3.4.1* (Philips et al., 2017) using parameters identified with *ENMeval* (Muscarella et al., 2014).

A 10% training threshold was used to turn models into a binary prediction of 1 (suitable habitat) or 0 (unsuitable). The model for each species was then limited to its EOO and the most recent forest cover estimate available (Vieilledent et al., 2018). They were resampled to 2 km^2 resolution, which is the IUCN standard for AOO, before the area was calculated.

2.3 RESULTS

We observed *E. flavifrons* in six of the 29 forest fragments surveyed (21% of sites; Table 2.1), and *E. macaco* in 17 fragments (59%). We observed *E. fulvus* at four sites, twice on its own and twice co-occurring with *E. macaco*. At four of the sites (14%) we found no individuals of any *Eulemur* species, and we never observed *E. flavifrons* and *E. macaco* in the same forest fragment. The lemurs we observed in previously unstudied areas near Antsahabilahy and along the Maherivaratra mountain range had pale eyes and no ruffs and appeared to be *E. flavifrons*, despite occurring north of the Andranomalaza River, within the IUCN-identified EOO for *E. macaco* (Figure 2.2 and Figure 2.4; Andriaholinirina et al., 2014b).

Table 2.1 Presence/absence of lemurs and threats at locations surveyed from 2015-2017. MNP: Madagascar National Parks-managed protected area, and NGO: non-profit Missouri Botanical Garden.

	Forest Fragment	**Year**	**Location**		**Protected area?**	***Eulemur* species**	**Traps or evidence of hunting**	***Tavy***
			Southing	**Easting**				
1	Ambavanambahatra	2016	13°58’1.74”	48°26'3.16"	MNP	*fulvus*	-	-
2	Ambodimanga	2015	14°14'21.12"	48°2'15.36"	-	*flavifrons*	+	+
3	Ambodivoahangy	2015	14°8'22.56"	48°22'57.00"	MNP	-	+	+
4	Ambohitsara	2016	14°3'36.7"	48°18'7.0"	MNP	*fulvus*	-	-
5	Ampapanabe	2015	14°5'28.68"	48°10'40.08"	-	-	+	+
6	Analafady	2015	14°6'5.76"	48°8'39.48"	-	*macaco*	-	+
7	Andokobe	2015	14°3'57.24"	48°12'1.02"	-	*macaco*	+	+
8	Andranomatavy	2015	13°39'59.37"	47°59'15.87"	NGO	*macaco*	+	+
9	Angodrahely	2017	14°16'14.88"	48°2'47.40"	-	*flavifrons*	+	+
10	Ankazomena	2016	13°55'20.86"	48°27'21.13"	MNP	*macaco*	-	-
11	Antsahabilahy A	2015	14°8'24.00"	48°15'25.56"	-	*flavifrons*	+	+
12	Antsahabilahy B	2015	14°7'53.40"	48°14'41.50"	-	-	+	+
13	Befalafa	2015	13°56'9.24"	48°27'10.80"	MNP	*macaco*	-	-
14	Bekiritsana	2015	13°55'45.37"	48°27'35.71"	MNP	*macaco*	-	+
15	Bemabaza	2015	14°5'42.72"	48°10'59.16"	-	*macaco*	+	+
16	Beraty	2015	14°1'57.72"	48°16'42.24"	MNP	*macaco, fulvus*	-	-
17	Bevazimba	2016	14°4'21.36"	48°17'15.36"	-	-	-	+
18	Bongomirahavavy	2015	13°45'48.06"	48°5'29.44"	NGO	*macaco*	+	-
19	Galoko	2015	13°35'25.55"	48°43'0.45"	NGO	*macaco*	+	+
20	Kalobinono	2015	13°40'53.70"	48°36'17.13"	NGO	*macaco*	+	+
21	Kapany	2015	14°5'46.32"	48°3'17.28"	MNP	*macaco*	-	-
22	Lokobe	2015	13°23'33.68"	48°20'29.13"	MNP	*macaco*	-	-
23	Mahadera	2016	14°6'37.80"	48°3'6.12"	-	*macaco*	-	+
24	Maherivaratra A	2015	14°8'33.72"	48°16'23.52"	-	*flavifrons*	+	+
25	Maherivaratra B	2016	14°7'4.44"	48°15'58.32"	-	*flavifrons*	-	+
26	Mandriranabe	2015	14°5'42.00"	48°10'41.16"	-	*macaco*	+	+
27	Manongarivo[1]	2015	13°58'21.00"	48°25'46.92"	MNP	*macaco, fulvus*	-	-
28	Nosy Komba	2015	13°26'38.70"	48°20'50.38"	-	*macaco*	-	-
29	Sahamalaza (Ankarafa)	2015	14°23'05.6"	47°45'56.9"	MNP	*flavifrons*	+	+

[1] *E. macaco* and *E. fulvus* were resting in the same tree together

Figure 2.4 Typical *Eulemur flavifrons* (left; a: male and d: female) and *E. macaco* (right; c: male and f: female), compared with a possible intermediate form from Beraty (centre; male: b). Note how the eye colour is similar to *E. macaco*, but the ruff is shorter. Putative *E. flavifrons* from Antsahabilahy A (centre; female: e). Note the pale eyes and lack of ruff.

The only lemurs we observed that did not resemble typical members of either species were a few male lemurs at Beraty in Manongarivo Special Reserve (Figure 2.4). These males had shorter ruffs than other male *E. macaco* we had seen previously. Given that the females all resembled typical *E. macaco* and these individuals occurred at the southern end of their range, they could simply be clinal variants. Thus, we tentatively identify them as *E. macaco*. On the north-eastern slopes of the Reserve, at another possible hybrid site we visited, we saw only typical *E. macaco* and *E. fulvus*. We were unable to locate any lemurs at Ambodivoahangy, the last possible hybrid site, despite extensive searching.

Although we observed *E. flavifrons* north of the Andranomalaza River, we never observed the species north of the Manongarivo River, nor did we observe *E. macaco* south of the Manongarivo. We therefore propose that the Manongarivo and its tributary, the Antsahakolana River, form the boundary between the two species. We generated a new EOO polygon for *E. flavifrons* based on these findings, increasing its EOO by 29%, from 2,700 to 3,475 km^2 (Andriaholinirina et al., 2014a; Figure 2.3).

In addition to changing the southern boundary of *E. macaco* to the Manongarivo River, we concluded the species is bounded in the east by the Ifasy River, as our literature search revealed no records east of the river (Figure 2.1and Figure 2.2). In the south-east *E. macaco* was bounded by the Tsaratanana Reserve for similar reasons, although such limited surveys have been conducted in this region that this should be considered a low-confidence boundary. This revision reduces the EOO for *E. macaco* to 6,510 km^2, only 55% of the previous estimate of 11,740 km^2 (Andriaholinirina et al., 2014b). When these EOOs are limited to suitable habitat and remaining forest cover, *E. macaco* has an AOO of ≤1,256 km^2 and *E. flavifrons* of ≤560 km^2.

Throughout their ranges these lemurs endure extensive habitat disturbance and other anthropogenic threats. Subsistence hunting and *tavy* were present in a majority of the 29 surveyed locations (69%, n=20; Table 2.1), including 100% of the *E. flavifrons* locations we visited. Only nine sites had no evidence of hunting or *tavy*. Eight were within protected areas managed by Madagascar National Parks and one was a heavily trafficked site on Nosy Komba where local people procure lemurs to entertain tourists. There was evidence of hunting, *tavy*, or both in all four protected areas managed by an NGO, whereas there were threats present in only three of the 11 sites managed by Madagascar National Parks (Table 2.1).

2.4 DISCUSSION

Although these changes in EOO do not warrant immediate adjustments to the species' IUCN Red List status, the range contraction for *E. macaco* is of concern and suggests the need for updated population estimates (Volampeno et al., 2010, 2015). A census of the newly identified population of *E. flavifrons* at Maherivaratra and Antsahabilahy is also needed urgently. The area it occupies south of Manongarivo Special Reserve is part of the largest continuous forest in this species' AOO (Figure 2.3), and this population may be the largest remaining without any protection.

We were unable to visually confirm the ongoing presence of hybrid lemurs, despite visiting three of the four sites where they had been reported previously. Surveys between the headwaters of the Antsahakolana and Sandrakota rivers are needed to determine a more accurate boundary between *E. flavifrons* and *E. macaco* and to collect genetic samples to address the question of any potential hybridisation.

During our surveys we encountered traps, hunters, livestock, and/or *tavy* in 20 of the 29 study sites (Table 2.1), indicating that disturbance is an ongoing concern both inside and outside protected areas. The recent pressures on these lemur populations were described by our local guide for the Bemabaza fragment, where we observed fewer than a dozen *E. macaco* in 2016. He informed us that there had been nearly 100 lemurs 5 years previously but most of these had been wiped out by hunting. This increased pressure may be partly a result of cultural shifts. This region was traditionally home to the Sakalava people, for whom lemur consumption is taboo (Ramanantsoa, 1976; Harpet et al., 2000); however, there has been a recent influx of Tsimihety people (Wilson, 1971; Feeley-Harnik, 1980), who consume primates (Golden & Comaroff, 2015).

Given the level of habitat exploitation and hunting we observed in this region we suggest a multifaceted approach to conserving both species, as well as protecting the remaining forest fragments. In the near-term, additional surveys are needed to measure population numbers accurately (Salmona et al., 2014), evaluate habitat quality in these fragments (Irwin et al., 2005), and assess the impact the various threats reported here have had on *E. flavifrons* and *E. macaco* (Rakotonirina et al., 2011; Ravaloharimanitra, et al., 2011).

In general, we observed fewer threats to lemurs in areas managed by Madagascar National Parks than in those managed by NGOs (Table 2.1); however, this dichotomy is confounded by a few factors. Well-protected sites within Manongarivo Special Reserve had one of two factors in their favour: frequent park staff presence or steep terrain unsuitable for rice cultivation or cattle ranching. All the NGO-managed protected areas we visited were closer to human settlements, arable, and unpatrolled by enforcement authorities.

The new-to-science population of *E. flavifrons* is in relatively accessible forest, but park patrols or on-site staff would help protect these animals. These options would be a possibility if the previously proposed expansion of Manongarivo Special Reserve happens (MEFT & MEM, 2008). This expansion would include the newly identified populations of *E. flavifrons* reported here and the largest block of suitable forest remaining for this species (Figure 2.3).

However, increasing of the size of the Reserve will not achieve protection for these lemurs until the ongoing problems at its current borders are addressed (Gardner et al., 2018). The remote, low-lying areas in and south of the Reserve, including Ambodivoahangy and the proposed area of expansion, are experiencing ongoing forest loss; and the *tavy* and poaching we report here have been a problem for the Reserve since at least 2010 (MEF & MNP, 2010). These incursions are in part because of the lack of boundary markers around the Reserve and the difficulty in patrolling remote areas. Additional funding to address these concerns, coupled with increased researcher presence in this region, would help to deter deforestation (Seiler et al., 2010; Campera et al., 2017). Poaching is probably driven by the lack of readily available protein in this region; we saw children with kwashiorkor in several of the towns we visited south of the Reserve. In Madagascar domestic meats are generally preferred to bushmeat; Therefore, providing chickens or fish stocks to these communities could reduce the poaching pressure on lemurs (Jenkins et al., 2011).

We also propose expanding the community-based conservation education initiative of the Association Européenne pour l'Etude et la Conservation des Lémuriens, which increases local support for conservation by teaching >2,000 students on the Sahamalaza Peninsula about *E. flavifrons*, the Association's flagship species (Randriatahina, 2013). We suggest expanding the programme westwards to include the communities near the newly discovered population of *E. flavifrons*, as well as incorporating aspects of community-based monitoring into the initiative. Community monitoring schemes have been established elsewhere in Madagascar to engage local people in forest management and the collection of species abundance and demographic data (Rakotonirina et al., 2011; Ravaloharimanitra, et al., 2011). Such a programme could promote conservation (Ratsimbazafy, 2003), foster positive attitudes towards the environment (Balestri et al., 2017), and reduce hunting in and around the Reserve (Nadhurou et al., 2017).

Continued surveying of Critically Endangered species such as *E. flavifrons* is vital for establishing range distributions and identifying anthropogenic pressures on taxa living in increasingly fragmented forests. However, our research and future work should serve as a foundation for urgent, practical efforts to conserve these species. We hope that the identification of new EOOs and AOOs for two priority lemur species, and the threats facing these species, will help the Association Européenne pour l'Etude et la Conservation des Lémuriens and community stakeholders as they collaborate to protect the few remaining populations of *E. flavifrons* and *E. macaco*.

Chapter 3

Social Organisation and Structure of *Eulemur flavifrons* and *E. macaco* During the Mating Season

ABSTRACT

The social systems of primates are diverse, with variation between genera, species, and populations possible. Research assessing social system variation is essential to gauge species' adaptability to novel selection pressures and to provide evidence of how species diverge. This study provides new details about the social systems of two closely-related species of lemurs, the Critically Endangered blue-eyed black lemur (*Eulemur flavifrons*) and the Vulnerable black lemur (*E. macaco*) during the mating season. Given the importance of group demographics and social relationships in understanding the social system of a population, I aimed to identify patterns of intersexual and intrasexual interactions and detect similarities and differences between the two taxa. Therefore, group composition and cohesion, the strengths of dyadic associations, and the rates of affiliative and agonistic interactions were measured at three distinct *E. flavifrons* locations (*Ef-1*, *Ef-2*, and *Ef-3* populations) and three distinct *E. macaco* locations (*Em-1*, *Em-2*, and *Em-3* populations). Group size and sex ratios were found to remain relatively consistent between the two species, but the levels of spatiotemporal distribution and fission-fusion dynamics, rates and initiation patterns of affiliative and agonistic interactions, and overall relationship tenor of the different dyad types differed within and between the species. Often these differences could be attributed to one population of *E. macaco*, *Em-1*. While preliminary, initial results suggest that local ecological conditions are contributing to the expression of these diverging behavioural characters within and between the two species, providing further support for behavioural plasticity in *E. flavifrons* and *E. macaco*.

3.1 INTRODUCTION

3.1.1 Primate social systems

Mammalian societies are complex and diverse, with numerous internal and external forces contributing to their intricacy (Kleiman & Eisenberg, 1973; Crook et al., 1976; Weckerly, 1998; Silk, 2007; Clutton-Brock, 2009). The order Primates is no exception; comparative studies focusing on the diversity in primate societies are pervasive (for review see Crook & Gartlan, 1966; Eisenberg et al., 1972; Clutton-Brock & Harvey, 1977; van Schaik & van Hooff, 1983; Smuts et al., 1987; Kappeler, 1997; Shultz et al., 2011; Opie et al., 2012; Kamilar & Cooper, 2013), with variation occurring between closely-related species (Kappeler, 1993b; Kappeler & Ganzhorn, 1993), within a single taxon (Pochron & Wright, 2003; Cronin et al., 2014), or even within one population (Sterck, 1999). Variation can be found in demographic composition, grouping arrangements, and spacing patterns (collectively known as social organisation); in social interactions and the quality of social relationships (social structure); or in mating behaviours and courtship rituals (mating systems, discussed in Chapter 4). Together these constructs form a species' social system (Kappeler & van Schaik, 2002).

Adaptation to novel selection pressures form the root of interspecies variation (Crook & Gartlan, 1966; van Schaik & van Hooff, 1983; Kappeler et al., 2103). Selection pressures were traditionally thought to be ecological, with factors affecting the distribution and abundance of resources and/or predators, which in turn would shape grouping patterns of individuals (Crook & Gartlan, 1966; Eisenberg et al., 1972; Hinde, 1976; Clutton-Brock & Harvey, 1977; van Schaik & van Hooff, 1983; Dunbar, 1996). Later research has also incorporated life-history traits (Kappeler, 1997), social behaviours (Emlen & Oring, 1977; Wittenberger, 1980, van Schaik, 1996), intersexual conflict (Nunn & van Schaik, 2009), and the adoption of local cultures (Whiten & van Schaik, 2007) into the paradigm explaining the variation found throughout primate societies (Figure 3.1).

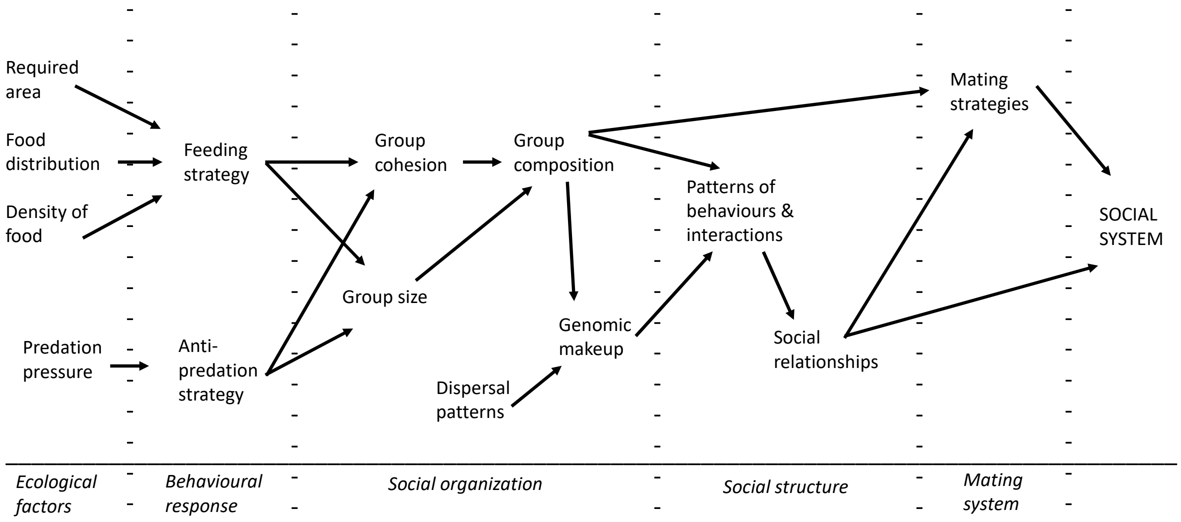

Figure 3.1 Schematic diagram depicting internal and external influences on a species' social system (adapted from van Schaik & van Hooff, 1983).

There also has been a shift in the approach to addressing intraspecific variation, due in part to the speed of certain recent ecological changes. Moving from purely describing diversity at the group or population level (Kamilar & Cooper, 2013; Strier et al., 2014), researchers now are focusing on the relationship between behavioural flexibility, adaptability, and anthropogenic changes to habitat (Kappeler et al., 2013; McFarland et al., 2014). This type of research is essential to assess species' risk of localised extirpation and adaptability (Schlaepfer et al., 2002; Robertson et al., 2013), and is useful for conservation management programmes (Ellwanger & Gould, 2011; Strier, 2017).

3.1.2 Social organisation

Social organisation refers to the demographic makeup of a social unit, including the group size, age/sex composition, unit cohesion, and genetic composition (Kappeler & van Schaik, 2002). There are three basic types of primate social organisations, ranging from solitary, to pair-living, to gregarious group-living. From these, seven different grouping patterns can be identified (Eisenberg et al., 1972; Kappeler, 1997) including: (1) species with individuals that feed alone, but join others for sleeping or mating purposes (e.g., *Galago zanzibaricus*: Harcourt & Nash, 1986; *Microcebus berthae*: Dammhahn & Kappeler, 2005; *Pongo pygmaeus*: van Schaik & van Hooff, 1996); (2) pair-living species comprised of one reproductive male and one reproductive female (e.g., *Pithecia pithecia*: Thompson et al., 2012; *Presbytis potenziani*: Tilson & Tenaza, 1976; *Tarsius lariang*: Driller et al., 2009); (3) species with groups containing a single adult male, but multiple females (e.g., *Gorilla* spp.: Harcourt, 1979; Parnell, 2002); (4) species with groups containing a single reproductive female, but multiple males (e.g., *Sanguinus weddelli*: Garber et al., 2016); (5) species in which males have strong associations and together defend females (e.g., *Ateles* spp. & *Pan* spp.: Symington, 1990); (6) species that form small one male-multiple female subunits, which exist collectively with other subunits (e.g., *Papio* spp.: Patzelt et al., 2014); and (7) species in which individuals live in groups containing multiple males and multiple females (e.g., *Cercopithecus aethiops*: Isbell & Young, 1993; *Presbytis entellus*: Borries et al., 1999). Multimale-multifemale groups can further be distinguished based on the dispersal patterns of individuals reaching sexual maturity. While in many of these species females are the philopatric sex and males disperse from the natal group (e.g., *Cebus capucinus*: Jack & Fedigan, 2009; *Papio hamadryas hamadryas*: Hammond et al., 2006; *Saimiri boliviensis*: Boinski et al., 2005*)*, there are also species in which males are philopatric (e.g., *Procolobus badius temminckii*: Starin, 1994; *Saimiri oerstedii*: Boinski et al., 2005) or both sexes disperse (e.g., *Alouatta palliata*: Glander, 1992; *Saimiri sciureus*: Boinski et al., 2005). Group living is thought to reduce potentially the risk of infanticide, predation, and/or feeding competition from non-group members, while at the same time providing greater mating opportunities (van Schaik, 1983; Dunbar, 1996; Silk, 2007; Kappeler & Fichtel, 2012).

Fission-fusion dynamics (FFD), in which groups frequently split into smaller subunits and eventually merge back together, can be a mark of social complexity (Aureli et al., 2008). It is typical of spider monkeys (*Ateles* spp.: Symington, 1990) and chimpanzees (*Pan troglodytes versus*: Lehmann & Boesch, 2004), and it has been documented in macaques (*Macaca* spp.: Sueur et al., 2010) as well as several taxa of lemurs (*E. mayottensis*: Tattersall, 1977, *Varecia rubra*: Vasey, 2006; *V. variegata*: Pereira et al., 1988; Moreland, 1993a; Holmes et al. 2016). It is seen as an evolutionarily adaptive strategy in response to ecological stressors and complex social demands, including unpredictable resource availability due to seasonality (Baden et al., 2016; Holmes et al., 2016), ecological time constraints (Lehmann et al., 2007a), the degree of communal infant care (Holmes et al., 2016), and increased mating competition, parasite loads, or predation (Krause & Ruxton, 2002). Such flexibility in grouping patterns allows individuals to maintain social bonds (Lehmann et al., 2007a, 2007b; Sueur et al., 2010) in times of increased competition or stress.

3.1.3 Social structure

The social structure of a species refers to the interactions and resulting relationships between two or more members of a social unit over time (Hinde, 1976; Kappeler & van Schaik, 2002; Kappeler et al., 2013). Examining dyads, or associations between two individuals, is one of the most robust ways to study social relationships (Silk et al., 2013). Dyadic interactions may be reciprocal, in which both individuals behave similarly, or complementary, in which the individuals behave differently (Hinde, 1976). The interactions can be affiliative or agonistic, with each individual contributing to the unique relationship network and community dynamics within the social unit. Due to variability in sex, age, rank, and kinship (social organisation), the dyadic interactions of conspecifics may vary in quality, content, and temporal patterns (Hinde, 1976). This suggests that behavioural flexibility can occur not only throughout an individual's life (Bernstein & Gordon, 1974; Kappeler et al., 2013), but also between individuals or social units (Koenig, 2000).

Primate social grooming (commonly referred to as either allogrooming, mutual grooming, or reciprocal grooming throughout the literature) is a behavioural interaction occurring between individuals in which one party member picks at debris or ecoparasites on another's body (Boccia, 1983). This is in contrast to self-grooming, in which an individual cleans his/her own body. Allogrooming is thought to serve a hygienic and utilitarian function (Barton, 1985), while also being an essential component of social relationships (Carpenter, 1942; Boccia, 1983; Saunders, 1988). Today, social grooming in gregarious primates is generally accepted as the most important affiliative interaction, responsible for fostering and maintaining social bonds in those taxa that groom (Seyfarth, 1977; Henzi & Barrett, 1999; Schino, 2001). It is perceived as a form of social currency, being used in exchange not only for reciprocal cleaning, but also for additional services such as agonistic support (Seyfarth & Cheney, 1984; Henzi & Barrett, 1999; Schino, 2007; Carne et al., 2011), reproductive access (Norscia et al., 2009; Port et al., 2009), or reconciliation after conflict (Ren et al., 1991; Aureli, 1997). Long-term studies on baboons have even suggested that an individual's fitness may be tied to the quality of their social bonds (Silk, 2006), with those that groom more frequently often having higher reproductive success (Silk et al., 2006a).

Grooming also is essential to group cohesion, with individuals choosing to maintain social bonds either with all group members (Dunbar, 1991; Lehmann et al., 2007b), or only with preferred partners (Sueur et al 2011; Roubová et al., 2015). Differences in grooming partnerships and durations have been attributed to group size (Lehmann et al., 2007b), age and kinship (Kanngiesser et al., 2010; Roubová et al., 2015), dominance rank (Schino, 2001; Roubová et al., 2015), female philopatry and sex ratios (Lehmann et al., 2007b), quality of grooming (Silk et al., 2006b), and/or levels of within group competition (Barrett et al., 1999). As such, the amount of time that an individual spends grooming is related to the quality and the quantity of their social bonds (Dunbar, 1991; Lehmann et al., 2007b).

Agonistic encounters between individuals also play an important role in shaping the overall structure of a social unit (Bernstein & Gordon, 1974). Agonistic signals are generally species-typical and can include forms of physical aggression and chasing (de Waal et al., 1976), facial gestures (Liebal et al., 2004), olfactory communication (Epple, 1972; Fornasieri & Roeder, 1992a, 1992b), or vocalisations (Ordóñez-Gómez et al., 2015), collectively forming a ritualized repertoire of agonistic behaviours (de Waal et al., 1976).

Aggression, competition, threatening behaviours, and subordination among group members function to create a system of social dominance within a group (Pereira & Kappeler, 1997. The analysis of these patterns can reveal the dominance relationships of dyads (Pereira & Kappeler, 1997) and help construct the social network for the group. Dominance and the use of ritualized aggression are noteworthy as they are thought to alleviate both the occurrence and gravity of potential physical conflicts (Tinbergen, 1953; Bernstein & Gordon, 1974). At the same time, dyadic agonistic interactions influence access to resources (Correia et al., 2013), mating opportunities (Nishida, 1983), and/or individual health (Archie et al., 2012). Without reconciliation and social tolerance (Patzelt et al., 2014; Fichtel et al., 2018), often in the form of social grooming (Aureli, 1997), gregariousness would not be achievable (Bernstein & Gordon, 1974; Harcourt & de Waal, 1992).

3.1.4 Lemur social systems

The social systems of lemurs are varied (Wright, 1999; Kappeler, 1997, 2000; Kappeler & Fichtel, 2015). There is a prevalence of female dominance (Jolly, 1966; Kappeler, 1993a) and female targeted aggression (Vick & Pereira, 1989; Digby, 1999); a lack of sexual dimorphism (Kappeler, 1991); and within the strict mating seasons (Wright, 1999), monogamy is relatively common (adult female and male live together and mate exclusively; e.g., *Avahi laniger*: Harcourt, 1991; *Cheirogaleus medius*: Fietz, 1999b; *Hapalemur grieseus alaotrensis*: Nievergelt et al., 2002; for review, see Kappeler, 2014). Even within *Eulemur*, a single genus of Lemuridae, there is great diversity in social systems. Species belonging to this genus can be pair-living (*E. mongoz* and *E. rubriventer*: Digby & Kahlenberg, 2002; Sussman, 2002; Kappeler & Fichtel, 2016) or group-living (*E. macaco*: Colquhoun, 1993); in some taxa, female dominance is the norm (*E. coronatus* & *E. rubriventer*: Marolf et al., 2007), while in others there is no discernible dominance pattern (*E. fulvus*: Kaufman, 1994; *E. mayottensis*: Roeder & Fornasieri, 1995; *E. rufus*: Pereira & Kappeler, 1997). Kappeler & Fichtel (2016) recently reviewed *Eulemur* social organisation and suggested that intrasexual female competition, coupled with interspecific competition between closely-related taxa, resulted in a shift from group-living to pair-living in this genus. In doing so, they demonstrated that comprehensive comparisons between congeners can lead to improved understanding regarding the evolution of social diversity within a genus.

Within the genus *Eulemur*, the blue-eyed black lemur (*Eulemur flavifrons*) and the black lemur (*E. macaco*) are medium-sized (Terranova & Coffman, 1997; Bayart & Simmen, 2005; Mittermeier et al., 2010; Randriatahina & Roeder, 2013) and sexually dichromatic lemurs (Koenders, 1989; Colquhoun, 1993; Mittermeier et al., 2010) restricted to the northwest of Madagascar (Andriaholinirina et al., 2014a, 2014b). Both taxa live in multimale-multifemale groups (*E. macaco*: Colquhoun, 1993; Bayart & Simmen, 2005). *E. flavifrons* groups generally contain 4-11 individuals (Volampeno, 2010), with a mean group size ranging from five to eight (Rakotondratsima, 1999; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004; Volampeno, 2010; Randriatahina & Roeder, 2013). A slight sex bias towards males has been observed in all surveyed locations (Rakotondratsima, 1999; Andrianjakarivelo, 2004; Volampeno, 2010). While it has been reported that *E. flavifrons* form stable groups (Randriatahina & Roeder, 2013), researchers in Ankarafa Forest observed grouping behaviour comparable to that of a fission-fusion society (Prodger, 2015; C. Schwitzer, pers. comm.), opening questions about the stability of the species' social organisation. In comparison, *E. macaco* groups are reported to have both a greater average and range in group size. Groups typically contain anywhere from 2-22 individuals, with a mean group size of 7-10 individuals (Colquhoun, 1993; Andrews & Birkinshaw, 1998; Bayart & Simmen, 2005). The sex ratio of *E. macaco* varies, with some researchers finding an even ratio of males to females (Andrews, 1990; Colquhoun, 1993) and others finding populations to be male-biased (Petter, 1962; Jolly, 1966; Bayart & Simmen, 2005).

The published home ranges for the two taxa are similar and report a mean size of approximately 5 ha (*E. flavifrons*: 5.28 ha, Volampeno, 2011a; *E. macaco*: 5.25 ha, Colquhoun, 1993), although Bayart & Simmen (2005) reported a distinctly larger mean home range in Ampasikely (18.2 ha). The group size and ranging patterns of both species fluctuate with forest quality (*E. flavifrons*: Schwitzer et al., 2007b; Volampeno et al., 2011a; Prodger, 2015, *E. macaco*: Colquhoun, 1993, 1998b; Bayart & Simmen, 2005) as well as seasonal changes in food availability (*E. flavifrons*: Volampeno et al., 2011a; *E. macaco*: Colquhoun, 1993; Colquhoun, 1998a; Bayart & Simmen, 2005).

Female dominance (Digby & Kahlenberg, 2002; Digby & Stevens, 2007) and clear group hierarchies (Prodger, 2015) have been consistently reported in *E. flavifrons*. Conversely, *E. macaco* has been reported as lacking a clear sexual dominance or hierarchical pattern (Koenders, 1989; Fornasieri & Roeder, 1992b), although other studies have found females are the dominant sex (Colquhoun, 1993; Bayart & Simmen, 2005). Juveniles of both sexes may disperse (Bayart & Simmen, 2005), while adult males frequently transfer between groups during the mating season (*E. flavifrons*: Schwitzer et al., 2006; *E. macaco*: Bayart & Simmen, 2005). Intragroup interactions vary by sex and season; strong intrasexual associations and a high frequency of intersexual agonistic interactions have been observed in *E. flavifrons* during non-mating/non-birth seasons (Prodger, 2015). Aggressive male-male interactions occur frequently during the mating and birth season, while female-female agonistic interactions occur frequently during the perinatal period (*E. flavifrons*: Digby, 1999, *E. macaco*: Andrews, 1998; Bayart & Simmen, 2005). But, despite these earlier studies assessing the social organisation of *E. flavifrons* and *E. macaco*, little research has focused on the types of behavioural interactions and the resulting relationships that occur between group members.

3.1.5 Aims, hypotheses, and predictions

Currently no published studies exist that compare comprehensively the social systems of the two taxa, which would greatly contribute to our understanding of *E. flavifrons* and *E. macaco* speciation and would help to clarify their potential hybridisation in the Manongarivo Special Reserve (MSR; Meyers et al., Andrianjakarivelo, 2004; Goodman & Schütz, 2000). Therefore, the goal of this study was to describe the social system of *E. flavifrons* and *E. macaco* across multiple geographic locations by (1) characterising their social organisations, (2) identifying patterns of intersexual and intrasexual interactions and associations, and (3) detecting similarities and differences between the two species.

I hypothesised that there are significant differences in various aspects of the social structure and social organisation of *E. flavifrons* and *E. macaco*, including but not limited to, group composition and cohesion, the strengths of dyadic associations, and/or the rates of affiliative grooming and agonistic interactions. Three potential patterns of social system divergence, which are not necessarily mutually exclusive, were predicted:

- Differences could be species-specific, reinforcing the species' separate taxonomic statuses as previously established by genomic studies (Fausser et al., 2000; Pastorini et al., 2000; Mittermeier et al., 2008). As such, it was predicted that the *E. flavifrons* populations would be similar to each other, as would the *E. macaco* populations. It was also predicted that the *E. flavifrons* populations would be significantly different from the *E. macaco* populations.
- Significant differences could be evident along a geographical cline, with populations observed in areas of species repartition predicted to be more dissimilar to each other than those populations in allopatric locations. Such evidence of character displacement would suggest that observed differences may have evolved to reinforce unique mate recognition systems and maintain reproductive isolation in areas of secondary contact (Bush, 1975; Kaneshiro, 1980; Templeton, 1981; Hendry et al., 2007). Conversely, convergence of characters in an area of close contact could suggest possible hybridisation between the two species (Brown & Wilson, 1956; Leary, 2001).
- Significant social system differences may be the result of differing ecological conditions at each location. Habitat fragmentation and anthropogenic disturbances are known to negatively affect various aspects of primate social organization and structure (Struhsaker et al., 2004; Arryo-Rodríguez & Dias, 2009; Boyle & Smith, 2010; Irwin et al., 2010; Schwitzer et al., 2011). Because of this, I predicted to find a relationship between forest quality, anthropogenic influences, and behavioural characters at each location.

Additionally, within each population, it was hypothesised that affiliative and agonistic interactions would differ between the sexes and that the different dyad classes (male-male, female-female, male-female) would exhibit relationships of various quality.

- Because data collection was to take place during the mating season (Petter-Rousseaux, 1964; Rasmussen, 1985; Bayart & Simmen, 2005; Volampeno et al., 2010, 2011b), and grooming has been suggested as a currency used in exchange for access to reproductive females in lemurs (Norscia et al., 2009), the rate of male-initiated intersexual grooming was predicted to be higher than both female-initiated intersexual grooming or intrasexual grooming.

- As male competition for access to reproductive females increases during the mating season (Bayart & Simmen, 2005), male-male aggression was predicted to be high and rates of grooming were predicted to be low.
- Female aggression was predicted to be directed towards males more often than the reverse (Roeder et al., 2002; Prodger, 2015) because females are generally considered the dominant sex in both species (Koenders, 1989; Colquhoun, 1993; Digby & Kahlenberg, 2002; Bayart & Simmen, 2005; Digby & Stevens, 2007).
- Additionally, because female-female aggression is relatively high and affiliative interactions relatively low among lemur species (Kappeler, 1993b; Kappeler & Fichtel, 2015), female-targeted aggression was predicted, especially if groups were female-biased or if there were a large number of reproductive females in that group (Vick & Pereira, 1989; Randriatahina & Roeder, 2013).

Thus, a pattern in the quality of dyadic interactions was predicted in which intersexual relationships would show higher rates of grooming and lower rates of aggression, while intrasexual relationships would show the opposite.

3.2 METHODS

3.2.1 Locations, study animals, and collection effort

Data on social organisation and social structure were collected over three mating seasons (2015-2017) from two populations of *E. flavifrons* (*Ef-1* and *Ef-3*) and two populations of *E. macaco* (*Em-1* and *Em-3*; Figure 3.2 and Table 3.1). In addition, group composition data were collected from a third *E. flavifrons* population (*Ef-2*) and a third *E. macaco* population (*Em-2*).

Three ecological measurements were included as basic quantifiers of habitat quality at each location. A perimeter-area ratio (PAR) was calculated for each location, comparing the fragment's perimeter to its overall area; fragments were measured in Google Earth. A higher PAR value suggests an elongated fragment with more core forest exposed to the fragment edge. In comparison, a fragment with a more uniformed and condensed shape will have less core forest exposed to the edge, and a lower PAR value. Therefore, higher PAR values would be indicative of lower-quality forest fragments (Helzer & Jelinski, 1999). A forest-type scale was created, with each location rated as being composed of either 1: highly degraded and fragmented forest, 2: a combination of old growth and regenerated forest, or 3: primarily old growth forest. This was an ordinal scale, with a categorisation of 1 considered lowest quality and a categorisation of 3 considered the highest quality. The number of anthropogenic threats at each location were counted as well. Threats included: hunting, cattle grazing, logging, *tavy*, bushfires, ecotourism, and proximity to villages and/or roads. Further detailed information regarding each location can be found in Chapter 1: Introduction and General Methodology.

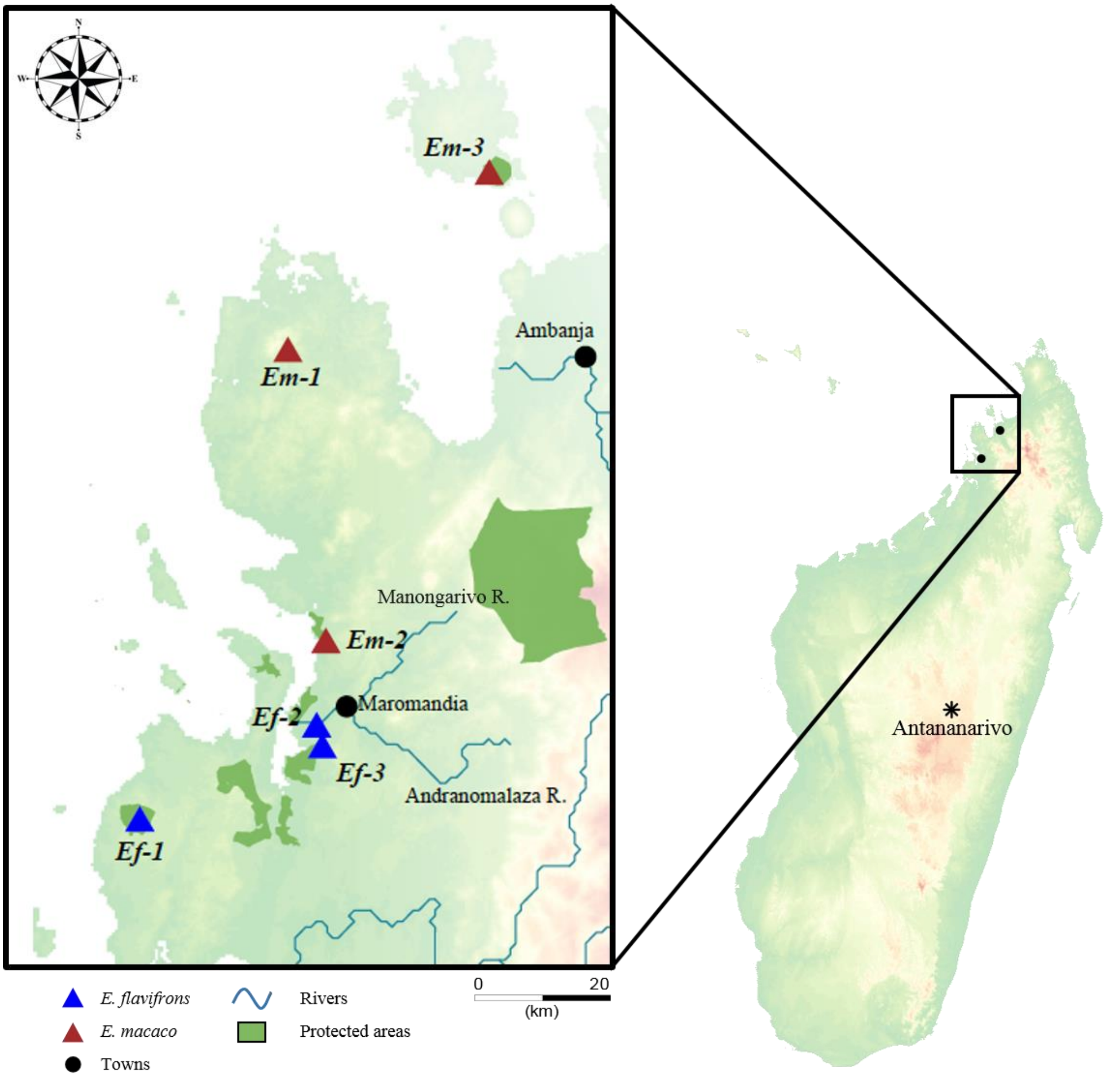

Figure 3.2 The locations of the six lemur populations studied in the northwest of Madagascar. *Ef-1*: Ankarafa, 2015; *Ef-2*: Ambodimanga, 2016; *Ef-3*: Angodrahely, 2017; *Em-1*: Andranomatavy, 2016; *Em-2*: Mahadera, 2016; and *Em-3*: Lokobe, 2017.

Table 3.1 Location details for the six study populations, split according to whether behavioural observations were or were not collected on the population. PAR represents the ratio of forest edge perimeter (km) to total fragment area (km^2). Forest type: 1=highly degraded and fragmented secondary forest, 2=combination of old growth and regenerated forest, and 3=primarily old growth forest. Anthropogenic pressure (AP), an ordinal score, was calculated as the total number of threats present at a location; the higher the score, the more threats observed.

	Population	**Species**	**Location**	**Loc. size (ha)**	**PAR**	**Forest**	**AP**	**Collection Effort**
Behav. obs.	*Ef-1*	*E. flavifrons*	Ankarafa Forest 14°22’64.2”S, 47°45’31.5”E	185	7.79	2	5[*]	14 April – 29 May 2015 216.3 hrs
	Ef-3	*E. flavifrons*	Angodrahely 14°16’14.8”S, 48°2’47.4”E	49	16.5	2	3[^]	11 April – 16 May 2017 290 hrs
	Em-1	*E. macaco*	Andranomatavy 13°40’26.4”S, 47°59’35.1”E	2,543	1.2	3	2[<]	4 April – 30 May 2016 557.5 hrs
	Em-3	*E. macaco*	Lokobe National Park 13°24’24.2”S, 48°18’17.9”E	740	1.5	3	4[>]	18 April – 26 May 2017 139 hrs
No behav. obs.	*Ef-2*	*E. flavifrons*	Ambodimanga 14°14’21.2”S, 48°2’15.4”E	2.7	63.1	1	6[+]	20 – 23 July 2016 12 hrs
	Em-2	*E. macaco*	Mahadera 14°6’44.0”S, 48°3’6.8”E	13.4	40.0	1	4[º]	21 June – 3 July 2016 24 hrs

[*] grazing, logging, *tavy*, bushfires, ecotourism
[^] logging, *tavy*, villages/roads
[<] grazing, logging
[>] logging, *tavy*, ecotourism, villages/roads
[+] hunting, grazing, logging, *tavy*, ecotourism, villages/roads
[º] grazing, logging, *tavy*, bushfires, villages/roads

3.2.2 Data collection and analyses

Data collection took place between 0530 and 1730 hrs, six days a week. Although *E. flavifrons* and *E. macaco* are cathemeral (Colquhoun, 1998a; Schwitzer et al., 2007a), lack of visibility in the evening prevented behavioural observations at night. Groups were typically located between 0545 and 0700 hrs; they were then monitored for the entire day, or until lost. Due to the unhabituated nature of some of the populations, the lack of prior monitoring, and the fact that these taxa do not have obvious morphological markings or patterns apart from sexual dichromatism, identification of individuals was not possible. To complicate matters, group identification also was not consistently feasible because of the level of FFD observed in certain locations. Data are therefore attributed to the sex of the individual and the overall population.

3.2.3 Social organisation: group composition and proximity patterns

Group size, demographics, and locations were recorded daily for the *Ef-1*, *Ef-2*, *Ef-3*, *Em-1*, *Em-2*, and *Em-3* populations. Due to the similar body sizes of adults and juveniles, especially in the *Em-3* population, characterizing the age class of an individual was not always possible. For this reason, the adult and juvenile age classes were grouped together (Volampeno et al., 2010). Descriptive statistics and Kruskal-Wallis tests were used to examine differences in the social organisation of groups within each population.

Five-minute scan samples (Altmann, 1974; Zuberbühler & Wittig, 2011) were used to record the proximity of group members for all visible individuals in the *Ef-1*, *Ef-3*, *Em-1*, and *Em-3* populations. Because Johnson (2002) found that in brown lemurs, the mean distance for nearest neighbour was approximately 1 m, lemurs in this study were recorded as either being together (within 1 m of each other) or apart (further than 1 m from each other) during each scan. Dyads, defined as two lemurs within 1 m of each other, were distinguished from groups, which were classified as containing more than two lemurs in 1 m proximity.

Only three classes of dyad associations were distinguished here—two intrasexual (male-male, female-female) and one intersexual (male-female). Following Pepper & colleagues (1999), the pairwise affinity index (PAI) for the frequency of dyad associations was then calculated for each population:

$$\mathrm{PAI}(f)_{\mathrm{xy}} = \frac{f_{xy}}{f_{\mathrm{x}} + f_{\mathrm{y}}}$$

where f_{xy} equals the number of observed dyads between sex X and Y, f_{x} represents the total number of observed dyads sex X, and f_{y} represents the total number of observed dyads containing sex Y.

3.2.4 Social structure: social behaviours and interactions

Behavioural data were collected through a combination of all-occurrence and *ad libitum* methods (Altmann, 1974; Zuberbühler & Wittig, 2011). Affiliative and agonistic interactions (Table 3.2) were recorded for all visible group members; those interactions falling which were part of a mating courtship have been removed from analyses here and are reviewed in Chapter 4. Both allogrooming (sex X grooms sex Y; unidirectional) and reciprocal grooming (sex X and Y groom either consecutively or concurrently) were scored as affiliative behaviours. If more than one minute lapsed after a grooming behaviour had ended, the interaction was considered finished. For each bout of social grooming, the groomer, the groomee, the type of grooming, and the duration were recorded. The aggressor, the recipient, and duration were recorded. Because nearly all agonistic interactions were extremely short (<2 s), durations were not considered in the analyses.

Table 3.2 Ethogram of affiliative and aggressive behaviours used to record social behaviours and interactions.

	BEHAVIOUR	DESCRIPTION
Affiliative	Allogrooming	One lemur picks at debris or ecoparasites on another lemur’s body. Unidirectional grooming.
	Reciprocal grooming	Two lemurs pick at debris or ecoparasites on each other. Grooming occurs either consecutively or concurrently.
Agonistic	Cuffing	One lemur hits or swats at a second lemur.
	Chasing	One lemur quickly pursues a second lemur. Often vocalisations accompany chasing.
	Lunging	One lemur suddenly thrust forward in the direction of a second lemur. Second lemur usually moves away.
	Fighting	Two or more lemurs engage in a combination of back-and-forth agonistic behaviours (cuffing, biting, chasing, scratching) for more than 3 s. Often accompanied by vocalisations.

Binomial tests compared the initiation of the different interactions by sex. Rates of affiliative and agonistic interactions were calculated as the number of bouts per observation hour. Rates of interactions and grooming durations were log transformed and Bartlett's tests were carried out to ensure that assumptions of an ANOVA were met prior to further analyses. Two-way ANOVAs were then carried out to test whether the rates of affiliative behaviours, durations of social grooming bouts, and rates of aggressive behaviours differed between species, populations, and/or dyad classes. Tukey's HSD post hoc tests were used to determine where differences lay.

The relationship tenor (RT; Silk et al., 2013), which measures relationship quality by comparing rates of affiliative interactions to agonistic interactions, was calculated for each dyad class at each location (Weaver & de Waal, 2002) using the following formula:

$$RT_{xy} = \left(\frac{\frac{AF_{xy}}{AF_{all}}}{\frac{AG_{xy}}{AG_{all}}} \right)$$

where AF_{xy} is the rate of affiliative interactions between sex X and Y, AF_{all} is the average rate of affiliative interactions occurring across all dyads, AG_{xy} is equal to the rate of agonistic interactions occurring between sex X and Y, and AG_{all} is the average rate of agonistic interactions occurring across all dyads in that population. Scores are relative to 1.00, which is the overall population's mean score. Therefore, dyads with RT scores greater than 1.00 have a relationship characterised by more socio-positive interactions, while those with scores lower than 1.00 have a relationship characterised by more socio-negative interactions. After scores were log transformed, a two-way ANOVA was used to test whether there were differences in the relationship tenor between populations and dyad classes.

3.2.5 Effects of ecological and anthropogenic factors

The effects of the measured ecological and anthropogenic factors on group size and FFD at each location were analysed using linear mixed-effects models (LMM). Fragment size and PAR were log transformed to achieve normality. Correlations between the four factors were then calculated; significant strong correlations were found between the three ecological factors, but not with anthropogenic pressures (Table 3.3). To accommodate the limitations associated with having a small sample size and too many correlated ecological factors, a cumulative ecological score was calculated for each population. This was done by ranking each ecological factor in terms of quality, with lower quality attributes receiving a lower ranking. Cumulative ecological scores could range from 3 (lowest quality location) to 15 (highest quality location). The final scores were: *Ef-2* (3), *Em-2* (5), *Ef-3* (8), *Ef-1* (10), *Em-3* (13), and *Em-1* (15). In each LMM, the random effect was the population and the fixed effects were the cumulative ecological score, the level of anthropogenic pressures, and the interaction between those two factors. Due to the fact that rates of affiliative and agonistic behaviours could only be collected for each population as a whole and not the groups, sample sizes were too small for models.

Table 3.3 Correlations between the different ecological and anthropogenic factors measured for each population's location (N=6). Significant results for Kendall's tau-b correlation coefficient are in bold. PAR= perimeter-to-area ratio.

	PAR	***Forest type***	***# of anthropogenic pressures***
Fragment size	**τ=-1.0, p=0.009**	**τ=0.89, p =0.029**	τ=-0.55, p=0.181
PAR		**τ=0.89, p=0.029**	τ= 0.55, p=0.181
Forest type			τ=-0.54, p=0.226

3.2.6 Statistical tests

With the exception of ANOVAs, alpha levels were set at α=0.05 for all tests. To accommodate a non-homogenous variance in one attribute and decrease the chances of a Type 1 Error, significance levels for ANOVA and accompanying Tukey's post-hoc tests were set at α=0.01 after applying a Bonferroni correction. All tests were carried out in R (R Core Team, 2013) using the ggplot2 (Wickham, 2016), dplyr (Wickham et al., 2018), Kendall (McLeod, 2015), lme4 (Bates et al., 2015), MASS (Venables & Ripley, 2002) and car (Fox & Wisberg, 2011) packages.

3.3 RESULTS

3.3.1 Group size and composition

Groups were easily identified in three of the *E. flavifrons* populations (*Ef-1*, *Ef-2* and *Ef-3*) and two of the *E. macaco* populations (*Em-2* and *Em-3*). Group size did not differ significantly between the six populations (Kruskal-Wallis test: X^2=8.89, df=5, p=0.116; Table 3.4). Mean group size ± SD in the *Ef-1* population

(N=7) was 12.9 ± 5.1, with a male-biased sex ratio of 1.04. There was only one group living in the *Ef-2* location; the group size was seven, with a female-skewed sex ratio (0.8). In the *Ef-3* population (N=3), mean group size was 9 ± 2.7, with a male-biased sex ratio of 1.5. In the *Em-2* population (N=3), the mean group size was 4.3 ± 2.3, with a male-biased sex ratio of 1.6. The mean group size for the *Em-3* population (N=3) was 13 ± 4.6, with a slight male-sex bias of 1.2. Neither group identification nor group size could clearly be established in the *Em-1* population because these lemurs were not actively monitored by researchers prior to the start of this study, coupled with the fact that individuals were often found on their own. Based on GPS points collected during behavioural observations, I concluded there were a minimum of six different *Em-1* groups observed in Andranomatavy. The maximum number of individuals observed together was 12. The average number of visible individuals was calculated from the proximity scans (N=6,161), which resulted in a mean score of 2.1 ± 1.3. Proximity scans also revealed a nearly even sex ratio of 1.1 (slight male bias).

Table 3.4 Group location and composition of *E. flavifrons* and *E. macaco* measured 2015-2017. It was impossible to accurately document group size in the *Em-1* population. The maximum number of lemurs found together in the morning and the mean number of individuals observed together during proximity scans are listed instead.

Species	**Year**	**Population**	**Location**	**Group Name**	**Group Total**	**Adult Male**	**Adult Female**	**Juvenile Male**	**Juvenile Female**
E. flavifrons	2015	*Ef-1*	Ankarafa Forest	Yellow	21	10	8	1	2
				Red	12	5	6	1	0
				Gila Maso	16	7	5	2	2
				Group 4	6	2	3	0	1
				MAT1	14	7	6	1	0
				MAT2	9	5	4	0	0
				MAT3	12	4	6	0	1
	2016	*Ef-2*	Ambodimanga	AMB1	7	3	3	0	1
	2017	*Ef-3*	Angodrahely	ANG1	8	5	3	0	0
				ANG2	12	7	4	1	0
				ANG3	7	3	4	0	0
E. macaco	2016	*Em-1*	Andranomatavy		12				
	2016	*Em-2*	Mahadera	MD1	7	4	2	1	0
				MD2	3	1	1	0	1
				MD3	3	2	1	0	0
	2017	*Em-3*	Lokobe	LKB1	12	6	5	1	0
				LKB2	18	8	7	2	1
				LKB3	9	4	4	0	1

The ecological score had a strong effect on group size (LMM: X^2=10.32, df=1, p=0.001). Typically, larger groups were found in larger fragments with smaller forest edge perimeters and better-quality core forest (Figure 3.3). No significant effect was found between group size and the number of anthropogenic pressures (LMM: X^2=2.43, df=1, p=0.12).

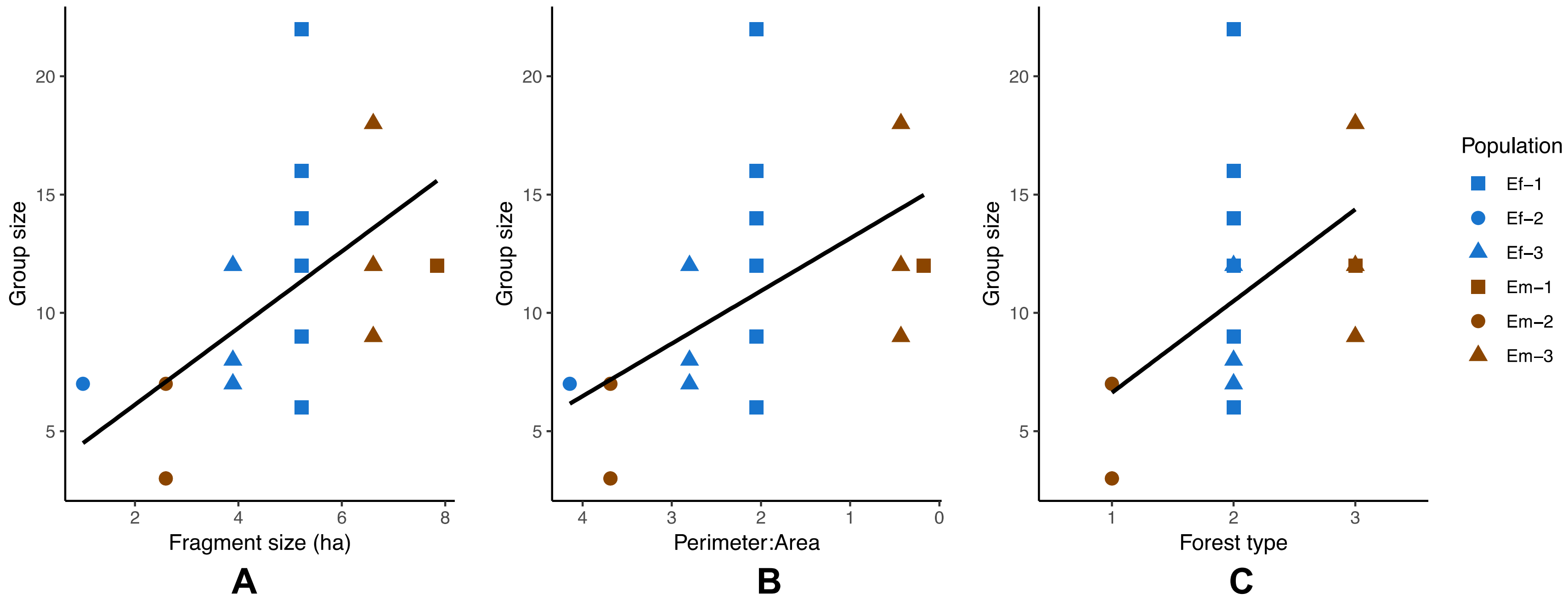

Figure 3.3 The relationship between group size and (A) fragment size, (B) forest perimeter-to-area ratio, and (C) forest type at each location. All ecological factors are presented in terms of lower to better quality. Forest type: (1) highly degraded and fragmented secondary forest, (2) a combination of old growth and regenerated forest, and (3) primarily old growth forest.

3.3.2 Proximity patterns and group cohesion

Group cohesion, determined by the frequencies of scans in which individuals were within 1 m of a group member (either as a dyad or a group) compared to more than 1 m from a group member, varied across the two species (Chi-square test: X^2= 1042.2, df=3, $p < 0.001$). The proximity patterns of both the *Ef-1* and *Em-3* populations showed lemurs spent significantly more time near another lemur than alone. Of the 2,334 proximity scans that occurred within the *Ef-1* population, lemurs were observed together in 54.8% of the instances and apart in 45.2% of the instances (Binomial test: $p < 0.001$). Of the 1,668 scans in the *Em-3* population, lemurs were observed alone in 46.2% and together in 53.8% of the cases (Binomial test: $p < 0.001$). In contrast, the *Ef-3* and *Em-1* populations spent significantly more time at a greater distance from group members. There were 3,476 scans in the *Ef-3* population, during which lemurs were observed together in 47.7% of the instances and alone in 52.3% of the scans (Binomial test: $p < 0.001$). Of the 6,161 proximity scans conducted on the *Em-1* population, lemurs were observed alone more often (64.2%) than together (35.8%; Binomial test: $p < 0.001$).

Proximity preferences were further broken down by sex (Figure 3.4). Males always spent more time alone than with group members, while females generally spent more time in proximity to other lemurs. A pairwise affinity index (PAI) was calculated for the three classes of dyads observed (Figure 3.5). Intersexual dyads had the highest PAI scores in all four populations. Male-male PAI scores were the lowest in *Ef-1* and *Em-1* populations, while female-female scores were lower in *Ef-3* and *Em-3*.

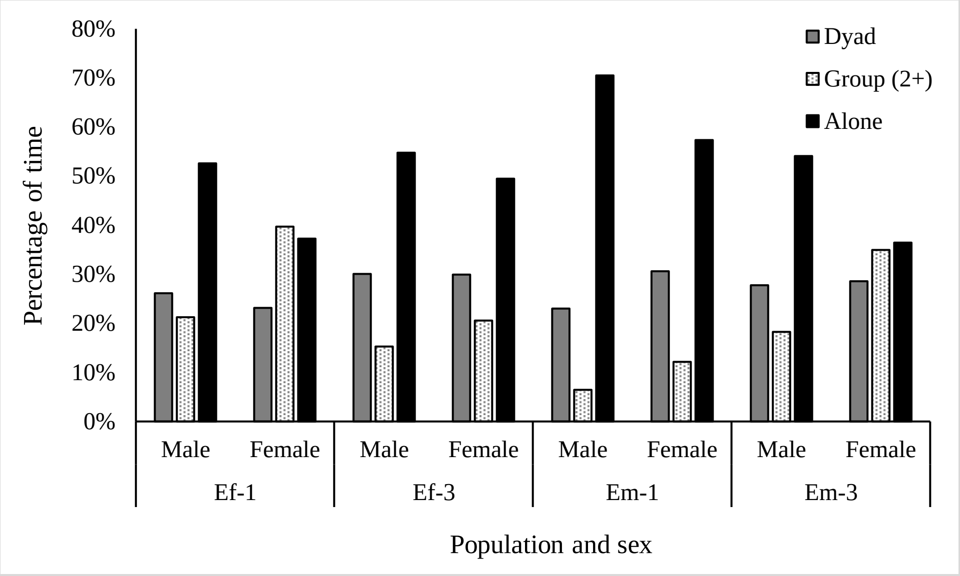

Figure 3.4 Percentage of time each sex spent in proximity (one meter or less) to a group member or apart.

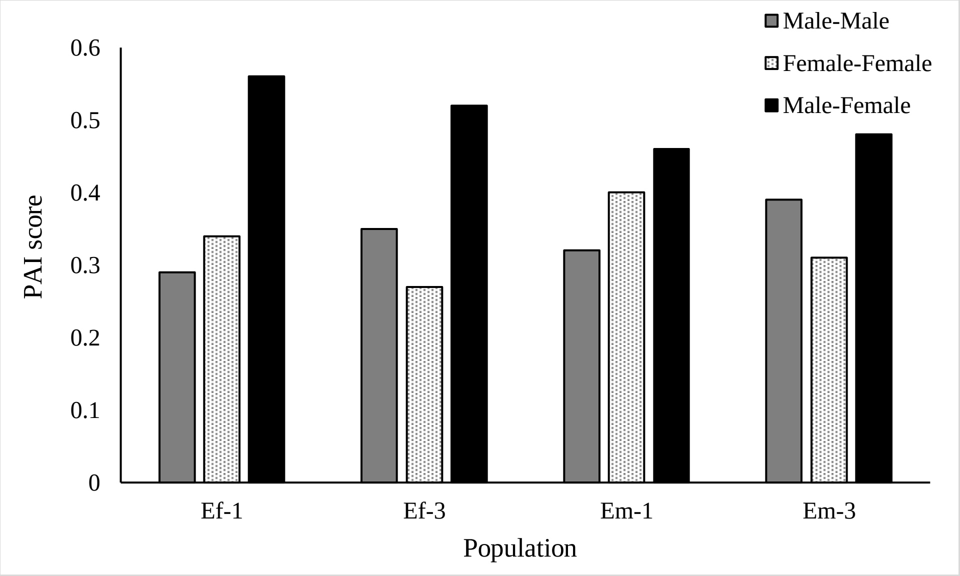

Figure 3.5 Pairwise affinity index (PAI) for dyad classes in each population. Individuals were classified as a dyad if within one meter of each other. A PAI score that approaches 1 suggests strong associations, while a PAI score that approaches 0 implies no association.

There was evidence of fission-fusion sociality in one *E. flavifrons* population (*Ef-1*) and two *E. macaco* populations (*Em-1* and *Em-3*), although not every group in these locations showed evidence of flexible group cohesion. In the *Ef-1* population, groups split into smaller subgroups throughout the day and joined in the evening; this occurred frequently in the group that contained 21 individuals. In addition, multiple groups would come together at feeding sites, with over 30 lemurs observed feeding in a single location. One *Em-3* group that contained 18 individuals frequently split into subgroups when feeding as well. Although the subgroup size was never consistent, the faction always remained near the core group. In comparison, *Em-1* individuals dispersed from group sleeping sites in the morning, often remaining solitary or in groups of two or three individuals throughout the day, and then reconvened in the evening. Fission-fusion dynamics were never observed in the remaining *E. flavifrons* (*Ef-2* and *Ef-3*) or *E. macaco* (*Em-2*) populations.

The number of group members had a significant effect on whether FFD occurred within that group (LMM: X^2=3.87, df=1, p=0.049). Larger groups (>13 individuals) were more likely to break apart into smaller subgroups throughout the day than groups containing fewer individuals (Figure 3.6). No statistical significance was found between FDD and the ecological score (LMM: X^2=1.41, df=1, p=0.234) nor between FDD and the number of anthropogenic pressures (LMM: X^2=0.02, df=1, p=0.898).

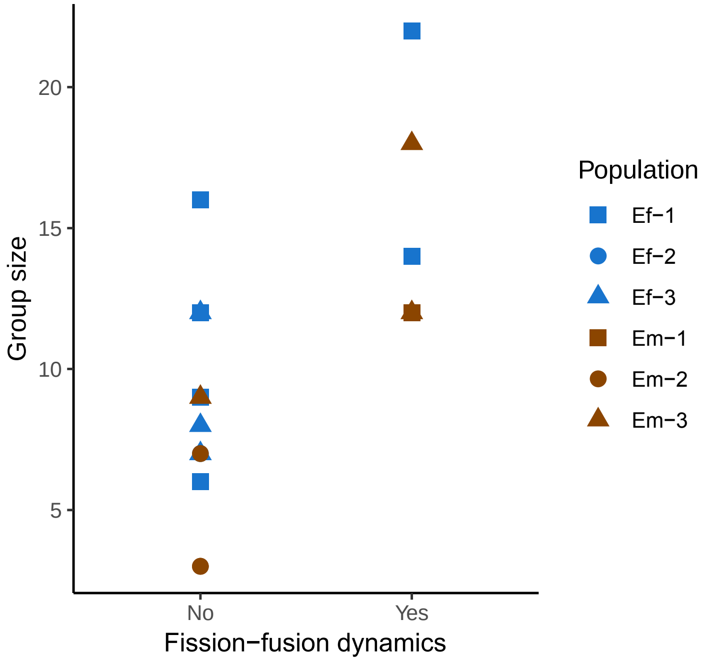

Figure 3.6 The relationship between FFD and group size for each population.

3.3.3 Affiliative interactions

There were 595 bouts of social grooming observed in the *Ef-1* population, 444 bouts in the *Ef-3* population, 386 bouts in the *Em-1* population, and 272 bouts in the *Em-3* population. Binomial tests indicated that females initiated significantly more grooming bouts than males in *Ef-1*, while in the *Ef-3* population, males were found to initiate significantly more grooming bouts than females. No differences were found in the two *E. macaco* populations. Females were the recipient of social grooming significantly more often than males in both species (Figure 3.7).

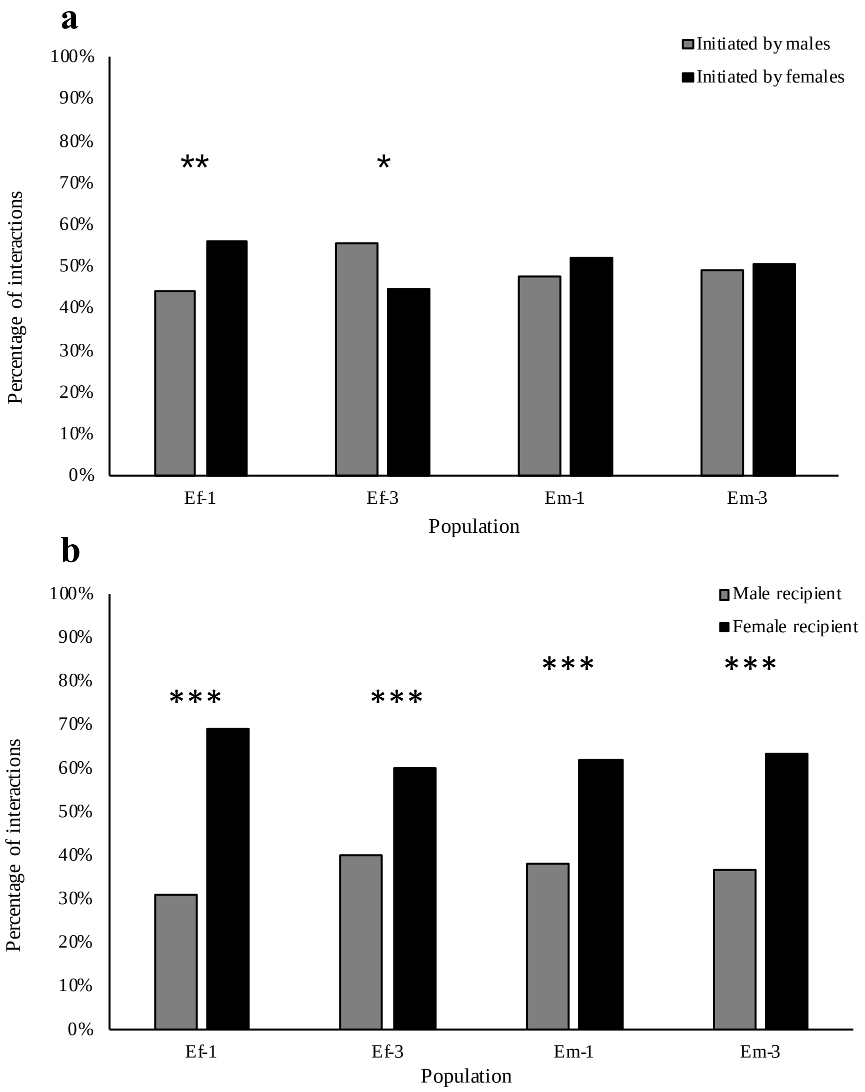

Figure 3.7 Patterns of affiliative interactions. Binomial test results comparing (a) male initiated vs. female initiated affiliative interactions and (b) the proportion in which each sex was the recipient in each population. Significant findings: $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

Overall population rates of social grooming ranged from 0.7 bouts/hour in *Em-1*, 1.5/hr in *Ef-3*, 2.0/hr in *Em-3*, to 2.8/hr in *Ef-1*. Affiliative rates were further investigated for the four dyad classes in each population (Figure 3.8). A two-way ANOVA found significant differences in the affiliative rates of the populations (F(3, 9)=17.75, p<0.001) and the different dyad classes (F(3, 9)=6.58, p=0.012). Tukey's HSD post hoc test revealed that the *Em-1* population had significantly lower rates of social grooming than the other three populations (*Ef-3*: p=0.011, *Ef-1* & *Em-3*: p ≤0.001). Post hoc tests showed that Male→Female and Female→Female dyads had significantly higher rates of affiliative behaviours in comparison to Male→Male dyads (p=0.017 and p=0.032, respectively).

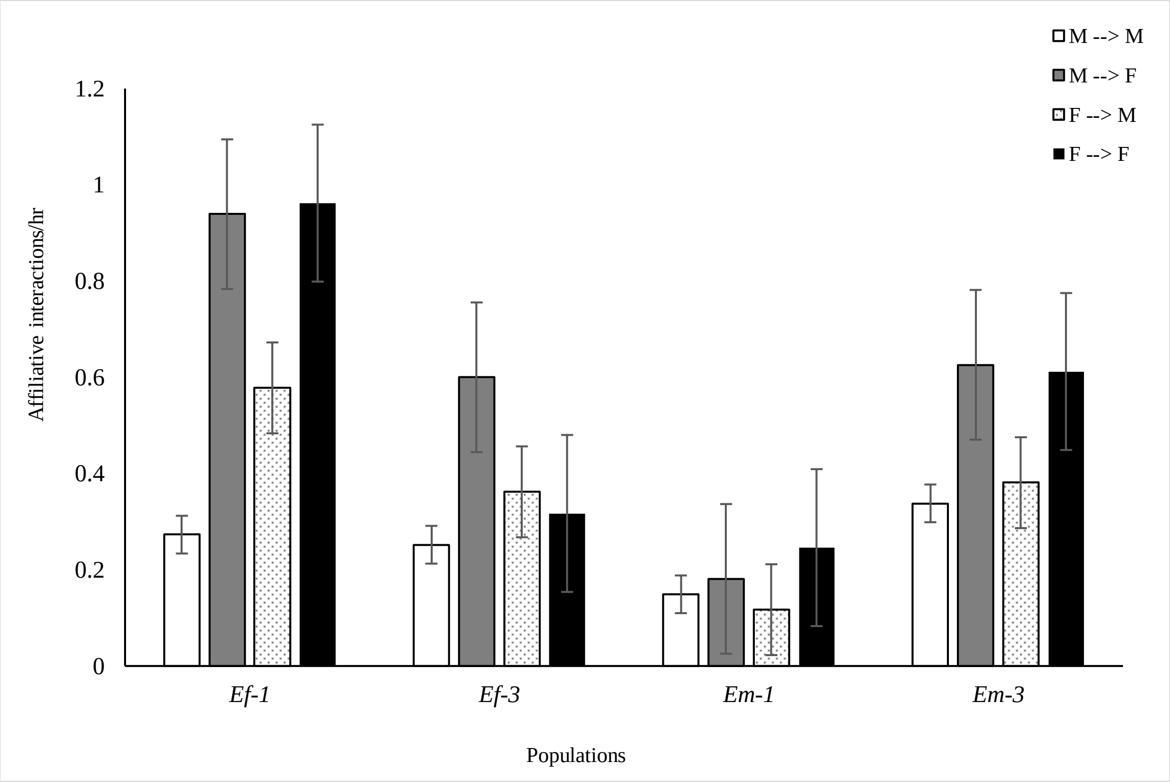

Figure 3.8 Affiliative interactions per hour of observation (± SE) for the different dyad classes. M is male, F is female, and groomer → groomee. The *Em-1* population was found to have significantly lower rates of grooming as compared to the other three populations, while M→F and F→F dyads had significantly higher rates of grooming as compared to M→M dayads.

There were more observed instances of allogrooming than reciprocal grooming in both of the *E. flavifrons* populations (*Ef-1*: N=312, N=283; *Ef-3*: N=311, N=133). In comparison, there were more observed instances of reciprocal grooming than allogrooming in the two *E. macaco* populations (*Em-1*: N=155, N=223; *Em-3*: N=109, N=160). Pairwise comparisons revealed significant differences in the proportions of grooming types that occurred in each population, except between the *Em-1* and *Em-3* populations (Table 3.5).

Table 3.5 Chi-squared comparisons looking at differences in the frequency of allogrooming and reciprocal grooming between populations. Significance levels were set at 0.008 with Bonferroni corrections.

	Ef-3	***Em-1***	***Em-3***
Ef-1	X^2=32.11 **p<0.001**	X^2=11.65 **p<0.001**	X^2=10.06 **p=0.002**
Ef-3		X^2=68.95 **p<0.001**	X^2=59.11 **p<0.001**
Em-1			X^2=0.002 p=0.97

A two-way ANOVA comparing the durations of allogrooming bouts (Figure 3.9, Appendix B.1) revealed significant differences between populations (F(3, 779)=9.58, p<0.001), but not between dyad classes (F(3, 779)=1.34, p=0.260). Tukey's HSD post hoc test revealed that allogrooming durations were significantly shorter in *Ef-3* than *Ef-1* (p<0.001), *Em-3* (p=0.007), and *Em-1* (p=0.016). The interaction between the effects of populations and dyad classes on the duration of allogrooming bouts was not statistically significant (F(9, 779)=1.32, p=0.222).

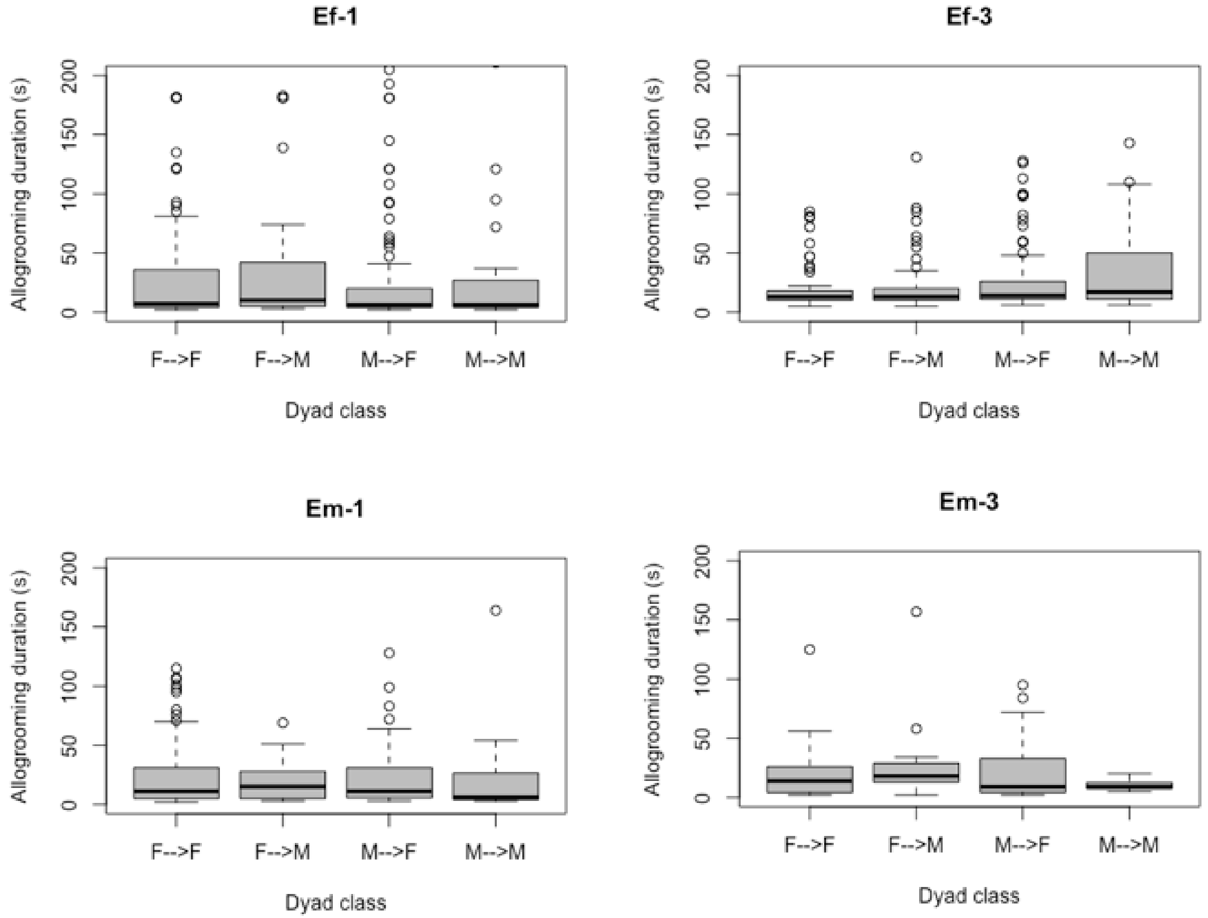

Figure 3.9 Allogrooming durations (s) by dyad class and population. Black bars indicate median durations, boxes represent upper and lower quartiles, and whiskers show data points falling within a 1.5x inter-quartile range.

A two-way ANOVA revealed significant differences when comparing the duration of reciprocal grooming bouts (Figure 3.10, Appendix B.2) occurring within each population (F(3, 742)=15.88, p<0.001), as well as when comparing between the different dyad classes (F(3, 742)=4.04, p=0.007). The interaction between the effects of populations and dyad classes on the duration of reciprocal grooming bouts was not statistically significant (F(9, 742)=1.05, p=0.401). Tukey's post hoc comparison of reciprocal grooming durations across the four populations revealed that *Ef-1*'s grooming bouts were significantly longer than grooming bouts of the other three populations (p<0.001). Tukey's HSD post hoc tests comparing reciprocal grooming durations by dyad class, regardless of population, revealed that Female→Female reciprocal grooming bouts were significantly longer than Male→Male bouts (p=0.004).

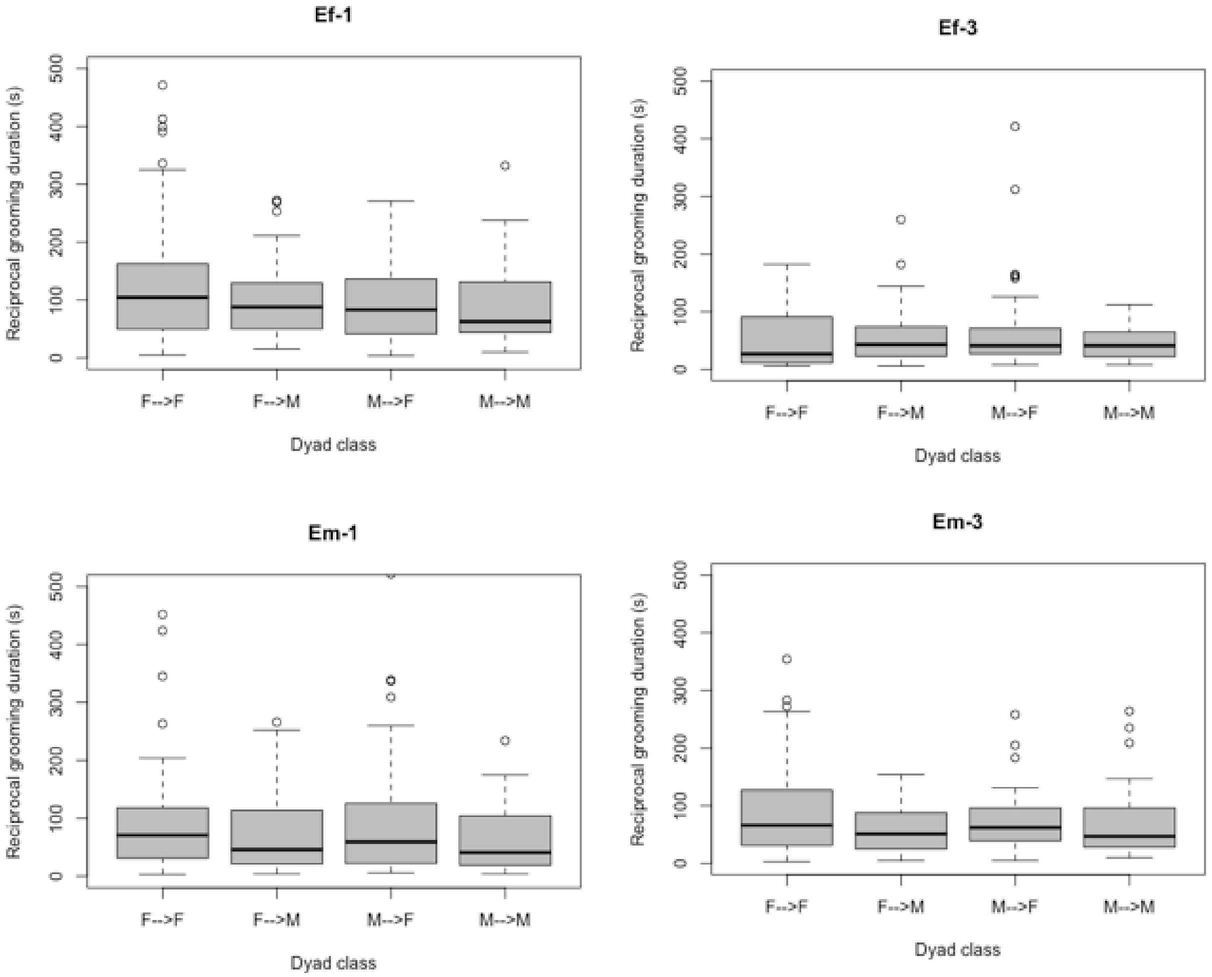

Figure 3.10 Reciprocal grooming durations (s) by dyad class and population. Black bars indicate median durations, boxes represent upper and lower quartiles, and whiskers show data points falling within a 1.5x inter-quartile range.

3.3.4 Agonistic interactions

Outside the mating context, there were 42 agonistic interactions observed in the *Ef-1* population, 55 interactions in the *Ef-3* population, 25 interactions in the *Em-1* population, and 119 in the *Em-3* population. Males were the aggressor more often in the *Ef-3* and *Em-3* populations, while females initiated more aggressive bouts in the *Ef-1* and *Em-1* populations (Figure 3.11). Binomial tests revealed that the proportion of male-initiated to female-initiated agonistic interactions was significantly different in *Em-1* only (p =

0.015). Males were the recipient of aggressive interactions significantly more often than females in every population besides *Ef-1*, where no significant difference between the sexes was observed (Figure 3.11).

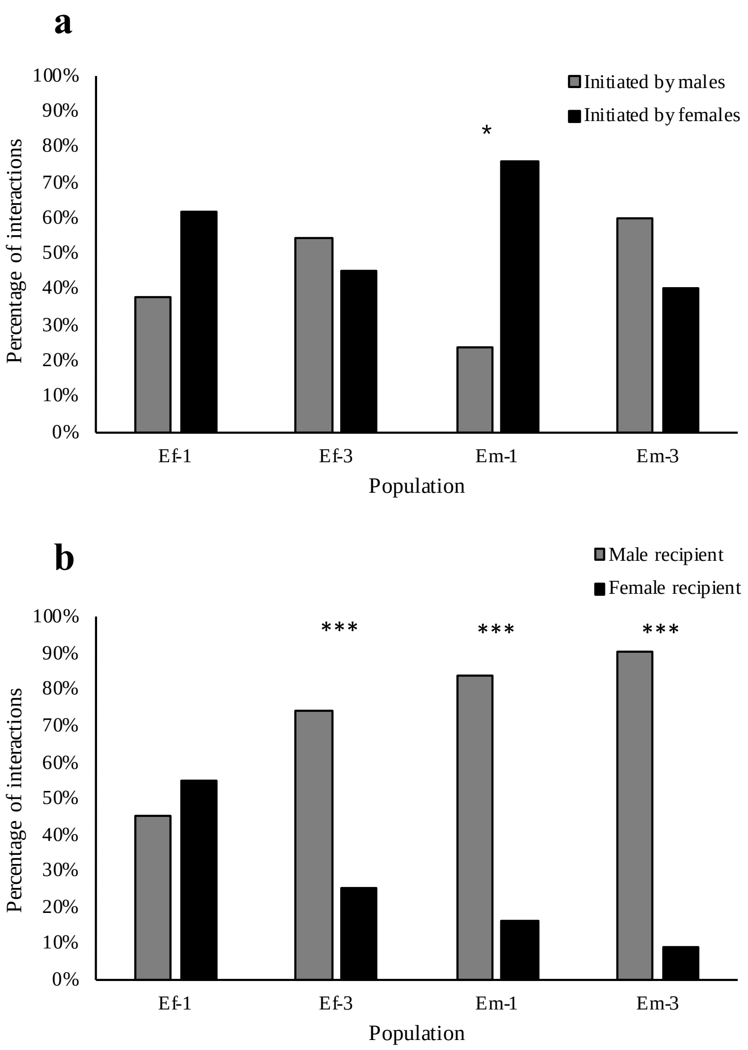

Figure 3.11 Patterns of agonistic interactions. Binomial test results comparing (a) male initiated vs. female initiated agonistic interactions and (b) the proportion in which each sex was the recipient of an agonistic interaction in each population. Significant findings: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

Overall group rates of agonism ranged from 0.05 interactions/hour (*Em-1*), to 0.2 interactions/hour (*Ef-1* and *Ef-3*), to 0.9 interactions/hour (*Em-3*). A comparison of the rates of agonistic interactions between the dyad types in the four populations revealed significant differences (ANOVA: F(3,9)=6.16, p=0.015), while comparison between the different dyad classes did not (Figure 3.12). Tukey's HSD post hoc found that there were population differences in *E. macaco* only. The *Em-1* population had significantly fewer agonistic interactions compared to the *Em-3* population (p=0.011).

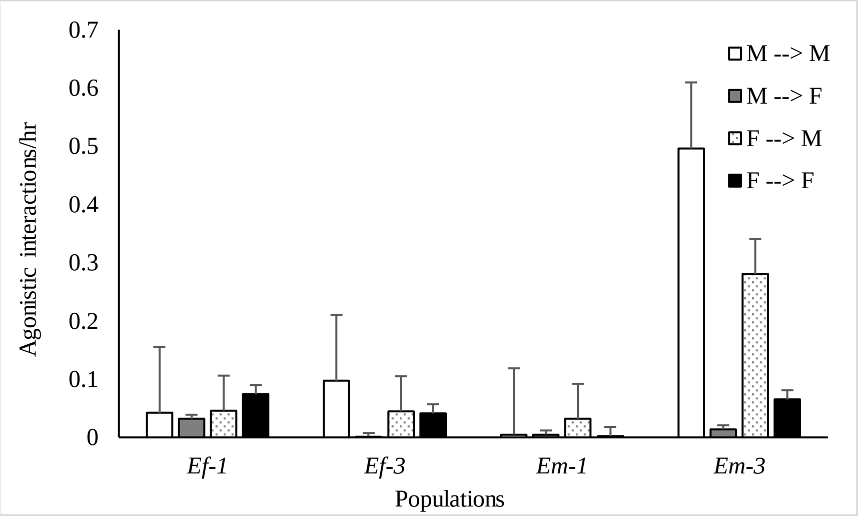

Figure 3.12 Agonistic interactions per hour of observation (+ SE) for the different dyad classes. M is male, F is female, and aggressor → aggressee. Significant differences were found between the two *E. macaco* populations (*Em-1* vs. *Em-3*, p=0.001).

3.3.5 Relationship tenor

When comparing the relative rate of social grooming and aggression that occurred in each of the four dyad classes, a clear pattern emerged (Figure 3.13). Dyad classes that had the highest relative rates of affiliative interactions exhibited the lowest relative rates of directional aggression. Conversely, the dyad classes with lower relative rates of social grooming generally also had higher relative rates of directional aggression.

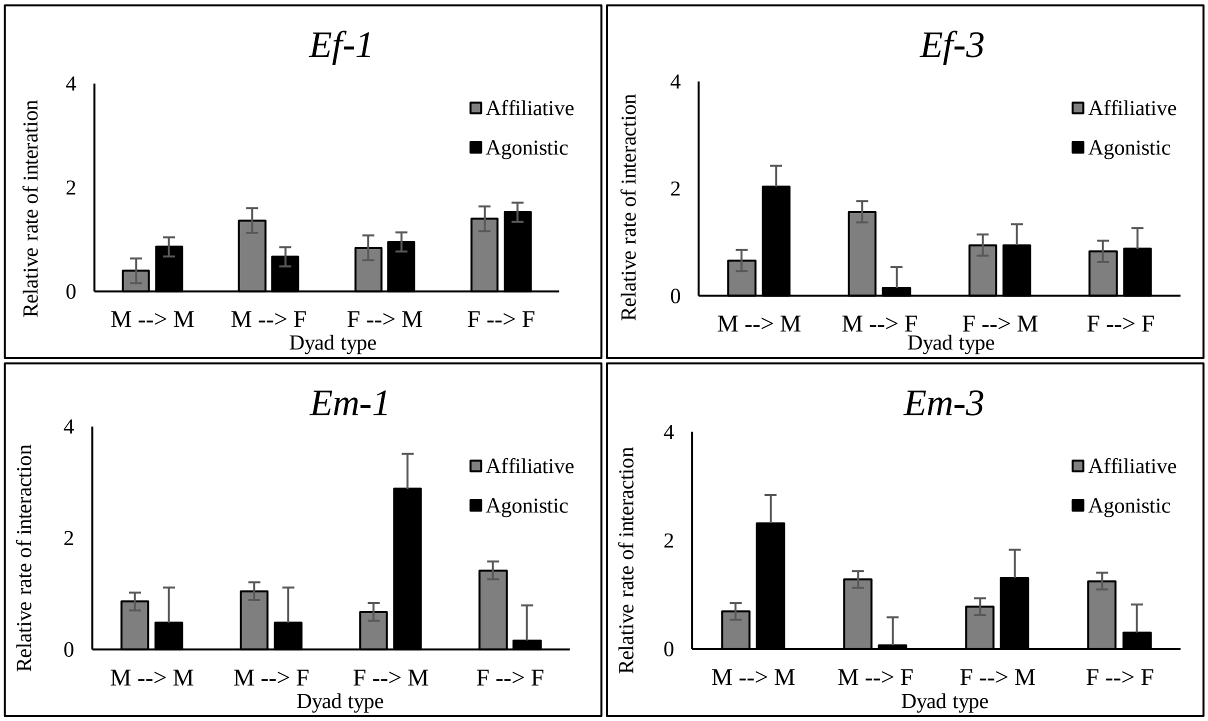

Figure 3.13 Relative rates (interaction/hr ± SE) of affiliative and agonistic interactions for each dyad class. Relative rates were calculated by comparing the dyad's interaction rate by the population's mean interaction rate. A relative interaction rate less than one means fewer interactions were observed in comparison to the populations' average; greater than one, and that dyad exhibited more interactions per hour as compared to the population's average. Arrows depict the direction of the interaction, from groomer to groomee or aggressor to aggressee. M is male, F is female.

From these rates, the overall tenor of the dyadic relationships (RT) was calculated; values ranged from 0.23 to 19.03 (Table 3.6). Again, because dyad rates were compared to each population's mean rates, score interpretations are always relative to 1. A score above this indicates that interactions were more affiliative, while a score lower than 1 indicates more agonistic interactions. Male→Female relationships were the most positive in all populations besides *Em-1*, where Female→Female relationships were of better quality. The same pattern was evident when looking at the lower quality relationships. Male→Male relationships were likely to be agonistic in *Ef-1*, *Ef-3*, and *Em-3*, while Female→Male relationships were more negative in the *Em-1* population. There were no significant differences between or within the two species (Kruskal-Wallis test: χ^2=0.55, df=3, p=0.907), although differences in dyad class approached significance (χ^2=9.46, df=3, p=0.023). Pairwise comparisons revealed that Male→Female dyads were typically more socio-positive than Female→Male and Male→Male dyads (both p=0.029).

Table 3.6 The relationship tenor (RT) of each dyad, as a measure of the quality of social relationships. Scores greater than 1 suggest a quality of social relationship better than the population's average. Scores lower than 1 depict an overall relationship that is more agonistic than population's average.

	E. flavifrons		***E. macaco***	
	Ef-1	***Ef-3***	***Em-1***	***Em-3***
Male→Male	0.46	0.32	1.79	0.30
Male→Female	2.05	10.78	2.18	19.03
Female→Male	0.88	1.00	0.23	0.59
Female→Female	0.92	0.95	8.87	4.13

3.4 DISCUSSION

The findings presented here provide new details about the social systems of *E. flavifrons* and *E. macaco* during the mating season. Although group size and sex ratios were relatively consistent between the two species, there appears to be some degree of flexibility in the levels of spatiotemporal distribution and fission-fusion dynamics, rates and initiation patterns of affiliative and agonistic interactions, and overall relationship tenor of the different dyad types. Often these differences can be attributed to one population of *E. macaco*, *Em-1*. Initial results suggest that local ecological conditions, specifically fragment size, perimeter-area ratio, and quality of forest-type are contributing to the expression of these diverging behavioural characters within and between the two species, providing further support for behavioural plasticity in *E. flavifrons* and *E. macaco*.

Congeneric species that also belong to the same ecological niche are expected to show similarities in aspects of their social system (Clutton-Brock & Harvey, 1977). Ossi & Kamilar (2006) assessed the phylogenetic and environmental correlates of behavioural and ecological variation in the *Eulemur* genus and found that closely-related taxa were more similar in terms of group size and sex ratio than distantly-related species. This suggests that these facets of social organisation may have strong evolutionary correlates making them likely to be conserved between species (Ossi & Kamilar, 2006). As predicted, mean group size in this study did not differ significantly within or between the two species and was within the previously established ranges for both *E. flavifrons* (Rakotondratsima, 1999; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004; Volampeno, 2010; Randriatahina & Roeder, 2013) and *E. macaco* (Colquhoun, 1993; Andrews & Birkinshaw, 1998; Bayart & Simmen, 2005). With this said, group cohesion varied both between and within the two taxa. Furthermore, although extensive ecological data collection was outside the scope of this study, there was a strong relationship between group size, fission-fusion dynamics, and the habitat quality at each location. Larger groups found in one *E. flavifrons* population (*Ef-1*) and two *E. macaco* populations (*Em-3* and *Em-1*) often fissioned into smaller subgroups throughout the day to feed and aggregated during resting periods. Fission-fusion dynamics have been reported previously in *Ef-1* (Prodger, 2015) and two further populations of *E. macaco* (Colquhoun, 1993; Bayart & Simmen, 2005), in addition to numerous other primate species (*Ateles* spp.: Symington, 1990; Asensio et al., 2008; *Cacajao* spp.: Bowler & Bodmner, 2009; *Eulemur* spp.: Tattersall, 1977; *Pan* spp.: Symington, 1990; *Pongo* spp.: van Schaik, 1999; *Propithecus diadema*: Irwin, 2007; *Varecia* spp.: Pereira et al., 1988; Moreland, 1993a; Vasey, 2006; Holmes et al., 2016). In this study, these three populations with observed flexible cohesion also occupied the largest forests of continuous, primary vegetation. Previous research conducted at Ankarafa (*Ef-1*) concluded that the large group sizes could be attributed to a preponderance of resting and feeding trees found in the primary forest parcels (Schwitzer et al. 2007b; Volampeno et al., 2010). Based on the behavioural similarities with *Ef-1*, it is plausible that the larger, continuous *Em-1* and *Em-3* locations also harbour an abundance of important *Eulemur* vegetation, thus supporting larger lemur groups. It is also possible that flexible social cohesion in *Ef-1*, *Em-1* and *Em-3* serves to either alleviate intragroup feeding competition (Lehmann et al., 2007a, 2007b; Aureli et al., 2008; Baden et al., 2016; Holmes et al., 2016) or aids in maintaining choice social bonds when group size precludes relationships with all group members (Lehmann et al., 2007b). Bayart & Simmen (2005) have suggested that the critical group size in *E. macaco* is 16 individuals, at which point groups will split in order to mitigate competition associated with patchy and/or scarce food sources (Colquhoun, 1993). Although this pattern of critical group size holds true for this study, as all groups larger than 16 were observed splitting into subgroups throughout the day, the proximate cause of this observed behaviour remains unclear without a precise understanding of resource distribution.

In comparison to the aforementioned groups, group membership was smaller and relatively stable in two of the *E. flavifrons* populations (*Ef-2* and *Ef-3*) and one *E. macaco* population (*Em-2*). These three locations were the most fragmented in the study, primarily secondary forest, and had humans living and working within them. Fragmentation and habitat destruction have been found to alter local abiotic and biotic conditions (Saunders et al., 1991), decrease canopy cover while increasing fragments' edges (Vieilledent et al., 2018), increase predator pressures through increased detection (Irwin et al., 2009), and modify the composition of vegetation and food resources (Arryo-Rodríguez & Mandujano, 2006; Schwitzer et al., 2011). The negative effects of fragmentation on group demographics, size, and ranging patterns are well documented in primates (*Allouatta* spp.: Arryo-Rodríguez & Dias, 2009; *Chiropotes* spp.: Boyle & Smith, 2010; *Procolobus gordonorum*: Struhsaker et al., 2004; for review, see Irwin et al., 2010; Schwitzer et al., 2011), with frugivores often being more susceptible to habitat disturbance than folivores (Johns & Skorupa, 1987; Wright et al., 2005). It is possible that a combination of (i) increased predation, whether from increased detection by natural predators or anthropogenic hunting (see Chapter 2), (ii) decreased available habitat and space, and (iii) fewer important fruit trees in the *Ef-2*, *Ef-3*, and *Em-2* locations limit the number of individuals in a group, while also increasing their cohesion. Collectively the findings presented here regarding group size and cohesion suggest that a combination of resource availability, existing habitat size and quality, critical group size, and a need to reduce intragroup competition may play roles in dictating spatiotemporal distribution in *E. flavifrons* and *E. macaco.*

As expected, all populations, apart from *Ef-2*, were found to have either an equal or slightly male-biased sex ratio, which is consistent with previous reports of the two species (Petter, 1962; Jolly, 1966; Andrianjakarivelo, 2004; Bayart & Simmen, 2005; Volampeno, 2010), as well as lemurs in general (Kappeler & Fichtel, 2015). Bias in group or population sex ratios in primates is fairly common and can be caused by a number of factors, including sex differences at birth, rates of sexual maturity, or mortality rates (Veran & Beissinger, 2009; Székely et al., 2014). A surplus of males, as was seen here, is thought to also potentially provide social benefits to female group members, including increased vigilance against predators (van Schaik & Hörstermann, 1994) or protection from infanticide by non-group members (Ostner & Kappeler, 2004). In the year subsequent to this study, the *Ef-2* group size and sex ratio changed; it grew from seven to nine individuals and shifted from a female-biased sex ratio (0.75) to a male-biased sex ratio (1.25) (J. van Mildert, pers. comm.). Without continual monitoring of the *Ef-2* group, it is impossible to say whether these changes in group composition were the result of male immigration, a greater number of male births, female death and/or dispersal, or a combination of these factors. Alterations in group composition can affect dispersal patterns, genomic makeup, group cohesion, predator avoidance strategies, social relationships, and the mating strategies of group members (van Schaik & van Hooff, 1983). The fact that aspects of the *Ef-2* population's social organisation fluctuated to the degree it did within a single year highlights how vulnerable small and isolated communities are and underscores the importance of continual monitoring of Critically Endangered species.

In looking at which sex was responsible for initiating an interaction and which sex was the recipient of that interaction, a picture emerges that is not necessarily consistent with typical patterns associated with female dominant societies, but one that instead may be explained by the added pressures associated with competition to secure breeding partners or by the methods of analyses used in this study. Generally, there were no differences in the sex that initiated a grooming bout, but females were the recipient significantly more often. It was initially surprising that, in this study, no differences were found between the rate of male and female initiated grooming interactions, as male primates have been found to initiate grooming interactions significantly more often than females during the mating season (*E. coronatus*: Kappeler, 1989; *Propithecus verreauxi*: Richard & Heimbuch, 1975; *Macaca fascicularis*: Gumert, 2007). However, the grooming data reported here represent bouts of *non-sexual* social grooming only. That is to say, the frantic and aggressive sexual grooming of genitalia that occurs during the context of mating, and which is documented in detail in Chapter 4, was removed from this study. If non-sexual and sexual social grooming were both taken into consideration, males would have been found to initiate significantly more bouts during the mating season. The only outlier in this was with *Ef-1*; females there initiated significantly more bouts than males. This too can be explained by the separation of sexual and non-sexual grooming. As sexual grooming occurred frequently in this population (see Chapter 4), it is possible that such behaviour was a strategy employed by males to solicit female partners. Similarly, there were no significant differences in which sex initiated an aggressive interaction in all populations apart from *Em-1*, although males were significantly more likely to be the recipient of that aggression. Typically, female *E. flavifrons* (Digby & Kahlenberg, 2002; Digby & Stevens, 2007; Prodger, 2015) and female *E. macaco* (Roeder et al., 2002) have been observed initiating more aggressive interactions than their male counterparts, as is consistent with species that are characterized by female dominance (Kappeler, 1989; Pollock, 1989). The lack of significant differences between which sex initiated an aggressive interaction may be due to the increased male-male aggression that occurred as males competed for access to reproductive females.

The prevalence of grooming type, i.e. allogrooming vs. reciprocal grooming, was found to map according to species. Allogrooming, which is unidirectional social grooming, occurred significantly more often in both *E. flavifrons* populations (*Ef-1* and *Ef-3*) than in either of the *E. macaco* populations (*Em-1* and *Em-3*). In comparison, reciprocal grooming (consecutive or concurrent social grooming) occurred more often in the two *E. macaco* populations (*Em-1* and *Em-3*). While this poses an interesting question regarding the role of grooming and its relationship to dominance, hierarchical ranking, and cooperation during the mating season (de Waal & Luttrell, 1988; de Waal, 2000; Silk et al., 2006b; Schino & Aureli, 2008), the limited timing of this study renders commentary on the importance of this finding premature. However, if this pattern remains consistent throughout the year, it may serve in fact as a possible isolation mechanism, reinforcing species-typical behaviours.

A comparison of the relationship tenor and dyad proximity preferences revealed similarities in the *Ef-1, Ef-3,* and *Em-3* populations. As predicted, inspection of proximity patterns revealed that male and female lemurs were characterised as having the greatest affinity. Male-initiated intersexual interactions had consistently higher rates of social grooming and lower rates of aggression in these three populations as well. The preponderance of socio-positive intersexual dyads may be due to the timing of this study. Maintenance of close physical proximity during mating periods has been shown to increase copulatory rates of males (*Alouatta pigra*: Van Belle et al., 2009; *Lophocebus albigena*: Arlet et al., 2008; *Macaca mulatta*: Manson, 1992). Mate-guarding was observed, to a greater or lesser extent, in each population (see Chapter 4 for details), with males actively following and maintaining close contact with a female leading up to and during periods of female oestrus. Focusing on collecting demographic data and identifying the reproductive success of individuals in the future would help clarify whether dyad pairings occur more frequently between certain individuals and whether these pairings are more likely to result in the production of offspring.

Intra-male bonds were the weakest in the *Ef-1*, *Ef-3* and *Em-3* populations. Males tended to spend less time together in dyads, have lower rates and durations of grooming, and increased rates of agonistic interactions compared to intersexual and intra-female dyads. Male aggression during this time was particularly hostile in these communities. Individuals continuously chased each other, often fell considerable heights from trees, and physically harmed their competitors. Increased male aggression as males compete for access to reproductive females during the mating season is common in species that live in multimale-multifemale groups (Ridley, 1986; Dunbar, 1988). This has been reported previously in *E. macaco* (Bayart & Simmen, 2005), as well as other species of primates (*E. fulvus*: Kaufman, 1994; *Lemur catta*: Jolly, 1966; *Macaca sylvanus*: Berghänel et al., 2011; *Propithecus verreauxi*: Vick & Pereira, 1989). This pattern of overtly negative male-male relationships is interesting when compared to findings from Prodger (2015). Outside the mating season, strong affiliative male-male relationships were observed in *E. flavifrons* (Prodger, 2015). These inconsistent patterns in male social bonds may highlight a mechanism developed to elicit intermale cooperation during a time of high male-male contest. By creating friendships during months of low competition, *E. flavifrons* and *E. macaco* males may be able to call upon these friends to form coalitions and limit the number of direct physical contests during the mating season. Males of both species were observed cooperatively working together to divert guarding males away from reproductive females. A similar pattern of male-male bonding during non-mating seasons coupled with increased aggression and the formation of coalitions for access to reproductive females has been observed in macaques (*Macaca* *assamensis*: Schülke et al., 2010; *M. sylvanus*: Berghänel et al., 2011). The difference in findings from this study compared to those conducted during different seasons suggests that, even within a single population, association preferences may fluctuate within and between years. The social relationships of *E. flavifrons* and *E. macaco* are likely to be flexible and may function differentially depending on changing group dynamics, individual histories, or social needs.

When there was a consistent pattern of divergence in social structure or organisation, it was the *Em-1* population that typically differed from the other three populations. The degree to which group fissioning occurred was greatest in *Em-1*, with both sexes spending the majority of their day ranging either in solitude or in small groups of 2-3 individuals, regardless of feeding. This propensity for solitude resulted in significantly lower rates of grooming and aggression as compared to the other populations. Relationship tenor also differed in this population. Intrasexual female bonds were the most socio-positive while female-initiated intersexual dyads were characterised as having the weakest quality relationships. This overall characterisation of the social structure of *Em-1* not only differs from the two populations of *E. flavifrons*, but it provides evidence of distinct intra-species differences within *E. macaco*. Social plasticity within a species is known to correlate with variation in phylogenetics, behavioural incongruities, cultural learning, or local ecological conditions, all of which can lead to environmental adaptations (for review, see Lott, 1984; Chapman & Rothman, 2009; Kappeler et al., 2013; Strier, 2017).

A similar pattern in the group cohesion and social associations of black-and-white ruffed lemurs (*Varecia variegata*) has been in observed in Mangevo, a section of undisturbed primary rainforest in Ranomafana National Park (Baden et al., 2015). Group fissions were common, and individuals were observed spending more than half of their time away from other group members. Additionally, Baden & colleagues (2015) found that female-female relationships were stronger than all other dyad types. This led them to suggest that the observations may be explained by the communal breeding strategies of *V. variegata*. Because infants are unable to cling to their mothers when first born, they are often "parked" in nests and monitored by female group members (Baden et al., 2013). Therefore, strong female relationships throughout the year may enable cooperative infant care during this time (Baden et al., 2015). While the similarities between the observations at Mangevo and those at Angodrahely cannot be denied, communal care of infants seems an unlikely explanation for the behaviours occurring in the *Em-1* population. *E. flavifrons* and *E.macaco* infants can cling directly after birth and spend all of their time with their mothers in the first weeks following parturition (Harrington, 1978; Volampeno et al., 2011b). Additionally, while there have been reports of alloparenting, these examples tend to occur more often with adult males than adult females (Volampeno et al., 2011b). As parental care in the *Em-1* population has not been studied, this subject warrants further attention.

Another hypothesis to explain the results from the *Em-1* population is the environmental conditions at this location. Considering that the local habitat at *Em-1* clearly differed from the other three study sites, it is easy to conclude that ecological factors are responsible at least in part for the behavioural patterns observed there. *Em-1,* the largest location included in this analysis, was composed of higher quality forest (low fragment perimeter to area ratio, primarily primary vegetation) and experienced the least anthropogenic pressure. Although hunting and logging have been documented across the Ampasindava peninsula (Tahinarivony, 2014), the *Em-1* forest is sacred (MBG, 2015), deterring local communities from using its resources.

Because better quality habitats are associated with lower levels of stress (*Alouatta* spp.: Martínez-Mota et al., 2007; Rimbach et al., 2013; *E. collaris*: Balestri et al., 2014), the combination of these more positive ecological factors at *Em-1* may have resulted in a population that was less stressed than its counterparts in three ways. First, fewer threats may have led to lower levels of group cohesion. As the risk of predation decreases in primates, so too does a group's need to collectively monitor and protect against these threats, which ultimately leads to decreased group cohesion (van Schaik, 1989). Second, the large and quality forest of *Em-1* is likely to harbour more abundant and evenly distributed food sources across a wider range of forest. This would reduce intragroup competition and could account for lower rates of aggression observed in this population. Detailed ecological surveys on important *E. macaco* vegetation are needed to test that this is the case. Lastly, when the need to alleviate stress (Aureli & Yates, 2010) or maintain social bonds in defence of limited resources or predator threats (Dunbar, 1988) is reduced, grooming may not be as important in the population. Captive studies on langurs (*Presbytis entellus*) found similar results; i.e., that when enclosures were more naturalistic, individuals were less stressed, engaged in fewer social interactions such as allogrooming and aggression, and increased their distance from other group members (Little & Sommer, 2002).

The effect of fewer ecological stressors resulted in an *Em-1* population with a decreased need to compete for and/or defend resources (i.e., lower rates of aggression), that was less vulnerable to predation (i.e., lower levels of group cohesion), and that required less maintenance of social bonds (i.e., lower rates of grooming), which lends credence to theories using the extreme conditions of Madagascar to explain incongruities in strepsirrhine societies as compared to anthropoids. In contrast to many anthropoid species (*Cebus* spp.: Perry, 1996; *Pan* spp.: Parish, 1996; Lehmann & Boesch, 2009; *Papio* spp.: Silk et al., 2006a, 2006b, 2010) in which females typically have strong bonds, socio-negative female-female relationships are common among lemurs (Kappeler, 1993b, 1997; Kappeler & Fichtel, 2015), with intense competition and female eviction well documented during the mating season (Vick & Pereira, 1989; Digby, 1999; Randriatahina & Roeder, 2013). The variable and inconsistent food availability in Madagascar is thought to have led to female dominant societies and increased intrasexual competition for limited resources among females, especially during important reproductive periods (Jolly, 1984; Young et al., 1990; Wright, 1999). Surprisingly though, *Em-1* females tended to groom each other more frequently, for longer durations, and engage in fewer agonistic interactions during a time when female competition is normally pronounced. The fact that intrasexual female interactions in the *Em-1* population were characterised as being the most socio-positive, especially considering it was the mating season, implies that strong female-female bonds may be advantageous for these lemurs. Perhaps it is only with unpredictable resources and resulting competitive stressors that those relationships are not fostered.

Several broad conclusions can be drawn from this study. The social organisation and social structure of *E. flavifrons* and *E. macaco* are very similar, with only the *Em-1* population showing consistent divergence of characters. This suggest that social behaviours and group relationships are not different enough to differentiate between the two species, at least in the *Ef-1*, *Ef-3*, and *Em-3* populations. Because of this, it seems unlikely that the social organisation and structure of *E. flavifrons* and *E. macaco* would prevent interbreeding in areas of range overlap. Within these defined parameters though, a degree of behavioural plasticity seems to be characteristic of both taxa. Depending on the specific behaviour assessed, a different pattern of divergence was possible either between or within the taxa. While there are no long-term observational data from most of these populations, comparisons between previous work at Ankarafa (*Ef-1*) and this study show that even within a single population, aspects of a social system can fluctuate seasonally. Plasticity in the social organisation, behaviours, and relationships within a single population has been attributed to a number of seasonal factors, including: fluctuations in ambient temperature and rainfall patterns, which can affect the daily ranging patterns and activity budgets of individuals (*Varecia variegata*: Moreland, 1993b); the reproductive states of group members, which can influence the levels of aggression (*Macaca mulatta*: Wilson & Boelkins, 1970) or play (*Saimiri sciureus*: Mendoza et al., 1978); changes in predator abundance, which can influence polyspecific associations (*Procolobus badius* and *Cercopithecus diana*: Noë & Bshary, 1997); and changes in food availability, which can impact group size and the level of group cohesion (Pazol & Cords, 2005).

Additionally, the differences in social structure found in the one *E. macaco* population (*Em-1* from Andranomatavy) suggest that differing ecological factors may be playing significant roles in influencing the social attributes of these communities. Although preliminary, results from this population provide further support for the role of limited resources in the development of what are deemed typical relationship patterns of lemurs. Although the lack of individual identification, kinship, life history, and detailed ecological data constrain interpretation of these findings and prevent an exhaustive understanding of the dynamic social relationships of *E. flavifrons* and *E. macaco*, this study should be viewed as a foundation for future work on the social systems of these closely-related species. The inclusion of detailed demographic, genomic, and local habitat data would undoubtedly elucidate the mechanisms contributing to behavioural flexibility documented here, especially at the *Em-1* location.

Understanding the root cause of intraspecific variation and a population's ability to adapt favourably to novel conditions is fundamental to conservation efforts (Strier, 2017). Animals that demonstrate increased plasticity in their foraging efforts, feeding and activity patterns, social organisation, or group relationships will be better able to adapt to and survive in novel settings (Poirier, 1969; Lott, 1984; Dehgan, 2003; Schwitzer et al., 2007b; De Vleeschouwer & Raboy, 2013; Williams-Guillén et al., 2013; Donati et al., 2016). Given the drastic loss of forest cover in Madagascar (Vieilledent et al., 2018) and the high levels of anthropogenic disturbances observed across these two taxa's ranges (see Chapter 2, but also Ruperti, 2007; Schwitzer et al., 2007b; Seiler et al., 2010; Seiler, 2012; Randriatahina & Volampeno, 2013), social flexibility will be an essential attribute for *E. flavifrons* and *E. macaco* moving forward. The plasticity detailed in both this and previous studies (Schwitzer et al., 2007b; Prodger, 2015) suggests that although the two species are at least reasonably resilient and can adapt to living in disturbed forest, some of the species-typical traits associated with the two taxa may be responses to living in increasingly stressful habitats. By elucidating how *E. flavifrons* and *E. macaco* respond to rapidly changing environmental conditions, conservation management programmes will be able to develop concrete methods to combat increased pressures, thereby supporting the long-term viability of the two species.

Chapter 4

The Mating Systems, Sexual Behaviours, and Mating Tactics of *Eulemur flavifrons* and *E. macaco*

ABSTRACT

The Critically Endangered blue-eyed black lemur (*Eulemur flavifrons*) and the black lemur (*E. macaco*) are two species for which relatively little is known regarding their mating systems. Compounding this are claims of hybridisation between the taxa. Mating activities of individuals from two distinct *E. flavifrons* locations (*Ef-1* and *Ef-3* populations) and two distinct *E. macaco* locations (*Em-1* and *Em-3* populations) were recorded through a combination of continuous and sequence sampling over three mating seasons (Apr-Jun 2015-2017). This resulted in an observed 81 *Ef-1* courtships, 8 *Ef-3* courtships, 52 *Em-1* courtships, and 51 *Em-3* courtships. I assessed the occurrence of sexual behaviours, identified key behavioural sequences within courtships, and characterised the mating tactics of males and females at each location. Although the sexual behaviour repertoire was not found to differ between the species, comparisons revealed that one population of *E. macaco* (*Em-1*) employed significantly different mating tactics compared to the other *E. macaco* population and the two *E. flavifrons* populations. Higher levels of courtship reciprocity, characterised by consensual mate-guarding, female copulation consent, and longer courtship durations, were found in the *Em-1* population. In comparison, male sexual coercion and female rejection were common mating tactics in the remaining three populations. Although the proximate causes of behavioural variation remain ambiguous, they may have resulted from differing social and/or ecological conditions at the *Em-1* location as opposed to distinct species-level diversity. These findings suggest that strong pre-mating behavioural isolation mechanisms do not exist between the two species and provide insight into hybridisation probabilities in areas of purported species sympatry.

4.1 INTRODUCTION

Collectively, primate mating systems, which form a core component of a species' social system, address the sexual behaviours, mating tactics, and reproductive strategies of that group of organisms (Clutton-Brock, 1989). Sexual selection for specific characters drives intrasexual competition and helps individuals secure mating partners or breeding opportunities (Darwin, 1871). The reproductive fitness, or an individual's ability to pass on their genes, is closely tied to the number of copulatory partners for males; by mating often, a male may increase his chances of siring more offspring. Conversely, having multiple mating partners does not significantly increase female fecundity, nor is a female's reproductive success closely tied to her ability to attract mates (Bateman, 1948).

As with many mammalian taxa (Trivers, 1972; Emlen & Oring, 1977), female parental investment tends to be greater in primates; as such, females are generally the limiting resource within a mating system (Clutton-Brock & Harvey, 1977). This relationship between reproductive fitness and mating partners favours competition between males as they vie for access to the limited sex, whether through ornamentation, physical strength, or services provided. At the same time, selectivity for superior male mating partners becomes more advantageous for females (Trivers, 1972; Andersson, 1994; Clutton-Brock, 2007). Therefore, primate mating systems can be described by the number of males the female mates with and the length of those interactions. There are currently five recognised primate mating systems: monogamy, polygyny, polyandry, polygynandry, and dispersed (Dixson, 2012). Although a species' mating system is not necessarily fixed and may change over time. Flexibility between a primary and secondary mating system can be related to fluctuations in the number of breeding females (Nievergelt et al., 2002), changes in parental care needs (Díaz-Muñoz, 2016), or desire to avoid inbreeding (Guo et al., 2010).

Monogamy has been documented in nearly a quarter of primate species (Opie et al., 2013) across each major clade (*Aotus* spp.: Fernandez-Duque, 2011; *Callicebus moloch*: Anzenberger, 1988; *Cheirogaleus medius*: Fietz, 1999b; *Hylobates* spp.: Tilson & Tenaza, 1976; Reichard, 1995; *Indri indri*: Pollock, 1975, 1986; Bonadonna et al., 2014; *Sanguinus* spp.: Díaz-Muñoz, 2016; Garber, et al. 2016). In a monogamous system, an adult female and male live together in a small group and mate exclusively for a prolonged period (van Schaik & Dunbar, 1990; Fuentes, 1999; Dixson, 2012; Kappeler, 2014; Díaz-Muñoz & Bales, 2016), although there is evidence to suggest that extra-pair copulations can occur (Reichard, 1995; Bonadonna et al., 2014).

Polygynous mating systems, in which one central male has long-term relationships with several females, are typically found in Old World monkeys (*Papio* spp.: Swedell et al., 2014; Fischer et al., 2017) and apes (*Nomascus hainanus*: Zhou et al., 2008). Intrasexual competition in polygynous species tends to favour sexual dimorphism and the selection for secondary sexual characters that aid in the ability to fight (Clutton-Brock, 1991; Plavcan & van Schaik, 1992; Mitani et al., 1996; Gomez et al., 2012).

A polyandrous mating system is one in which one female forms long-term mating relationships with multiple male partners. In such mating systems, the males often provide infant care (Sussman & Garber, 1987). Polyandry is uncommon and inadequately understood both in primates and other vertebrates (Dixson, 2012), although it relatively common in callitrichids (Sussman & Garber, 1987; Goldizen, 1988; Díaz-Muñoz, 2016).

In polygynandrous mating systems, also referred to as multimale-multifemale, polygamous, or promiscuous, both males and females will mate with multiple partners. Common among primates (Brockman, 1999; Pfefferle et al., 2008; van Belle et al., 2009; Guo et al., 2010), males and females form temporary relationships characterised by high levels of reciprocity, or consortships (Manson, 1997). During the mating season, intrasexual competition is often pronounced (Dixson, 2012; Plavcan, 2015).

In a dispersed mating system, a female mates with a number of males despite not living in permanent social groups. Dispersed mating systems are primarily found in non-gregarious, nocturnal strepsirrhines (*Microcebus* spp.: Fietz, 1999a; Eberle & Kappeler, 2004a, 2004b; *Lepilemur* spp.: Méndez-Cárdenas & Zimmermann, 2009; Mandl, 2017; *Galago moholi*: Pullen et al., 2000), as well as orangutans (Utami Atmoko et al., 2009; Spillmann et al., 2017).

Sexual behaviour is not always synonymous with reproduction in primates and can occur outside of the mating season (Campbell, 2011). Anthropoids often engage in sociosexual behaviours, which serve other functions besides reproduction such as stress reduction or the maintenance of dominance hierarchies (Beach, 1976), while strepsirrhines generally engage in sexual behaviour only during times when conception is possible (Campbell, 2011). During periods of oestrus, females may show enhanced levels of receptivity and proceptivity (Beach, 1976). Receptivity refers to the ability and willingness to copulate. A proceptive female may actively alert males to their readiness to mate (Beach, 1976) with vocalisations, facial cues, presenting, and/or following (Boinski, et al., 2003; Barelli et al., 2008; Campbell, 2011). This behavioural signalling can be important in primates that form vaginal blocks or closures during non-receptive periods, as it indicates a physical ability to copulate (Boskoff, 1977). Reproductive seasonality is closely tied to predictable seasonal variations; important reproductive events are hypothesised to occur in synchrony with peak environmental conditions (Negus & Berger, 1972). During this time, females may or may not synchronise their oestrus cycles (Sauther, 1991; Schwab, 2000; Campbell, 2011). If multiple females come into oestrus at the same time, one male will be incapable of monopolising all reproductive females at the same time; this will increase intrasexual competition between males and the number of mating partners for females (Dunbar, 1988; Dixson, 2012).

In order to increase chances of reproductive success, primates may use varying mechanisms of sexual selection and behavioural tactics, all with the aim of gaining access to a mating partner and promoting copulatory opportunities (Andersson, 1994; Clutton-Brock, 2007; Kappeler & van Schaik, 2015; Plavcan, 2015). Traditionally, male competition by way of secondary sexual characters was viewed as the dominant avenue through which to accomplish this endeavour (Darwin, 1871), partly due to the fact that intrasexual male competition is generally quite conspicuous. Now researchers know that both sexes are subject to sexual selection, conflict can be intra- or interspecific, and it can occur pre- or post-copulation (Andersson, 1994; Andersson & Iwasa, 1996; Birkhead & Møller, 1998; Birkhead & Pizzari, 2002; Andersson & Simmons, 2006). In addition to intrasexual competition to improve their chances of access to a reproductive female, males may employ possessive techniques where they form consortships or actively guard the female from access by other males (Girard-Buttoz et al., 2014). In such situations, alternative reproductive tactics may be employed by lower-ranking males to increase their chances of mating success (Brockmann, 2001; Setchell, 2008). This can include coercive behaviours (Smuts & Smuts, 1993; Arlet et al. 2008; Swedell et al., 2014), interruption of courtships by male coalitions (Gachot-Neveu et al., 1999), surreptitious matings (Alberts et al., 2006), or dispersal from their group (Strier, 1996).

Despite its prominence in current studies on primate reproduction, sexual selection theory has not always highlighted female mate choice (Andersson & Simmons, 2006); early theorists even went so far as to suggest that female choice might simply boil down to a fear-based decision (West-Eberhard, 1979). Today it is known that female mate choice, which can be viewed as accepting, rejecting, or initiating a courtship with a specific male, and female mating tactics play a significant role in sexual selection and can greatly influence the structure and genetic makeup of a population (Andersson & Simmons, 2006; Eberle & Kappeler, 2004a). When choosing a partner, females either select one male or choose to mate with multiple individuals; these choices are driven by risks and payoffs associated with possible mates (Izar et al., 2009). Having a single partner will either benefit the female directly or indirectly by increasing the fitness of genes passed onto offspring (van Schaik & Kappeler, 2003). Conversely, having multiple partners, whether within or outside the social group, may serve to increase genetic diversity through extra-group copulations (Guo et al., 2010; Kowalewski & Garber, 2010), foster sperm competition (Harcourt et al., 1981; Harvey & Harcourt, 1984; Birkhead & Hunter, 1990; Curtsinger, 1991; Harcourt et al., 1995; Fietz, 1999a), reduce the risk of infanticide through paternity confusion (Hrdy, 1979; van Schaik & Dunbar, 1990; Reichard, 1995; Jolly et al., 2000; Guo et al., 2010; Kowalewski & Garber, 2010), provide greater access to food sources (Overdorff, 1998; Guo et al., 2010), and/or aid in territory defence (van Schaik & van Hoof, 1983).

4.1.1 *E. flavifrons* and *E. macaco* mating systems

The mating systems of the closely-related *E. flavifrons* and *E. macaco* (Fausser et al., 2000; Meyer et al., 2015) are poorly understood. Little research has focused on their mating behaviours in the wild, although Volampeno (2009) spent considerable time studying the birthing season of *E. flavifrons*. Bimaturation, or a difference in development timing of the sexes, is not observed in either species (Leigh & Terranova, 1998). While the reported age of maturation varies slightly between studies, consensus ranges between one-and-a-half and two years for *E. macaco* (Petter-Rousseaux, 1964; Bogart et al., 1977a; Colquhoun, 1993; Leigh & Terranova, 1998; Bayart & Simmen, 2005) and around two to two-and-a-half years for *E. flavifrons* (Leigh & Terranova, 1998; Randriatahina & Roeder, 2013). The age of first reproduction is around two-and-a-half years in *E. macaco* (Petter-Rousseaux, 1964; Bogart et al., 1977a; Bayart & Simmen, 2005) and three years in *E. flavifrons* (Volampeno, 2009; Volampeno et al., 2011b).

As with most lemurs (Wright, 1999), *E. flavifrons* and *E. macaco* are seasonal breeders. Research suggest that in the wild, their short mating season begins as early as April and lasts through mid-June, with the majority of copulations occurring in May (Petter-Rousseaux, 1964; Rasmussen, 1985; Bayart & Simmen, 2005; Volampeno, 2009; Volampeno et al., 2010, 2011b). Due to changes in latitude and photoperiod, captive individuals in the northern hemisphere experience a shift in their mating season of approximately six months (Rasmussen, 1985). Captive studies suggest that females are typically in oestrus for only 3-5 days during their mating season (Bogart et al., 1977a). Unlike some other primate species (Sauther, 1991; Street et al., 2016), oestrus is not marked by conspicuous changes in female anatomy (Bogart et al., 1977a). Prior to the start of the mating season, male testes size increases significantly in *E. macaco* (Bogart et al., 1977a, 1977b). During the mating season an increase in intrasexual aggression has been reported in both taxa (*E. flavifrons*: Digby, 1999; *E. macaco*: Bayart & Simmen, 2005).

Gestation length for both species is estimated to be between 108 days and 128 days (*E. flavifrons*: Volampeno, 2009; Volampeno et al., 2011b; *E. macaco*: Bogart et al., 1977a; Rasmussen, 1985; Colquhoun, 1993; Kappeler & Ganzhorn, 1993; Asa et al., 2007). Females of both species commonly give birth to one offspring at a time, although twin births have been reported (*E. flavifrons*: Volampeno, 2009. *E. macaco*: Rasmussen, 1985; Asa et al., 2007). In Lokobe National Park, the birth rate of female *E. macaco* has been recorded to be 100% (Bayart & Simmen, 2005), while in the Ankarafa population of *E. flavifrons*, the birth rate fluctuated between 44% and 100% across different years (Randriatahina & Roeder, 2013). The sex ratio at birth has been found to have a slight male-bias in both *E. flavifrons* (Volampeno 2009: male-biased but did not significantly differ from 1:1; Randriatahina & Roeder, 2013: ranged 0.8-7.0 male/female over two consecutive birth seasons) and *E. macaco* populations (Bayart & Simmen, 2005:

ranged from 1.2-1.67 male/female over two consecutive birth seasons). In the first year, infant mortality rates range from 22% (*E. flavifrons*: Volampeno, 2009) to 27% (*E. macaco*: Colquhoun, 1993; Bayart & Simmen, 2005). Deaths were attributed to predation, disease (Bayart & Simmen, 2005; Volampeno, 2009), and infanticide by a female lemur (Andrews, 1998).

4.1.2 Hybridisation, mate recognition, and reproductive isolation

Reports of hybridisation between the two species have come from areas in and around the Manongarivo Special Reserve (Meyers et al., 1989; Rabarivola et al., 1991; Anrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004). These claims were based on morphological variation, with individuals appearing as intermediate forms between the two species. These reports have not been fully substantiated with genomic or behavioural evidence. In order for *E. flavifrons* and *E. macaco* to remain reproductively isolated in areas of contact, they must develop unique mate recognition systems and reproductive isolation mechanisms, or occupy ecologically different niches (Kaneshiro, 1980; Templeton, 1981; Hendry et al., 2007). Mate recognition systems, which are a fundamental force driving behavioural differences in visual, auditory, and olfactory communication (Shaw & Parsons, 2000; Pillay & Rymer, 2012), can act as reproductive barriers at the pre-mating, mating, or post-mating level (Templeton, 1981). Therefore, understanding the factors that affect sex-based and taxa-based differences in sexual behaviours and mating strategies is critical to understanding the role of isolation mechanisms in the speciation of these closely-related species (Shaw & Parsons, 2002; Pillay & Rymer, 2012; Garber et al., 2016).

4.1.3 Aims, hypotheses, and predictions

To gain further insight into the mating systems and potential reproductive isolation mechanisms between *E. flavifrons* and *E. macaco*, this study assessed the sexual behaviours and mating courtships of lemurs across four geographic locations, i.e. two populations of each species, during the mating season.

I hypothesised that *E. flavifrons* and *E. macaco* would have the same mating system classification. For many lemurs, the mating season is short and female oestrus synchrony is common (Wright, 1999). Because of this, coupled with the fact that both species live in multimale-multifemale groups (Colquhoun, 1993; Randriatahina & Rabarivola, 2004; Bayart & Simmen, 2005; Randriatahina et al., 2014), I predicted that both *E. flavifrons* and *E. macaco* would have a polygynandrous mating system. Based on this classification, I further predicted that mating would occur between April and June (Petter-Rousseaux, 1964; Rasmussen, 1985; Bayart & Simmen, 2005; Volampeno, 2009; Volampeno et al., 2010, 2011b), that multiple females would come into oestrus at the same time, and that one male would be unable to monopolise access to several reproductive females at the same time. As a result, it was predicted that both sexes would have multiple mating partners over the course of the mating season.

In addition, I hypothesised that both males and females would employ sex-specific behavioural mating tactics to gain access to preferred mating partners and/or to secure chances of a successful mating courtship. I predicted males would show signs of pre-copulatory competitive behaviours and preferential mate choice as they vied for access to reproductive females (Andersson, 1994; Bayart & Simmen, 2005; Clutton-Brock, 2007; Kappeler & van Schaik, 2015; Plavcan, 2015). Similarly, females were predicted to use mate choice, as determined by consenting/rejecting solicitations or initiating courtships, to mate with preferred partners (Andersson, 1994; Clutton-Brock, 2007). Female-female competition to limit the mating success of other females was also predicted (Digby, 1999).

Lastly, I sought to investigate the levels of intra- and interspecies variation by highlighting similarities and/or differences between the four populations. Although both *E. flavifrons* and *E. macaco* were predicted to both have polygynandrous mating systems, I further hypothesised that there would be species-specific behavioural differences within their mating systems. I predicted significant differences in reproductive behaviours and/or mating tactics employed by the two species. These differences would serve as behavioural isolation barriers, aiding in species recognition and limiting the chances of interbreeding (Phillips-Conroy et al., 1991) between the two genetically distinct taxa (Fausser et al., 2000; Pastorini et al., 2000; Mittermeier et al., 2008).

4.2 METHODS

4.2.1 Locations and collection effort

Across three mating seasons (2015–2017), mating data were collected for two populations of *E. flavifrons* (*Ef-1* and *Ef-3*) and two populations of *E. macaco* (*Em-1* and *Em-3*; Table 4.1 and Figure 4.1). Although it was impossible to confirm that no mating events occurred outside of the study's time frame, typically there was at least a week's buffer at the beginning and end of data collection where no mating behaviours were observed. For further details regarding the locations, see Chapter 1: Introduction and General Methodology.

Table 4.1 Group location and collection effort for the 2015-2017 field seasons.

Species	Year	Pop	Location	Dates Followed	Observation hours
E. flavifrons	2015	*Ef-1*	Ankarafa Forest 14°22’64.2”S and 47°45’31.5”E	14 April – 29 May 2015	216.3
	2017	*Ef-3*	Angodrahely 14°16’14.8”S, 48°2’47.4”E	11 April – 16 May 2017	290
E. macaco	2016	*Em-1*	Andranomatavy 13°40’26.4”S and 47°59’35.1”E	4 April – 30 May 2016	558.5
	2017	*Em-3*	Lokobe 13°24’24.2”S and 48°18’17.9”E	18 April – 26 May 2017	139

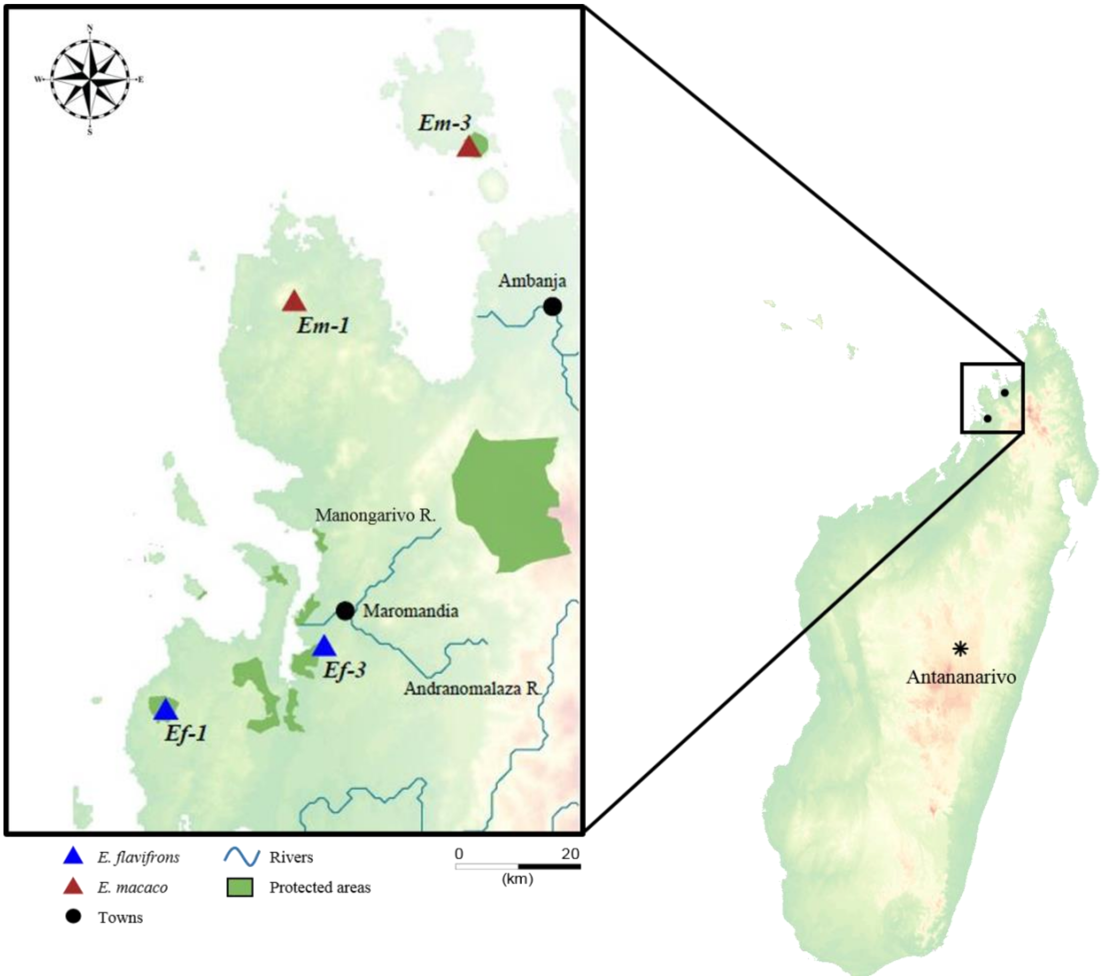

Figure 4.1 Locations of study populations in northwest Madagascar. *Ef-1*: Ankarafa Forest, 2015; *Ef-3*: Angodrahely, 2017; *Em-1*: Andranomatavy, 2016; and *Em-3*: Lokobe, 2017.

4.2.2 Study animals

Group identification was always possible in the *Ef-3* and *Em-3* populations and usually possible in the *Ef-1* population. Identification became difficult when individuals were found after splitting from the core group (FFD). Mean group size ± SD was 12.9 ± 5.1 (N=7) in the *Ef-1* population, 9 ± 2.7 (N=3) in the *Ef-3* population, and 13 ± 4.6 (N=3) in the *Em-3* population. Due to low levels of group cohesion in *Em-1*, group identification and composition were impossible. The maximum number of individuals observed together was 12, while the average number of visible individuals together was 2.1 ± 1.3. Details of various aspects of each populations' social system can be found in Chapter 3: Social Organisation and Structure (see Table 3.4 for group size and composition).

4.2.3 Data collection

Behavioural observations occurred between the hours of 0530 and 1730, six days a week. Once located, groups were monitored for the entire day, or until lost. Reproductive and mating behaviours were recorded through a combination of continuous and sequence sampling of the consort male and reproductive female. After an initial one-week observation period an ethogram was developed, with components of courtships grouped by their perceived function (Table 4.2). A mating courtship was defined as a temporary intersexual dyadic interaction between a male and a reproductive female that included one or more of the sexual behaviours detailed in the ethogram. A courtship was considered to be finished when one individual left the event and the second individual did not follow, if one or both individuals went out of sight, or if the pair associated with other individuals for more than 15 minutes. This window of time was chosen due to the increased difficulty in reliably identifying individuals as time spent interacting with other group members increased. With sequence sampling, the focus was the order of occurrence of interactions within a mating event, as opposed to a particular individual (Altmann, 1974; Lehner, 1996). The initiator, all resulting interactions including conspecific involvement, and the terminator of the mating event, were noted. Due to the distance between lemurs and researchers, ejaculation could not always be visually confirmed; therefore intromission (thrusting) served as a proxy for mating success.

Table 4.2 Ethogram of sexual behaviours observed during mating courtships. Behaviours were grouped according to their function.

FUNCTION	BEHAVIOUR	DESCRIPTION
Sexual Arousal	*Sexual Grooming:*	Male inspects, smells, and/or licks female's genitalia and back in a frantic and vigorous manner. Females often respond aggressively. This type of grooming is distinctly different than observed during typical social grooming and it typically happens in the beginning of a courtship.
	Non-sexual Grooming	Typical social grooming; relaxed in nature. Either the female grooms the male, the male grooms the female, or the male and female simultaneously groom each other. Social grooming can happen at any point during a courtship.
	Coercion	Males pin, restrain, and bite uninterested females in order to mount them; or they aggressively chase a retreating female, trying to force copulation.
	Mate-guarding	Male continuously follows female around, preventing other males from accessing the female. Mate-guarding can be consensual or unwanted by the female. If consensual, the female waits for guarding male to catch up when travelling and alerts the guarding male of other satellite males' advances in unwanted.
	Scent marking	The individual rubs their forehead, hands, or anogenital region on another individual or substrate. This is typically a male behaviour during a courtship, although females also may scent mark.
Copulation	*Clasp*	The male uses his front legs to grab and hold on to the female. This can occur in conjunction with another behaviour, such as mounting or grooming. At times, clasping was aggressive, where the male pins the female down to attempt to coerce her into mating.
	Mount	The male braces on his back two legs, climbing onto the female from behind. This is the preparatory step to intromission.
	Intromit	The male enters the female and thrusts.
	Ejaculate	Copulation occurs; seminal fluid is released. This can result in the formulation of a copulatory plug in the female, although not always visible to researchers.
	Female proceptivity	A female behaviour that signals willingness, or proceptiveness, to copulate with a male. The female braces on her front two legs while lifting her anogenital region in the direction of a male, thereby attempting to initiate copulation.
Interruption	*Male harassment and coalitions*	Two or more males band together to form a coalition aimed at diverting the guarding male's attention away from the reproductive female. Often one harassing male approached the female, which resulted in the guarding male chasing after him. When this happened a second harassing male quickly approached the female and tried to copulate with her. This could lead to surreptitious matings.
	Female interference	A second female places herself in between the guarding male and reproductive female, halting copulation. Actions can include: climbing on top of the guarding male, aggressively hitting and pulling at guarding male, blocking access to the reproductive female's vagina by lying across her back.
	Juvenile male interference	Juvenile males interrupted copulation by sitting on top of the reproductive female and grooming her. Neither the mating male nor the reproductive female was ever seen retaliating against the interfering individual.
	Agonistic female response	An unreceptive female rejects the advances of a male by hitting, baring teeth, biting, vocalising, and desperately trying to get away.

4.2.4 Analysis

Reliable identification of individuals was not possible due to the lack of distinguishing morphological features, apart from dichromatic sex differences, and the short window available for collecting mating data. Because of this, each courtship was considered an independent observation for these analyses. Given the low number of courtships observed in Angodrahely, the only mating data presented on the *Ef-3* population is timing of the mating season, the number of potential mates, courtship durations, and initiations/terminations of courtships. Additional mating data on the *Ef-3* population can be found in Appendix C. Behavioural and sequence analyses results are therefore presented for the three remaining populations only (*Ef-1*, *Em-1*, and *Em-3*).

4.2.4.1 Frequency of sexual behaviours

Statistical tests carried out in R (R Core Team, 2013) were applied to the frequency data to look at population-level and species-level differences in courtship durations and sexual behaviours. An independent samples T-test was used to compare species' differences in mating season lengths. From the number of available males and females, a potential mate partner ratio (MPR) was calculated. Kruskal-Wallis and Mann-Whitney U tests were used to assess differences in courtship durations and to assess whether the MPR differed between populations. Kendall's tau test was used to determine the relationship between the number of potential partners and the duration of a courtship. Significance levels for Kruskal-Wallis, Mann-Whitney U, and Kendall's tau tests were set at $\alpha = 0.05$.

Hinde's Index, which is a measure of proximity between two individuals (Hinde & Atkinson, 1970), was used to determine the sex responsible for maintaining the courtship. This score ranges from -1 to 1. A positive score suggests that males are the sex responsible for maintaining proximity in dyad pairs, while a negative score suggests that females are the responsible sex.

A chi-square test of proportions was used to compare population differences in the frequency of sexual behaviours. In each population, the proportion of behaviour 'x' relative to the total observed sexual behaviours was calculated. Due to the pairwise comparisons of the chi-square tests, significance levels were set at $\alpha = 0.02$ with a Bonferroni correction.

4.2.4.2 Sequence analysis of courtships

To determine whether there were unique behavioural patterns within the courtships of each species, and whether any of these patterns were predictors of mating success, I analysed the sequential order of all behaviours occurring in a courtship. Following a method developed by Bode *et al.* (2017), each behavioural interaction in a courtship was represented as a series of ten different behavioural states, labelled ‘0’ through ‘9’ (Table 4.3). These discrete categories were identified through the development of the ethogram (Table 4.2).

Table 4.3 Behavioural states used to construct courtship sequences.

Code	Behaviour
0	Start or stop of interaction
1	Approach
2	Groom
3	Rest
4	Males: Clasp, mount, and/or intromit Females: proceptive behaviour
5	Ejaculation
6	Scent mark
7	Aggression
8	Interference/coalitions
9	Move away

For this analysis, it was essential to know the behavioural state of both the male and female throughout the duration of the courtship. Behaviours occurring at the same time were listed in brackets, with the male’s behavioural state first and the female’s behavioural state listed after the comma (Figure 4.2). Each bracket represents a change in one or both individuals’ behaviour. Changes in behaviour could occur simultaneously or consecutively. Representing courtships this way allowed me to distinguish changes in the dyad’s behavioural states.

Male mate-guarding in a courtship was defined as a male approaching a female {1}, the female moving away {9}, and the male following and once again approaching the female {1}. In other words, I investigated whether courtship sequences contained the following pattern: {1,-}{-,9}{1,-}. For this particular pattern, the partner’s co-occurring behaviour was not the focus and therefore the pattern could include any of the identified behavioural states (represented by “-” here). I also allowed for other sequence elements to occur between the three elements of this pattern. Two types of social grooming patterns, allogrooming and mutual grooming, were also coded. Within the category of allogrooming, either the male could groom the female

{2,3}, or the female could groom the male {3,2}. Mutual grooming, where both individuals groomed each other simultaneously, was coded as {2,2}.

In the sequence example (Figure 4.2), the first sequential element is {1,3}. This communicates that a male approaches {1} a resting female {3}. The male then begins grooming the female, who remains resting {2,3}. After a bout of mutual grooming {2,2}, the male mounts the female {4,3}. Intromission {4,4} follows, which would classify this as a successful mating sequence. Then the female aggressively removes herself from the male; this is represented as {4,79}. By combining more than one behavioural state at a time for one individual, as seen with the female's behavioural state of {79}, it is possible to increase the depth of information conveyed in an element.

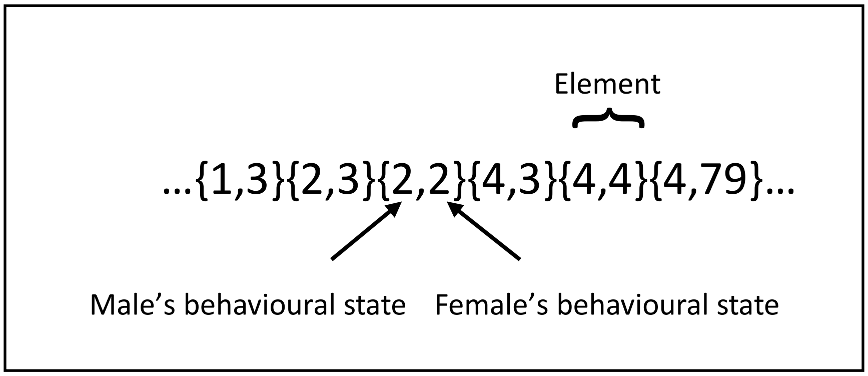

Figure 4.2 A representative sequence within a courtship.

I first looked for frequently occurring patterns, or strings of elements that occur in the same order repeatedly, within the courtships. To do this, a modified version of a database mining technique (Bode et. al., 2017), known as the 'Apriori algorithm' (Agrawal & Srikant, 1995), was used in R (R Core Team, 2013). When a low threshold of occurrence was set (requiring sequences of elements to occur in only a small number of courtships in order to classify as a pattern), many patterns were obtained. A threshold any lower than 20% did not return useful patterns on the population; rather it reported back information on individual courtships. If the occurrence threshold was increased, the number of patterns found drastically decreased. At a threshold of 50% or higher, only patterns with a sequence length of one element were found. An element length of one described the dyad's simultaneous behaviours but conveys no information about how these behaviours changed over time, nor any information about the sequential order of multiple behaviours. For this reason, I set a low occurrence threshold, requiring that sub-sequences occur in at least 20-50% of the observed courtships in order to classify as a frequently occurring pattern.

From this dataset, I focused on patterns with a length of two elements or longer. This means that {1,3}{2,3} would constitute a pattern, while {1,3} would not. Permutation tests were performed to assess if the occurrence of these frequent patterns was higher than we would expect based on a random ordering of the sequence's elements. To do this the element order within a courtship sequence was randomly shuffled, and then reassessed to determine how frequently a certain pattern was observed in the permutated courtship sequences. This procedure was repeated 1,000 times; the proportion of times the occurrence frequency in the permutated data was higher or equal to the observed data represents the p-value. Due to the large number of tests performed on this data set, a Bonferroni correction for the number of patterns examined was used to reduce the likelihood of obtaining results with a type I error. Significance level for identifying common behavioural sequences was set at $\alpha = 0.001$.

Once courtships were represented as a series of changes in behavioural states, a variety of nonparametric tests were applied to the data to reveal characteristic differences in sequence length (Kruskal-Wallis test), the presence/absence of key patterns (Chi-square test), and the duration of successful and non-successful courtships (Kruskal-Wallis test and Mann-Whitney U test) at the population and species-level. Significance levels were set at $\alpha = 0.05$ for all tests; if a Bonferroni correction was applied, it is stated in the results.

4.3 RESULTS

4.3.1 Characterisation of mating season

4.3.1.1 Timing of mating season

For all four populations, mating behaviours began in April and ended in May (

Table 4.4). The mean length $\pm$ SD of the two *E. flavifrons* mating seasons was 26 ± 1.4 days, while the mean length of the two *E. macaco* mating seasons was 27 ± 4.2 days. There were no significant species differences in mating season durations (T-test: t(1.22)= -0.32, p=0.797). During this time, I observed 81 *E. flavifrons* courtships in Ankarafa (*Ef-1*), 8 *E. flavifrons* courtships in Angodrahely (*Ef-3*), 52 *E. macaco* courtships in Andranomatavy (*Em-1*), and 51 *E. macaco* courtships in Lokobe (*Em-3*).

Table 4.4 First and last observed instances of mating across the four populations.

	Ef-1 **Ankarafa** **2015**	***Ef-3*** **Angodrahely** **2017**	***Em-1*** **Andranomatavy** **2016**	***Em-3*** **Lokobe N.P.** **2017**
First observed mating behaviour	27 April	18 April	13 April	19 April
Last observed mating behaviour	21 May	14 May	12 May	12 May
Number of days in mating season	25	27	30	24

Observed courtships were concentrated in the end of April and the beginning of May (Figure 4.3). There was a pronounced peak in mating attempts for the *Em-3* population between 25 April and 2 May; outside of this window there were few observed courtships. In comparison, courtships were observed occurring over a wider range of days for the *Ef-1*, *Ef-3*, and *Em-1* populations. For the *Ef-1* and *Em-1* populations, courtships tended to occur in greater frequency in May.

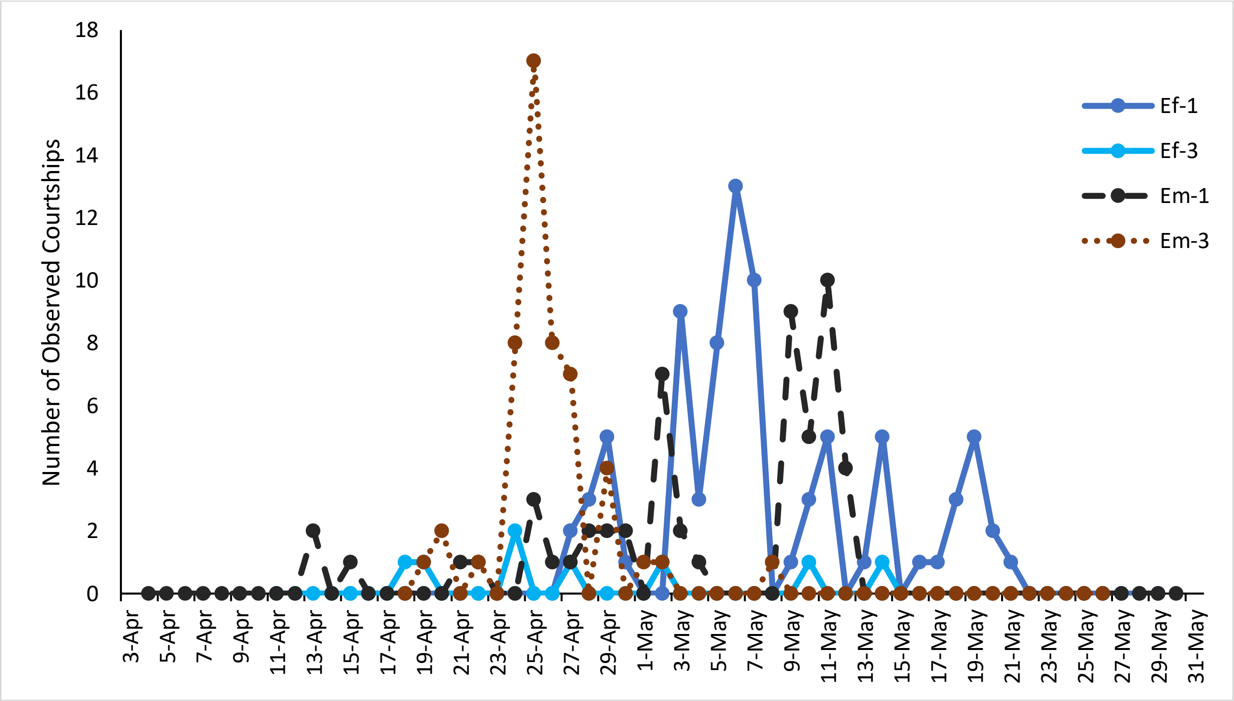

Figure 4.3 The number of courtships observed each day, across the mating season, for each population. *Ef-1* data were collected in 2015, *Em-1* data were collected in 2016, and *Ef-3* and *Em-3* data were collected in 2017.

4.3.1.2 Available potential partners during a courtship

Group size was not always an accurate reflection of the number of males or females during a courtship. On average, there were more potential male partners present during a courtship in the *Em-3* population than in the other three populations (Table 4.5). A comparison of potential males found significant differences between the three populations (Kruskal-Wallis test: X^2=27.84, df =3, p<0.001). Post hoc tests with Bonferroni corrections revealed that pairwise differences lay between all populations (Mann-Whitney U test: *Ef-1* and *Em-1*, p=0.017; *Ef-1* and *Em-3*, p=0.009; *Em-1* and *Em-3*, p<0.001) apart from *Ef-3* (p=1.0 for *Ef-1* comparison and *Em-1* comparison, and p=0.143 for *Em-3* comparison). In each population, the potential mate partner ratio (MPR) comparing the number of adult males to adult females during a courtship was always skewed towards males; there were no significant differences between the populations (Kruskal-Wallis test: X^2=0.83, df =3, p=0.842).

Table 4.5 The number of available male and female partners during observed courtships (N) for each population. The potential mate partner ratio (MPR) is the ratio of potential male mating partners to potential female mating partners for all courtships in a population. An MPR greater than 1 implies more males were present, while a ratio less than 1 implies more females were present. All populations were male-biased, and no significant differences were found in the population's ratios.

		Max Number of Individuals	**Mean ± SD**	**MPR (♂: ♀)**
Ef-1 (N=81)	Males	9	4.26 ± 2.09	1.32
	Females	7	3.22 ± 1.65	
Ef-3 (N=8)	Males	7	3.7 ± 1.8	1.23
	Females	3	3.01±01.9	
Em-1 (N=52)	Males	7	3.12 ± 1.62	1.29
	Females	7	2.42 ± 1.46	
Em-3 (N=51)	Males	11	5.59 ± 2.56	1.26
	Females	9	4.45 ± 1.88	

4.3.1.3 Courtship durations

Of the 192 observed courtships, durations varied considerably between and within the two species (Figure 4.4). Mean duration ± SD for *Ef-1* was 670±1,544 s, for *Ef-3* was 690±1,027 s, for *Em-1* 3,047±3,885 s, and for *Em-3* 509±1,132 s. A comparison of durations found significant differences between the four populations (Kruskal-Wallis test: X^2=32.76, df =3, p<0.001). Further pairwise comparisons with Bonferroni corrections revealed that *Em-1* had significantly longer courtships compared to than two populations (Mann-Whitney U test: *Em-1* and *Ef-1*, p<0.001; *Em-1* and *Em-3*, p<0.001). There were no other significant differences found between the populations (*Ef-1* and *Em-3*, p=1.00; *Ef-1* and *Em-3*, p=1.00; *Ef-3* and *Em-1*, p=0.42; and *Ef-3* and *Em-3*, p=1.00). No significant correlations were found between the duration of a courtship and the number of potential male partners present in the *Ef-1* (Kendall's tau: τ=0.09, p=0.259), *Ef-3* (τ= -0.04, p=1.00), *Em-1* (τ=0.03, p=0.803) or *Em-3* (τ=0.08, p=0.468) populations. Similar results were found when comparing courtship durations and the number of adult females present (*Ef-1:* τ=0.15, p=0.066; *Ef-3*: τ= -0.04, p=1.00; and *Em-3*: τ=0.04, p=0.703). Only in the *Em-3* population was there found to be a weak positive relationship between these two variables, with courtships lasting longer with the more females present (τ=0.22, p=0.038)

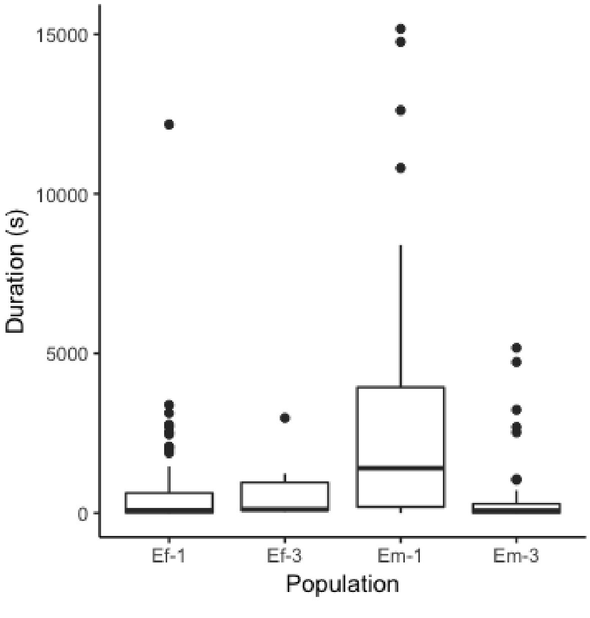

Figure 4.4 Duration (seconds) of observed courtships across the three populations. The duration of *Em-1* courtships were significantly longer than the *Ef-1* and *Em-3* populations only (p<0.001). Black bars indicate median durations, boxes represent upper and lower quartiles, and whiskers show data points falling within a 1.5x inter-quartile range.

4.3.1.4 Initiation and termination of courtships

Males predominately initiated courtships, although there were instances of female initiation in both species (Table 4.6). *Ef-1* males initiated 98.8% of the courtships recorded, and no female-initiated courtships were observed. *Ef-3* males initiated 87.5% of recorded courtships and females initiated 12.5% of courtships. In comparison, *Em-1* males initiated 82.7% and females initiated 7.7% of courtships, while *Em-3* males initiated 90.2% and females initiated 7.8%. For all three populations, there were a small number of courtships where the initiator was unknown (*Ef-1*: 1; *Em-1*: N=5; *Em-3*: N=1).

Regardless of population or species, more than half of all courtships were terminated by females (*Ef-1*: 87.7%; *Ef-3: 75%; Em-1*: 69.2%; *Em-3*: 54.9%), while males were less likely to end a courtship (*Ef-1:* 12.3%; *Ef-3*: 25%; *Em-1*: 30.8%; *Em-3*: 45.1%). Hinde's Index scores were greater for males than females in each population, suggesting that males were the sex primarily responsible for maintaining proximity during a courtship (Table 4.6).

Table 4.6 Initiation and termination of courtships by sex. A high Hinde's Index (HI) score suggests that sex is responsible for the maintenance of dyadic proximity in that population.

	N	**♀ Initiate**	**♂ Initiate**	**♀ Terminate**	**♂ Terminate**	**♀ HI**	**♂ HI**
Ef-1	81	0	80	71	10	44.1	55.9
Ef-3	8	1	7	6	2	43.8	56.2
Em-1	52	4	43	36	16	40.4	59.6
Em-3	51	4	46	28	23	31.7	68.3

4.3.2 Characterisation and frequency of sexual behaviours

E. flavifrons and *E. macaco* were observed displaying a similar range of sexual behaviours within a courtship. There were 416 counts of different sexual behaviours (identified from the ethogram, Table 4.2) recorded in the *Ef-1* population, 528 in the *Em-1* population, and 273 in the *Em-3* population. Apart from behaviours that occurred during copulation, which were generally more stereotyped, sexual behaviours did not follow a prescribed order of occurrence (Figure 4.5).

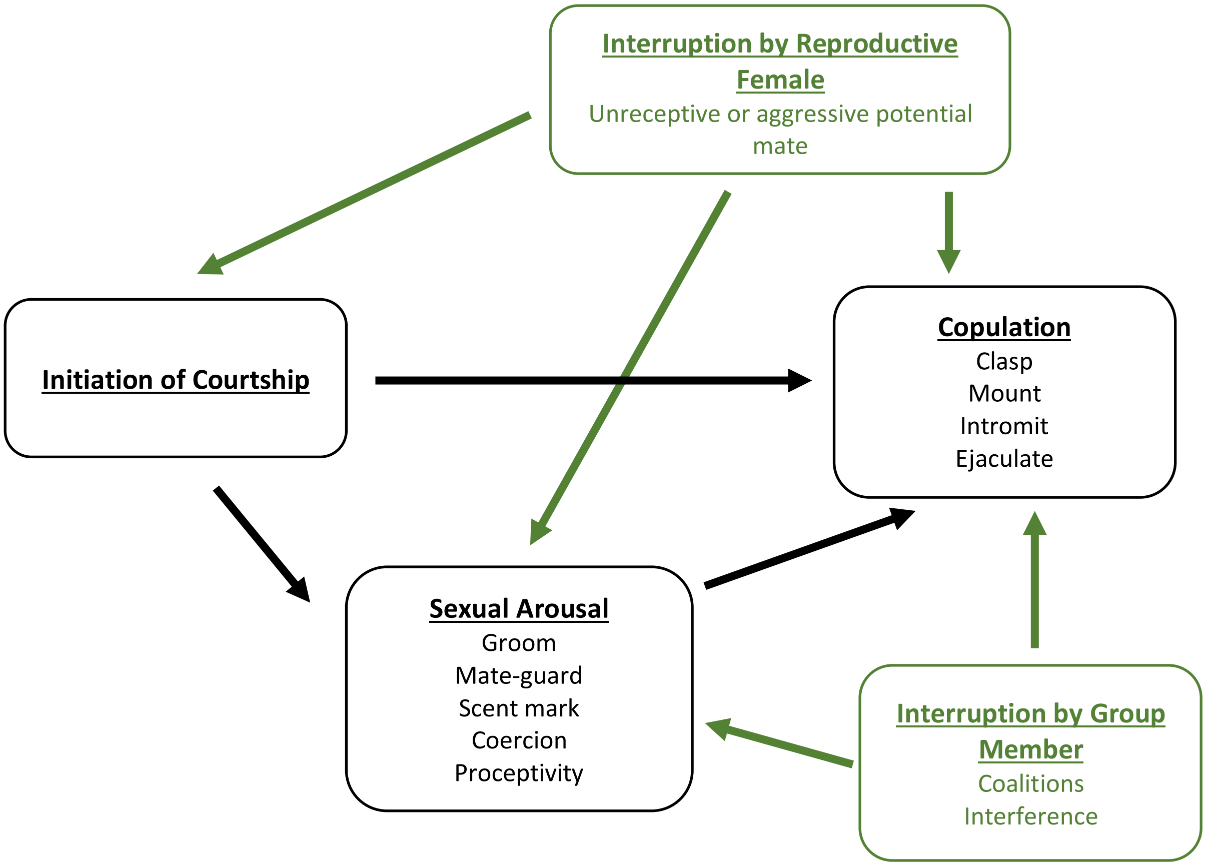

Figure 4.5 Schematic diagram depicting the progression of sexual behaviours during typical *E. flavifrons* and *E. macaco* mating courtships, grouped by their function. Black arrows depict directional flow of behaviours between a male and reproductive female. Green arrows represent points at which the courtship may be interrupted, either by the reproductive female or a group member.

4.3.2.1 Sexual arousal function

Grooming: Grooming during a courtship was observed in every population. There were 88 counts of sexual grooming (frantic grooming of the anogenital region) in *Ef-1*, 46 counts in *Em-1*, and 23 counts in *Em-3*. *Ef-1* counts of sexual grooming represented a significantly greater proportion of that population's total observed sexual behaviours than either *Em-1* (Chi-square test: X^2=28.56, df=1, p<0.001) or *Em-3* (X^2=18.83, df=1, p<0.001). There was no significant difference between the proportion of sexual grooming found in the *Em-1* and *Em-3* populations (X^2<0.001, df=1, p=0.996).

Coercion: Males aggressively tried to force, or coerce, females to mate with them on multiple occasions. *Ef-1* males forcefully attempted to mate with females 60.5% of the time (49/81 courtships), while *Em-3* males employed this strategy 49.0% of the time (25/51 courtships); differences in the prevalence of coercion within a courtship did not differ between these two populations (Chi-square test: X^2=1.24, df=1, p=0.266). *Em-1* males displayed coercive behaviours 23.6% of the time (12/52 courtships), which was significantly less often than *Ef-1* males (X^2=16.38, df=1, $p<0.001$) or *Em-3* males (X^2=6.44, df=1, p=0.011).

Mate-guarding: There were 14 observed instances of mate-guarding in the *Ef-1* population, 87 instances in the *Em-1* population, and 33 instances in the *Em-3* population. Chi-square tests revealed no differences when comparing between the two *E. macaco* populations (X^2=2.39, df=1, p=0.122). Mate-guarding during courtships occurred significantly less often in *Ef-1* population as compared to the *Em-1* population (X^2=40.51, df=1, $p<0.001$) or the *Em-3* population (X^2=18.38, df=1, $p<0.001$).

Scent marking: Male scent marking played a role in the courtships of most of the populations. Males were observed marking on or near females with their hands, anogenital glands, or their foreheads 32 times in the *Ef-1* population, 128 times in the *Em-1* population, and 34 times in the *Em-3* population. Pairwise comparisons revealed that this behaviour represented a significantly larger proportion of *Em-1* courtship behaviours than *Ef-1* behaviours (Chi-square test: X^2=44.11, df=1, $p<0.001$) or *Em-3* behaviours (X^2=14.78, df=1, $p<0.001$). Due to Bonferroni corrections, there was no significant difference between the *Ef-1* and *Em-3* populations (X^2=3.78, df=1, p=0.052).

Although females were observed scent marking during the mating season, there was only one instance of a female scent marking during an *Em-1* courtship. This behaviour occurred during an episode of male mate-guarding. After the guarding male approached the female, she anogenitally marked the male's back.

Female proceptivity: Female presenting was only observed in *E. macaco*. Within four *Em-1* courtships, female proceptivity occurred six times. This behaviour was observed in the *Em-3* population on four separate courtships as well. In one *Em-3* courtship, in which the female assumed the proceptive posture, she also clasped and mounted the male from behind. The male responded aggressively, hitting the female as he struggled to escape. This behaviour was reminiscent of male coercive behaviour. Due to the infrequent nature of this behaviour, no statistical tests were carried out.

4.3.2.2 Copulation mechanism

Clasping: Clasping could occur during copulation, but it was not a required behaviour in the sequence. Clasping was observed 23 times in *Ef-1*, 53 times in *Em-1*, and 37 times in *Em-3*. Clasping in *Ef-1* represented a significantly smaller proportion of observed sexual behaviours than *Em-1* (Chi-square test: $X^2=5.80$, df=1, p=0.016) or *Em-3* ($X^2=12.36$, df=1, $p<0.001$). No differences were found between *Em-1* and *Em-3* ($X^2=1.89$, df=1, p=0.169).

Mounting: Although there were far more observed instances of males mounting a female in *Ef-1* (N=99) than in either of the *E. macaco* populations (*Em-1*: N=46; *Em-3*: N=51), these counts did not represent significantly different percentages of the overall sexual behaviours observed in the *Ef-1* and *Em-3* populations (Chi-square test: $X^2=2.24$, df=1, p=0.134). In comparison, the number of mountings observed in *Em-1* represented significantly less of all the observed sexual behaviours relative to *Ef-1* ($X^2=39.58$, df=1, $p<0.001$) or *Em-3* ($X^2=15.88$, df=1, $p<0.001$).

Intromission: There were 38 instances of intromission in *Ef-1*, 34 instances in *Em-1*, and 14 instances in *Ef-3*. No significant differences were found in the pairwise comparisons of populations (Chi-square test: *Ef-1—Em-1*: $X^2=2.03$, df=1, p=0.154; *Ef-1—Em-3*: $X^2=3.24$, df=1, p=0.072; *Em-1—Em-3*: $X^2=0.34$, df=1, p=0.559). What is perhaps more interesting is the comparison of the number of mountings that led to intromission. Although *Em-1* was observed to have the fewest instances of mounting, 73.9% of these led to intromission. Only 38.4% of *Ef-1* mountings resulted in intromission, while only 27.5% of *Em-3* mountings resulted in intromission.

4.3.2.3 Interruptions

Male coalitions and sneak matings: Conspecific harassment was observed in all three populations. Fifteen instances of male coalitions were observed in the *Ef-1* population, 99 instances in the *Em-1* population, and 13 instances in the *Em-3* population. The *Em-1* population's frequency was significantly greater than either the *Ef-1* population (Chi-square test: $X^2=48.84$, df=1, $p<0.001$) or the *Em-3* population ($X^2=28.12$, df=1, $p<0.001$). There was no significant difference found when comparing *Ef-1* and *Em-3* ($X^2=0.31$, df=1, p=0.579).

Occasionally, these coalitions resulted in a sneak mating by a satellite, non-guarding male. Surreptitious matings were characterised by an approach and attempted copulation by a male while the guarding male was busy fending off coalitions, or when the guarding male was feeding. Of the 81 observed *Ef-1* courtships, two were sneak matings. One sneak courtship was also observed in the *Em-1* population, and five sneak courtships were observed in the *Em-3* population.

Female and juvenile interference: Direct interference during the copulation sequence by a group member was observed in all populations. Interferences in *Em-1* (N=7) occurred significantly less frequently than in *Ef-1* (N=27; Chi-square test: X^2=16.42, df=1, p<0.001) or *Em-3* (N=12; X^2=6.06, df=1, p=0.014). There were no observed differences between *Ef-1* and *Em-3* (X^2=0.99, df=1, p=0.320). In all populations, adult and juvenile females were seen disrupting mating (*Ef-1*: N=24; *Em-1*: N=4; *Em-3*: N=11) more often than juvenile males (*Ef-1*: N=3; *Em-1*: N=3; *Em-3*: N=1). Adult males were not observed physically interfering in copulation; rather they would harass in the form of coalitions (see above).

Agonistic female response: Female response to a male's advances was often agonistic, especially in the *Ef-1* and *Em-3* populations. Female aggression towards a male during a courtship was observed 80 times in *Ef-1* and 45 times in *Em-3*; the proportion of these frequencies was not significantly different (Chi-square test: X^2=0.039, df=1, p=0.852). In comparison, *Em-1* females only responded aggressively 23 times to males' advance; this finding was significantly lower compared to the *Ef-1* population (X^2=61.75, df=1, p<0.001) and the *Em-3* population (X^2=47.26, df=1, p<0.001).

4.3.3 Sequence analysis of *E. flavifrons* and *E. macaco* courtships

4.3.3.1 Courtship sequences lengths

The courtship sequences of *Em-1* contained more elements (mean ± SD: 22±23) than the courtships of either *Ef-1* (11±18; Wilcoxon rank sum test: W=3484, p<0.001) or *Em-3* (13±16; W=1791, p<0.01). *Em-3* courtships had significantly more elements than *Ef-1* courtships (W=2750.5, p=0.035). This indicates that both populations of *E. macaco* exhibited more changes in the behaviours of one or both individuals throughout the observed courtships than *Ef-1*.

4.3.3.2 Common behavioural patterns in courtship sequences

Forty-eight behavioural patterns, between two and four elements in length, were found to occur in a minimum of 20-50% of *E. flavifrons* and *E. macaco* courtships (Table 4.7). Permutation tests revealed that most of these patterns occurred significantly more often than expected by chance. There were 14 frequently occurring patterns identified in *Ef-1* courtships, 16 patterns identified in *Em-3* courtships, and 34 patterns identified in *Em-1* courtships. *Ef-1* and *Em-3* courtships share many of the same frequent patterns, while the frequent patterns found in *Em-1* courtships were often different. Only two pattern types were common across *Ef-1*, *Em-1*, and *Em-3*: those where the male approaches a female and then either grooms her or mounts her. Patterns including agonistic behaviours by the female occurred frequently in both *Ef-1* and *Em-3* courtships, but not *Em-1* courtships. In comparison, patterns associated with male scent marking, male mate-guarding, and satellite male harassment were common in *Em-1* courtships, but not in *Ef-1* or *Em-3* courtships

Table 4.7 Commonly occurring courtship behavioural patterns. To be included here, a pattern must occur in minimum of 20% of the courtships in at least one population. Patterns occurring ≥20% are in bold, those <20% are not in bold. Patterns with * were found to occur significantly more often than would be expected based on a random ordering of the sequence's elements. Behaviours: aggression (AG), ejaculation (E), grooming (G), male harassment (H), intromission (I), mate-guarding (MG), mounting (MT), resting (R), and scent marking (SM).

Pattern	**Behavioural Sequence**	**Behaviours**	***Ef-1***	***Em-1***	***Em-3***
{1,3}{2,3}	♂ approaches resting ♀, ♂ grooms ♀	G	**40%***	**32%***	**21%***
{1,3}{3,3}	♂ approaches resting ♀, ♂ and ♀ rest	R	12%	**42%***	**25%***
{1,3}{4,0}	♂ approaches resting ♀, ♂ mounts ♀	MT	**50%***	**36%***	**49%***
{1,3}{6,3}	♂ approaches resting ♀, ♂ scent marks ♀	SM	6%	**30%***	**23%***
{1,9}{1,3}	♂ follows ♀ (mate-guarding), ♂ approaches resting ♀	MG	13%	**26%***	11%
{1,9}{8,8}	♂ follows ♀ (mate-guarding), satellite ♂ harassment	H, MG		**28%***	
{2,2}{3,3}	♂ mutually grooms ♀, ♂ and ♀ rest	G, R	8%	**23%***	11%
{2,3}{2,7}	♂ grooms ♀, ♀ responds aggressively	AG, G	**23%***	9%	11%
{2,3}{2,9}	♂ grooms ♀, ♀ moves away	G	**25%***	9%	6%
{2,3}{3,3}	♂ grooms ♀, ♂ and ♀ rest	G, R		**30%***	5%
{3,3}{2,3}	♂ and ♀ rest, ♂ grooms ♀	G, R	6%	**26%***	7%
{3,3}{3,9}	♂ and ♀ rest together, ♀ moves away	R		**42%***	**27%***
{3,3}{9,3}	♂ and ♀ rest together, ♂ moves away	R	8%	**40%***	15%
{3,9}{1,9}	♀ moves away, ♂ follows (mate-guarding)	MG		**44%***	11%
{4,0}{4,4}	♂ mounts ♀, ♂ intromits	I, MT	16%	**28%***	13%
{4,0}{4,7}	♂ mounts ♀, ♀ responds aggressively	AG, MT	**37%***	11%	**39%***
{4,0}{4,9}	♂ mounts ♀, ♀ moves away	MT	**40%***	13%	**35%***
{4,0}{4,79}	♂ mounts ♀, ♀ aggressively moves away	AG, MT	**28%***	7%	**29%***
{4,4}{5,5}	♂ intromits, ♂ ejaculates	E, I	11%	**23%***	9%
{4,79}{0,0}	♀ aggressively moves away from mounting male, courtship ends	AG, MT	14%		**21%***
{4,9}{0,0}	♀ moves away from mounting male, courtship ends	MT	**25%***	4%	**27%***
{5,5}{9,3}	♂ ejaculates, then ♂ moves away from ♀	E		**21%***	7%

Pattern	**Behavioural Sequence**	**Behaviours**	***Ef-1***	***Em-1***	***Em-3***
{6,3}{3,3}	♂ scent marks ♀, ♂ and ♀ rest	R, SM		**26%***	9%
{8,8}{1,3}	Satellite ♂ harassment, ♂ approaches resting ♀	H	12%	**42%***	9%
{9,3}{3,3}	♂ moves away, ♂ and ♀ rest	R	12%	**25%***	15%
{9,3}{8,8}	♂ moves away, satellite ♂ harassment	H		**36%***	19%
{1,3}{2,3}{2,9}	♂ approaches resting ♀, ♂ grooms ♀, ♀ moves away	G	**23%***	7%	
{1,3}{3,3}{3,9}	♂ approaches ♀, ♂ and ♀ rest together, ♀ moves away	G		**25%***	15%
{1,3}{3,3}{9,3}	♂ approaches ♀, ♂ and ♀ rest together, ♂ moves away	G		**25%***	7%
{1,3}{4,0}{4,4}	♂ approaches resting ♀, ♂ mounts ♀, ♂ intromits	I, MT	12%	**23%***	11%
{1,3}{4,0}{4,7}	♂ approaches ♀, ♂ mounts ♀, ♀ responds aggressively	AG, MT	**29%***	5%	**31%***
{1,3}{4,0}{4,9}	♂ approaches ♀, ♂ mounts ♀, ♀ moves away	MT	**32%***	9%	**31%***
{1,3}{4,0}{4,79}	♂ approaches ♀, ♂ mounts ♀, ♀ aggressively moves away	AG, MT	**22%***		**25%***
{1,9}{8,8}{1,3}	♂ follows ♀ (mate-guarding), satellite ♂ harassment, ♂ approaches resting ♀	H, MG		**26%***	
{3,3}{3,9}{1,9}	♂ and ♀ rest together, ♀ moves away, ♂ follows ♀ (mate-guarding)	MG, R		**40%***	11%
{3,3}{9,3}{8,8}	♂ and ♀ rest together, ♂ moves away, satellite ♂ harassment	H, R		**25%***	75
{3,9}{1,9}{8,8}	♀ moves away, ♂ follows ♀ (mate-guarding), satellite ♂ harassment	H, MG		**26%***	
{4,0}{4,4}{5,5}	♂ mounts ♀, ♂ intromits, ♂ ejaculates	E, I, MT	11%	**21%***	9%
{4,0}{4,79}{0,0}	♂ mounts ♀, ♀ aggressively moves away, courtship ends	AG, MT	14%		**21%***
{4,0}{4,9}{0,0}	♂ mounts ♀, ♀ moves away, courtship ends	MT	**25%***	5%	**25%***
{4,4}{5,5}{9,3}	♂ intromits, ♂ ejaculates, ♂ moves away	E, I		**21%***	7%
{8,8}{1,3}{3,3}	satellite ♂ harassment, ♂ approaches resting ♀	H		**28%***	7%
{9,3}{8,8}{1,3}	♂ moves away from ♀, satellite ♂ harassment, ♂ approaches ♀	H		**30%***	9%
{1,3}{3,3}{3,9}{1,9}	♂ approaches ♀, ♂ and ♀ rest together, ♀ moves away, ♂ follows ♀ (mate-guarding)	MG, R		**25%***	
{1,3}{3,3}{9,3}{8,8}	♂ approaches ♀, ♂ and ♀ rest together, ♂ moves away, satellite ♂ harassment	H, MG		**21%***	5%
{1,3}{4,0}{4,9}{0,0}	♂ approaches ♀, ♂ mounts ♀, ♀ moves away, courtship ends	MT	**20%***		**23%***
{3,3}{3,9}{1,9}{8,8}	♂ and ♀ rest together, ♂ moves away, ♂ follows ♀ (mate-guarding), satellite ♂ harassment	H, MG, R		**25%***	
{3,3}{9,3}{8,8}{1,3}	♂ and ♀ rest together, ♂ moves away, satellite ♂ harassment, ♂ approaches ♀	H, R		**21%***	7%

* Significant results at $p \leq 0.001$

Sequences identified in Table 4.7 were grouped according to their overarching behaviour, i.e., all sequences that contained instances of mate-guarding. Four of these behavioural measures were selected for further comparison between the three populations due to their potential as mating tactics. These included mate-guarding, grooming, scent marking, and male harassment. In addition, although not a common behavioural sequence, female proceptivity and/or female courtship initiation was also investigated due to its reproductive significance as a female mating tactic. Comparisons between the three populations revealed significant differences between the occurrence of some, but not all, of the behaviours. The *Ef-1* and *Em-3* populations were quite similar (Table 4.8; three out of five categories non-significant), as well as the *Em-1* and *Em-3* populations (Table 4.9; three out of five categories non-significant). In comparison, the *Ef-1* and *Em-1* populations were the most different (Table 4.10; one out of five categories non-significant).

Table 4.8 Percentage of courtships in which various behavioural patterns were observed for *Ef-1* and *Em-3* populations. N= total number of observed courtships and n= number of courtships that contained that specific behavioural measure. Significant findings* are in bold.

Behavioural measure	***Ef-1* (N=81)**		***Em-3* (N=51)**		
	n	**Percent**	**n**	**Percent**	**X^2 results**
Mate-guarding *{1,-} {-,9} {1,-}*	26	32.1%	15	29.4%	*X*2=0.02, p=0.895
Grooming *{-,2}, {2,- } or {2,2}*	50	61.7%	21	41.2%	X^2=4.52, p=0.033
Scent marking *{-,6} or {6,- }*	11	13.6%	17	33.3%	**X^2=6.17, p=0.013**
Male harassment *{8,8}*	12	13.5%	11	21.6%	X^2=0.58, p=0.447
Female proceptive/initiate *{-,1} or {-,4}*	0	0%	8	15.7%	**X^2=9.14, p=0.003**

*Alpha level set to p= 0.01 due to Bonferroni corrections.

Table 4.9 Percentage of courtships in which various behavioural patterns were observed for *Em-1* and *Em-3* populations. N= total number of observed courtships and n= number of courtships that contained that specific behavioural measure. Significant findings* are in bold.

Behavioural measure	***Em-1* (N=52)**		***Em-3* (N=51)**		
	n	**Percent**	**n**	**Percent**	**X^2 results**
Mate-guarding *{1,-} {-,9} {1,-}*	31	59.6%	15	29.4%	**X^2=8.32, p=0.004**
Grooming *{-,2}, {2,- } or {2,2}*	35	67.3%	21	41.2%	**X^2=6.07, p=0.014**
Scent marking *{-,6} or {6,- }*	27	51.9%	17	33.3%	X^2=2.92, p=0.088
Male harassment *{8,8}*	24	46.2%	11	21.6%	X^2=5.88, p=0.015
Female proceptive/initiate *{-,1} or {-,4}*	13	25.0%	8	15.7%	X^2=0.86, p=0.353

*Alpha level set to p= 0.01 due to Bonferroni corrections.

Table 4.10 Percentage of courtships in which various behavioural patterns were observed for *Ef-1* and *Em-1* populations. N= total number of observed courtships and n= number of courtships that contained that specific behavioural measure. Significant findings* are in bold.

Behavioural measure	***Ef-1* (N=81)**		***Em-1* (N=52)**		
	n	**Percent**	**n**	**Percent**	**χ^2 results**
Mate-guarding *{1,-} {-,9} {1,-}*	26	32.1%	31	59.6%	**X^2=8.7, p=0.003**
Grooming *{-,2}, {2,- } or {2,2}*	50	61.7%	35	67.3%	X^2=0.22, p=0.639
Scent marking *{-,6} or {6,- }*	11	13.6%	27	51.9%	**X^2=20.97, p<0.001**
Male harassment *{8,8}*	12	13.5%	24	46.2%	**X^2=14.21, p<0.001**
Female proceptive/initiate *{-,1} or {-,4}*	0	0%	13	25.0%	**X^2=14.19, p<0.001**

*Alpha level set to p= 0.01 due to Bonferroni corrections.

4.3.4 Successful courtships

4.3.4.1 Duration of successful courtships

Within each population, successful courtships were significantly longer than non-successful courtships (Table 4.11). A comparison of the durations of successful courtships between groups found significant differences between *Em-1* and *Ef-1* populations only (Kruskal-Wallis test: X^2= 7.08, df=2, p=0.029; Mann-Whitney U test: *Ef-1- Em-1*: p=0.049, *Ef-1- Em-3*: p=1.00, *Em-1- Em-3*: p=0.138).

Table 4.11 Successful versus non-successful courtship durations (s). Successful courtships were significantly* longer than non-successful courtships in each population.

	# of Courtships	**Range**	**Mean duration + SD (s)**	**Mann-Whitney U Test**
Ef-1 Successful	14	7 – 12,172	1,934 + 3,142	**W=224, p=0.002**
Ef-1 Non-successful	68	3 – 3,384	405 + 730	
Em-1 Successful	13	80 – 15,192	5,148 + 4,896	**W=146, p=0.012**
Em-1 Non-successful	39	3 – 12,600	2,276 + 3,175	
Em-3 Successful	7	38 – 4,752	1,598 + 1,792	**W=79, p=0.047**
Em-3 Non-successful	44	2 – 5,148	344 + 914	

* Alpha level set to p= 0.05

4.3.4.2 Occurrence of key behaviours in successful courtships

In order to determine whether specific behaviours were needed for mating success, I evaluated successful courtships for the key behaviours established above (Table 4.12). Male harassment, in which satellite males form coalitions with the aim of distracting the consort male, was removed from this analysis because it was not considered a direct action of the consort male, but rather a reaction to satellite males' behaviours. Mounting was also removed as it was impossible to have a successful courtship without mounting occurring.

Across all three groups, nearly every successful courtship contained mate-guarding by the male (Table 4.12). Some form of grooming was found in most *Ef-1* and *Em-1* courtships as well, although the predominance of grooming type differed. Female-initiated intersexual grooming was observed in two successful courtship in the *Em-1* population only. On average, successful *Ef-1* and *Em-3* courtships contained a combination of two key behaviours, while the successful *Em-1* courtships contained, on average, a combination of three behaviours. Due to the limited number of successful courtships in relation to unsuccessful courtships, further analyses were not possible.

Table 4.12 Percentage of successful courtships that contain specific key behaviours. N= total number of successful courtships in that population, while n= the number of successful courtships that contained the key behaviour.

	Mate-guarding	**All grooming types**	**Mutual grooming**	**♂ grooming ♀ only**	**♀ grooming ♂ only**	**Scent marking**	**♀ proceptive behaviour**
Ef-1 **(N=14)**	78.6% (n=11)	64.3% (n=9)	21.4% (n=3)	64.3% (n=9)	0.0% (n=0)	35.7% (n=5)	0.0% (n=0)
Em-1 **(N=13)**	92.3% (n=12)	92.3% (n=12)	46.2% (n=6)	46.2% (n=6)	15.4% (n=2)	30.8% (n=4)	30.8% (n=4)
Em-3 **(N=7)**	85.7% (n=6)	42.9% (n=3)	28.6% (n=2)	14.3% (n=1)	0.0% (n=0)	42.9% (n=3)	14.3% (n=1)

4.4 DISCUSSION

In this study, both *E. flavifrons* and *E. macaco* were found to have polygynandrous mating systems, as well as exhibit similar repertoires of sexual behaviours. Apart from female proceptivity, which was not seen in *E. flavifrons*, all mating behaviours observed in this study occurred in both species. Conspicuous differences were found between the populations regarding the observed frequency of specific behaviours and the mating tactics employed by the two sexes. Interestingly, the largest differences did not fall along species lines. Instead, the *Ef-1* (*E. flavifrons*, Ankarafa Forest) and *Em-3* populations (*E. macaco*, Lokobe National Park) were found to be similar to each other, while the *Em-1* population (*E. macaco*, Andranomatavy) tended to use different tactics to gain access to preferential mating partners. Even within a population, individuals of the same sex were observed using different strategies to access preferred partners. Cumulatively, these findings suggest that the mating tactics males and females employ can vary both between and within species, as well as within a population.

The variability in tactics observed in this study may have resulted from a combination of ecological and social conditions at each location (Clutton-Brock, 1989), including habitat quality and food availability, individual life histories, social ranking, demographics, dispersal and transfer patterns, and oestrus cycling. It has been proposed that intra-population variation in the mating tactics of one sex, referred to as alternative reproductive tactics, is the result of lower-quality individuals finding adaptive solutions to gain otherwise limited mating opportunities (Brockmann, 2001; Setchell, 2008). Many of these variables remain elusive within the scope of this study, confounding the ability to uncover the proximate mechanisms driving sexual selection and the resulting mating tactics at each location. In addition, there was no way to correlate mating success to reproductive success in this study. Paternity identification would have shed considerable insight into the effectiveness of the different observed mating tactics.

Regardless, the relationship between male and female tactics are intricate (Setchell & Kappeler, 2003), with the strategies employed by one sex influencing the counter-strategies of the other.

4.4.1 Sexual behaviour repertoire

Males and females of both species were observed mating with multiple partners, indicating that *E. flavifrons* and *E. macaco* have polygynandrous mating systems. Polygynandry is typical of taxa that live in multimale-multifemale societies (Dixson, 2012), although extra-male matings have been observed in species that are typically characterised as monogamous or polygynous (e.g. *Rhinopithecus roxellana*: Guo et al., 2010). Thirteen different behaviours, grouped according to their perceived function, were found to make up the repertoire of sexual interactions that occurred during mating. These included behaviours associated with sexual arousal, behaviours associated with copulation, and behaviours associated with the interruption of a mating event. When the sexual behaviour repertoires of *E. flavifrons* and *E. macaco* are compared to those of other lemurs, as well as haplorrhine species, there are striking similarities. During the mating season, sexual grooming of females by males, including the sniffing and inspecting of females' anogenital regions, has been observed in *Lemur catta* (Palagi et al., 2003), *Varecia variegata* (Pereira et al., 1988; Moreland, 1993), and *E. fulvus* (Colquhoun, 1987). Mate-guarding by central and/or high-ranking males is common in *Lemur catta* (Sauther, 1991), *Lophocebus albigena* (Arlet et al., 2008), and *Microcebus murinus* (Eberle & Kappeler, 2004). Male coalitions have been observed in *Macaca sylvanus* (Berghänel et al., 2011), while direct male-male competition occurs in *Lophocebus albigena* (Arlet et al., 2008), *E. fulvus* (Gachot-Neveu et al., 1999), and *Varecia variegata* (Moreland, 1993). Male-directed female agonistic behaviour, such as cuffing and chasing, is common in *Lemur catta* (Sauther, 1991) and *Varecia variegata* (Moreland, 1993). Coercive behaviours by males have been recorded in *Proithecus verreauxi* (Brockman, 1999), *Gorilla gorilla gorilla* (Breuer et al., 2016) and *Pan troglodytes schweinfurthii* (Feldblum et al., 2014). Female posturing, with females raising their anogenital region of the male and looking over their shoulder, occurs in *Lemur catta* (Sauther, 1991), while female solicitation takes a slightly different form in *Lagothrix poeppigii*, with head shaking and grinning being used to entice males (Di Fiore & Fleischer, 2005). Females have been observed mating with multiple partners in *Alouatta caraya* (Kowalewski & Garber, 2010) and *Propitheus verreauxi* (Brockman, 1999). Copulation often includes multiple bouts of intromission, separated by periods of rest (*Cheirogaleus major*: Petter-Rousseaux, 1964; *Varacia variegata*: Pereira et al., 1988; Moreland, 1993). Interruption of a mating pair by either juveniles and adult females has been observed in *Papio ursinus* (Huchard & Cowlishaw, 2011), *Lemur catta* (Jolly, 1967), and *Lagothrix poeppigii* (Di Fiore & Fleischer, 2005). This list, while in no way exhaustive, highlights the similarities in certain mating behaviours throughout the different primate taxa, although each species' repertoire is more nuanced than presented here. What is perhaps more interesting is the degrees to which *E. flavifrons* and *E. macaco* made use of these behaviours, and how these different mating tactics could increase the chances of mating success of males and females in each population.

4.4.2 Characterisation of the mating season

Unlike many catarrhine species (*Hylobates* spp.: Barelli et al., 2008; *Mandrillus sphinx*: Setchell & Wickings, 2004; *Pan troglodytes*: Emery & Whitten, 2003; *Papio cynocephalus*: Domb & Pagel, 2001), lemurs tend not to show conspicuous signs of sexual colouring and swelling to signal oestrus cycling. While the vaginas of several taxa of lemurs (*Microcebus murinus*, *Cheirogaleus major*: Petter-Rousseaux, 1964; *Varecia variegata*: Bogart et al., 1977a; Foerg, 1982) open only during oestrus, thus marking a pronounced change in anatomy, the vaginas of female *E. flavifrons* and *E. macaco* remain open throughout the year and exhibit very minimal changes during fertile periods (Bogart et al., 1977a). Because of this, the mating season in the studied populations was identified by the onset of sexual behaviours only. This included aggressive sexual grooming and sniffing of females' anogenital regions and attempted mountings (Colquhoun, 1987). In *Lemur catta*, anogenital sniffing and inspection is hypothesised to facilitate males' ability to determine the onset of female oestrus (Jolly, 1967), and is likely to serve the same role in *E. flavifrons* and *E. macaco*.

The reproductive period in this study lasted from 24 to 30 days between mid-April and mid-May 2015-2017 and was consistent with the previously reported mating seasons of both *E. flavifrons* (Volampeno et al., 2010, 2011b) and *E. macaco* (Petter-Rousseaux, 1964; Rasmussen, 1985; Bayart & Simmen, 2005), as well as other lemur species (Sauther, 1991; Wright, 1995). As predicted, females and males were observed mating with multiple partners during this time, suggesting a polygynandrous mating system for both species. The possibility remains that additional matings occurred at night and that non-conceptive matings could have occurred outside of the research window because the populations were not studied in the months leading up to or following the mating season.

Although female oestrus synchronisation was predicted in both species, it was impossible to visually confirm oestrus because of this lack in overt changes to *E. flavifrons* and *E. macaco* female anatomy (Bogart et al., 1977a). Nearly all reproductive behaviours in the *Em-3* population were found to occur between 23 April and 01 May. In the *Em-1* population there were two concentrated peaks, with one occurring 01-03 May and the second occurring 09-13 May. In comparison, courtships were spread out further in *Ef-1*, consistently occurring throughout much of April and May. Due to infrequent observation of *Ef-3* courtships, discerning patterns in this population's peak reproductive periods was not possible. Previous research on lemur reproductive behaviour suggests that the females of many taxa are only in oestrus for 1-4 days (Jolly, 1966; Bogart et al., 1977a; Foerg, 1982; Sauther, 1991; Moreland 1993; Wright, 1995). If this is the case for both *E. flavifrons* and *E. macaco*, then the fact that courtships were spread out further in the two *E. flavifrons* populations could suggest that the oestrus cycles of those females were less synchronised, while the narrower window of *E. macaco* courtships implies increased synchronisation. Variation in the degree of synchronicity between lemur populations is not uncommon. Within a single population of *Propithecus verreauxi* at Beza Mahafaly Special Reserves, females in one group were found to have synchronous oestrus cycles, while the oestrus cycles in a second group were asynchronous (Brockman & Whitten, 1996). Oestrus synchronisation can reduce a single male's ability to monopolise all reproductive females. This, in turn, increases the number of male partners a female can have, which can ultimately confuse paternity and reduce the chances of male infanticide (Dunbar, 1988; Dixson, 2012). In comparison, asynchronous oestrus cycling can be a mechanism to moderate levels of intra-female competition while also ensuring access to favoured males (Sauther, 1991). It is therefore possible that the observed variation in oestrus synchronisation is reflective of differing female mating strategies in the study populations.

4.4.3 Mating tactics used in the different populations

4.4.3.1 The Ef-1 and Em-3 populations

Mate choice was the predominate mating tactic employed by females in this study. Female mate choice is well documented in primates and is characterised as either rejecting, consenting, or initiating a courtship with a specific male (Janson, 1984; Sauther, 1991; Soltis et al., 1997; Jolly et al., 2000; Carosi & Visalberghi 2002). Although these mechanisms of selection were observed in this study, there were distinct differences in how females exercised their choice depending on the population in question. There were no instances of female proceptivity in *Ef-1* and only four instances in the *Em-3* population. Interestingly, during one instance in the *Em-3* population, the female was observed mounting the male from behind. This "reverse mounting" behaviour has been observed in tufted capuchins (*Cebus apella*: Carosi & Visalberghi, 2002) and is a proceptive behaviour that occurs after the male has already mounted the female (Janson, 1984). This behavioural sequence was true for the *Em-3* event as well. Intromission between the male, which appeared to be the dominant male, and the female had been observed prior to the reverse mounting. This female behaviour did not result in any additional copulations though. Instead, the male promptly moved away from the female and began pursuing a second female. As such, this reverse mounting behaviour may have been a form of female-female competition as opposed to a proceptive behaviour.

More often though, *Ef-1* and *Em-3* females were seen actively avoiding and/or rejecting the sexual advances of males. Upon approach, *Ef-1* and *Em-3* females responded aggressively, cuffing, biting, baring their teeth, or chasing away the male. Female avoidance also included lying down to block vaginal access. These forms of active rejection seem to be typical of female *Propithecus verreauxi* (Brockman, 1999), *Microcebus murinus* (Petter-Rousssseaux, 1964), *Lemur catta* (Sauther, 1991), and *Varecia variegate* as well (Moreland, 1993). In both of these populations, male coercion was pronounced, occurring in 60.5% of *Ef-1* courtships and 49% of *Em-3* courtships. During a coercive courtship, males approached and forcibly pinned and mounted a female. Females typically countered this type of aggressive behaviour by fighting back or frantically trying to flee, abruptly ending the courtship; often this behaviour was accompanied by high-pitched distress vocalisations. This interrelated pattern of behaviours between the coercive males and the rejecting females contributed to the short courtship durations (mean ± SD) observed in *Ef-1* and *Em-3* (670 ± 1,501 s and 509±1,132 s, respectively). Although infrequent, copulation via coercion was observed in both the *Ef-1* and *Em-3* populations. Similar coercive behaviours have been observed in *Propithecus verreauxi*, but hormonal assays showed that females were anoestrus during this time, leading researchers to conclude that coercion had no effect on males' reproductive success (Brockman, 1999). So, while coercion was at least a moderately effective method for achieving mating success for male *E. flavifrons* and *E. macaco*, the effectiveness of this tactic in terms of reproductive success, i.e. successful paternity, remains unclear. In both *Ef-1* and *Em-3* mate-guarding occurred infrequently (in 32.1% and 29.4% of courtships, respectively). As a result, there were few observed instances of male coalitions competing against a guarding male for female access (in 13.5% and 21.6% of courtships, respectively).

The observed sexual conflict in *Ef-1* and *Em-3* may have arisen from distinct differences in reproductive interests; when sexual interests do not align, males may try to coerce reluctant females into mating (Smuts & Smuts, 1993). There are several factors that may have contributed, either partially or wholly, to frequent female rejection and the use of sexual force by males. If *Ef-1* and *Em-3* males were attempting to copulate with females during anoestrus periods, female refusal may be common. Non-oestrus female *Lemur catta* often reject male mating solicitations either with vocalisations (Koyama, 1988) or aggressive behavioural responses (Sauther, 1991). These responses are similar to the rejections observed in the *Ef-1* and *Em-3* populations. Recently, it was demonstrated that male *Papio ursinus* who aggressively target females in the days prior to oestrus cycling were more likely to monopolise those females during reproductive periods (Baniel et al., 2017). Similarly, although aggression during periods of female swelling increased copulatory events in male *Pan troglodytes schweinfurthii*, only those males that were aggressive outside of this period increased their paternity rates; this finding was particularly true for high-ranking males, suggesting that long-term coercion can be a successful strategy (Feldblum et al., 2014). It therefore may be possible that *Ef-1* and *Em-3* males were using aggressive sexual behaviours during anoestrus periods as an intimidation strategy to later gain access to an oestrus female. This may be particularly true for the *Ef-1* population where mating behaviours occurred over a longer period and where instances of female rejection were more common. To better identify the role of oestrus cycling in the sexual behaviours of male and female *E. flavifrons* and *E. macaco*, hormonal data should be considered. Endocrinological assessment has been used in several reproductive studies of *Pan troglodytes* to better understand and predict fertile periods, copulatory behaviour, conception, and aging in females (Emery & Whitten, 2003; Thompson, 2005; Videan et al., 2006). This type of methodology would provide the data needed to determine how oestrus timing, female rejection/consent, and coercive behaviours are linked in *E. flavifrons* and *E. macaco*.

The ratio of potential male mating partners to potential female partners, termed here as MPR, may have influenced the sexual behaviours of these lemurs by increasing levels of intrasexual competition. The MPR was male-biased in the *Ef-1* and *Em-3* populations, suggesting that females had more than one partner to choose from. On several occasions, persistent males were observed ultimately mating with the sought-after female. In these instances, it is possible that the female no longer had the energy to reject the male. Another possibility is that *Ef-1* and *Em-3* females used the persistence and stamina required to maintain coercive behaviours as a measure of male fitness; those males able to withstand continual rejection could be deemed higher-quality. This hypothesis has been suggested to explain heightened levels of male coercion and male-directed aggression by females during the mating season in other lemur taxa (*Propithecus verreauxi*: Brockman, 1999; *Varecia variegata*: Moreland, 1993a). After observing *Varecia variegata* during the mating season, Foerg (1982) concluded that female aggression served to monitor male fitness; those males that were able to withstand assault were more likely to be chosen as a mating partner. If this was the case, coercive behaviours could be seen as a form of endurance rivalry, in which certain males were able to energetically out-compete rivals for access to reproductive females (Andersson, 1994). Endurance rivalry is known to contribute to male mating success in rhesus macaques (*Macaca mulatta*) when competition is high; those individuals that were able to access oestrus females for longer periods sired more offspring (Bercovitch, 1997). Therefore, this may be an approach to showcase fitness in these lemur populations, with forceful persistence used as a method by males to non-aggressively out-compete rivals while also allowing females to select for higher-quality males.

It is also likely that female choice against unattractive mates drove lower-quality males to use force to guarantee copulation opportunities (*Pan troglodytes*: Muller et al., 2011; *Pongo pygmaeus*: Knott et al., 2009; *Propithecus verreauxi*: Brockman, 1999). In *Lemur catta*, females try to solicit matings by presenting to specific males. In such cases, less preferred males (i.e. those not presented to) often attempt to force copulations with these females, which can increase their mating success as compared to more preferred males (Parga & Henry, 2008). In *Pongo pygmaeus*, female preference leads some females to reject a potential partner, even if no other option exists (Utami Atmoko et al., 2008). In such instances, coercion by rejected males also has been observed (Knott et al., 2009). Counter to this though, Soltis & colleagues (1997) found that in *Macaca fuscata*, female choice for preferred males increased their time spent in proximity, resulting in higher levels of coercion by those preferred males. Thus, coercion was a by-product of choosing a more attractive male.

Although difficult to quantify what constituted an attractive partner in this study, it is conceivable that genetic relatedness contributed to mate choice. Inbreeding avoidance is common in primates. Female *Varecia variegata* and *Lemur catta* are known to avoid mating with their sons and will respond aggressively to their approaches (Pereira et al., 1988). Because inbreeding can affect the populations' overall reproductive fitness (Pusey & Wolf, 1996; Duthie & Reid, 2015), primates have developed strategies to avoid mating with closely-related individuals. During the mating season, the olfactory secretions of male *Lemur catta* convey genetic signals, which may facilitate female choice for more fit mates while also avoiding relatives (Charpentier et al., 2008; Boulet et al., 2009; Charpentier et al., 2010). In *Propithecus edwardsi*, non-natal males were found to scent mark at higher rates than natal males (Pochron et al., 2005), potentially conveying information regarding not only their natal status, but also their level of relatedness. Dispersal and/or transfer from natal groups has been documented in *Cebus capucinus* (Muniz et al., 2006), *Pan troglodytes* (Pusey, 1980), *Papio anubis* (Packer, 1979b) and *Presbytis thomasi* (Sterck et al., 2005), although there is debate to whether this is an inbreeding avoidance mechanism or an way to avoid intrasexual aggression (Pusey, 1990). In the *Ef-1* and *Em-3* populations, habitat fragmentation, edge effects, and anthropogenic disruptions have resulted in small fragments with few remaining corridors of connected forest patches; dispersal and transfer from natal groups may be difficult in these populations (Prodger, 2015). Therefore, *Ef-1* and *Em-3* females may have been refusing particular males based on close kinship in an attempt to circumvent inbreeding. In this scenario, non-related partner options might have been so limited that males were forced to use aggressive tactics in order to have any chance at mating success (Duthie & Reid, 2015).

Social hierarchies may influence mate choice in these populations as well. Although adult female *E. flavifrons* and *E. macaco* are dominant to males (Koenders, 1989; Colquhoun, 1993; Digby & Kahlenberg, 2002; Bayart & Simmen, 2005; Digby & Stevens, 2007), juvenile females have lower social ranks than adult males (Prodger, 2015). In *Lemur catta*, which are also characterised by female dominance (Jolly, 1966), lower-quality males employ coercion as a strategy when attempting to copulate with younger females (Parga & Henry, 2008). Therefore, adult females in the *Ef-1* and *Em-3* populations may have also refused males based on social rank, with preference given to more high-ranking individuals. This, in turn, could have resulted in the less-preferred males trying to force copulations with subadult or juvenile females before those females became fully dominant to the males. Such research demonstrates that coercive behaviours can be caused and motivated by very different social situations. Long-term information on demographics, life histories, and social relationships of group members is needed to identify the proximate causes of female rejection and male coercion in these populations.

Satellite adult and juvenile females, and very rarely juvenile males, were observed directly interfering in coercive *Ef-1* and *Em-3* courtships. During events where the reproductive female was actively trying to flee forced copulation, satellite females sat on the male's back and pulled, bit, and hit him. Although the male never countered with aggression, he always ended up dismounting and moving away from the reproductive female. After a male disengaged, the satellite female would often groom the reproductive female, presumably either to relieve stress (Aureli & Yates, 2010) and/or reinforce social bonds

(Dunbar, 1988). Satellite females were only observed interrupting coercive courtships and these females always directed their aggression towards the coercive male and never the distressed female. Because of this, this behaviour was deemed distinctly different from the targeted female-female aggression previously reported in *Eulemur* species (Vick & Pereira, 1989; Digby, 1999; Randriatahina & Roeder, 2013) as well as other primate taxa (*Lagothrix poeppigii*: Di Fiore & Fleischer, 2005; *Presbytis entellus*: Sommer, 1989; *Rhinopithecus roxellana*: Li & Zhao, 2007). Classic targeted aggression, such as that observed in *E. fulvus* and *E. albifrons*, is known to be particularly intense, can last for several weeks, and often results in the expulsion of the targeted female from the group. This type of behaviour is hypothesised to reduce conception possibilities of rival females during the mating season (Vick & Pereira, 1989). Although female coalitions formed to defend food sources or against aggression are common among primates (*Cebus capucinus*: Perry, 1996; *Pan troglodytes*: Newton-Fisher, 2006; *Papio cynocephalus*: Silk et al., 2004; *Saimiri sciureus*: Mitchell et al., 1991), efforts aimed at defending reproductive females from unsolicited and aggressive courtships have been reported less frequently (*Pan troglodytes*: de Waal, 1998; for review, see Smuts & Smuts, 1993). Gouzoules (1974) suggested that harassment by adult female *Macaco arctoides* may distract males from aggressively attacking female mating partners, although this hypothesis is contested (Niemeyer & Chamove, 1983). Interestingly, in the Ankarafa population (*Ef-1*), *E. flavifrons* females were found to have strong socio-positive bonds outside of the mating season (Prodger, 2015). Perhaps it is these friendships that were called upon during instances of forced copulation to help defend the coerced female. In addition, related individuals may come to the defence of the reproductive female, especially if she is their mother, such as with *Presbytis johnii* (Poirier, 1970). Given that juvenile females and males were also observed interrupting these forced copulations, kinship ties may play a role in the decision to interfere as well.

4.4.3.2 The Em-1 population

In comparison, the mating tactics of the *Em-1* population could be characterised by higher levels of reciprocity and coordination between male and female mating partners, consistent with some definitions of consortships in other species (for review of species forming consortships see Manson, 1997 or Dixson, 2012). Female receptivity, in which a female accepted a male's advances, was more pronounced in the *Em-1* population than the other two populations. This was evident not only in fewer instances of female aggression during a courtship (23 instances over the course of the mating season), but also in the lengths of those interactions. Courtships lasted significantly longer in the *Em-1* population (mean ± SD: 3,044 ± 3,885 s) than either the *Ef-1* (670 ± 1,501 s) or *Em-3* (509 ± 1,132 s) populations despite there also being a surplus of potential male partners. Although coercion was observed in the *Em-1* population (23.6% of the events), it occurred significantly less often than the other populations (*Ef-1*: 60.5% and *Em-3*: 49%). Instead, mate-guarding, in which one male followed and blocked another male's access to a reproductive female, was frequently observed in the *Em-1* population (in 59.6% of the courtships). Male mate-guarding of reproductive females is often associated with multimale-multifemale societies (Clutton-Brock & Harvey, 1977, 1978) and is known to be intense in lemurs (Sauther, 1991; Curtis & Zaramody, 1999; Wright, 1999; Eberle & Kappeler, 2004b). In *Em-1*, where female consent was more common, females were also more likely to actively participate in the mate-guarding. When travelling, the reproductive female would often wait for the guarding male to catch up before continuing to move. Females were also observed grunting to vocally alert the guarding male of an approaching satellite male.

Male scent marking on or near a reproductive female occurred significantly more often in the *Em-1* population during this time as well (51.9% of courtships). Typically, males only marked females with their anogenital region, while they would also mark substrates, such as tree limbs, with their hands. Scent marking is a hallmark of lemurs, especially during the mating season (*E. fulvus* and *Microcebus murinus*: Petter-Rousseaux, 1999; *Lemur catta*: Asa, 2008; *Propithecus edwardsi*: Pochron et al., 2005). Olfactory signals in the *Eulemur* clade are chemically complex and not only differ by species, but also by sex and season; based on this, they are hypothesised to aid in, among other things, reproductive advertisement (DelBarco-Trillo et al., 2012). With increased instances of mate-guarding and scent marking, there were also more occurrences of satellite male coalitions. They were typically formed by two to eight males and aimed to divert the guarding male's attention and gain access to the reproductive female. This alternative mating tactic (Setchell, 2008) is found in primates that live in a multimale society where mate-guarding by a dominant male is common (*Macaca sylvanus*: Bissonnette et al., 2011; *Theropithecus gelada*: le Roux et al., 2013). Surreptitious copulations by harassing males, presumably of a lesser-quality, occurred occasionally in this population, as well as *Ef-1* and *Em-3*, suggesting that these sneak matings may be an effective method to gain access to reproductive females when monopolisation by one male occurs.

The high levels of reciprocity and coordination observed during *Em-1* courtships suggest reduced levels of sexual conflict between the sexes during the mating season as compared to the other two populations. As such, the reproductive interests of males and females are more likely to be aligned in this population. Possessive tactics are indicative of male choice for attractive females, which can be based on female fertility, social rank, or ability to produce viable offspring (Setchell & Wickings, 2006). Mate-guarding is often closely tied to dominance, which can be an important factor in the reproductive success of male primates (Colquhoun, 1987; Cowlishaw & Dunbar, 1991; Sauther, 1991; Gachot-Neveu et al., 1999; Lynch Alfaro, 2005; Arlet et al., 2008; Kappeler & Port, 2008; Izar et al., 2009). To date, our understanding of the social hierarchies of male *E. macaco* is limited, although Prodger (2015) found that male hierarchies exist in *E. flavifrons*. It is therefore plausible that male dominance is tied to ability to guard in these populations. Given the proactive involvement of females in their own guarding (i.e. alerting the guarding male of approaching satellite males and coordinating travel), it is conceivable that female partner choice is related to male dominance in *Em-1*. In a species where females are generally considered the dominant sex, it seems unlikely that selection of a dominant male would provide increased access to food (although the opposite has been suggested in *Eulemur rufus* by Overdorff, 1998) or would contribute significantly to offspring care (Volampeno, 2009). Rather, if females were selecting males based on social rank, it could be for advantageous genes (Emlen & Oring 1877; Lynch Alfaro, 2005; Arlet et al., 2008) or as a method to prevent infanticide from other males (van Schaik & Dunbar, 1990; van Schaik & Kappeler, 1993, 1997; Opie et al., 2013).

The possibility also exists that males and females in this population develop intersexual friendships or pair-bonds, with mating preference given to those partners. Formed during the mating season, male-female associations in *Papio hamadryas ursinus* (Moscovice et al., 2010) and in *Macaca assamensis* (Ostner et al., 2013) have resulted in higher rates of mating success, suggesting that such friendships may have important consequences for the reproductive fitness of involved individuals. In several *Eulemur* species, central males and pair-bonded males have been found to mate with females first and more often (*E. rufus*: Pereira & McGlynn, 1997; Overdorff, 1998; Ostner & Kappeler, 1999; *E. mongoz*: Curtis & Zaramody, 1999). As identified in Chapter 3, the lemurs in the *Em-1* population spent considerable time in small subgroups of 2-3 individuals, with intersexual dyads occurring frequently. This population also resides in the largest study location experiencing the lowest levels of habitat disturbance and anthropogenic threats. The relationship between primate mating systems and ecological conditions is well studied, with the trend towards monogamy thought to be related to a decrease in home range overlap and feeding competition among females (Lukas & Clutton-Brock, 2014; Kappeler, 2014), and/or increased risk of male infanticide (van Schaik & Dunbar, 1990; van Schaik & Kappeler, 1997; Opie et al., 2013). While no published behavioural or ecological data from outside the mating season exist for this population, it is plausible that the observed reciprocity in *Em-1* is influenced by the quality of their local environment or is reflective of key intersexual friendships that were established prior to the mating season. If this was the case, guarding could be a tactic used to ensure mating success with a preferred partner. Scent marking, which was also more pronounced in this population of *E. macaco*, has been suggested not only to be a form of indirect male mating competition (*Lemur catta* and *E. rufus*: Gould & Overdorff, 2002), but also a method for strengthening bonds during the mating season (*E. rubriventer*: Overdorff & Tecot, 2006). Although scent marking was not found to be predictive of mating success in the study, this behaviour undeniably was serving some communicatory function, whether simply conveying levels of elevated excitement or signalling to rivals a male's claim on a particular female. Although too preliminary to derive any comprehensive conclusions regarding whether this population of *E. macaco* do in fact form intersexual friendships that result in preferential mate partners, this observed tendency towards more consensual courtships warrants further investigation.

4.4.3.3 Successful courtships

Despite the differences in tactics used, success rate of courtships in this study were low for all the populations. Only 13.7% of *Em-3* courtships, 17.3% of *Ef-1* courtships, and 25% of *Em-1* courtships were found to result in intromission, although this number could be under-representative because determining whether intromission occurred was not always visually possible. Unsurprisingly, durations of successful courtships were longer and contained more behavioural interactions than unsuccessful courtships for all populations. Although it is feasible that successful courtships required more complex behavioural interactions directly related to mating, it is more likely that increased levels of reciprocity increased courtship duration, which increased the number of behaviours that could occur during this time. Sequence analysis did not reveal any one behaviour that was predictive of successful courtship, although mate-guarding was the behaviour present most often. Over 90% of successful *Em-1* courtships contained mate-guarding, while this behaviour was found in approximately 86% of *Em-3* and 79% of *Ef-1* successful courtships. Males that guard and form consorts with reproductive females have been found to have higher mating and reproductive success rates than those males that did not (*Alouatta pigra*: Van Belle et al., 2009; *Macaca* spp.: Bercovitch, 1997; Engelhardt et al., 2006; *Mandrillus sphinx*: Setchell et al., 2005; *Propithecus verreauxi*: Mass et al., 2009). Therefore, mate-guarding may be one of the most important behavioural tactics that *E. flavifrons* and *E. macaco* males can employ to ensure both mating and reproductive success. It is also interesting that female presenting was not associated with mating success in this study. Often, males ignored or moved away from females that presented to them, especially if this behaviour occurred while they were pursuing a different female. In such instances, proceptivity could potentially be seen as a form of female-female competition for attractive males. Although this behaviour was observed only in the *E. macaco* populations, it seems unlikely that it represents a species-level difference in their behavioural repertoire as female presenting has been observed in a number of other lemur taxa (*Lemur catta*: Sauther, 1999; *Mirza coquereli*: Stranger et al., 1995; *Propithecus verreauxi*: Brockman, 1999). Presenting easily could have occurred in the *Ef-1* population when lemurs were out of sight.

4.4.4 Implications for species hybridisation

This study found that neither the timing of the mating seasons nor the sexual behaviours of the two species, as identified in the study populations, are different enough to serve as strong pre-mating isolation barriers between the taxa. Based on the fact that the different mating tactics employed by the sexes were more likely to be caused by local social or ecological conditions as opposed to species-specific differences, it is feasible that if a contact zone did exist, or still does exist, hybridisation could occur between the two taxa. This conclusion is further supported by recordings of hybrid individuals resulting from captive *E. flavifrons* x *E. macaco* matings (when they were still housed together prior to their classification as separate species; Rabarivola et al., 1991). Although the fertility of these hybrid individuals is unknown, the presence of F1 offspring implies that mechanical or gametic incompatibility does not exist between the species. Additionally, reports of morphological intermediates from the Manongarivo Special Reserve (MSR; Meyers et al., 1989; Rabarivola et al., 1991; Goodman & Schütz, 2000; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004) provide support, albeit limited, of hybridisation in the wild. Despite multiple attempts, I was unable to locate an area of contact between the two species during this study and the presence of a third closely-related congeneric species in MSR further complicates previous *E. flavifrons* x *E. macaco* hybrid claims (*E. fulvus*, see Chapter 2 and Goodman & Schütz, 2000). Despite this, these findings provide heuristic support for the *possibility* of hybridisation due to a lack of divergent mating systems. Further work should consider the importance of olfactory signals (Harrington, 1976, 1977; delBarco-Trillo et al., 2012) and morphological variation in both species' recognition and assortative mating.

Chapter 5

A Comparison of *Eulemur flavifrons* and *E. macaco* Vocal Communication During the Mating Season

ABSTRACT

Acoustic studies are an important non-invasive method for assessing phylogenetic relationships of closely-related species, with the divergence of acoustic signals in primates providing support for the classification of distinct taxa. To address the role that acoustic communication plays in species identification as well as reproductive isolation of two genetically distinct species of lemurs, the vocal communication of the blue-eyed black lemur (*Eulemur flavifrons)* and the black lemur (*E. macaco*) were assessed. Spectral and temporal features from 862 vocalisations, recorded at two *E. flavifrons* locations (*Ef-1* and *Ef-3* populations) and three *E. macaco* locations (*Em-1*, *Em-2*, and *Em-3* populations), were measured and compared. This resulted in the identification of 15 distinct call types, three of which had not previously been reported in these two species—chatter, modulated squeals, and tonal-grunt calls. Classical stepwise discriminant function analysis (DFA) with leave-one-out cross validation was performed to test whether each population's calls are distinct and to identify the acoustic predictor variables that maximise population and/or species differences. Although the sample size of many of the call types was too small for statistical analyses, calls from two frequently occurring categories, grunts and alarm calls, revealed no clear or consistent species-level differences. This lack of overt divergence in the vocalisations of *E. flavifrons* and *E. macaco* has distinct implications for our current understanding of the role of vocal communication in the speciation of these two taxa and lends acoustic support to their putative natural hybridisation.

5.1 INTRODUCTION

Communication refers to the production of a signal and the transfer of that signal from the signaller to the receiver (Bradbury & Vehrencamp, 1998), with the receiver gathering information not only from the signal itself but also from the context that resulted in the signal's production (Kershenbaum et al., 2016). This 'production-transmission-reception' paradigm serves as the foundation for studying and interpreting communication in social animals such as primates. Although a variety of different modalities are employed to communicate with conspecifics, including visual, olfactory, and tactile, auditory communication in primates remains one of the most widely studied.

5.1.1 Sound production and acoustic communication

Much of primate acoustic research is grounded in the source-filter theory of sound production (Fant, 1960). Bursts of air deriving from the lungs pass through opening and closing vocal folds (glottis) at the larynx. The rate at which airflow oscillates through the glottis produces the fundamental frequency of that phonation. These tonal sounds have a clear harmonic structure, with energy peaks not only at the fundamental frequency (F0), but also at integer multiples of F0. In contrast, noisy sounds, which lack clear harmonic structure, are produced by a 'rapid release' of air. This results in sounds without clearly defined energy peaks; instead there is similar energy across all the frequencies. The production of sound stemming from the lungs and larynx is known as the source signal. This signal then travels through the vocal tract to leave the body. Operating as a filter, the vocal tract moderates the sound by removing specific frequencies and amplifying others. Frequencies that have maximum energy are known as formants (Fant, 1960; Gamba & Giacoma, 2005). This physiological structure in primates allows the production of both noisy and tonal sounds, as well as gradations between these (Fitch & Hauser, 1995; Kershenbaum et al., 2016).

An acoustic element represents the fundamental unit of a vocalisation (Kershenbaum et al., 2016); multiple elements can be strung together to create sequences or compound calls. Spectral analysis of vocalisations allows the identification of vocalisations that may sound the same to an observer, but differ structurally (Snowdon, 1982). Such analysis of the structural differences of primate vocalisations was first conducted on *Macaca mulatta* (Rowell & Hinde, 1962) and focused on fundamental frequency and pitch, perhaps to the detriment of sounds that are filtered; such a narrow focus could restrict researchers' ability to discriminate between meaningful differences, thus limiting the perceived size of the vocal repertoire (Fitch & Hauser, 1995). Now researchers readily examine duration, formants, syntax, fundamental frequencies, pitch contours, pulses, and nonlinearities in vocalisations (Robinson, 1979; Macedonia & Taylor, 1985; Fitch et al., 2002; Arnold & Zuberbühler, 2006).

Once a sound is produced, it must reach the receiver; anything that constrains or disrupts a signal can threaten its effectiveness. Habitats influence and distort sound propagation of primate calls. Surrounding noise, ambient temperature, attenuation of calls, and degradation due to reverberation and turbulence all impact how far the calls will be heard (Wiley & Richards, 1978; Waser & Brown, 1986; Bradbury & Vehrencamp, 1998). In tropical rainforests, calls originating in the canopy with low frequency ranges (200-1000 Hz) propagate the best and have the lowest attenuation rates, while calls with higher frequencies tend to serve better as short-range signals (Waser & Brown, 1984).

The final step in effective auditory communication is the perception and interpretation of the signal (Wiley & Richards, 1978). Selective pressures have resulted in the evolution of a hearing structure in primates, the ear, that allows the detection of a range of frequencies, amplitudes and temporal changes, as well as the ability to localise the origination of the sound (Heffner, 2004). Answering the question of what is being conveyed through these signals can be complicated. The conservative approach to extracting meaning from vocalisations is to hypothesise that the calls serve to affect the behaviours of those receiving the signal, without encoding specific contextual information (Owren & Rendall, 2001; Rendall et al., 2009). This stems from the fact that many vocalisations contain acoustic features—such as striking fluctuations in frequency and intensity or calls with noisy structures—that result in an immediate nervous system response (Owen & Rendall, 2001; Rendall et al., 2009).

5.1.2 Primate acoustic communication

There is now widespread evidence from studies looking at the acoustic structure of calls (Semple, 2001), call-behavioural responses (Gosset et al., 2001), and playback experiments (Seyfarth et al., 1980; Fichtel & van Schaik, 2006) to suggest that primates can also understand information related to caller identity and specific social and environmental situations (for a review, see Seyfarth et al., 2010). Long-distance loud calls can serve to alert conspecifics of potential dangers. These calls can be functionally referential and convey information regarding predator type (Fichtel & Kappeler, 2002; Arnold & Zuberbühler, 2006; Kirchhof & Hammerschmidt, 2006; Cäsar et al., 2013) or deter predator advancement (Zuberbühler et al., 1997; Zuberbühler et al., 1999). Loud calls can also be used in territory defence and intergroup spacing (Waser, 1975; da Cunha & Byrne, 2006; Rasoloharijaona et al., 2006) because a signaller advertises its presence and location (Busnel, 1977), conveying information regarding the distance between the signaller and the receiver (Snowdon & Hodun, 1981; Oda, 1996; Rendall et al., 2000). Contact calls, which typically have lower frequencies and amplitudes, are important in maintaining group cohesion (Braune et al., 2005; Digweed et al., 2007), fostering social bonds (Rendall et al., 2000), and conveying functionally referential information regarding food sources (Watson et al., 2015). Sexual advertisement calls and duetting can be used to attract mates (Geissman & Orgeldinger, 2000; Delgado, 2006), strengthen pair bonds (Geissmann, 1986; Méndez-Cárdenas & Zimmermann, 2009), or discourage competitors (Masters, 1991; Steenbeek & Assink, 1998). The syntax of call sequences has also been demonstrated to convey semantic information (Zuberbühler, 2002; Arnold & Zuberbühler, 2006; Cäsar et al., 2013).

Quantitative and qualitative analyses of primate vocal repertoires have been useful in assessing phylogenetic relationships (Struhsaker, 1981; Zimmermann, 1990; Bearder et al., 1995), especially of unhabituated and/or Critically Endangered individuals. Due to the relative stability of acoustic elements (Kummer, 1970; Owren et al., 1993), direct comparisons between individuals, populations, and species are possible. Such work has identified acoustic differences in populations of *Varecia* spp. (Macedonia & Taylor, 1985), *Galago* spp. (Ambrose, 2003), *Lepilemur* spp. (Méndez-Cárdenas et al., 2008), *Presbytis* spp. (Meyer et al., 2012), *Pongo* spp. (Ross & Geissmann, 2007), and *Microcebus* spp. (Zimmermann, 2016), lending to the classification of and support for new species and subspecies.

Divergence of acoustic signals between closely-related species is generally thought to be driven by ecological variation or differing sexual preferences, although a combination of the two mechanisms or drift may also contribute to the phenomenon (Wilkins et al., 2013). Ecological differences can result in the natural selection of signals that are best suited to propagate in that particular environment (Waser & Brown, 1986; Brown & Waser, 1988; Mitani et al., 1999; Braune et al., 2008). Alternatively, sexual selection can result in diverging preferences for specific acoustic signals or the intensity of selection; an outcome of this can be vocal repertoires that are species-specific and serve as a pre-mating isolation mechanism between sympatric or parapatric species (Mayr, 1963; Wilkins et al., 2013). In areas where insufficient barriers exist to exclude interbreeding, the convergence of acoustic characters and adoption of parental characters has been observed (*Hylobates* spp.: Tenaza, 1985; *Presbytis* spp.: Hohmann, 1988; *Cercopithecus* x *Chlorocebus*: de Jong & Butynski, 2010).

The use of acoustic studies to assess the phylogenetic relationship between members of the *Eulemur* genus is of value (Petter & Charles-Dominique, 1979; Macedonia & Stranger, 1994). *Eulemur* are found throughout Madagascar (Ossi & Kamilar, 2006; Mittermeier et al., 2010; Blair et al., 2013), and while most species now exist in allopatry, there remain areas of possible contact between several taxa (Mittermeier et al., 2008). As a result, the genus has been the focus of numerous behavioural ecology (Overdorff, 1993, 1996; Roeder et al., 2002; Cooper & Hosey, 2003; Johnson et al., 2005; Ossi & Kamilar, 2006; Jacobs et al., 2011; Blair et al., 2013) and genomic studies (Wyner et al., 1999; Yoder & Irwin, 1999; Pastorini et al., 2000; Wyner et al., 2002; Johnson et al., 2008) all contributing to identification of the individual taxa.

5.1.3 *E. flavifrons* and *E. macaco vocalisations*

Of particular interest within the *Eulemur* genus is the murky relationship between the blue-eyed black lemur (*Eulemur flavifrons*) and the closely-related black lemur (*Eulemur macaco*). Initially considered morphological variants of the same species (Koenders et al., 1985), and later different subspecies (Fausser et al., 2000), *E. flavifrons* and *E. macaco* are now perceived as distinct taxa (Mittermeier et al., 2008). While this classification has genetic support (Pastorini et al., 2000), this matter is complicated by reports of hybridisation occurring in areas of plausible range overlap (Meyers et al., 1989; Rabarivola et al., 1991; Goodman & Schütz, 2000; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004; Schwitzer & Lork, 2004). Any sort of comprehensive ethological comparisons to support their unique taxonomic status though, including studies of their acoustic communication, are lacking.

Early analysis of *E. flavifrons* and *E. macaco* vocalisations proved difficult in classifying distinct call types. Calls were categorised based on the context in which they were uttered (threat, aggression, etc.), resulting in multiple call types being grouped together (Petter & Charles-Dominique, 1979). Macedonia & Stranger (1994) identified eight call types in captive *E. macaco*, while Gosset & colleagues (2001) characterised 16 different call types. While these types of analyses are useful to understand call context and emotional states of individuals, they do not fully take into consideration possible structural differences in calls. Both Pozzi & colleagues (2010) and Gamba & colleagues (2015) used discriminant function analyses for *a priori* classification of the two species' vocal repertoires and showed that at least 10 calls within each species' repertoire were structurally and/or temporally unique.

E. flavifrons and *E. macaco* vocalisations are a combination of low-pitched grunts, tonal calls, and loud calls (Gamba et al., 2015). Low-pitched calls with a chaotic, or noisy, structure typically have easily recognisable formant bands, although the F0 and harmonics can be very difficult to calculate (Gamba & Giacoma, 2005). Low pitch tonal calls, which are typically given in close contact to conspecifics, display a clear fundamental frequency and harmonic structure (Macedonia & Stranger, 1994). High-pitch tonal calls that fluctuate drastically in frequency occurred in stressful situations (Petter & Charles-Dominique, 1975). Loud calls, which serve to alert conspecifics to aerial predators, space groups, advertise, etc., tend to be both harsh and noisy. In instances of heightened arousal, all *Eulemur* species emit a call that is a combination of a low-pitched grunt and tonal call (Gamba & Giacoma, 2005).

5.1.4 Aims, hypotheses, and predictions

Although vocal repertoires of *E. flavifrons* and *E. macaco* have been published, these studies have focused mainly on discrimination of call types from captive populations and have not thoroughly assessed vocalisations for interspecific or intraspecific variation. Only one study by Gamba & Giacoma (2008) has made direct comparisons of these species' vocalisations, focusing simply on long grunts. A widespread comparison of multiple call types across several populations remains an important step in addressing the role that acoustic communication can play in species identification for *E. flavifrons* and *E. macaco* (Fichtel, 2014). Therefore, the goals of this study were (1) to add to the known vocal repertoire by contributing any newly characterised calls from wild populations during the mating season, (2) to identify those acoustic variables that are typical for each taxon, and (3) to determine whether there exists significant variation in these variables so that calls may function as a pre-mating barrier or reinforce assortative mating between the two taxa.

Because vocalisations are an innate feature in primate species and remain highly conserved through generations (Winter et al., 1973; Geissmann, 1984; Macedonia & Stranger, 1994; Seyfarth & Cheney, 1997) and because species that are closely-related typically produce calls with similar signalling functionality (Macedonia & Taylor, 1985; Fichtel & van Schaik, 2006; Fichtel, 2014), I hypothesised that there would be no significant differences in the vocal repertoires of *E. flavifrons* and *E. macaco*. As such, I predicted I would observe the same call types in all five of my sampled populations.

I also hypothesised that if certain calls are important for species recognition and/or to attract/locate mates, those specific vocalisations would show significant variation of variables when comparing between the two species. Although currently there are no data on the vocalisations that occur within the mating context, grunts are hypothesised to be species-specific and convey the identity of the vocaliser in *Eulemur* species (Gosset et al., 2001; Gamba & Giacoma, 2008; Gamba et al., 2012a, 2012b). I therefore predicted that there would be significant differences in the duration, fundamental frequency, and/or formant frequencies of grunt calls in the *E. flavifrons* populations as compared to the *E. macaco* populations. Similarly, the acoustic structure of long-distance loud calls has been found to differ significantly between closely-related primate species (Macedonia & Taylor, 1985; Mitani & Gros-Louis, 1995; Braune et al., 2008; Méndez-Cárdenas et al., 2008; Thinh et al., 2011). I therefore hypothesised that the loud alarm calls of *E. flavifrons* and *E. macaco* would also be species-specific. Differences were predicted in both the spectral and temporal features of these call types.

5.2 METHODS

5.2.1 Locations, study animals, and collection effort

Vocalisations were recorded from two populations of *E. flavifrons* (*Ef-1* and *Ef-3*) and three populations of *E. macaco* (*Em-1*, *Em-2*, and *Em-3*) across the 2015-2017 mating seasons (Figure 5.1). Specifically, 14 April – 29 May 2015 and 07 July – 11 July 2016, calls were recorded from the *Ef-1* population, while calls from the *Ef-3* population were recorded 11 April – 16 May 2017. Acoustic data collection took place from 4 April – 30 May 2016 for the *Em-1* population and 21 June – 3 July 2016 for the *Em-2* population. From 18 April – 26 May 2017 the *Em-3* population was monitored.

For further details regarding the locations, see Chapter 1: Introduction and General Methodology. Details of various aspects of each populations' social system can be found in Chapter 3: Social Organisation and Structure (see Table 3.4 for group size and composition).

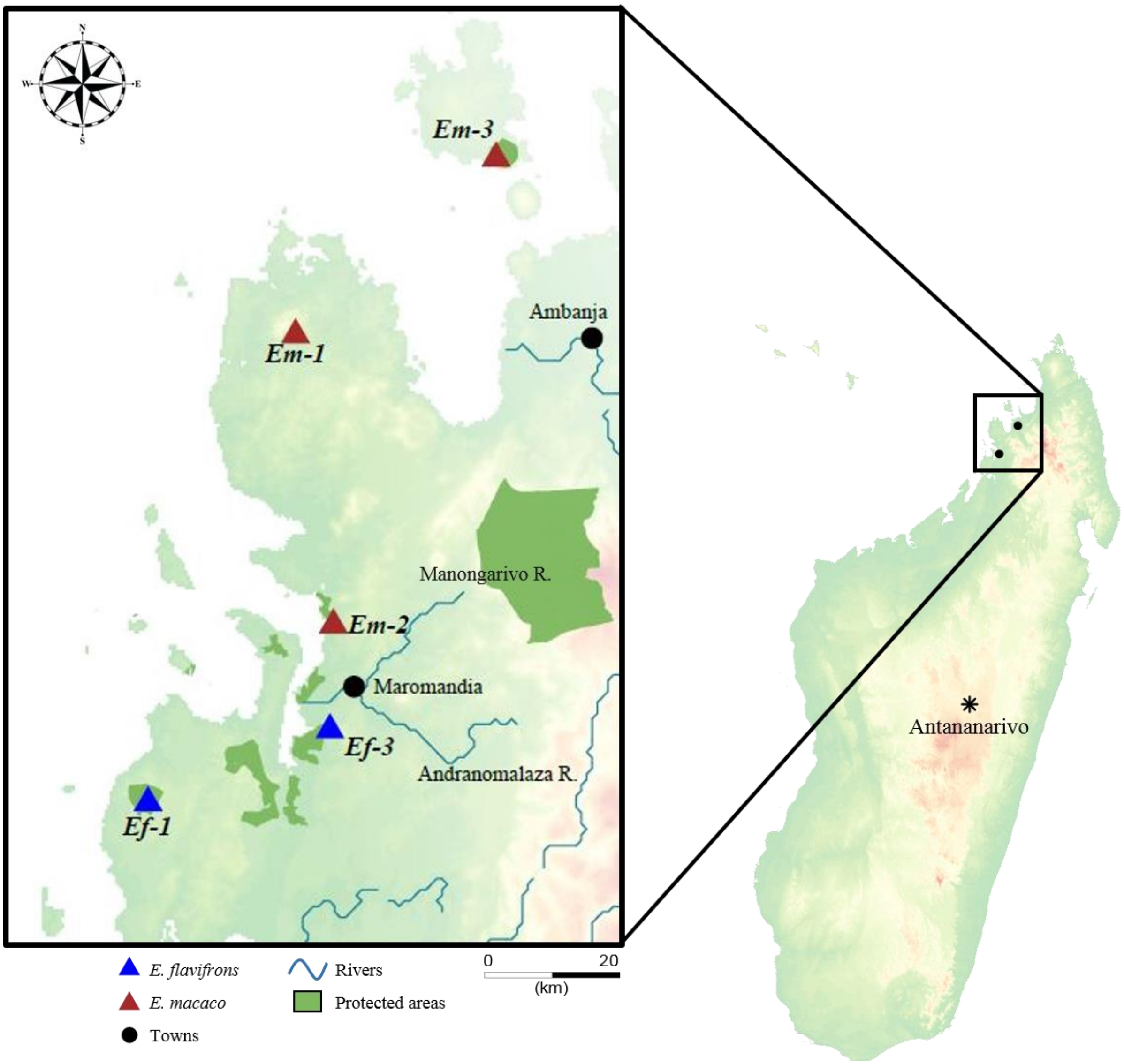

Figure 5.1 Locations of the five populations from which acoustic data were collected. *Ef-1*: Ankarafa Forest, 2015; *Ef-3*: Angodrahely, 2017; *Em-1*: Andranomatavy, 2016; *Em-2*: Mahadera, 2016; and *Em-3*: Lokobe, 2017.

5.2.2 Acoustic data collection

Opportunistic recording of acoustic communication took place during daily follows, which typically occurred between 0530 and 1730 hrs. Two handheld Roland R-05 WAVE/MP3 Recorders and accompanying Sennheiser ME66 directional microphones with K6 powering modules were used to record vocalisations *ad libitum* (Lehner, 1996) at a sampling rate of 44.1 kHz. This resulted in 310 *Ef-1* recordings, 50 *Ef-3* recordings, 213 *Em-1* recordings, 41 *Em-2* recordings, and 89 *Em-3* recordings. For each, the location, the time, sex, call context, and accompanying behaviours were logged when possible (Geissmann & Parsons, 2011). Due to the gregarious nature of both species, it was often difficult to assign caller identification or behavioural context to vocalisations. It was also common for the vocaliser to be ‘out of sight’ or for multiple individuals to call at the same time. For this reason, no quantitative analyses were conducted on contextual or caller identity data.

5.2.3 Acoustic analysis

Recordings were first visualised using Avisoft SASLab Pro (Version 5.2.12, Avisoft Bioacoustics 2015, Glienicke, Germany). Within each recording, high quality calls were selected and saved based on their amplitude, suitably low levels of background noise (signal-background ratio greater than 12dB, following Gamba et al., 2015), and a lack of overlap of multiple calls. Due to the lower frequency range of many *Eulemur* vocalisations (typically no higher than 14 kHz), insect and bird vocalisations often overlapped with higher formants that therefore could not always be measured accurately. In addition to the aforementioned requirements for suitability of the recordings, a minimum of the first two formants needed to be measurable for a call to be entered to further analysis.

Unless multiple individuals were clearly vocalising in a single recording, only one or two calls per call type were saved per recording for further analysis. Each isolated recording was therefore either a single acoustic element or a compound call comprised of two or more elements. Calls were then grouped together based on the spectral features and vocal categories recognised by earlier *E. flavifrons* and *E. macaco* studies (Petter & Charles-Dominique, 1979; Macedonia & Stanger, 1994; Gosset et al., 2001; Gamba & Giacoma, 2007; Gamba et al., 2015). Calls not falling into previously identified categories were grouped based on their structure and behavioural context and are introduced here as new call types.

The acoustic parameters of the refined recordings were then measured in Praat (Version 6.0.40, Boersma & Weenink 2018, Institute of Phonetic Sciences, University of Amsterdam) using Fast Fourier transforms and a Hamming window (Frequency range: 0-22,000 Hz; maximum: 50 dB/Hz; dynamic range: 30-70 dB; pre-emphasis: 6.0 dB/Oct; dynamic compression: 0.0). Following the source-filter theory (Fant, 1960) and established methodology to analyse *Eulemur* calls (Gamba & Giacoma, 2007; Gamba & Giacoma, 2008; Gamba et al., 2012a, 2012b; Gamba et al., 2015; Nadhurou et al., 2016), the call duration, five larynx-based call features related to fundamental frequency, and four vocal tract-based features related to formant structure were measured (Table 5.1). If a vocalisation was comprised of more than one acoustic element, the number of elements and the inter-element interval durations were also recorded. For certain calls the number of elements was highly variable, so measurements were restricted to four elements only.

Formant settings (Maximum formant: 14,000-22,000 Hz, Number of formants: 6-14, Window length: 0.06 s, Dynamic range: 45 dB) and fundamental frequency settings (Pitch range: 50-7,000 Hz, Analysis method: autocorrelation) in Praat differed depending on call type analysed. Formants were estimated and tracked using Linear Predictive Coding (LPC), which assesses resonance frequencies that are produced by the vocal tract (Owren & Bernacki, 1998). Praat calculates mean values by averaging all points along the identified contours. Fundamental and formant frequency plots were compared to the spectral slices to ensure accurate identification, and manually adjusted when necessary. Histograms of formant frequencies for call types were plotted to ensure that there was minimal overlap of outlier formant measurements.

Table 5.1 Description of the various acoustic parameters measured for *E. flavifrons* and *E. macaco* calls.

Acoustic Parameter	**Code**	**Description**
Structure		The structure of each acoustic element was classified as one of the following: tonal: visible harmonic structure, also referred to as clear call noisy: no visible harmonics or formants, harsh structure combination: tonal and noisy components both present in a call
For single element calls		
Duration	Dur	The time from the start to the end of the acoustic element
Mean fundamental frequency	F0mean	The mean fundamental frequency, or pitch, across the acoustic element
Minimum fundamental frequency	F0min	The minimum fundamental frequency during the call
Maximum fundamental frequency	F0max	The maximum fundamental frequency during the call
Start fundamental frequency	F0s	The fundamental frequency at the start of the call
End fundamental frequency	F0e	The fundamental frequency at the end of the call
Change in fundamental frequency	F0dif	The difference between fundamental frequency at the start of the call and at the end of the call
First formant	F1	The mean frequency of the first band of concentrated energy in the call
Second formant	F2	The mean frequency of the second band of concentrated energy in the call
Third formant	F3	The mean frequency of the third band of concentrated energy in the call
Fourth formant	F4	The mean frequency of the fourth band of concentrated energy in the call
Harmonics	H	The number of visible harmonics in the element
For composite (two types of elements) or compound calls (multiple elements or pulses)		
Number of elements	E	The number of acoustic elements within a call
Interval duration	IDur	The time between the end of one acoustic element and the start of the following acoustic element
Pulse duration	PDur	The time from the start to end of a pulse
Rate	R	The number of pulses per second
Period	P	The number of cycle modulations per second
Grunt duration	GDur	The time from start to end of grunt component of the composite call
Tonal duration	TDur	The time from start to end of tonal component of the composite call

5.2.4 Statistical analysis

Data were log-transformed to meet normality assumptions required for analyses. MANOVAs were carried out to determine whether acoustic variables differed significantly between populations for each call type. The resulting list of exhaustive univariate tests of differences can be found in the Appendices D-I. Classical stepwise discriminant function analysis (DFA) with leave-one-out cross validation was performed on call types to test whether each population's vocalisations are distinct and to identify the acoustic predictor variables that maximise population differences (Lachenbruch & Goldstein, 1979). Six call types were analysed for interspecies and intraspecies differences. Only calls in which every predictor variable was measured were included in these analyses. Because the fundamental frequency was difficult to measure in grunt calls and including this variable in the DFAs would have greatly reduced the sample size, all grunt F0 measurements were excluded from the models. An additional eight calls could not be compared between populations due to their limited occurrence or poor recording quality. Significance levels were set at $\alpha = 0.05$ for all tests. Analyses were carried out in R (R Core Team, 2013) using the car (Fox & Wisberg, 2011), ggplot2 (Wickham, 2016), dplyr (Wickham et al., 2018), and MASS (Venables & Ripley, 2002) packages.

5.3 RESULTS

5.3.1 Statistical analyses

Grunt Calls

Prior synonyms—Short grunts and Simple long grunts: Gosset et al., 2001; Grunts and Long grunts: Macedonia & Stranger, 1994; Gamba & Giacoma, 2008; Pozzi et al., 2010

Grunts are low-pitched vocalisations that are structurally noisy, with visible glottis pulses and five to ten detectable formant bands. When discernible, the fundamental frequency was relatively low in comparison to other calls. Following the temporal categorisation by Gamba & Giacoma (2008) and Pozzi & colleagues (2010), three types of grunts were identified based on their duration—short grunts, intermediate grunts, and long grunts. Short grunts were characterised as being shorter than 0.15 s, long grunts were longer than 0.35 s, and intermediate grunt durations were between the aforementioned two. Frequency and amplitude modulation were common in both the intermediate and long grunts, but less often in the short grunts. Collectively, grunts were used in a variety of social contexts while in contact with group members including locomoting, feeding, or approaching a conspecific.

Short grunts: The duration, fundamental frequency, and first four formants were measured from 175 short grunts (Table 5.2; Figure 5.2). Of those, 104 calls were further tested. Multivariate analysis found significant differences between the populations (MANOVA: Wilk's λ=0.58, F(4,99)=2.8, p<0.001).

The DFA revealed overlap in the five populations' short grunts, with the first discriminant function (LD1) explaining 52.8% of the variance between populations and the second discriminant function (LD2) explaining 29.4% of the variance (Figure 5.3). Important predictor variables include F3 and F2. Leave-one-out cross validation assigned 49% of the short grunts to the correct population (Table 5.3**)**. Pairwise comparisons of each variable can be found in Appendix D.

Table 5.2 Mean, standard deviation, and ranges of short grunt call acoustic parameters for each population. For each call, duration (Dur), mean fundamental frequency (F0mean), minimum fundamental frequency (F0min), maximum fundamental frequency (F0max), 1st formant (F1), 2nd formant (F2), 3rd formant (F3), and 4th formant (F4) were measured. The means and standard deviation are in bold, the range below. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-2= E. macaco*, Mahadera; *Em-3= E. macaco*, Lokobe.

Call type	**Pop**	**Dur (s)**	**F0mean (Hz)**	**F0min (Hz)**	**F0max (Hz)**	**F1 (Hz)**	**F2 (Hz)**	**F3 (Hz)**	**F4 (Hz)**
Short grunt	*Ef-1*	**0.094±0.033**	**114±19**	**105±20**	**118±18**	**715±100**	**1910±183**	**3628±408**	**4838±559**
	(N=50)	0.033-0.150	87-154	77-151	90-157	442-980	1485-2267	2706-4463	3869-6506
	Ef-3	**0.063±0.030**	**112±29**	**107±26**	**115±31**	**646±69**	**1707±230**	**3416±326**	**4809±392**
	(N=26)	0.015-0.142	76-159	75-149	77-164	550-841	1321-2165	3027-4033	4288-5325
	Em-1	**0.087±0.032**	**96±12**	**93±13**	**98±13**	**804±93**	**1998±252**	**3841±503**	**5138±440**
	(N=31)	0.018-0.150	78-113	75-111	80-115	679-1008	1554-2482	2575-4849	4666-6438
	Em-2	**0.078±0.036**	**99±24**	**95±22**	**102±28**	**720±119**	**1872±160**	**3768±502**	**5223±391**
	(N=15)	0.028-0.150	71-135	69-127	72-146	540-981	1724-2337	3188-4627	4738-5778
	Em-3	**0.089±0.026**	**106±19**	**102±20**	**109±19**	**724±94**	**1833±204**	**3687±265**	**4881±314**
	(N=53)	0.016-0.150	86-150	76-149	87-151	528-910	1426-2450	2920-4166	4054-5520

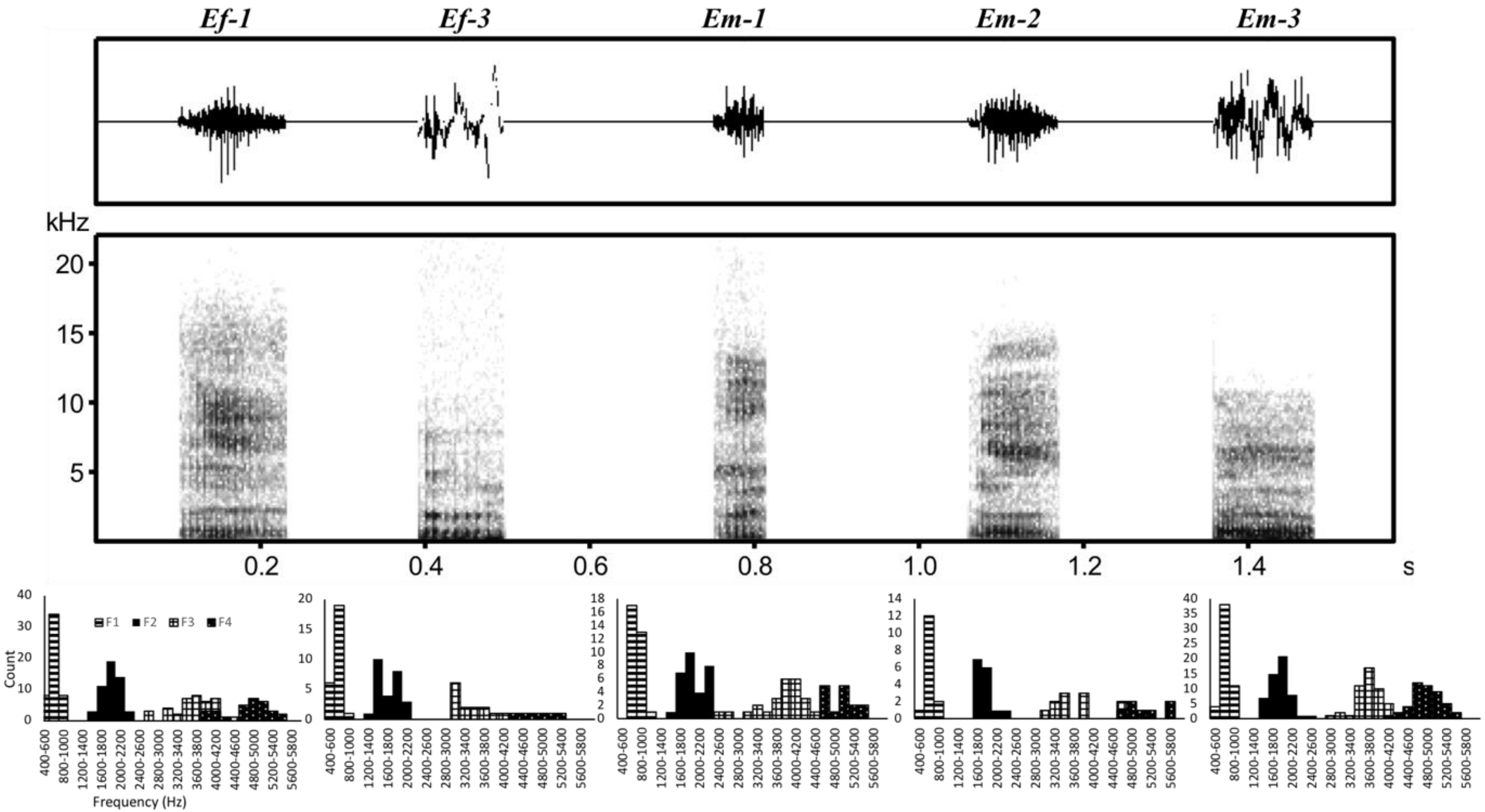

Figure 5.2 Spectrograms and oscillograms of an exemplar short grunt from the five different populations. Below each spectrogram are histograms of formant frequencies (F1-F4) for all short grunts measured in each population. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

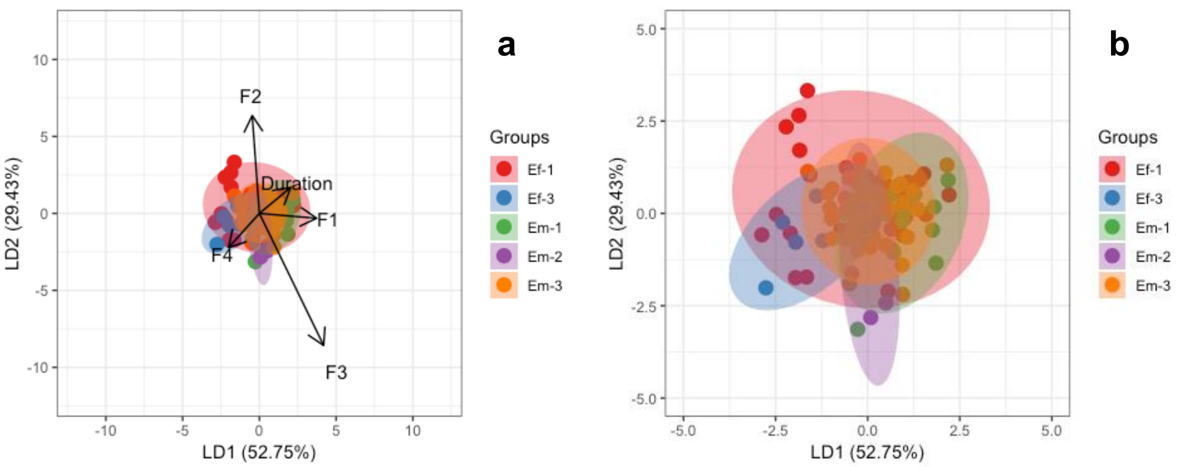

Figure 5.3 Biplot of the first two discriminants (LD1 and LD2) of the DFA classification for short grunts. Plot b is a zoomed in version of plot a, highlighting the overlap in the populations. Vectors represent the variation in the acoustic variables. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

Table 5.3 Percentages (and counts) of correctly (in bold) and incorrectly assigned short grunts from stepwise discriminant function analysis with leave-one-out cross validation. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

	Assigned population				
Population	*Ef-1*	*Ef-3*	*Em-1*	*Em-2*	*Em-3*
Ef-1	**43.8** **(7)**	25 (4)	12.5 (2)	12.5 (2)	6.2 (1)
Ef-3	50 (1)	**50** **(1)**			
Em-1	22.2 (2)		**33.3** **(3)**	33.3 (3)	11.1 (1)
Em-2					100 (2)
Em-3	36.4 (4)		27.3 (3)	27.3 (3)	**9** **(1)**

Intermediate grunts: 208 grunts of intermediate length (0.151-0.349 s) across the five populations were measured (Table 5.4, Figure 5.4). Of those, the acoustic variables of 154 calls were further tested, finding significant differences between the populations (MANOVA: Wilk's $\lambda=0.71$, $F(4,149)=2.61$, $p<0.001$). The DFA revealed overlap in the five populations' intermediate grunts, with *Ef-3* having a wider spread of points. The first and second discriminant functions explain 76.5% of the between-population variance (Figure 5.5). Important predictor variables include F4 and F2. Leave-one-out cross validation correctly assigned 37% of the calls (Table 5.5). Pairwise comparisons of each variable can be found in Appendix E.

Table 5.4 Mean, standard deviation, and ranges of intermediate grunt call acoustic parameters for each population. For each call, duration (Dur), mean fundamental frequency (F0mean), minimum fundamental frequency (F0min), maximum fundamental frequency (F0max), 1st formant (F1), 2nd formant (F2), 3rd formant (F3), and 4th formant (F4) were measured. The means and standard deviation are in bold, the range below. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-2= E. macaco*, Mahadera; *Em-3= E. macaco*, Lokobe.

Call type	Pop	Dur (s)	F0mean (Hz)	F0min (Hz)	F0max (Hz)	F1 (Hz)	F2 (Hz)	F3 (Hz)	F4 (Hz)
Interm. grunt	*Ef-1*	**0.234±0.050**	**113±17**	**102±16**	**124±21**	**743±74**	**1858±281**	**3747±399**	**5000±392**
	(N=77)	0.153-0.349	67-147	63-134	72-178	574-959	1388-2498	2916-4430	4003-5870
	Ef-3	**0.262±0.038**	**118±13**	**113±8**	**130±14**	**658±71**	**1767±71**	**3257±419**	**4359±302**
	(N=9)	0.215-0.329	101-135	106-121	117-144	558-779	1454-2099	2882-3856	4081-4697
	Em-1	**0.261±0.047**	**131±15**	**119±14**	**144±17**	**767±91**	**1928±202**	**3944±346**	**5133±368**
	(N=56)	0.166-0.348	91-162	83-149	98-170	557-976	1492-2577	2926-4575	4148-5689
	Em-2	**0.234±0.059**	**124±18**	**114±17**	**137±23**	**733±110**	**1822±197**	**3830±608**	**4899±596**
	(N=17)	0.151-0.332	102-158	96-149	106-173	540-958	1532-2130	2794-4498	4078-5697
	Em-3	**0.249±0.062**	**115±17**	**106±17**	**127±20**	**722±104**	**1922±170**	**3802±380**	**4884±371**
	(N=49)	0.152-0.348	90-150	77-144	92-173	545-1018	1612-2450	2801-4365	4203-5520

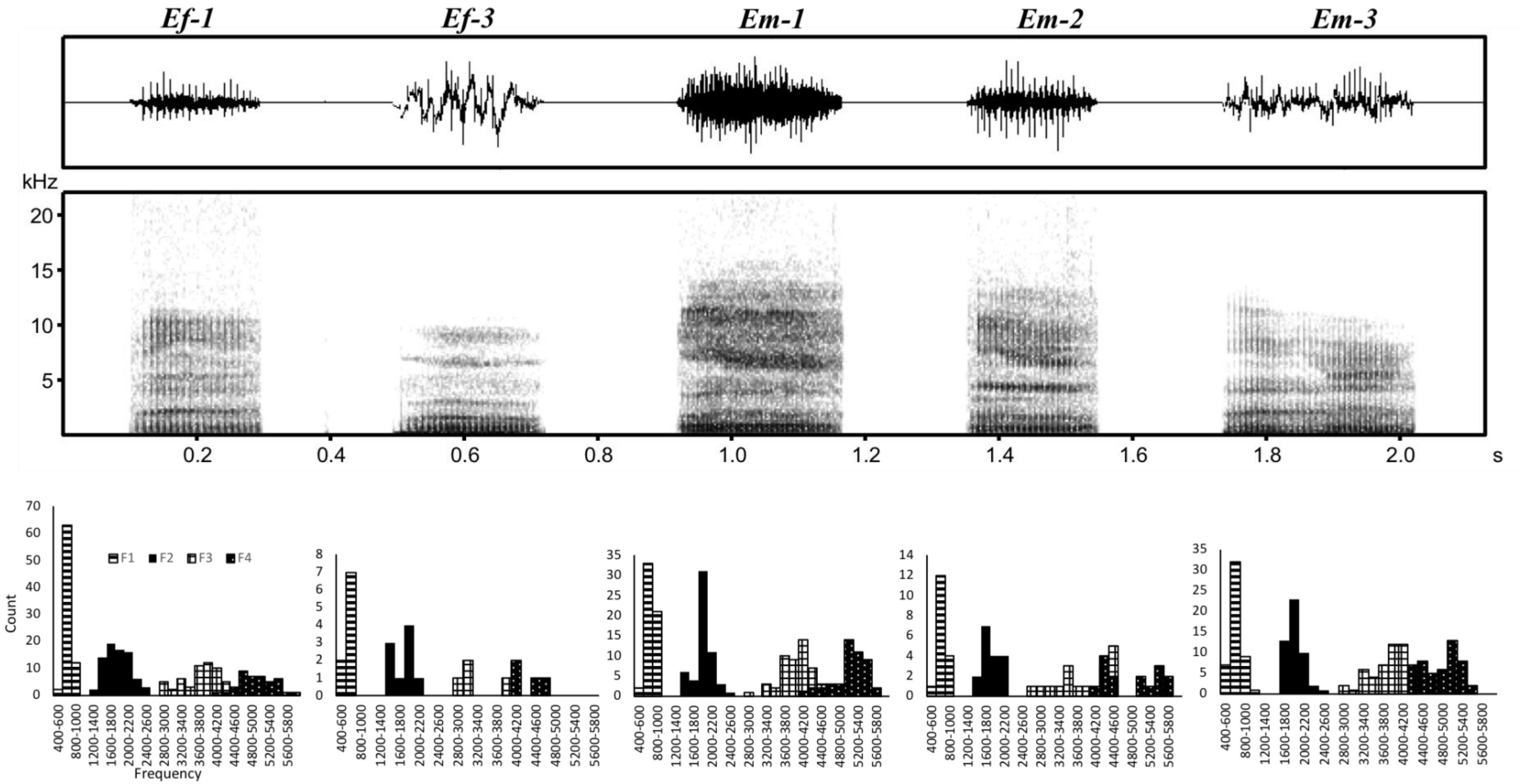

Figure 5.4 Spectrograms and oscillograms of an exemplar intermediate grunt from the five different populations. Below each spectrogram are histograms of formant frequencies (F1-F4) for all intermediate grunts measured in each population. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

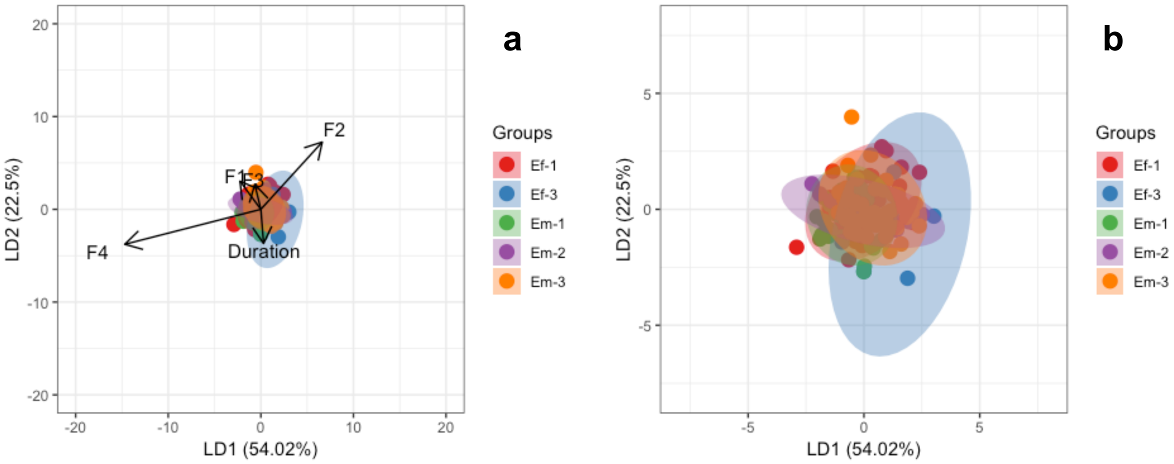

Figure 5.5 Biplot of the first two discriminants (LD1 and LD2) of the DFA classification for intermediate grunts. Plot b is a zoomed in version of plot a, highlighting the overlap in the populations. Vectors represent the variation in the acoustic variables. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

Table 5.5 Percentages (and counts) of correctly (in bold) and incorrectly assigned intermediate grunt calls from stepwise discriminant function analysis with leave-one-out cross validation. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

	Assigned population				
Population	*Ef-1*	*Ef-3*	*Em-1*	*Em-2*	*Em-3*
Ef-1	**12.2** **(5)**	2.4 (1)	31.7 (13)	4.9 (2)	48.8 (20)
Ef-3			25 (1)		75 (3)
Em-1	26.7 (12)		**51.1** **(23)**	2.2 (1)	20 (9)
Em-2			46.7 (7)	**13.3** **(2)**	40 (6)
Em-3	22.4 (11)		22.4 (11)		**55.2** **(27)**

Long grunts: Acoustic variables were measured from 248 grunts with durations over 0.35 s (Table 5.6, Figure 5.6). Of those, 170 calls were compared, finding significant differences between the populations (MANOVA: Wilk's $\lambda=0.74$, $F(4, 165) = 2.53$, $p<0.001$). The DFA revealed overlap in the populations, apart from *Ef-3* (Figure 5.7). LD1 explained 70.8% of the between-population variance and LD2 explained a further 18%, with F4 and F3 serving as important predictor variables. Leave-one-out cross validation correctly classified 48.8% of the long grunts (Table 5.7). Pairwise comparisons of each variable can be found in Appendix F.

Table 5.6 Mean, standard deviation, and ranges of long grunt call acoustic parameters for each population. For each call, duration (Dur), mean fundamental frequency (F0mean), minimum fundamental frequency (F0min), maximum fundamental frequency (F0max), 1st formant (F1), 2nd formant (F2), 3rd formant (F3), and 4th formant (F4) were measured. The means and standard deviation are in bold, the range below. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-2= E. macaco*, Mahadera; *Em-3= E. macaco*, Lokobe.

Call type	**Pop**	**Dur (s)**	**F0mean (Hz)**	**F0min (Hz)**	**F0max (Hz)**	**F1 (Hz)**	**F2 (Hz)**	**F3 (Hz)**	**F4 (Hz)**
Long grunt	*Ef-1*	**0.517±0.179**	**106±17**	**93±14**	**114±22**	**778±96**	**2097±252**	**4001±241**	**5265±267**
	(N=25)	0.356-1.024	81-134	75-123	82-158	611-992	1447-2491	3358-4294	4657-5523
	Ef-3	**0.515±0.121**	**112±11**	**109±13**	**124±7**	**657±67**	**1953±216**	**3695±253**	**4644±422**
	(N=20)	0.370-0.883	92-122	90-117	117-130	565-823	1530-2239	3360-4099	3984-5030
	Em-1	**0.508±0.135**	**113±16**	**103±15**	**123±20**	**806±88**	**2181±234**	**4093±348**	**5315±387**
	(N=134)	0.352-1.059	80-146	75-141	81-167	632-1112	1683-2753	2623-5001	4031-6692
	Em-2	**0.606±0.193**	**91±12**	**84±12**	**96±15**	**785±63**	**2125±182**	**4102±343**	**5333±342**
	(N=16)	0.373-0.989	65-111	60-104	70-118	644-870	1804-2507	3252-4485	4636-5917
	Em-3	**0.547±0.144**	**102±12**	**93±11**	**115±14**	**809±124**	**2148±183**	**4021±244**	**5203±308**
	(N=53)	0.362-0.929	67-128	75-128	86-156	559-1215	1748-2560	3044-4477	4147-6275

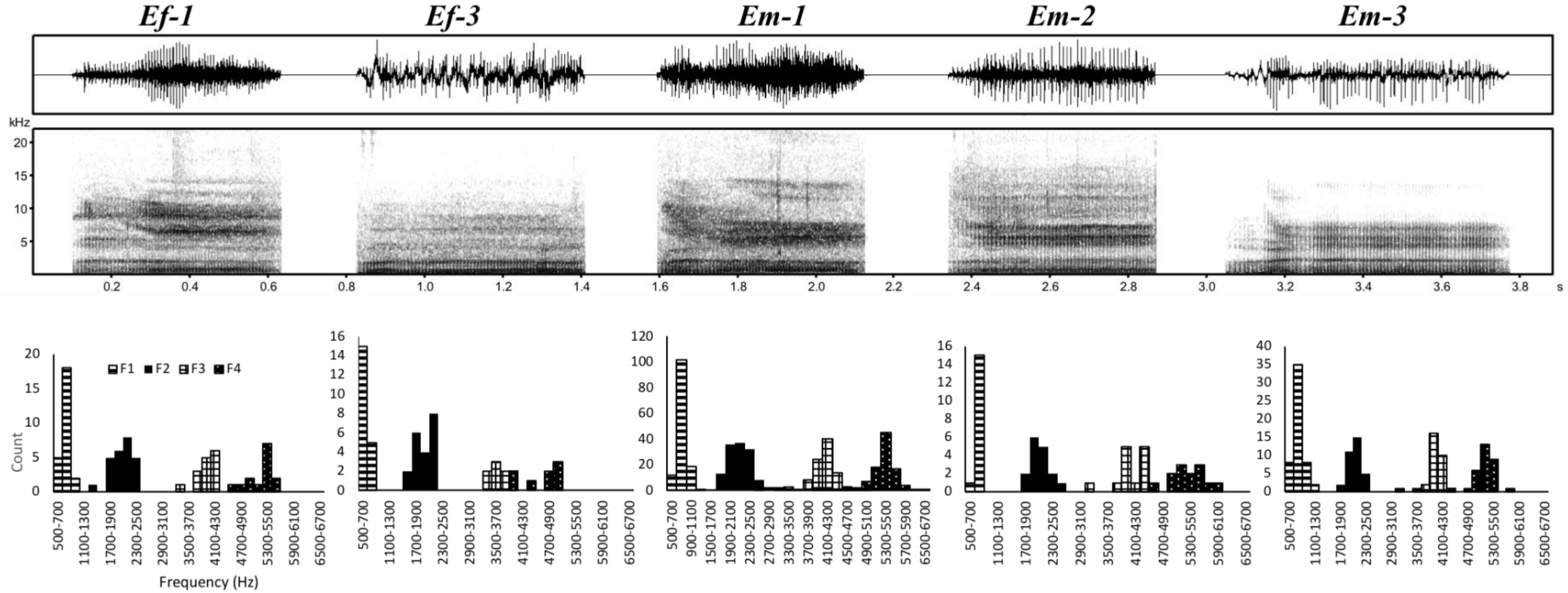

Figure 5.6 Spectrograms and oscillograms of an exemplar long grunt from the five different populations. Below each spectrogram are histograms of formant frequencies (F1-F4) for all long grunts measured in each population. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-2= E. macaco*, Mahadera; *Em-3= E. macaco*, Lokobe.

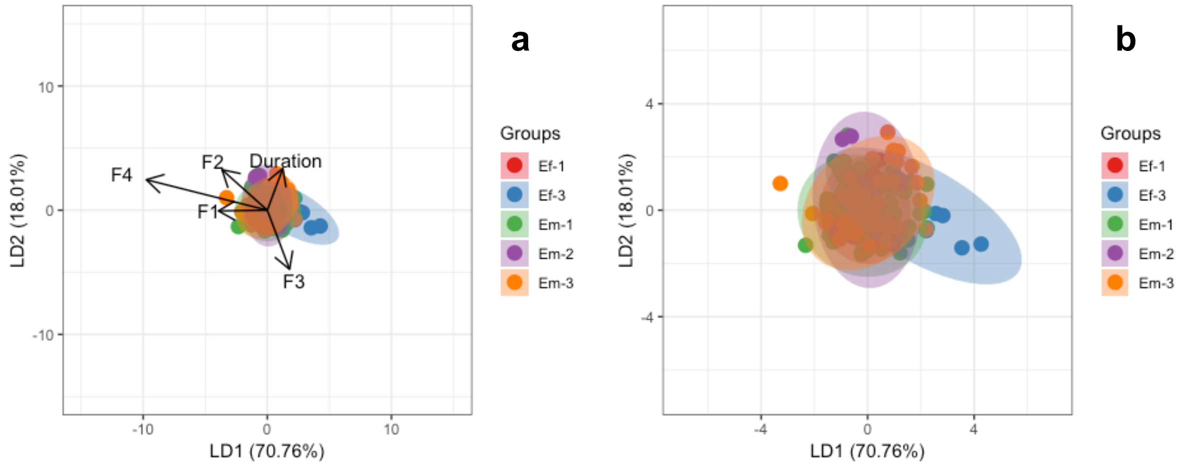

Figure 5.7 Biplot of the first two discriminants (LD1 and LD2) of the DFA classification for long grunts. Plot b is a zoomed in version of plot a, highlighting the overlap in the populations. Vectors represent the variation in the acoustic variables. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

Table 5.7 Percentages (and counts) of correctly (in bold) and incorrectly assigned long grunts from stepwise discriminant function analysis with leave-one-out cross validation. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

	Assigned population				
Population	*Ef-1*	*Ef-3*	*Em-1*	*Em-2*	*Em-3*
Ef-1			85.7 (12)		14.3 (2)
Ef-3		**50** **(4)**	25 (2)		25 (2)
Em-1		4.8 (4)	**81** **(68)**		14.2 (12)
Em-2			61.5 (8)		38.5 (5)
Em-3		3.9 (2)	74.5 (38)		**21.6** **(11)**

Trains of long grunts (TLG)

Prior synonyms—Recognition signals/Meueu-meueu-meueu-meume-meume-me: Petter & Charles-Dominique, 1979; Train of modulate long grunts: Gosset et al., 2001

All five populations were observed stringing intermediate grunts and long grunts together in a quasi-stereotyped way (Figure 5.8). These trains of long grunts (TLG) typically ranged 4-8 acoustic elements in length, with the length of the grunt increasing in duration until the ultimate utterance was a long grunt. This compound signal was predominately vocalised by males, although a female was observed emitting this pattern on one occasion. Both group members and non-group members often responded. These calls occurred throughout the day, although they were concentrated both in the morning and at dusk, after intergroup encounters, or during peak mating period.

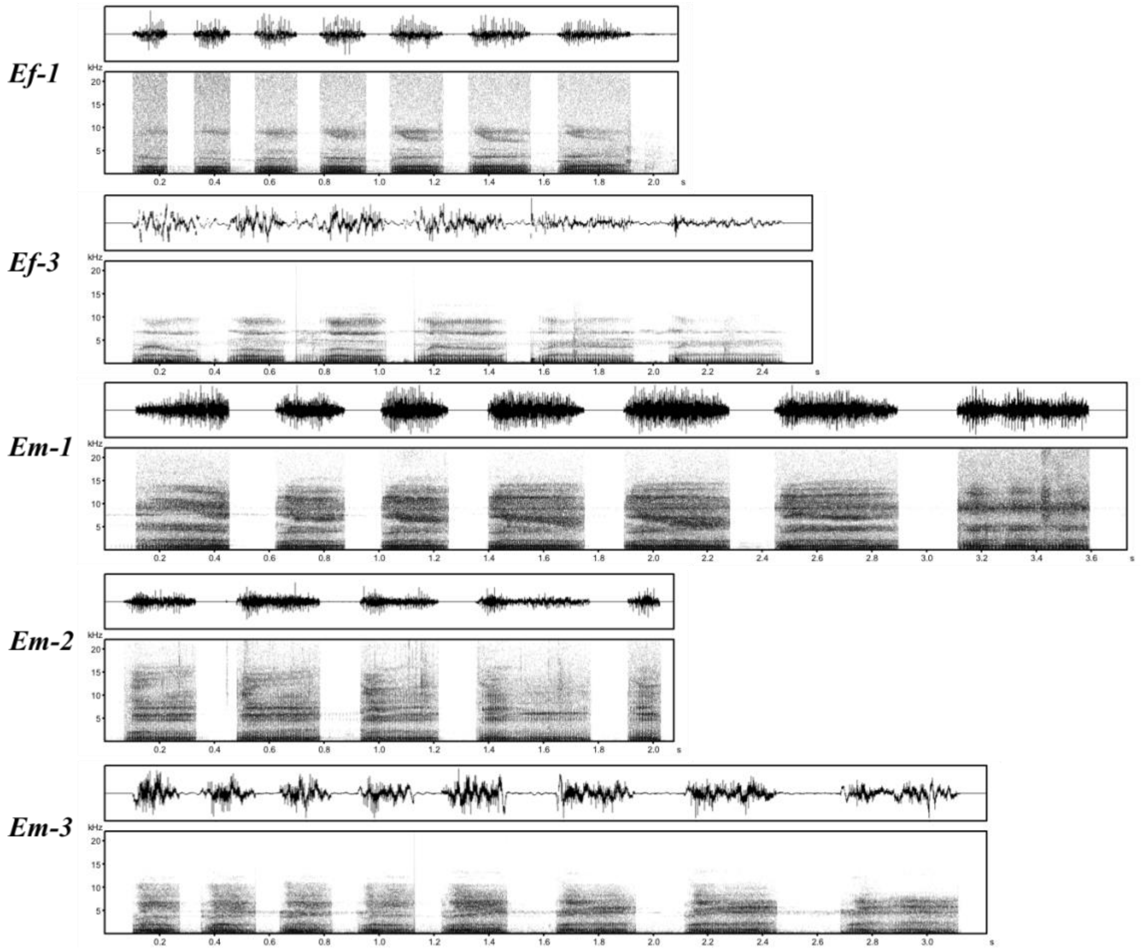

Figure 5.8 Spectrograms and oscillograms of an exemplar compound call, train of long grunts, from the five different populations. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-2= E. macaco*, Mahadera; *Em-3= E. macaco*, Lokobe

Alarm Calls

Two types of alarm calls were identified—a group alarm call and an individual alarm call. Both types of vocalisations represent heightened levels of excitement. These calls have a dense broadband structure, with harmonics briefly visible at the start, followed by a longer noisy segment. Due to this structure, formants were sometimes difficult to locate. These calls have long durations (typically over 0.6 s) and relatively high fundamental frequencies (>1200 Hz) compared to other call types.

Group Alarm Calls

Prior synonyms—Terrestrial disturbance/Advertisement calls: Macedonia & Stranger, 1994; Melodic croucroucrou: Gosset et al., 2001; Cuiiiii ciot ciot: Petter & Charles-Dominique, 1979; Alarm call: Pozzi et al., 2010; Gamba et al., 2015

Group alarm calls often occurred in choruses by all members, making it difficult to tease apart individual calls. These calls typically occurred in the morning after waking up or in the late afternoon, and neighbour groups would respond. They were also given during intergroup encounters or disturbances in the environment throughout the day. Scent marking was frequently observed in conjunction with the choruses.

Comparisons of the acoustic variables from 22 group alarm calls (Table 5.8, Figure 5.9) found significant interpopulation differences (MANOVA: Wilk's $\lambda=0.06$, $F(2,18)=3.56$, $p=0.004$). The first linear discriminant explained 71.1% of the between-population variance and the second linear discriminant explained the remaining 28.9% (Figure 5.10). F3, F0mean, and F0min were important predictor variables. Leave-one-out cross validation correctly classified 71.4% of the group alarm calls (Table 5.9). Pairwise comparisons of each variable can be found in Appendix G.

Table 5.8 Mean, standard deviation, and ranges for the acoustic parameters of group alarm calls for each population. For each call, duration (Dur), mean fundamental frequency (F0mean), minimum fundamental frequency (F0min), maximum fundamental frequency (F0max), first formant (F1), second formant (F2), third formant (F3), and fourth formant (F4) were measured. The means and standard deviation are in bold, ranges below. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-2= E. macaco*, Mahadera; *Em-3= E. macaco*, Lokobe.

Call type	**Pop.**	**Dur (s)**	**F0mean (Hz)**	**F0min (Hz)**	**F0max (Hz)**	**F0s (Hz)**	**F0f (Hz)**	**F1 (Hz)**	**F2 (Hz)**	**F3 (Hz)**	**F4 (Hz)**
Group alarm	*Ef-1*	**1.411±0.266**	**1328±64**	**1198±61**	**1430±61**	**1230±70**	**1355±94**	**1409±62**	**3497±112**	**4399±260**	**5847±499**
	(N=4)	1.145-1.778	1273-1420	1127-1253	1373-1513	1127-1282	1266-1488	1350-1495	3363-3636	4095-4730	5465-6533
	Ef-3	**1.184±0.533**	**1236±48**	**1124±49**	**1364±113**	**1184±68**	**1243±72**	**1347±185**	**3001±219**	**4412±132**	**6452±248**
	(N=12)	0.406-2.044	1147-1338	1047-1223	1249-1591	1051-1278	1124-1388	1246-1918	2569-3265	4221-4617	6081-6921
	Em-1	**1.562±0.421**	**1356±36**	**1238±84**	**1468±62**	**1225±76**	**1359±54**	**1436±75**	**3019±278**	**4608±193**	**6342±208**
	(N=6)	1.036-2.157	1302-1410	1123-1351	1375-1562	1135-1305	1272-1413	1336-1554	2635-3285	4257-4800	6001-6572

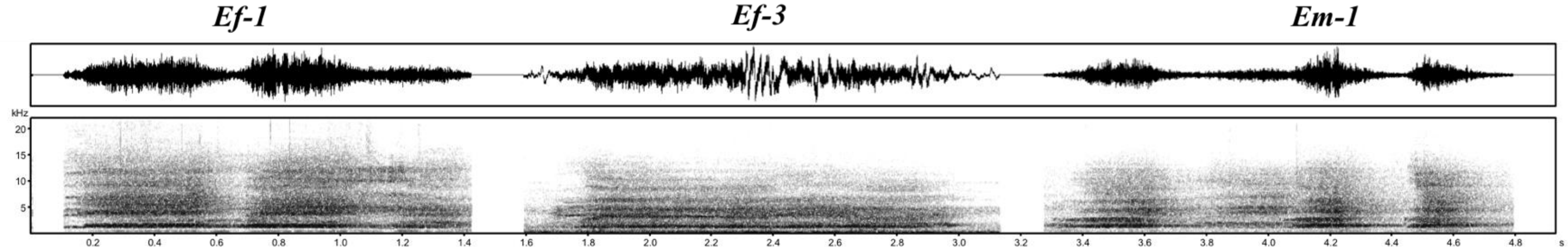

Figure 5.9 Spectrograms and oscillograms of an exemplar group alarm call from three different populations. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; and *Em-1= E. macaco*, Andranomatavy.

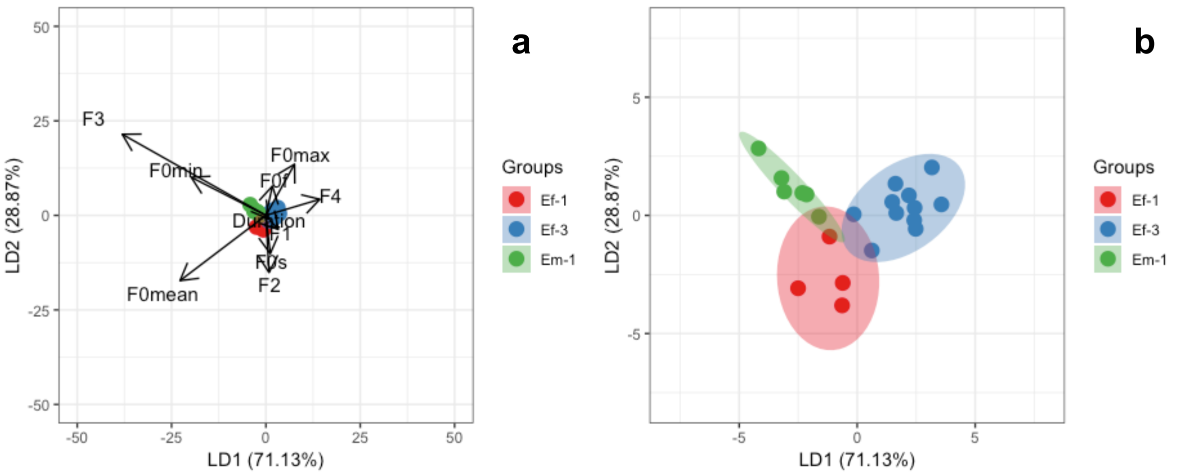

Figure 5.10 Biplot of the first two discriminants (LD1 and LD2) of the DFA classification for group alarm calls. Plot b is a zoomed in version of plot a, highlighting the overlap in the populations. Vectors represent the variation in the acoustic variables. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy.

Table 5.9 Percentages (and counts) of correctly (in bold) and incorrectly assigned group alarm calls from stepwise discriminant function analysis with leave-one-out cross validation. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*.

Population	**Assigned population**		
	Ef-1	*Ef-3*	*Em-1*
Ef-1	**50** **(2)**	25 (1)	25 (1)
Ef-3	18.2 (2)	**72.7** **(8)**	9.1 (1)
Em-1	16.7 (1)		**83.3** **(5)**

Individual alarm calls

Prior synonyms—Terrestrial disturbance/advertisement call: Macedonia & Stranger, 1994; Crou-crou-crouii: Petter & Charles-Dominique, 1979; Gosset et al., 2001; Creeeee: Petter & Charles-Dominique, 1979

Individual alarm calls were typically given by one or two individuals in the group and represented individual agitation or distress caused by an environmental disturbance including researcher presence. Tail swinging, peering, and jumping frequently accompanied these vocalisations. Although very similar both structurally and acoustically, there are two features that distinguish individual alarm calls from group alarm calls. First is the temporal pattern; an individual alarm call could be given on its own or could be combined with a series of preliminary pulses that increased in duration and intensity until culminating in the full alarm call. Second is the characteristic upswing of the fundamental frequency and the first formant. For this analysis, only the final element was analysed and not the introductory pulses.

Of the 64 calls measured (Table 5.10, Figure 5.11), 43 were used in the multivariate analyses. Although significant interpopulation differences were found (MANOVA: Wilk's λ=0.1, F(4,38)=2.35, $p<0.001$), leave-one-out cross validation correctly assigned less than half of the individual alarm calls (47.6%; Table 5.11). Apart from *Ef-3*, there was considerable overlap in the individual alarm calls of the populations (Figure 5.12). LD1 explained 67.2% of the between-population variance and LD2 explained and additional 12.3%, with F0min, F0max, and F0f acting as important predictor variables. Pairwise comparisons of each variable can be found in Appendix H.

Table 5.10 Mean, standard deviation, and ranges for the acoustic parameters of individual alarm calls for each population. For each call, duration (Dur), mean fundamental frequency (F0mean), minimum fundamental frequency (F0min), maximum fundamental frequency (F0max), first formant (F1), second formant (F2), third formant (F3), and fourth formant (F4) were measured. The means and standard deviation are in bold, ranges below. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

Call type	**Pop**	**Dur (s)**	**F0mean (Hz)**	**F0min (Hz)**	**F0max (Hz)**	**F0s (Hz)**	**F0f (Hz)**	**F1 (Hz)**	**F2 (Hz)**	**F3 (Hz)**	**F4 (Hz)**
Individual alarm	*Ef-1*	**0.765±0.401**	**1367±220**	**1205±146**	**1525±287**	**1239±145**	**1477±317**	**1560±272**	**2795±533**	**4379±606**	**5640±610**
	(N=19)	0.092-1.678	1049-1779	944-1456	1167-2090	959-1465	1098-2044	1164-2512	1963-4470	3379-5758	4760-7031
	Ef-3	**0.731±0.224**	**1495±184**	**880±126**	**2001±329**	**937±66**	**1979±304**	**1829±225**	**2910±419**	**4244±605**	**5694±860**
	(N=11)	0.473-1.054	1234-1783	806-1102	1449-2330	842-1234	1449-2221	1317-2158	2130-3492	3209-4964	4190-7205
	Em-1	**0.624±0.212**	**1418±93**	**1311±84**	**1533±138**	**1340±110**	**1520±133**	**1688±291**	**3107±794**	**4537±889**	**5708±920**
	(N=13)	0.311-1.018	1208-1584	1126-1430	1232-1699	1126-1512	1211-1652	1203-2124	2312-4781	3249-6553	4813-7681
	Em-2	**0.497±0.117**	**2168±572**	**1804±816**	**2315±500**	**1815±832**	**2251±470**	**1500±178**	**2685±10**	**3734±148**	**5105±339**
	(N=3)	0.365-0.587	1763-2572	1226-2381	1962-2669	1226-2403	191-2583	1386-1705	2673-2692	3629-3838	4865-5345
	Em-3	**0.620±0.158**	**1365±236**	**1125±201**	**1568±378**	**1151±211**	**1553±377**	**1631±231**	**2766±287**	**3977±442**	**5140±526**
	(N=18)	0.396-0.985	1053-1943	945-1566	1155-2610	945-1587	1155-2610	1289-2296	2321-3430	3268-4669	4431-5997

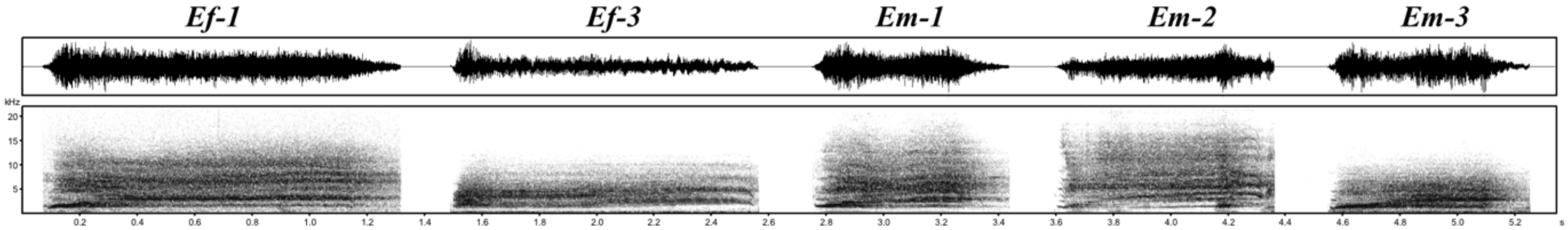

Figure 5.11 Spectrograms and oscillograms of an exemplar individual alarm call from the five different populations. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

Table 5.11 Percentages (and counts) of correctly (in bold) and incorrectly assigned individual alarm calls from stepwise discriminant function analysis with leave-one-out cross validation. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

	Assigned population				
Population	*Ef-1*	*Ef-3*	*Em-1*	*Em-2*	*Em-3*
Ef-1	**27.3** **(3)**		27.3 (3)		45.4 (5)
Ef-3		**40** **(2)**	20 (1)	20 (1)	20 (1)
Em-1	25 (3)		**58.3** **(7)**		16.7 (2)
Em-2					
Em-3	28.6 (4)		14.3 (2)		**57.1** **(8)**

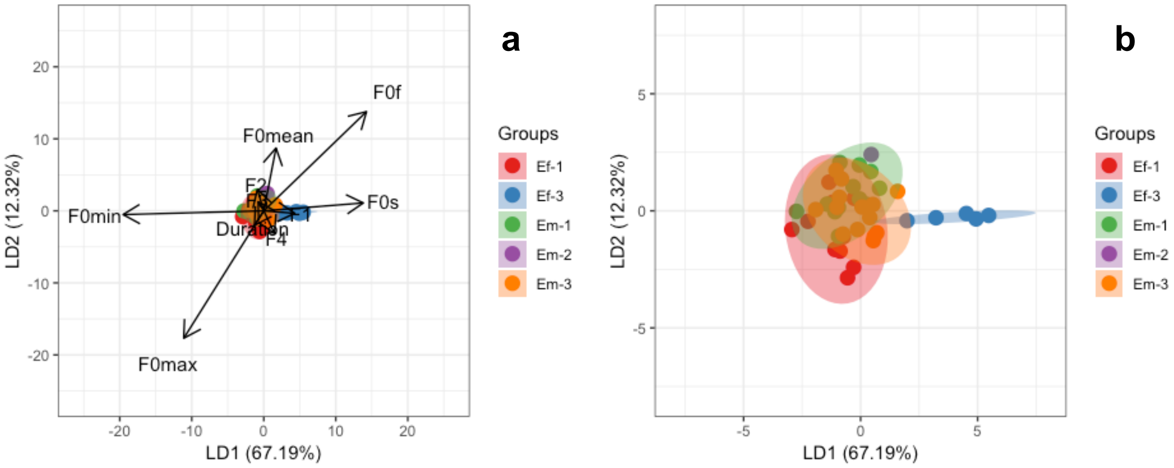

Figure 5.12 Biplot of the first two discriminants (LD1 and LD2) of the DFA classification for individual alarm calls. Plot b is a zoomed in version of plot a, highlighting the overlap in the populations. Vectors represent the variation in the acoustic variables. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

Hoot

Prior synonyms—Hoot: Pozzi et al., 2010; Gamba et al., 2015; Macedonia & Stranger, 1994; Oum (possibly): Gosset et al. 2001

These low amplitude tonal calls were short, with population means ranging 0.079-0.11 s (Table 5.12, Figure 5.13). They were characterised by a low fundamental frequency (>350 Hz), a narrow frequency range (>2000 Hz), and minimal frequency modulation resembling a flattened U-shape. One to four harmonics were typically visible. Hoots were given in low-stress situations while locomoting or resting with the group.

Table 5.12 Mean, standard deviation, and ranges for the acoustic parameters of hoot calls for each population. For each call, duration (Dur), mean fundamental frequency (F0mean), minimum fundamental frequency (F0min), maximum fundamental frequency (F0max), start fundamental frequency (F0s), and end fundamental frequency (F0f) were measured. The means and standard deviation are in bold, ranges below. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-2= E. macaco*, Mahadera; *Em-3= E. macaco*, Lokobe.

Call type	Pop	Dur (s)	F0mean (Hz)	F0min (Hz)	F0max (Hz)	F0s (Hz)	F0f (Hz)
Hoot	*Ef-1*	**0.110±0.064**	**288±38**	**269±37**	**303±42**	**269±37**	**277±42**
	(N=10)	0.055-0.236	224-357	196-332	238-381	196-332	206-2365
	Ef-3	**0.111±0.062**	**308±48**	**283±43**	**325±53**	**294±49**	**293±44**
	(N=19)	0.056-0.251	234-404	205-357	242-434	221-373	225-380
	Em-1	**0.103±0.025**	**336±37**	**309±37**	**356±37**	**317±42**	**322±36**
	(N=6)	0.068-0.131	286-384	268-353	303-408	270-371	275-373
	Em-2	**0.079±0.023**	**284±51**	**251±37**	**305±63**	**267±47**	**264±45**
	(N=6)	0.059-0.116	213-333	205-295	220-385	205-327	204-311
	Em-3	**0.082±0.026**	**282±34**	**253±44**	**299±37**	**260±47**	**276±35**
	(N=10)	0.054-0.148	195-309	156-286	198-323	156-307	195-310

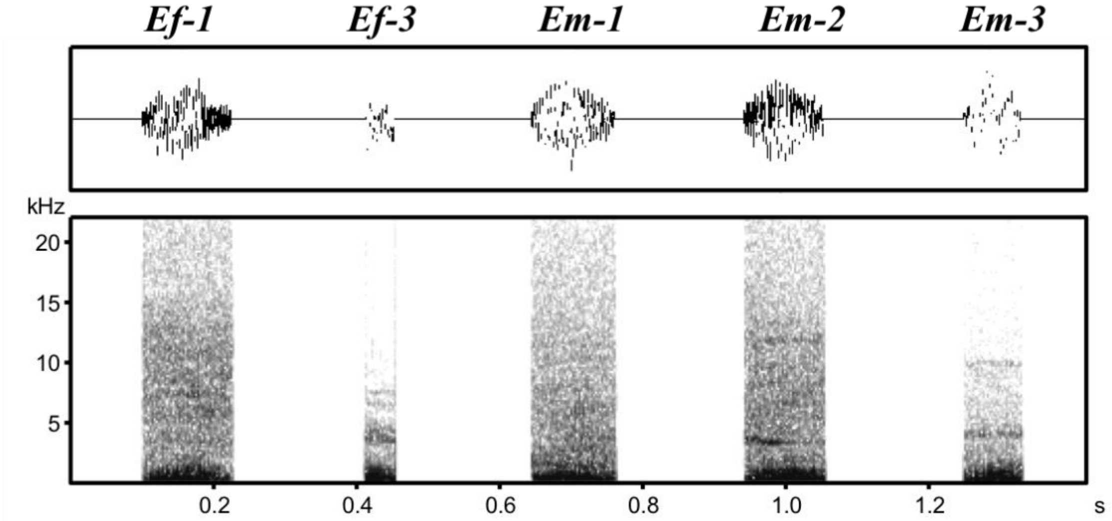

Figure 5.13 Spectrograms and oscillograms of hoot exemplars from the five popualtions. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-2= E. macaco*, Mahadera; and *Em-3= E. macaco*, Lokobe.

While multivariate tests revealed significant interpopulation differences in acoustic variables of hoot calls (MANOVA: Wilk's λ= 0.43, F(4,45)=1.63, p=0.043), the DFA showed considerable overlap in the populations (Figure 5.14). LD1 explained 70.9% of the between-population variance and LD2 explained the remaining 14%. F0min, F0s, and F0f were found to be important predictor variables. Leave-one-out cross validation assigned 22% of the hoot calls to the correct populations (Table 5.13). Pairwise comparisons of each variable can be found in Appendix I.

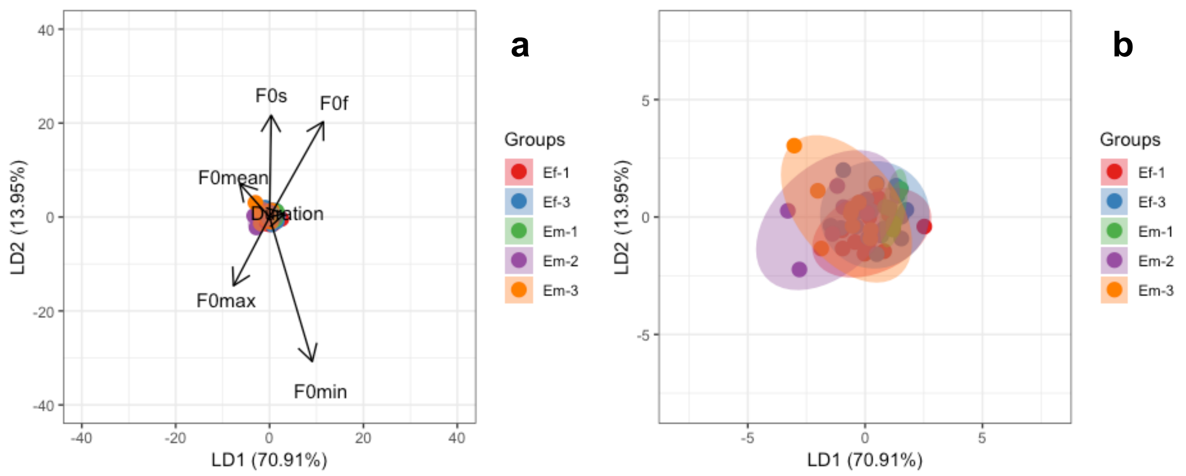

Figure 5.14 Biplot of the first two discriminants (LD1 and LD2) of the DFA classification for hoot calls. Plot b is a zoomed in version of plot a, highlighting the overlap in the populations. Vectors represent the variation in the acoustic variables. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

Table 5.13 Percentages (and counts) of correctly (in bold) and incorrectly assigned hoot calls from stepwise discriminant function analysis with leave-one-out cross validation. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

Population	**Assigned population**				
	Ef-1	*Ef-3*	*Em-1*	*Em-2*	*Em-3*
Ef-1	**20** **(2)**	60 (6)		10 (1)	10 (1)
Ef-3	11.1 (2)	**44.5** **(8)**	11.1 (2)	11.1 (2)	22.2 (4)
Em-1		100 (6)			
Em-2		50 (3)		**16.7** **(1)**	33.3 (2)
Em-3	10 (1)	60 (6)		30 (3)	

5.3.2 Additional calls

Chatter

Ten chatter calls were measured from four populations (Table 5.14, Figure 5.15). Chatter was characterised as a low-amplitude composite call comprised of a series of short, grunt-like pulses strung together in quick succession. On average, pulse duration and inter-pulse intervals were around 0.02-0.035 s in duration, with rate of 21.45-25.39 pulses/s across four populations. Between 2 and 4 formants were typically visible within a pulse. Chatter was emitted by a male during the mating context, either while engaging in sexual grooming (frantic anogenital sniffing and licking) or when mounting a female. This call was only heard when the female was refusing to mate with the male and often occurred at the same time as a high-pitched squeal call.

Table 5.14 Acoustic parameters of measured chatter calls. For each call, total call duration (Dur), four individual pulse durations (PDur), three inter-pulse intervals (IDur), the number of pulses/second (Rate), 1st formant (F1), 2nd formant (F2), and 3rd formant (F3) were measured. The means and standard deviation are in bold, the range below. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-3*= E. *macaco*, Lokobe.

Call type	**Pop**	**Rate (pulse/s)**	**PDur (s)**	**IDur (s)**	**F1 (Hz)**	**F2 (Hz)**	**F3 (Hz)**
Chatter	*Ef-1*	**21.45±1.19**	**0.023±0.006**	**0.032±0.007**	**441±86**	**1248±94**	**3223±325**
	(N=7)	19.40-22.66	0.013-0.039	0.017-0.043	283-610	1039-1502	2632-3672
	Ef-3	**23.91**	**0.022±0.009**	**0.026±0.005**			
	(N=1)		0.013-0.035	0.020-0.0297			
	Em-1	**25.04**	**0.025±0.005**	**0.023±0.012**	**749±29**	**1850±77**	**3784±223**
	(N=1)		0.021-0.031	0.014-0.031	716-768	1785-1935	3641-4040
	Em-3	**25.39**	**0.021±0.002**	**0.025±0.002**	**383±80**	**1289±174**	
	(N=1)		0.019-0.022	0.023-0.026	297-455	1087-1460	

Note: due to low noise-background ratio, some formants could not be measured from the *Ef-3* and *Em-3* calls.

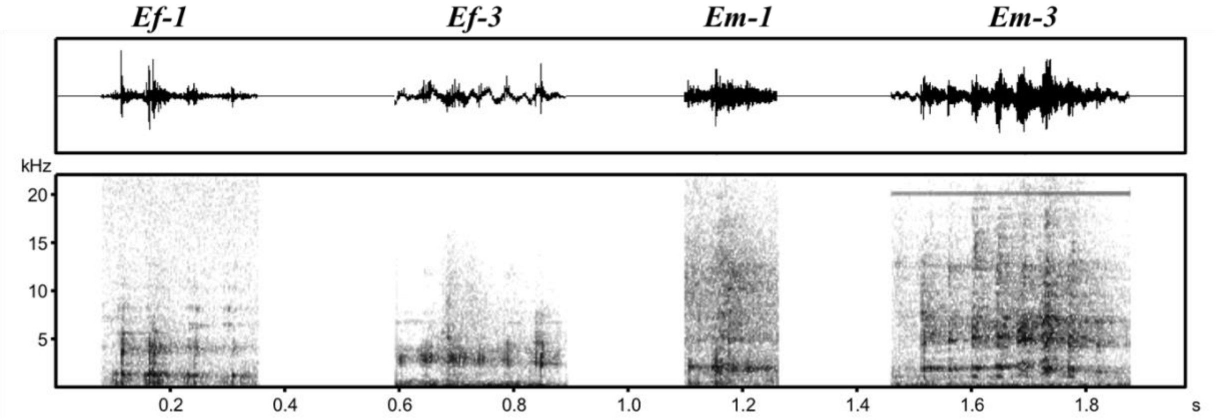

Figure 5.15 Spectrograms and oscillograms of an exemplar chatter call from four different populations. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-3*= *E. macaco*, Lokobe.

Squeals and Modulated Squeals

Prior synonyms—Distress: Petter & Charles-Dominique, 1979; Scream: Gamba et al., 2015

These calls were loud tonal calls representing sizable gradation in their structure (tonal to tonal-noisy), fundamental frequencies (ranging from 2500-6500 Hz), fundamental frequency contours (minimal quavering of harmonics to rapid, sweeping fluctuation), and durations (Table 5.15, Figure 5.16). On one end of the spectrum were squeals. Squeals had sparse harmonics with an initial upsweep in frequency at the onset of the call, followed by minimal frequency modulation, and ending with a down sweep in frequency. There were usually three to four harmonics visible, and in two instances harmonics went above 22000 Hz and outside the sampling frequency. Rapid frequency modulation resulted in modulated squeal calls with waveform or chevron-shaped frequency contours and a distinctly chirping/warbling sound. Depending on the length of the call, only the upward or downward contour of the waveform may be visible. The harmonics of this call type were denser, irregular and complex (subharmonics were often visible), and structurally noisier. Because squeals and modulated squeals were not stereotyped calls and gradations between the two were common, simple statistics are reported here for the collective call category. These calls were given by both sexes. Males emitted these calls only in socionegative situations, such as fights over access to food and during male-male competition for access to females. In addition to these scenarios, females were also observed emitting calls during an unwanted mating attempt.

Table 5.15 Mean, standard deviation, and ranges for the acoustic parameters of squeal and modulated squeal calls for each population. For each call, duration (Dur), the number of visible harmonics (H), mean fundamental frequency (F0mean), minimum fundamental frequency (F0min), maximum fundamental frequency (F0max), start fundamental frequency (F0s), end fundamental frequency (F0f), the change in fundamental frequency (F0dif), and the number of cycle modulations/second (Period) were measured. The means and standard deviation are in bold, ranges below. *Ef-1= E. flavifrons*, Ankarafa; *Em-1= E. macaco*, Andranomatavy; *Em-3= E. macaco*, Lokobe.

Call type	Pop	Dur (s)	H	F0mean (Hz)	F0min (Hz)	F0max (Hz)	F0s (Hz)	F0f (Hz)	F0dif (Hz)	Period (c/s)
Squeal/modulated squeal calls	*Ef-1*	**0.248±0.201**	**3.3±0.9**	**5010±1120**	**4043±1171**	**5643±1107**	**4982±1528**	**4893±1080**	**1600±241**	**18.46±3.83**
	(N=10)	0.058-0.661	2-5	2501-6324	1584-5231	3288-6911	1614-6332	3279-6209	1178-2051	14.05-20.85
	Em-1	**0.216±0.174**	**4.4±0.5**	**3747±1041**	**3017±1423**	**4357±926**	**4192±971**	**3532±1128**	**1339±1325**	**19.58±2.35**
	(N=5)	0.036-0.411	4-5	2784-5036	1298-4904	2881-5100	2976-5029	2504-5036	196-3545	17.92-21.24
	Em-3	**0.337±0.208**	**4.3±1**.1	**3897±871**	**2944±963**	**4653±825**	**3831±960**	**4031±978**	**1709±797**	**18.23±2.49**
	(N=9)	0.104-0.712	3-6	2588-4869	1634-4055	3423-5470	2026-5341	2536-5098	341-3261	15.26-20.69

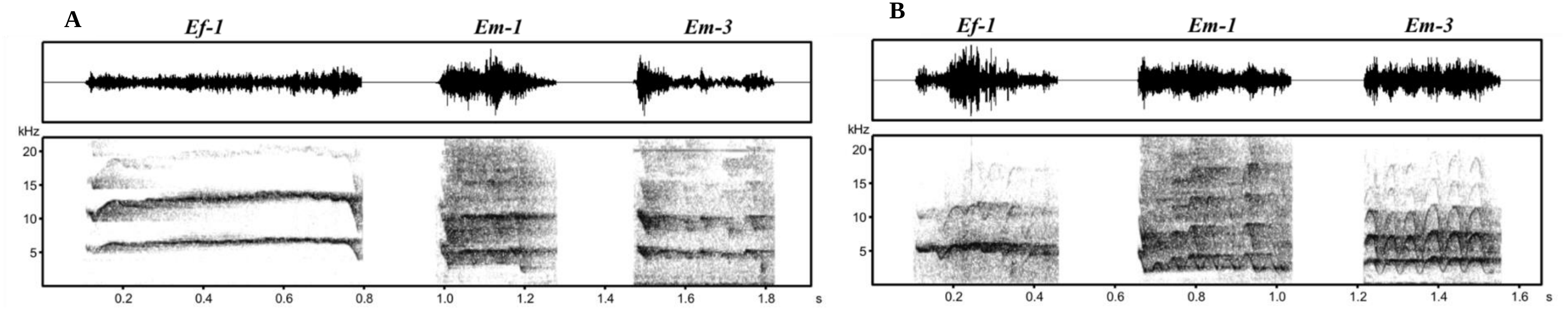

Figure 5.16 Spectrograms and oscillograms of exemplars of squeals (A) and modulated squeals (B). *Ef-1= E. flavifrons*, Ankarafa; *Em-1= E. macaco*, Andranomatavy; and *Em-3= E. macaco*, Lokobe.

Clear calls

Prior synonyms—Group cohesion clear call, tonal close contact call, or trills: Macedonia & Stranger, 1994; Moan: Gosset et al., 2001; Tonal call: Gamba et al., 2015; Clear call: Pozzi et al., 2010

Clear calls were another type of tonal call with significant variation in call duration, structure, fundamental frequency, modulation, and harmonics (Table 5.16, Figure 5.17). The structural pattern of tonal calls included chevron-shaped, down sweep, upsweep, and constant frequencies. In comparison to hoots, clear calls had distinctly higher fundamental frequencies and sparser harmonics. These calls occurred across a variety of different contexts including group locomotion, resting, and social distress.

Table 5.16 Mean, standard deviation, and ranges for the acoustic parameters of tonal calls for each population. For each call, duration (Dur), mean fundamental frequency (F0mean), minimum fundamental frequency (F0min), maximum fundamental frequency (F0max), and the number of visible harmonics (H) were measured. The means and standard deviation are in bold, ranges below. *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-3*= *E. macaco*, Lokobe.

Call type	**Pop**	**Dur (s)**	**F0mean (Hz)**	**F0min (Hz)**	**F0max (Hz)**	**H**
Clear call	*Ef-1*	**0.116+0.077**	**1029+380**	**949+359**	**1196+369**	**6+0.81**
	(N=4)	0.008-0.190	633-1535	548-1415	831-1662	5-7
	Ef-3	**0.031**	**514**	**476**	**554**	**4**
	(N=1)					
	Em-1	**0.283+0.101**	**832+111**	**776+117**	**887+108**	**8+1**
	(N=3)	0.189-0.390	704-909	641-854	769-979	7-9
	Em-3	**0.078+0.049**	**1006+203**	**886+190**	**1187+306**	**9.33+4.12**
	(N=12)	0.021-0.194	767-1469	552-1330	840-1691	5-16

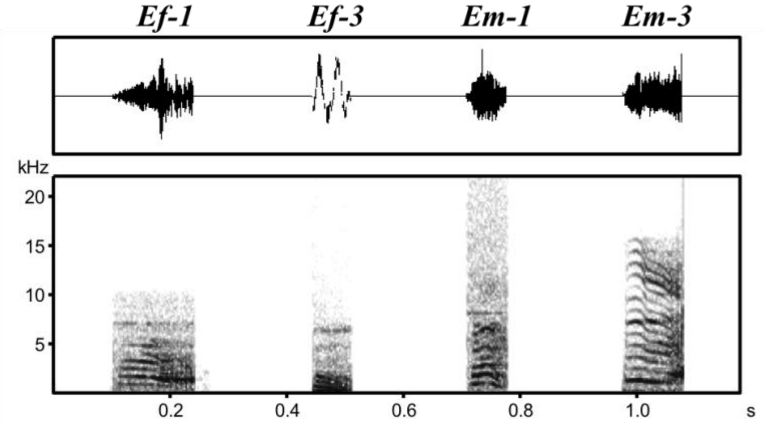

Figure 5.17 Spectrograms and oscillograms of an exemplar clear call from four populations. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; and *Em-3= E. macaco*, Lokobe.

Puffed grunt

Prior synonyms—Puffed grunt: Gosset et al., 2001

Puffed grunts were similar in structure to a typical grunt but were comprised of a series of pulsed elements given at a rate of 165.8-175.2 elements/s (Table 5.17, Figure 5.18). Element durations were very short (0.003-0.004 s) as were the inter-element durations (0.002-0.003). These calls had lower maximum frequencies, near 10000 Hz. To the human ear, puffed grunts sound like grunts emitted in concurrence with a rush of air. Puffed grunts happened infrequently in socio-negative behavioural contexts.

Table 5.17 Acoustic parameters of measured puffed grunts. For each call, total call duration (Dur), three individual pulse durations (PDur), two inter-pulse intervals (IDur), the number of pulses/second (Rate), the mean fundamental frequency (F0mean), 1st formant (F1), 2nd formant (F2), 3rd formant (F3), and 4th formant (F4) were measured. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-3= E. macaco*, Lokobe.

Call type	**Pop**	**Rate (p/s)**	**Dur (s)**	**PDur (s)**	**IDur (s)**	**F0mean (Hz)**	**F1 (Hz)**	**F2 (Hz)**	**F3 (Hz)**	**F4 (Hz)**
Puffed grunt	*Ef-3* (N=1)	165.8	0.060	0.004	0.002	175	521	2773	4590	5990
	Em-3 (N=1)	175.2	0.074	0.003	0.003	339	892	1870	2948	4408

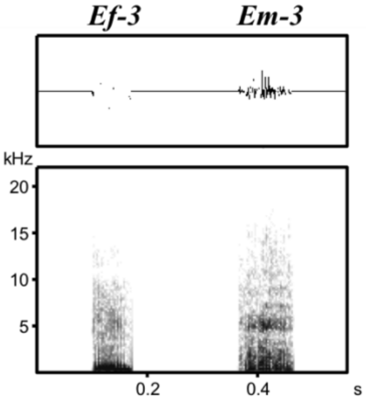

Figure 5.18 Spectrograms and oscillograms of a puffed grunt exemplar from two populations. *Ef-3*= *E. flavifrons*, Angodrahely and *Em-3*= *E. macaco*, Lokobe.

<u>Grunt-hoot, grunt-tonal call, tonal call-grunt</u>

Prior synonyms—Grunt-tonal call: Macedonia & Stranger 1994; Gamba & Giacoma, 2005; Long grunt-clear call: Pozzi et al., 2010

Grunts, hoots, and tonal elements could be combined into a single composite element (Table 5.18, Figure 5.19). This class of calls were under 0.6 s, with the grunt representing a longer duration than either the hoot or tonal component, regardless of order. Fundamental frequencies of the hoot or tonal component were typically under 1000 Hz, although this was not the case in the one *Ef-1* grunt-tonal call measured. The exact nature of these calls could not be determined, although they did tend to occur during situations with elevated levels of arousal.

Table 5.18 Acoustic parameters of measured of combination calls. For each call, total call duration (Dur), the grunt component duration (GDur), 1st formant (F1), 2nd formant (F2), the tonal component duration (TDur), the mean fundamental frequency (F0mean), the minimum fundamental frequency (F0min), and maximum fundamental frequency (F0max) were measured. The means and standard deviation are in bold, ranges below. *Ef-1= E. flavifrons*, Ankarafa; *Ef-3= E. flavifrons*, Angodrahely; *Em-1= E. macaco*, Andranomatavy; *Em-3= E. macaco*, Lokobe.

Call type	**Pop**	**Dur (s)**	**GDur (s)**	**F1 (Hz)**	**F2 (Hz)**	**TDur (s)**	**F0mean (Hz)**	**F0min (Hz)**	**F0max (Hz)**
Grunt-hoot	*Ef-1*	**0.113**	**0.044**	**545**	**1271**	**0.069**	**471**	**437**	**495**
	(N=1)								
	Ef-3	**0.359±0.135**	**0.207±0.157**	**426**	**1358**	**0.152±0.130**	**300±35**	**242±82**	**351±16**
	(N=3)	0.213-0.479	0.085-0.384			0.061-0.301	260-328	149-301	332-361
	Em-1	**0.125**	**0.044**	**442**	**1234**	**0.081**	**303**	**235**	**326**
	(N=1)								
Grunt-tonal call	*Ef-1*	**0.418**	**0.350**	**64**	**2436**	**0.068**	**1945**	**849**	**2013**
	(N=1)								
	Ef-3	**0.497±0.176**	**0.460±0.187**	**766±19**	**2274±37**	**0.038±0.011**	**766±38**	**722±26**	**787±54**
	(N=2)	0.373-0.622	0.328-0.592	752-779	2248-2300	0.030-0.045	739-793	704-741	748-825
	Em-1	**0.505±0.072**	**0.443±0.065**	**770±85**	**2294±141**	**0.062±0.019**	**750±122**	**695±129**	**810±169**
	(N=7)	0.346-0.561	0.301-0.488	600-842	2007-2456	0.041-0.093	592-967	585-966	605-1086
	Em-2	**0.476±0.164**	**0.401±0.230**	**832±43**	**2307±183**	**0.074±0.069**	**591±121**	**563±121**	**625±124**
	(N=4)	0.242-0.627	0.065-0.582	771-865	2044-2462	0.027-0.177	411-657	382-637	440-708
	Em-3	**0.501±0.104**	**0.437±0.108**	**862±128**	**2419±186**	**0.064±0.015**	**817±92**	**817±92**	**962±204**
	(N=15)	0.33-0.754	0.282-0.698	650-1074	2011-2748	0.045-0.089	659-985	659-985	680-1554
Tonal call-grunt	*Em-2*	**0.346**	**0.256**	**642**	**2097**	**0.090**	**2050**	**2018**	**2063**
	(N=1)								
	Em-3	**0.209±0.108**	**0.132±0.055**	**1139±88**	**2344±291**	**0.077±0.055**	**933±64**	**833±36**	**1058±1**
	(N=3)	0.084-0.284	0.068-0.167	1076-1201	2139-2550	0.017-0.124	864-990	808-859	1057-1059

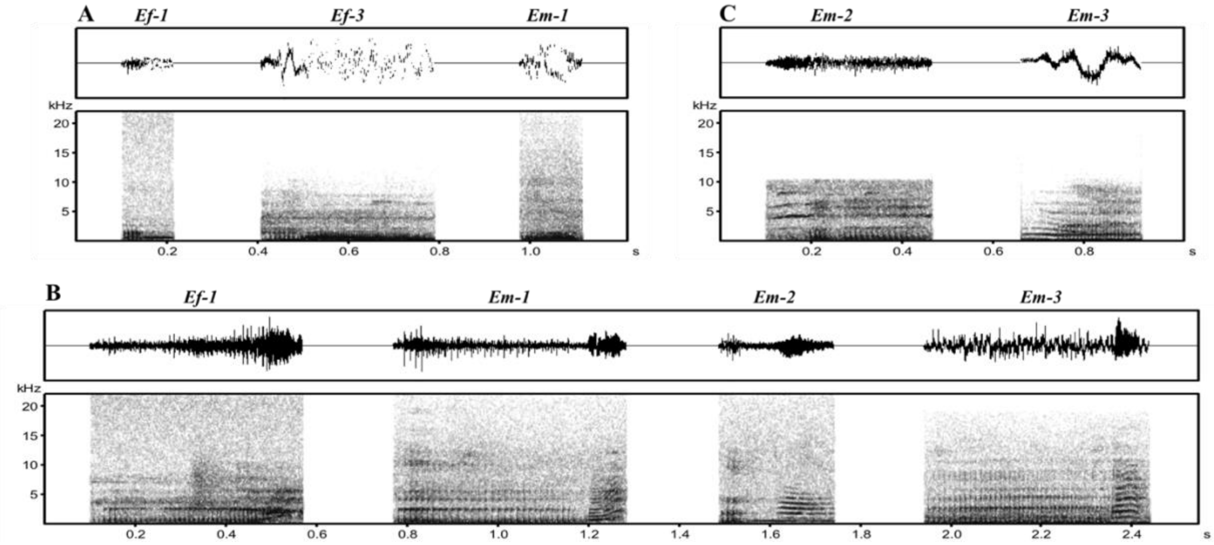

Figure 5.19 Spectrograms and oscillograms of exemplars from three types of composite calls, grunt hoot (A), grunt-tonal call (B), and tonal call-grunt (C). *Ef-1*= *E. flavifrons*, Ankarafa; *Ef-3*= *E. flavifrons*, Angodrahely; *Em-1*= *E. macaco*, Andranomatavy; *Em-2*= *E. macaco*, Mahadera; *Em-3*= *E. macaco*, Lokobe.

5.4 DISCUSSION

The results of this study not only corroborated previously documented calls for these species, but also identified three new call types that have not been recorded in either *E. flavifrons* or *E. macaco*. These include chatter, modulated squeals, and tonal-grunt calls. Interestingly, the comparison of calls from two frequently occurring categories, grunts and alarm calls, revealed that there were no clear and consistent species differences in many of the acoustic parameters measured. This finding has distinct implications for our current understanding of whether acoustic communication played a role in the speciation process of these two taxa and suggests that vocalisations currently would be unlikely to serve as a full reproductive barrier preventing interbreeding. Therefore, this study lends acoustic support for their putative natural hybridisation.

5.4.1 Evidence of acoustic divergence

The most striking finding from this study is the limited evidence of divergence in call types and their acoustic parameters between the two species. Nearly all of the 15 call types documented here were heard, if not also measured, from each of the five populations. In the *Em-2* population, chatter, squeals/modulated squeals, and tonal calls were not recorded; this may be due to the limited time spent at this location (13 days) and the fact that no instances of mating were observed during this time. *Em-2* aside, this finding suggests that there are no notable differences in the vocal repertoires of the two species, which is to be expected for closely-related taxa that have similar social systems (Macedonia & Taylor, 1985; McComb & Semple, 2005; Fichtel & van Schaik, 2006). In addition, the repertoire of captive (Gosset et al., 2001; Gamba & Giacoma, 2005; Pozzi et al., 2010; Gamba et al. 2015) and wild *E. flavifrons* and *E. macaco* populations appears to be relatively stable, supporting the hypothesis that vocalisations are an innate feature in primate species and remain highly conserved through generations (Winter et al., 1973; Geissmann, 1984; Macedonia & Stranger, 1994; Seyfarth & Cheney, 1997).

Grunts, irrespective of their duration, were the most ubiquitous calls produced by both *E. flavifrons* and *E. macaco*. The quality of the louder long grunts, which also have low fundamental frequencies (less than 160 Hz) and first formants (typically 600-800 Hz), make these calls less susceptible to signal attenuation and degradation over long distances (Waser & Brown, 1984). In fact, it was the intermediate and long grunts that were typically strung together in a series to produce a stereotyped Train of Long Grunts (TLG) that could be heard over long distances. These compound calls were almost exclusively given by males and both group members and non-members responded in kind. Calls occurred throughout a variety of behavioural contexts, including in the morning and evening as a group spacing signal, during intergroup encounters, and at seemingly random times. In the *Em-1* population, where lemurs spent more time apart from group members, this call was recorded more often. Interestingly, after mating subsided later in the season so did the frequency at which these vocalisations were heard.

Given this, it is possible that the TLG could not only signal individuality and group membership (Petter & Charles-Dominique, 1979; Gosset et al., 2001; Gamba et al., 2012a), but also serve as a form of male advertisement to attract reproductive females during the mating season. Long-distance advertisement calls can showcase the fitness of the caller, be used to evaluate rival males, or facilitate female choice in mates (Wich & Nunn, 2002; Delgado, 2006). These calls may be energetically costly to produce (Cowlishaw, 1996), which would account for the drop in TLGs after mating subsided. Use of loud calls as an ‘acoustic probe’ has been documented previously in *Microcebus* spp. (Zimmermann et al., 2000; Braune et al., 2008), *Cercopithecus* spp. (Zuberbühler, 2004), *Macaca nigra* (Neumann et al., 2010), and several Neotropical taxa (for review, see Oliveira & Ades, 2004). *Lemur catta* have been observed travelling to the edge of their territory, emitting a loud call, and then listening for responses during the mating season. Like *E. flavifrons* and *E. macaco*, this call is also thought to function as a contact, spacing, and advertisement signal (Jolly, 1966; Koyama, 1988; Macedonia, 1993). The addition of data assessing the individuals that produce TLGs most often, their dominance rank, and their mating success rate will help test if these calls are used to advertise and attract females during the mating season.

Previous studies looking at long grunts of mostly captive individuals have found interspecies differences in formant frequencies between not only *E. flavifrons* and *E. macaco* (Gamba & Giacoma, 2008), but between other *Eulemur* species as well (Gamba et al., 2012a, 2012b), concluding that these calls may be important in species-specific recognition. Because of this, the grunts of *E. flavifrons* and *E. macaco* populations in this study were predicted to differ as well. Surprisingly, this was not the case, especially given their potential role in individual advertisement during the mating season. Although there were multiple population differences in the fundamental frequency of long grunts, which are known to fluctuate considerably within individuals (Gamba et al., 2012a), there were no other significant differences in the formant frequencies, apart from the *Ef-3* population. Playback experiments with *Microcebus* spp. (Braune et al., 2008) and *Tarsius* spp. (Nietsch & Kopp, 1998) have shown that vocalisations that are species-specific can contribute to assortative mating and prevent interbreeding in areas of sympatry. At the same time, convergence of call features has been found in *Alouatta* spp. (Kitchen et al., 2017), suggesting that in certain cases, vocalisations may not be a sufficient mechanism to prevent hybridisation in sympatric primates. Given the minimal acoustic differences found between the *E. flavifrons* and *E. macaco* populations in this study, it is unlikely that long grunts would significantly contribute to species recognition in the wild, nor would they necessarily serve as a pre-mating isolation barrier.

Contrary to what was predicted, the acoustic parameters of alarm calls, which can alert conspecifics of aerial predators and/or terrestrial disturbances or monitor conspecific group location (Colquhoun, 1993), typically did not differ between species. This may be because having similar predator pressures lends itself to similar alarm call features (Zimmermann et al., 2000), especially if these types of calls are not under sexual selection pressures (Zimmermann, 2016). Additionally, interspecific recognition of other species' alarm calls may be beneficial in avoiding predators (Oda & Masataka, 1996; Fichtel & van Schaik, 2006). The solitary *Lepilemur sahamalaza* was found to respond differentially to areal and terrestrial calls of sympatric species, including *E. flavifrons* and two species of birds (Seiler et al., 2013). Similarly, *E. rufus* and *Propithecus verreauxi* were found to respond appropriately to each other's referential alarm calls (Fichtel, 2004). Therefore, important acoustic features to garner listeners' attention, such as broad amplitudes and harsh structure, are more likely to have been conserved between heterospecifics, whereas appropriate responses are more likely to have been learned (Fichtel, 2004; Seiler et al., 2013).

Differences in acoustic parameters of similar call types have been shown to be a good measure/indicator of phylogenetic divergence in primates (Zimmermann, 1990; Ambrose, 2003; Ross & Geissmann, 2007; Braune et al., 2008; Méndez-Cárdenas et al., 2008; Meyer et al., 2012) and can function as an effective pre-mating barrier between closely-related species (Braune et al., 2008). Because no clear and consistent differences in either the repertoire or acoustic features of common calls were found between the populations or the species, vocalisations may not fully support species-specific recognition or serve as a pre-mating isolation mechanism. There remains a caveat to this conclusion, the *Ef-3* population. This was the closest sampled *E. flavifrons* population to the *E. flavifrons-E. macaco* border (less than 4 km from the Andranomalaza River), and it also happened to be the population that differed from the others most consistently. No less than 50 years ago both species were visible on either side of the Andranomalaza River (pers. comm., mayor of Maromandia). Based on the location of this population, this finding lends tentative support to the idea that while allopatric populations of the two species show no divergence in acoustic features, differences are accentuated in areas of close contact (i.e., character displacement, Brown & Wilson, 1956). It is also possible that the *Ef-3* population may be displaying atypical acoustic features as a result of ecological (Brown & Waser, 1988; Petter & Charles-Dominique, 1979), cultural (Green, 1975), or genetic variations (Fichtel & van Schaik, 2006) present at this location (for review see Snowdon, 2009). In order to more thoroughly identify whether this is in fact an example of character displacement or simply an artefact localised to this population only, it is necessary to gather and assess the vocalisations of additional populations of lemurs along the area of repartition for the two species.

5.4.2 Newly identified calls

Three newly described calls for *E. flavifrons* and *E. macaco* are detailed here. Tonal-grunt calls, which have been documented in *E. fulvus* (Gamba et al. 2015), were recorded in the *Em-2* and *Em-3* populations for the first time. Although not heard frequently, these calls occurred during periods of elevated excitement or during group travel. It is well documented that, unlike other species of lemurs, *Eulemur* give grunt-tonal calls (Gamba & Giacoma, 2005). It is possible then that the tonal-grunt call is just a syntactic variant of the typical grunt-tonal call and therefore serves the same function. Also feasible is that the order of this signal conveys different semantic information. By combining call types from a fixed vocal repertoire, the number of distinct messages able to be communicated increases. Vocal flexibility has been demonstrated in several species of primates including *Cercopithecus nictitans* (Arnold & Zuberbühler, 2006), *Hylobates lar* (Clarke et al., 2006), *Saguinus oedipus oedipus* (Cleveland & Snowdon, 1982), *Pan paniscus* (Schamberg et al., 2016), and *Pan troglodytes* (Crockford & Boesch, 2005). Because this vocalisation was not recorded in either *E. flavifrons* populations, or in the third *E. macaco* location, it is conceivable that this may be a local dialect (Zuberbühler, 2011).

Produced by males in all populations apart from *Em-2* (which was observed outside the mating season), chatter is a low-pitched, grunt-like call that was heard only in the context of mating, when the male was trying to mate with an unreceptive female. Chatter and a squeal/modulated squeal call were often heard together. In studies of *Ateles geoffroyi*, it was found that aggressors use vocalisations that are lower in frequency, while the victims use higher frequencies (Ordóñez-Gómez et al., 2015). As larger bodies tend to produce calls with lower frequencies (Bowling et al., 2017), and size is tied to fitness (Gaulin & Sailer, 1983; Mitani et al., 1996), this may be an adaptive signal that males are using to persuade females with regard to their fitness, thereby enticing them into copulating. It is also possible that this is a submissive signal. Subordinate *Varecia variegata* give a spectrally similar call during agonistic interactions with more dominant individuals (Pereira et al., 1988).

Two variants of a high-pitched tonal call, the squeal and modulated squeal, were recorded during three types of socio-negative interactions—rejected mating attempts, aggressive encounters over food, and male-male competition for access to a reproductive female. Recorded in the *Ef-1*, *Em-1*, and *Em-3* populations, these calls had varying levels of frequency modulation and structural complexity and seem to represent extreme forms, with gradations in between. Squeals had less frequency modulation, while the frequency contours in modulated squeals were wave and chevron-shaped; this gave the call a chirp-like quality. Graded signals also have been reported in *Macaca sylvanus* (Fischer & Hammerschmidt, 2002), *Papio ursinus* (Meise et al., 2011), *Pan troglodytes* (Slocombe & Zuberbühler, 2007), *E. fulvus* (Fichtel & Hammerschmidt, 2002), *Circopithecus campbelli* (Keenan et al., 2013), and *Macaca mulatta* (Fitch et al., 2002) and are thought to convey differences in emotional states or arousal levels (Fitch et al., 2002; Fischer & Price, 2016). Vocalisations tend to become higher-pitched, have longer durations, and become noisier with increased arousal (Morton, 1977; Scherer et al., 2003; Briefer, 2012). Therefore, different levels of distress could account for the observed variation in both modulation and call length of squeals and modulated squeals in this study.

Because squeals and modulated squeals were not limited to one behavioural context nor to a specific sex suggests that they are not simply copulation calls. Several species of female mouse lemurs are also known to give high-pitched, chevron-shaped calls to reject approaching males during courtship (Zimmermann, 2010). In the mating context, not only could these calls function to deter males from approaching a female, but they could also conceivably serve to alert conspecifics and elicit their help. Often *E. flavifrons* and *E. macaco* females were unreceptive to male copulation solicitations and these high-pitched squealing signals were given in response. In several instances, satellite females and juvenile males were then observed disrupting mating by approaching the mating pair and sitting on the male, and cuffing or biting him, thereby stopping the event.

5.4.3 Comparisons to previous studies

Gamba & Giacoma (2008) found that the mean formants (± SD) of captive *E. flavifrons* were significantly lower (F1: 544 ± 225, F2: 2401 ± 346, and F3: 4792 ± 243) than for captive *E. macaco* (F1: 1035 ± 247, F2: 3108 ± 351, and F3: 5165 ± 303), concluding these features may be important in species-specific recognition. Formant frequencies measured from all populations in this study, regardless of species, closely resemble the acoustic features of *E. flavifrons* grunt calls from the study by Gamba & Giacoma (2008). Discrepancies in the measured formant frequencies of *E. macaco* grunt calls between this study and the aforementioned one may be due to different recording techniques, the equipment used to record these calls, hidden formants not being detected by spectral settings, or the quality of captive versus wild recordings. If any of these were the case though, one would expect to see discrepancies across both species and not just in the *E. macaco* calls. Another plausible explanation could be related to the physical size of captive and wild individuals. Because formants are produced by resonance in the vocal tract (Fant, 1960), and vocal tract length is a morphological attribute that has been shown to be correlated with body size, with larger animals having longer vocal tracts and producing lower frequency calls (Ey et al., 2007; Bowling et al., 2017), it is conceivable that body size is an important predictor of frequency variation within these taxa. Although there are no morphometric data from most of these populations, lemurs from the *Em-3* population at Lokobe are known to be smaller than conspecific populations on the mainland (Junge & Louis, 2007). A more thorough understanding of the morphometric characters of compared populations therefore would be useful.

A third possibility is related to problems associated with gathering data from individuals living in unnatural conditions. *Ex situ* data, although easier to obtain, may fail to accurately depict the flexibility that is typical for that particular taxon or may overrepresent captive characters that have deviated from their wild counterparts (Lambrechts et al., 1999). Identifying levels of variability in acoustic features of wild populations can provide important information on morphological and genetic variation (Macedonia & Taylor, 1985) or cultural dialects (Green, 1975; Mitani et al., 1992; de la Torre & Snowdon, 2009) that may be missing from captive populations. To my knowledge there are few studies comparing the vocalisations of *in situ* and *ex situ* individuals; those that exist tend to show few repertoire discrepancies, but often find differences in spectral features (Kalcounis-Rueppell et al., 2010). This comparison of findings highlights the importance of including wild populations in acoustic behavioural studies, especially when defining what is characteristic for a species.

5.4.4 Conclusion

Based on the fact that the two species were found to have diverged only recently (160 Kya: Meyer et al., 2015), that only minimal genomic differences exist (Pastorini et al., 2000), and there now there is limited evidence to support significant acoustic differences, it is possible that the two taxa are still in the process of speciation. So, although I can only speculate on the importance of vocalisations in species recognition and the role they may play in mate selection, it seems rather unlikely that acoustic communication serves as a full-formed reproductive isolation barrier, nor would it serve as the main driving force separating the two taxa. Rather ecological factors may have played a larger role in their initial division (Blair et al., 2013), as these species exist on an ecological cline that transitions between dry deciduous forests and sub-humid forests (Mittermeier et al., 2008). Given the ongoing and continual forest loss throughout Madagascar (Vieilledent et al., 2018), it is probable that these two taxa will eventually be irrevocably isolated from each other, at which point multiple characters, including acoustic, may rapidly diverge (Wilkins et al., 2013). By continuing to monitor changes in both their repertoire and the features of their calls over time, we may be able to document both the effects of habitat loss and isolation on the behaviours of these two taxa, while also contributing to what is known about the speciation process in lemurs.

Chapter 6

Synthesis, Conclusions, and Future Work

Through the contribution of new information to the growing body of research detailing the behavioural ecology of the Critically Endangered blue-eyed black lemur (*Eulemur flavifrons*) and Vulnerable black lemur (*E. macaco*), this study endeavoured to identify species-specific behaviours as potential pre-mating isolation mechanisms and to illuminate possible modes of speciation between the two taxa. While the previous chapters have detailed the similarities and differences in the two species' social systems and vocal communication, this final chapter concludes by synthesising these previous findings and addressing the likelihood of the natural hybridisation of *E. flavifrons* and *E. macaco* from an evolutionary perspective. Future work and conservation actions are also presented.

6.1 Updated range distributions of *E. flavifrons* and *E. macaco*

The first question this study sought to answer was the proposed parapatry of *E. flavifrons* and *E. macaco* and whether this resulted in the two species interbreeding. Despite extensive searching in previously speculated areas of range overlap in the Manongarivo Special Reserve (Meyers et al., 1989; Rabarivola et al., 1991; Goodman & Schütz, 2000; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004), a contact zone was not established during survey work. This does not mean conclusively that one does not, or at one time did not exist. Nor does this allow for conclusions to be reached regarding the two taxa's hybridisation. The inability to survey every conceivable region of possible overlap was due to time constraints of this study and complications in navigating this region of Madagascar. Furthermore, seasonal variations in ranging patterns could have contributed to the difficulty in locating lemur groups. Villagers in Ambodivoahangy informed us that lemurs can be difficult to find in the foothills of MSR during the dry season. Because food scarcity has been found to increase lemurs' home ranges and daily travelling (Volampeno et al., 2011a), it is possible that the lemurs in Ambodivoahangy may have moved further into MSR to exploit available resources during the dry season. In order to further clarify the area of repartition in MSR, surveys across all seasons are needed in this location. In addition, the polyspecific association between *E. macaco* and *E. fulvus* reported in Chapter 2 further confounds reports of *E. flavifrons* x *E. macaco* hybrids from this region, as morphological intermediates may actually be *E. macaco* x *E. fulvus* hybrids. Therefore, genomic samples from all observed *Eulemur* in MSR, including *E. fulvus*, are highly recommended.

During the survey work, new populations of *E. flavifrons* were observed to the southwest of MSR, but north of the Andranomalaza River. This river has served as the purported geographic barrier between the two species (Koenders et al., 1985; Andriaholinirina et al., 2014a, 2014b), but based on this survey work, the more likely barrier is the more northern Manongarivo River and its tributary. In collaboration with Jen Tinsman at Columbia University, Extent of Occurrence (EOO) and Area of Occupancy (AOO) for the two species were updated. The EOO of *E. flavifrons* was increased by 28.7%, while the EOO of *E. macaco* was decreased by 44.5%. When these EOOs are limited to suitable habitat and remaining forest cover, *E. macaco* has an AOO of less than 1,256 km^2 and *E. flavifrons* of less than 560 km^2. Compounding this finding are the ongoing anthropogenic pressures to this region; forest fragmentation, habitat exploitation, and hunting remain widespread. To address these concerns, we suggest that MSR should be expanded to protect these newly identified populations of *E. flavifrons*. In addition, the extension of AEECL's community-based education programme and the inclusion of a community-led lemur and forest monitoring programme off the Sahamalaza Peninsula would serve as practical conservation measures for the *Eulemur* species located here.

6.2 Behavioural characters of *E. flavifrons* and *E. macaco*

The next overarching question this study sought to answer was whether species-specific variations in behavioural characters exist and whether they are distinct enough to potentially serve as pre-mating isolation mechanisms between the two species. To do this, the social organisations, social structures, mating systems, and vocalisations of *E. flavifrons* and *E. macaco* were compared across multiple locations. Collectively, the individual studies found little evidence to support consistent species-level divergence of any of these characters.

Several important lower-level findings and population nuances from each chapter are worth reiterating here. Group size and sex ratios were relatively consistent between the populations, while fission-fusion dynamics, rates and patterns of grooming and aggression, and the relationship tenor of dyads showed evidence of intraspecific plasticity. In most of the studied populations (*Ef-1*, *Ef-3*, and *Em-3*), intersexual interactions initiated by males were found to be more socio-positive (i.e. have higher rates of grooming and lower rates of aggression) while intrasexual interactions tended to be more socio-negative. The quality of these dyadic relationships may be due to the timing of this study and might reflect strategic methods to ensure mating success during courtships, with males possibly trying to exchange grooming for access to reproductive females. In *Propithecus verreauxi*, males that groomed females more often also were found to mate more frequently, suggesting that grooming may play an integral role in shaping reproductive strategies in this taxon (Norscia et al., 2009).

In comparison to the findings from the *Ef-1*, *Ef-3*, and *Em-3* populations, consistent differences were found in one population of *E. macaco*, *Em-1*. In this population group cohesion was low, and lemurs spent most of their day alone or in small groups of 2-3 lemurs. Female-female interactions were the most socio-positive in this population, as females tended to groom each other more often, for longer periods of time, and engage in fewer agonistic interactions. This finding is surprising, given that inter-female aggression is typically pronounced in lemurs (Kappeler, 1993b, 1997; Roeder et al., 2002; Kappeler & Fichtel, 2015), especially during the mating season (Vick & Pereira, 1989; Digby, 1999; Randriatahina & Roeder, 2013). Additional research is needed to determine if this is typical in this population throughout the year, or an adaptive strategy that is advantageous for some reason during the mating season. All populations in this study would further benefit from long-term behavioural research in which individuals, and not just their sex, can be identified readily. Such studies are needed to better understand the influence of kinship, social hierarchies, and individual preferences on group dynamics, as well as their role in individual mating tactics and reproductive success.

Similarly, although the two species' sexual behaviour repertoires did not differ, the *Em-1* population was found to used different mating tactics compared to the other populations. Courtships in the *Em-1* population were characterised as having high levels of reciprocity. This included consensual mate-guarding, female copulation consent, and significantly longer courtship durations. As an alternative mating tactic, satellite males were frequently observed trying to divert the guarding male's attention away from the reproductive female to mate surreptitiously. In comparison, sexual coercion by males and female rejection were commonly observed tactics in the other three populations. These findings demonstrate the variability in sexual behaviours and mating strategies, even within a single taxon, and highlight the importance of adaptability to novel social and ecological conditions. The use of alternative reproductive tactics to gain access to mating partners is well documented in male and female primates, and can occur pre-, during, or post-mating (for review see Setchell, 2008). Similar reproductive behaviours to those observed in this study include coercion by *Lophocebus albigena* (Arlet et al. 2008), interruption of courtships by *E. fulvus* coalitions (Gachot-Neveu et al., 1999), and female mate choice/proceptive behaviours in *Cebus apella* (Janson, 1984). The addition of needed demographic data mentioned previously, as well as data on oestrus cycling of females, would greatly aid in identifying the factors affecting mating decisions in these populations. Furthermore, genomic research would elucidate the connection between mate choice, mating tactics, mating success, and reproductive success.

Understanding a species' reproductive biology is essential for successful captive breeding programmes of endangered primates (Kappeler, 1989; Asa et al., 2007; Campbell, 2011). Captive populations of both *E. flavifrons* and *E. macaco* can be found currently throughout Europe and North America, where they are typically housed in intersexual pairs (S. Gedman, pers. comm.). There is growing concern regarding the level of genetic diversity of these lemurs, especially for *E. flavifrons* which has a smaller founding population. Breeding programmes are therefore looking for strategies to maintain diversity levels (B. Lefaux, pers. comm.). This study can inform decisions on best practices to ensure mating success. Chapter 3 concluded that in the wild, both *E. flavifrons* and *E. macaco* are found in multimale-multifemale groups, while Chapter 4 highlighted the importance of consensual female mate choice in determining courtship success. As such, providing captive lemurs with a social environment that more closely resembles that found naturally, i.e. multiple potential mates, would result in more flexibility in mating choices and enhanced preferential partnering. This in turn could foster the development of consensual courtships and increase chances of successful reproduction.

As with the two species' social systems, one population exhibited consistent differences in the acoustic features of vocalisations. In this case, it was a *E. flavifrons* population, *Ef-3*. This was the closest sampled population to the *E. flavifrons-E. macaco* border, so it is possible that the observed differences are indicative of character displacement to aid in species recognition. This is the first, and only, finding that would lend support to a potential pre-mating barrier in an area of parapatry. This conclusion is preliminary, especially given the small *Ef-3* sample size and a lack of data from a similarly located parapatric *E. macaco* population. Additional recordings are needed from this area to determine if acoustic parameters do in fact differ in areas of near contact in a direction that would reinforce species-specific recognition and assortative mating of the two species.

Three new call types were described during this study as well—chatter, squeals/modified squeals, and tonal-grunt calls. The former two were heard during coercive mating interactions. The high-pitched squealing is thought to convey female distress and deter male advances, while the low-pitched chatter call may be used to persuade and/or assuage females. The infrequency in which tonal-grunt calls were heard, made it difficult to identify conclusively their function, although it appears that these calls are given in similar contexts as the grunt-tonal calls. As such it is possible that these calls are a syntactic variant of the same call type, although the possibility remains that the order of the call's components conveys important semantic information.

Understanding the root cause of observed intraspecific variation is fundamental to conservation efforts for both species (Strier, 2017). It appears that the flexibility in the species' social systems may be at least partially due to differing ecological conditions. Increased forest fragmentation has been shown to result in increased foraging efforts in *E. fulvus* (Dehgan, 2003). Provisioning and clumping of *Macaca radiata* food sources have been found to alter group structure from multimale-multifemale to single-male units, where males are able to monopolise females more readily (Sinha et al., 2005). Similarly, *Erythrocebus patas* will adopt linear dominance hierarchies when food sources are clumped, thus changing the social relationships of group members (Nakagawa, 2008). Therefore, variation in behaviours may indicate adaptability and/or increased chances of survival in novel situations. Most of the observed populations in this study live in small and severely fragmented and disturbed forest patches. Often there are also numerous anthropogenic pressures, including hunting, trapping, livestock grazing, logging, bushfires, roads, and ecotourism. Collectively, these conditions may be contributing to elevated levels of stress in the populations, which can alter the behaviours of individuals. The plasticity detailed here, as well as in previous studies (Schwitzer et al., 2007b; Prodger, 2015), suggests that although the two species seem reasonably resilient to environmental modifications and can adapt to living in disturbed fragments, some of the species-typical traits associated with *E. flavifrons* and *E. macaco* may be responses to living in these increasingly stressful habitats. Given the continual forest loss throughout Madagascar (Vieilledent et al., 2018), ongoing research is needed to assess how *E. flavifrons* and *E. macaco* respond to rapid habitat changes. In the meantime, reforestation corridors in the *Ef-1*, *Ef-2*, *Ef-3*, and *Em-2* locations may be an immediate action that could help mitigate stress associated with fragment isolation. By connecting available forest patches, these populations could have better access to more resources as well as increased dispersal and transfer options, which could improve mating choices.

6.3 Conclusions regarding possible modes of speciation for *E. flavifrons* and *E. macaco*

The final question this study sought to answer was how the data could inform our understanding of possible modes of speciation for the two taxa. In fact, this is a relatively common line of inquiry for researchers as they try to make sense of the unique distribution and high levels of diversification in Madagascar's lemur taxa (Martin, 1972; Pastorini et al., 2003; Goodman & Ganzhorn, 2004; Ganzhorn et al., 2006; Wilmé et al., 2006; Blair et al., 2013). By identifying barriers to gene flow, primate speciation events can be classified based on genetic, geographic, and/or environmental origins (Godfrey & Marks, 1991; Coyne & Orr, 2004).

One hypothesis attributes the high levels of lemur endemism in Madagascar to the country's extensive river systems. Based on their prevalence throughout the country (Pastorini et al., 2003), the elevation of headwaters (Goodman & Ganzhorn, 2004), the direction of drainage systems (Ganzhorn et al., 2006), and the isolation of watersheds (Wilmé et al., 2006), rivers are thought to act as physical barriers to dispersal for lemurs. Under such conditions, the geographic isolation of lemur populations may have led to their genetic isolation, ultimately resulting in allopatric speciation (Coyne & Orr, 2004). Rivers in Madagascar are hypothesised to contribute to the geographic isolation and speciation of *Indri indri* (the Mangoro and Bemarivo Rivers: Goodman & Ganzhorn, 2004; Mittermeier et al., 2008, 2010), *Varecia rubra* and *V. variegata* (the Antainambalana River: Mittermeier et al., 2008), *E. rufus* and *E. rufifrons* (Tsiribihina River: Pastorini et al., 2003; Blair et al., 2013), *E. collaris* and *E. cinereiceps* (Mananara River: Wyner et al., 2002; Goodman & Ganzhorn, 2004; Blair et al., 2013), and several taxa belonging to the genus *Propithecus* (Goodman & Ganzhorn, 2004; Ganzhorn et al., 2006). This postulated role of rivers in species distribution has been extended to platyrrhines and catarrhines as well. It is hypothesised that the current distribution of Amazonian primates is the result of the region's prevalent river systems, with river size and flow acting as good predictors of whether primates could potentially cross over the body of water (Ayres & Clutton-Brock, 1992). Similarly, in central and west Africa, rivers and the contracting and expanding forests also have been suggested to act as barriers to dispersal, contributing to the geographical range patterns of primates in these regions (Kamilar et al., 2009; Harcourt & Wood, 2012).

A second common hypothesis relies on Madagascar's unique climatic and phytogeographic regions (Humbert, 1955; Martin, 1972; Tattersall & Sussman, 1975), and the partitioning of ecological niches (Martin, 1972; Blair et al., 2013) to explain the adaptive radiation of extant lemur taxa. Under this hypothesis, parapatric speciation is thought to have occurred as populations ventured into new habitats along environmental clines (Bush, 1975; Coyne & Orr, 2004). Kamilar & Muldoon (2010) found support for the climatic variation in lemur diversity in Madagascar by correlating rainfall and temperature to phylogenetic distance of taxa.

Updated range distributions and behavioural character findings from this current study can inform the likelihood of either of these two hypotheses in explaining the speciation of *E. flavifrons* and *E. macaco*. While the Andranomalaza River was the purported physical boundary separating the two species (Koenders et al., 1985; Andriaholinirina et al., 2014a, 2014b), this study did not find support for this hypothesis. The fact that lemurs resembling *E. flavifrons* were found north of this river implies that it never served as a complete barrier to dispersal or that, due to changes in climate/landscape/etc., this river no longer prevents dispersal. While it was proposed in Chapter 2 that the more northern Manongarivo River and its tributary, the Antsahakolana River, are likely to serve as the delimiting river in the western part of their ranges, it still remains unlikely that these rivers serve as full physical barriers between the two species. This conclusion is based on previous researchers' reported observations detailing potential hybrid lemurs to the east of these rivers' headwaters in the Manongarivo Special Reserve (Meyers et al., 1989; Rabarivola et al., 1991; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004). While it is impossible to discredit the significance of rivers in the speciation of *E. flavifrons* and *E. macaco*, given the updated distributions in this study, it seems unlikely that their speciation resulted solely based on geographic isolation.

Instead, it may be that parapatric speciation via ecological niche divergence was the primary mode of speciation for *E. flavifrons* and *E. macaco*. These two species exist on an ecological cline, transitioning from the more northern and humid forests of the Sambirano region to the more dry and deciduous forests of the western region (Cornet, 1974; Schwitzer et al., 2006; Moat & Smith, 2007). Blair & colleagues (2013) recently created ecological niche models based on climate data to test both the ecological divergence and potential speciation process of pairs of *Eulemur* species. While they concluded that allopatric speciation was the likely cause of diversification between other *Eulemur* sister species (*E. collaris - E. cinereiceps, E. rufus - E. rufifrons,* and *E. albifrons - E. sanfordi*), they found stronger support for the parapatric speciation of *E. flavifrons* and *E. macaco*. The distance between occurrence records for these two species was small yet their ecological niches were found to differ significantly, with precipitation values varying along the transitional environmental cline. In addition, the reported potential hybridisation between *E. flavifrons* and *E. macaco* (Meyers et al., 1989; Rabarivola et al., 1991; Andrianjakarivelo, 2004; Randriatahina & Rabarivola, 2004) occurs in an area of overlap in the ecological niche models of the two species, further supporting the role of the diverging habitats in their speciation. In comparison, if geographic isolation had played a significant role, Blair & colleagues (2013) had predicted to find niche conservatism between *E. flavifrons* and *E. macaco*. The theory behind this is that an uninhabitable physical barrier is needed to split a single species previously occupying a single niche into two populations (Wiens, 2004).

As aforementioned, despite genetic differences (Fausser et al., 2000; Pastorini et al., 2000; Mittermeier et al., 2008), I did not find consistent species-specific differences in the social behaviours, mating systems, or vocalisations of *E. flavifrons* and *E. macaco*. Based on this, I concluded that, in their current state, none of the measured behaviours differed enough to serve as complete pre-mating barriers. While evidence from population genetics would greatly help to clarify this, these findings do lend further tentative support for the role of divergent niches in the speciation of the two taxa. This conclusion is based on current understanding of the modes of character change and evolution in primates. By modelling phenotypic characters and phylogenetic data across a range of primate species, Magnuson-Ford & Otto (2012) found strong evidence to suggest that diversification in primate social behaviours and mating systems tends to occur gradually over time along a lineage, while changes in habitat are more often associated with speciation events. Therefore, the collective lack of consistent species-level differences found here, coupled with Blair & colleagues (2013) findings of niche divergence across the two species' ranges, is consistent with overarching patterns of parapatric speciation. This conclusion is unsurprising when the evolutionary history of the taxa also is considered. These sister species are thought to have diverged relatively recently, around 160,000 years ago (Meyer et al., 2015). Given that the median duration of speciation in primates is approximately 1.1 million years (Curnoe et al., 2006), *E. flavifrons* and *E. macaco* may still be in the early stages of diverging aspects of their social systems. This would suggest that character displacement and/or reproductive isolation mechanisms may not have evolved completely yet for these two taxa.

Although a contact zone was not located, nor was hybridisation confirmed for *E. flavifrons* and *E. macaco*, this study can still inform the likelihood of such an event. The lack of an observed complete geographic barrier separating the two taxa, coupled with relatively similar social behaviours and mating systems, suggests that hybridisation could be a possibility along MSR's geographical cline. In fact, evidence from one of the best well-documented primate hybrid zones, the *Papio anubis* x *P. hamadryas* in the Awash National Park, suggests that behavioural differences do not automatically serve as reproductive isolation mechanisms; there, despite significant differences in the social systems of these baboon species, interbreeding still occurs (Alberts & Altmann, 2001; Bergman & Beehner, 2003; for review see Detwiler et al., 2005**).**

In comparison, if assortative mating is occurring throughout a contact zone along MSR, morphological features may be facilitating this behaviour. *E. flavifrons* has blue to blue-grey eyes and no ruffs, whereas *E. macaco* has yellow-orange eyes and exhibits ear ruffs (Mittermeier et al., 2010). In controlled experiments, Marechal (2010) found that *E. macaco* was able to discriminate based on morphological differences, showing significant preference for photographs of conspecifics as compared to photographs of *E. fulvus*. This suggests that *E. macaco* can recognise conspecifics based on visual clues, although whether these findings extend to its more closely-related and morphologically-similar sister species remains to be investigated. Similarly, the distinct facial patterns of *Cercopithecus* taxa may also play an important role in species recognition, especially in areas of parapatry or sympatry (Gautier, 1989; Allen et al., 2014). This is an example of character displacement where a visual signal helps to maintain reproductive isolation of coexisting species that have come back into contact (Allen et al., 2014). Furthermore, olfactory communication may play a role in assortative mating if a contact zone exists between *E. flavifrons* and *E. macaco*. The chemical signals of *Eulemur* taxa differ in composition (delBarco-Trillo et al., 2012), with at least one species, *E. fulvus*, demonstrating the ability to discriminate between the scents of sexes and conspecifics (Harrington, 1976, 1977). Assessing olfactory signalling in relation to reproductive isolation is necessary as this additional line of evidence could further contribute to the current understanding of species recognition in *E. flavifrons* and *E. macaco*.

I started this dissertation discussing the usefulness of relying on reproductive isolation as an important species criterion from a conservation perspective (Frankham et al., 2012). While none of my findings provide support for species-level differences based on behaviour, nor was there evidence of hybridisation, the updated data on their EOOs and AOOs is particularly alarming. Decreased suitable and available habitat poses a serious concern for their long-term conservation. Increased fragmentation and habitat destruction (Vieilledent et al., 2018), and high levels of anthropogenic disturbances (Godfrey & Iriwn, 2007; Seiler et al., 2010; Junge et al., 2011; Seiler, 2012; Balestri et al., 2014) are contributing to increasingly threatened species throughout Madagascar. It remains possible that due to increased isolation of populations, that behavioural differences will be amplified as populations of *E. flavifrons* and *E. macaco* evolve and adapt to local conditions. As such, continued research into range distributions, anthropogenic impacts, conspecific differences, and population adaptations to rapidly changing environments, such as that presented here, will provide a unique opportunity to not only provide diagnostic support of threatened species, but also study an evolutionary process that is rarely observed in real time.

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Appendix A. Morphological identification of *Eulemur flavifrons* and *Eulemur macaco* (adapted from Mittermeier et al., 2010).

	Male *E. flavifrons*	**Female *E. flavifrons***	**Male *E. macaco***	**Female *E. macaco***
Total length	90-100 cm	90-100 cm	90-110 cm	90-110 cm
Mass	1.8-1.9 kg	1.8-1.9 kg	1.8-1.9 kg	1.8-1.9 kg
Dorsal coat	Black	Reddish-tan to reddish-grey	Dark chocolate-brown to black	Golden-brown to chestnut-brown
Ventral coat	Black	White to grey	Dark chocolate-brown to black	White
Tail colour	Black	Reddish-tan to reddish-grey, often two toned	Dark chocolate-brown to black	Golden-brown to chestnut-brown, often darker than the body
Face colour	Black	Grey muzzle, with a rufus-tan face and crown	Dark chocolate-brown to black	Grey to black, with charcoal grey crown
Eye colour	Blue to blue-grey	Blue to blue-grey	Yellow-orange to deep orange	Yellow-orange to deep orange
Ear ruffs	No	No	Yes, black	Yes, white

Appendix B. Durations of grooming bouts.

B.1 Descriptive statistics for the durations of allogrooming bouts for each dyad class across the four populations. All durations are reported in seconds.

Population	Dyad	Range	Mean ± SD
Ef-1	M→M (N=27)	1 - 210	27 ± 47
	M→F (N=104)	1 - 580	32 ± 263
	F →M (N=65)	2 - 420	40 ± 76
	F → F (N=101)	1 - 600	34 ± 72
Ef-3	M→M (N=46)	1 - 143	35 ± 36
	M→ F (N=97)	5 - 219	27 ± 32
	F →M (N=61)	4 - 130	21 ± 23
	F → F (N=56)	4 - 84	19 ± 19
Em-1	M→M (N=19)	2 - 163	22 ± 37
	M→ F (N=35)	2 - 127	24 ± 30
	F →M (N=31)	2 - 68	18 ± 17
	F → F (N=59)	1 - 240	29 ± 42
Em-3	M→M (N=7)	4 -19	10 ± 6
	M→ F (N=38)	1 - 94	19 ± 24
	F →M (N=16)	1 - 156	29 ± 35
	F → F (N=33)	1 - 124	19 ± 24

B.2 Descriptive statistics for the durations of reciprocal grooming bouts for each dyad class across the four populations. All durations are reported in seconds.

Population	Dyad	Range	Mean ± SD
Ef-1	M→M (N=30)	9 - 331	91 ± 72
	M→ F (N=87)	3 - 270	94 ± 65
	F →M (N=57)	14 - 271	99 ± 63
	F → F (N=104)	4 - 470	122 ± 95
Ef-3	M→M (N=21)	7 -111	44 ± 31
	M→ F (N=47)	7 - 420	65 ± 74
	F →M (N=27)	5 - 259	61 ± 58
	F → F (N=22)	5 - 181	53 ± 56
Em-1	M→M (N=51)	3 - 233	64 ± 58
	M→ F (N=60)	5 - 521	94 ± 101
	F →M (N=29)	3 - 265	78 ± 77
	F → F (N=70)	2 - 451	93 ± 88
Em-3	M→M (N=29)	9 -263	71 ± 67
	M→ F (N=42)	4 - 257	68 ± 452
	F →M (N=33)	4 - 153	60 ± 43
	F → F (N=49)	2 - 353	90 ± 80

Appendix C. Mating behaviours observed in the eight *Ef-3* courtships.

Behaviour	**Number of observations**
Sexual grooming	5
Coercion	1
Mate-guarding	2
Scent marking	0
Female proceptivity	0
Clasping	10
Mounting	4
Intromission	3
Male coalitions	0
Female/juvenile interruptions	0

Appendix D. Multivariate and univariate comparison of short grunt calls.

D.1 A comparison of short grunt acoustic variables (MANOVA: Wilk's $\lambda=0.58$, $F(4,99)=2.8$, $p<0.001$). Significant findings are in bold.

Duration	ANOVA: F(4,99)=5.65, **p<0.001**
F1	ANOVA: F(4,99)=4.49, **p<0.001**
F2	ANOVA: F(4,99)=1.77, p=0.14
F3	ANOVA: F(4,99)=4.15, **p=0.004**
F4	ANOVA: F(4,99)=2.28, **p=0.066**

D.2 Tukey's HSD post hoc test comparing the F0mean of the short grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.985	**p=0.025**	p=0.28	p=0.575
Ef-3		p=0.404	p=0.686	p=0.976
Em-1			p=0.999	p=0.61
Em-2				p=0.876

D.3 Tukey's HSD post hoc test comparing the F0min of the short grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.999	p=0.247	p=0.709	p=0.964
Ef-3		p=0.478	p=0.767	p=0.974
Em-1			p=0.999	p=0.711
Em-2				p=0.933

D.4 Tukey's HSD post hoc test comparing the F0max of the short grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.951	**p=0.01**	p=0.246	p=0.482
Ef-3		p=0.365	p=0.733	p=0.985
Em-1			p=0.999	p=0.5
Em-2				p=0.885

D.5 Tukey's HSD post hoc test comparing the F1 of the short grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p=0.035**	**p<0.001**	p=0.999	p=0.978
Ef-3		**p<0.001**	p=0.155	**p=0.008**
Em-1			**p=0.045**	**p=0.004**
Em-2				p=0.999

D.6 Tukey's HSD post hoc test comparing the F2 of the short grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p<0.001**	p=0.465	p=0.98	p=0.311
Ef-3		**p<0.001**	p=0.058	**p=0.049**
Em-1			p=0.42	**p=0.009**
Em-2				p=0.949

D.7 Tukey's HSD post hoc test comparing the F3 of the short grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.436	p=0.284	p=0.861	p=0.915
Ef-3		**p=0.018**	p=0.202	p=0.135
Em-1			p=0.991	p=0.691
Em-2				p=0.991

D.8 Tukey's HSD post hoc test comparing the F4 of the short grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.999	p=0.129	p=0.178	p=0.965
Ef-3		p=0.509	p=0.424	p=0.994
Em-1			p=0.992	p=0.273
Em-2				p=0.312

Appendix E. Multivariate and univariate comparison of intermediate grunt calls.

E.1 A comparison of intermediate grunt call variables (MANOVA: Wilk's $\lambda=0.71$, F(4,149)=2.61, p<0.001). Significant findings are in bold.

Duration	ANOVA: F(4,149)=1.22, p=0.3
F1	ANOVA: F(4,149)=2.38, **p=0.054**
F2	ANOVA: F(4,149)=1.75, p=0.143
F3	ANOVA: F(4,149)=3.26, **p=0.013**
F4	ANOVA: F(4,149)=5.14, **p<0.001**

E.2 Tukey's HSD post hoc test comparing the F0mean of the intermediate grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.951	**p<0.001**	p=0.122	p=0.979
Ef-3		p=0.514	p=0.962	p=0.992
Em-1			p=0.676	**p<0.001**
Em-2				p=0.392

E.3 Tukey's HSD post hoc test comparing the F0min of the intermediate grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.675	**p<0.001**	p=0.056	p=0.811
Ef-3		p=0.992	p=1.0	p=0.895
Em-1			p=0.899	**p=0.009**
Em-2				p=0.431

E.4 Tukey's HSD post hoc test comparing the F0max of the intermediate grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.968	**p<0.001**	p=0.197	p=0.948
Ef-3		p=0.856	p=0.997	p=0.996
Em-1			p=0.765	**p=0.008**
Em-2				p=0.591

E.5 Tukey's HSD post hoc test comparing the F1 of the intermediate grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p=0.037**	p=0.643	p=0.978	p=0.561
Ef-3		**p=0.006**	p=0.238	p=0.261
Em-1			p=0.608	p=0.068
Em-2				p=0.993

E.6 Tukey's HSD post hoc test comparing the F3 of the intermediate grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.102	p=0.13	p=0.993	p=0.972
Ef-3		**p=0.009**	p=0.09	p=0.059
Em-1			p=0.744	p=0.438
Em-2				p=1.0

E.7 Tukey's HSD post hoc test comparing the F4 of the intermediate grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p=0.016**	p=0.546	p=0.862	p=0.666
Ef-3		**p=0.002**	p=0.115	p=0.067
Em-1			p=0.224	**p=0.027**
Em-2				p=0.999

Appendix F. Multivariate and univariate comparison of long grunt calls.

F.1 A comparison of long grunt call variables (MANOVA: Wilk's λ=0.74, F(4,165)=2.61, p<0.001). Significant findings are in bold.

Duration	ANOVA: F(4,165)=2.41, **p=0.051**
F1	ANOVA: F(4,165)=4.1, **p=0.003**
F2	ANOVA: F(4,165)=3.92, **p=0.005**
F3	ANOVA: F(4,165)=3.24, **p=0.014**
F4	ANOVA: F(4,165)=6.3 **p<0.001**

F.2 Tukey's HSD post hoc test comparing the F0mean of the long grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.781	p=0.163	**p=0.045**	p=0.939
Ef-3		p=0.999	**p=0.018**	p=0.438
Em-1			**p<0.001**	**p=0.002**
Em-2				p=0.125

F.3 Tukey's HSD post hoc test comparing the F0min of the long grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.195	**p=0.019**	p=0.309	p=0.999
Ef-3		p=0.897	**p=0.013**	p=0.179
Em-1			**p<0.001**	**p=0.003**
Em-2				p=0.228

F.4 Tukey's HSD post hoc test comparing the F0max of the long grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.795	p=0.259	**p=0.027**	p=0.998
Ef-3		P=0.999	**p=0.035**	p=0.855
Em-1			**p<0.001**	p=0.257
Em-2				**p=0.006**

F.5 Tukey's HSD post hoc test comparing the F1 of the long grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p<0.001**	p=0.616	p=0.997	p=0.734
Ef-3		**p<0.001**	**p<0.001**	**p<0.001**
Em-1			p=0.945	p=0.999
Em-2				p=0.966

F.6 Tukey's HSD post hoc test comparing the F2 of the long grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.173	p=0.371	p=0.986	p=0.799
Ef-3		**p<0.001**	p=0.097	p=0.004
Em-1			p=0.908	p=0.94
Em-2				p=0.996

F.7 Tukey's HSD post hoc test comparing the F3 of the long grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.157	p=0.901	p=0.947	p=0.999
Ef-3		**p<0.006**	**p=0.037**	**p=0.043**
Em-1			p=0.999	p=0.808
Em-2				p=0.952

F.8 Tukey's HSD post hoc test comparing the F4 of the long grunts. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p<0.001**	p=0.995	p=0.992	p=0.978
Ef-3		**p<0.001**	**p<0.001**	**p<0.001**
Em-1			p=0.999	p=0.459
Em-2				p=0.8

Appendix G. Multivariate and univariate comparison of group alarm calls.

G.1 A comparison of group alarm call variables from *Ef-1*, *Ef-3*, and *Em-1* (MANOVA: Wilk's λ=0.06, F(2,18)=3.56, p=0.004). Significant findings are in bold.

Duration	ANOVA: F(2,18)=1.04, p=0.375
F0mean	ANOVA: F(2,18)=14.23, **p<0.001**
F0min	ANOVA: F(2,18)=9.4, **p=0.002**
F0max	ANOVA: F(2,18)=2.32, p=0.127
F0s	ANOVA: F(2,18)=1.06, p=0.367
F0f	ANOVA: F(2,18)=7.62, **p=0.004**
F1	ANOVA: F(2,18)=13.65, **p<0.001**
F2	ANOVA: F(2,18)=6.19, **p=0.009**
F3	ANOVA: F(2,18)=2.65, p=0.098
F4	ANOVA: F(2,18)=6.51, **p=0.007**

G.2 Tukey's HSD post hoc test comparing the F0mean of group alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*
Ef-1	**p=0.01**	p=0.656
Ef-3		**p<0.001**

G.3 Tukey's HSD post hoc test comparing the F0min of group alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*
Ef-1	p=0.057	p=0.578
Ef-3		**p=0.002**

G.4 Tukey's HSD post hoc test comparing the F0f of group alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*
Ef-1	**p=0.027**	p=0.994
Ef-3		**p=0.008**

G.5 Tukey's HSD post hoc test comparing the F1 of group alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*
Ef-1	**p=0.008**	p=0.771
Ef-3		**p<0.001**

G.6 Tukey's HSD post hoc test comparing the F2 of group alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*
Ef-1	**p=0.009**	**p=0.021**
Ef-3		p=0.993

G.7 Tukey's HSD post hoc test comparing the F3 of group alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*
Ef-1	**p=0.006**	**p=0.027**
Ef-3		p=0.895

Appendix H. Multivariate and univariate comparison of individual alarm calls.

H.1 A comparison of individual alarm call variables from *Ef-1*, *Ef-3*, *Em-1*, *Em-2,* and *Em-3* (MANOVA: Wilk's λ=0.1, F(4,38)=2.35, p<0.001). Significant findings are in bold.

Duration	ANOVA: F(4,38)=2.16, p=0.092
F0mean	ANOVA: F(4,38)=1.95, p=0.121
F0min	ANOVA: F(4,38)=8.69, **p<0.001**
F0max	ANOVA: F(4,38)=3.9, **p=0.009**
F0s	ANOVA: F(4,38)=6.3, **p<0.001**
F0f	ANOVA: F(4,38)=3.87, **p=0.01**
F1	ANOVA: F(4,38)=2.12, p=0.098
F2	ANOVA: F(4,38)=0.57, p=0.689
F3	ANOVA: F(4,38)=1.01, p=0.413
F4	ANOVA: F(4,38)=2.0, p=0.114

H.2 Tukey's HSD post hoc test comparing the F0mena of individual alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	p=0.509	p=0.905	**p<0.001**	p=0.999
Ef-3		p=0.936	**p=0.018**	p=0.496
Em-1			**p=0.004**	p=0.894
Em-2				**p<0.001**

H.3 Tukey's HSD post hoc test comparing the F0min of individual alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p<0.001**	p=0.463	**p=0.015**	p=0.594
Ef-3		**p<0.001**	**p<0.001**	**p=0.018**
Em-1			p=0.119	**p=0.031**
Em-2				**p=0.002**

H.4 Tukey's HSD post hoc test comparing the F0max of individual alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p=0.034**	p=0.999	**p=0.024**	p=0.998
Ef-3		p=0.065	p=0.865	p=0.061
Em-1			**p=0.037**	p=0.999
Em-2				**p=0.036**

H.5 Tukey's HSD post hoc test comparing the F0s of individual alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p=0.005**	p=0.597	**p=0.037**	p=0.556
Ef-3		**p<0.001**	**p<0.001**	p=0.085
Em-1			p=0.196	**p=0.046**
Em-2				**p=0.005**

H.6 Tukey's HSD post hoc test comparing the F0f of individual alarm calls. Significant findings after Bonferroni corrections are in bold.

	Ef-3	*Em-1*	*Em-2*	*Em-3*
Ef-1	**p=0.023**	p=0.964	**p=0.029**	p=0.956
Ef-3		p=0.088	p=0.924	p=0.08
Em-1			p=0.07	p=1.0
Em-2				p=0.067

Appendix I. Multivariate and univariate comparison of hoot calls.

H.I A comparison of hoot call variables from *Ef-1*, *Ef-3*, *Em-1*, *Em-2*, and *Em-3* (MANOVA: Wilk's λ=0.43, F(4,45)=1.63, p=0.0427). Significant findings are in bold.

Duration	ANOVA: F(4,45)=0.81, p=0.523
F0mean	ANOVA: F(4,45)=1.8, p=0.146
F0min	ANOVA: F(4,45)=2.15, p=0.09
F0max	ANOVA: F(4,45)=1.63, p=0.184
F0s	ANOVA: F(4,45)=1.79, p=0.148
F0f	ANOVA: F(4,45)=1.83, p=0.141