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UNIVERSITY OF CALGARY

Predictors of agonism and affiliation in black-and-white ruffed lemurs (Varecia

variegata)

by

Li-Dunn Chen

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF ARTS

GRADUATE PROGRAM IN ANTHROPOLOGY

CALGARY, ALBERTA

AUGUST, 2020

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ABSTRACT

Agonism and affiliation work complementarily to influence social ranking in primate social systems, which ultimately impacts reproductive success. In this two-part study, I investigated social behaviour of the Critically Endangered blackand-white ruffed lemur, a highly frugivorous species characterized by female dominance, fission-fusion dynamics, synchronized breeding, and communal care of offspring. Although ruffed lemur sociality has been described in the literature, specific seasonal and ecological predictors of agonism and affiliation have not been quantitatively investigated. Behavioural data were collected in the Kianjavato commune of southeastern Madagascar. I first investigated fluctuating food availability and reproductive season as predictors of agonism. Food availability had no effect on group-wide agonism rates, but subgroup size and breeding seasons were highly predictive of increased agonism. Increased agonism rates were observed when subgroups were larger as well as during the mating and birthing seasons, but only in years when mating and birthing occurred. I speculate these patterns of agonism function as reproductive strategies, as male-male competition for access to mates is expected to increase during the mating season, and parents likely exhibit more agonism while guarding their offspring during the birthing season. In order to better understand the behavioural strategies employed by ruffed lemurs during the mating season, I also investigated how male individuals vary in their expression of agonistic and affiliative behaviours with respect to reproductive season and dominance rank. I

found that males exhibited higher rates of both agonism and affiliation during the mating season compared to the post-mating season, and that dominant males expressed higher rates of agonism but not affiliation compared to lowranking males. I also evaluated female agonism rates and ranks to determine if they were higher in females compared to males. Although females occupied the highest ranks within their subgroups, males surprisingly exhibited higher rates of agonism than females throughout the study period, which was unexpected in this female-dominated species. Overall, the results presented here indicate that agonism and affiliation may function as reproductive strategies, but additional research is required to better understand the patterns observed.

PREFACE

This thesis is composed of original, unpublished, independent work by the author, Li-Dunn Chen. The research reported in Chapters 2 and 3 were covered by ACC Certificate number AC19-0026, issued by the University of Calgary Animal Care Committee for the project "Functional diversity in lemur communities" on April 24, 2019.

Chapter 2 (in preparation for publication) is a collaborative effort comprised of intellectual input, assistance in statistical modeling and analyses, and data wrangling from Dr. Steig Johnson, Dr. Sheila Holmes, and Devin Chen. Letters of permission to use this work as a chapter for this thesis are provided below (Appendix A).

ACKNOLWEDGEMENTS

Sincere thanks to all of Team Varecia, especially Tracy Wyman and Malagasy field team members: Cressant Razafindravelo, Ferdinand DaKely Mbana, Jean Pierre Marolahy, Emilys Edgarçon Rakotoson, Jean Clement Lala Razafiarisona, Michel Patrick Zakamanana, Etienne Rabearivelo, Prosper Ramanananihaja, Rasolo Mampionana, Benjamin Rafidson Nomenjanahary, as well as the many Madagascar Biodiversity Partnership (MBP) personnel from 2014 to 2017 who volunteered their time to enrich the conservation efforts for black-and-white ruffed lemurs in Madagascar. I'd like to recognize Liva and Stephan of the MBP and personnel from the Omaha Henry Doorly Zoo's Grewcock Center for Conservation & Research in helping me to safely and legally transport, export, and import ruffed lemur feces from Madagascar to the United States. I am grateful to Dr. Ed Louis, director of the MBP, for making research in Kianjavato possible. Also, a huge thanks for Drs. "Al" Hays and Stacey Tecot of the Laboratory for the Evolutionary Endocrinology of Primates for their direction and long hours of help (including weekends and holidays) in the lab. This research was supported by Conservation International, Primate Conservation, Inc., the Calgary Zoo, Omaha's Henry Doorly Zoo & Aquarium, and the Natural Sciences and Engineering Research Council of Canada. Lastly, my sincerest gratitude to Dr. Steig Johnson for advising and supporting me through this incredible two-year journey financially and intellectually.

DEDICATION

To my lab associate, friend, and partner, Devin Chen for her support throughout this academic journey. Although we occasionally initiate bouts of agonism towards one another, I regard these interactions as necessary for strengthening our bonds as primates. Like our non-human primate cousins, I hope we can continue these quarrels for years to come, but I also aspire that love will conquer hate in all contexts- whether it be over access to food resources or otherwise, I appreciate and dedicate this to you as my dearest conspecific.



TABLE OF CONTENTS

I.	Abstract	ii
II.	Preface	iv
III.	Acknowledgements	v
IV.	Dedication	vi
V.	Table of Contents	vii
VI.	List of Tables	viii
VII.	List of Figures	ix
VIII.	Chapter 1: Introduction	1
	a) Reproductive Season, Rank, and Agonism in Primates	2
	b) Feeding Competition, Fission-Fusion Dynamics, and Agonism in	า
	Primates	3
	c) Black-and-White Ruffed Lemurs of Madagascar	5
	d) Hypotheses	6
	e) Figures	8
IX.	Chapter 2: Effects of reproductive season on agonism rates in bla	ick-
	and-white ruffed lemurs (Varecia variegata)	9
	a) Abstract	9
	b) Introduction	11
	c) Methods	16
	d) Results	21
	e) Discussion	23
	f) Conclusion	29
	g) Tables	32
	h) Figures	35
Х.	Chapter 3: Mating season and rank differences on ruffed lemur a	gonism
	and affiliation	36
	a) Abstract	36
	b) Introduction	38
	c) Methods	41
	d) Results	47
	e) Discussion	50
	f) Tables	56
	g) Figures	58
XI.	Chapter 4: Discussion	64
	a) Summary of Findings	64
	b) Limitations	67
	c) Future Directions	68
XII.	Literature Cited	72
XIII.	Appendix A: Permissions.	83
XIV.	Appendix B: Fecal Collection & Processing Protocol	86
XV.	Appendix C: Hormone Analysis Preliminary Results	88

LIST OF TABLES

Table 2.1. Rates of agonism (acts/hr) across the mating, birthing, and non-
breeding seasons. The non-breeding season encapsulates the time period
following the birthing and lactation season (September-December) and before
the mating season (May-July)32
Table 2.2. Top five models based on AICc values. Models within 2 AICc units of
the top model are considered the "best" models (Δ AIC values in bold font). All
models shown share the inclusion of five variables: average subgroup size, birth
year, mating season, mating year, mating season and mating year interaction
term
Table 2.3. Results of the full GLM with Poisson distribution. The model included
the number of hours of observation as an offset
Table 3.1. Average hourly rates of agonism, grooming, win/loss ratios, and
rankings for all focal lemurs throughout the study period. Site: TT = Tsitola, SS =
Sangasanga, VV = Vatovavy. A dash (-) indicates insufficient data to calculate
values56
Table 3.2. Frequency of affiliative interactions observed between male-male,
male-female, female-male, and female-female dyads57
Table 3.3. Frequency of agonistic interactions observed between male-male,
male-female, female-male, and female-female dyads57

LIST OF FIGURES

Figure 3.4. Comparison of mating season agonism between males and females. A		
t-test indicated no significant difference in agonism rates between males and		
females (<i>p</i> = 0.81)59		
Figure 3.5. Comparison of post-mating season agonism between males and		
females. A Kolmogorov-Smirnov test indicated no significant difference in		
agonism rates between males and females (p = 0.84)60		
Figure 3.6a. Elo-rating plot indicating a nonlinear and unstable dominance		
hierarchy among lemurs in Tsitola group throughout the study period. Higher		
Elo-ratings represent higher dominance ranks60		
Figure 3.6b. Elo-rating plot indicating a nonlinear and unstable dominance		
hierarchy among lemurs in Vatovavy group throughout the study period. Higher		
Elo-ratings represent higher dominance ranks61		
Figure 3.6c. Elo-rating plot indicating a partially linear and stable dominance		
hierarchy among lemurs in Sangasanga group throughout the study period.		
Higher Elo-ratings represent higher dominance ranks61		
Figure 3.7. Scatter plot with regression line indicating that male rank is strongly		
correlated with agonism rate (p < 0.05)62		
Figure 3.8. Scatter plot indicating that female rank is not associated with		
agonism rates (<i>p</i> > 0.05)62		

CHAPTER 1: INTRODUCTION

Agonistic behaviours play an important role in social animals, with the potential for large fitness benefits, including monopolization, defense, or greater access to various limiting resources such as food, shelter, space, and mates (Huchard & Cowlishaw, 2011; Muller & Wrangham, 2004; Mumby & Wabnitz, 2002; Plavcan, 2012). Agonism is defined as the suite of behaviours exhibited between members of the same species during moments of conflict and competition (Fedigan, 1992). Agonism includes displacement-causing behaviours such as grabbing, chasing, and vocalizations in addition to submissive behaviours such as retreating, submissive chatters, and fear grins (Higham et al., 2013). The term aggression may fit under agonism, but more specifically refers to behaviour with the outcome of inflicting bodily harm from one individual to another; examples include biting, fighting, and prolonged chases (Sussman & Garber, 2004). The benefits of engaging in agonistic behaviour, whether it be aggressive in nature or not, must outweigh large potential costs, including injury or death in some cases (Bernstein & Gordon, 1974).

While many primate species live in gregarious social groups, group living may exacerbate competition for resources, particularly access to food and mates (van Schaik, 1983). Individuals may enhance or monopolize access to defensible limiting resources through agonism in the form of contest competition (Majolo et al., 2012) or territoriality (Mitani et al., 2010). Individuals also exhibit agonistic behaviour in order to increase mating opportunities via guarding access to mates

1

(Girard-Buttoz et al., 2015; Mass et al., 2009), sexual coercion (Smuts & Smuts, 1993), or male infanticide to return females to cycling and gain access to reproductive females sooner (Ebensperger, 1998; Hrdy, 1979). Agonism may also be used as a counterstrategy to the strategies outlined above (Baniel et al., 2018; Smuts & Smuts, 1993).

Reproductive Season, Rank, and Agonism in Primates

Increased agonism within the context of breeding seasons (i.e., mating and birthing) has been well documented in primates (Beehner et al., 2005; Brockman et al., 1998; Cavigelli & Pereira, 2000; Fruth & Hohmann, 2003; Huchard & Cowlishaw, 2011; Kappeler, 1989; Muller & Wrangham, 2004). It is through agonistic interactions that a dominance hierarchy is established between conspecifics, which is known to affect individual fitness outcomes (Cowlishaw & Dunbar, 1991). Obtaining and maintaining high dominance ranks through increased agonism has been reported in many primates, particularly in males who, as a result of winning agonistic bouts, are often afforded enhanced mating privileges and reproductive success (Beehner et al., 2005; van Noordwijk & van Schaik, 2004). Additionally, male rates of agonism are known to increase during the mating season when females are conceptive, and successful initiators of agonism are often conferred with greater reproductive and fitness-related benefits (Fruth & Hohmann, 2003; Majolo et al., 2012). Similarly in females, social rank has been found to be a major factor affecting survival and fitness, especially in female primates with slow and long life histories (Blomquist et al.,

2011). Additionally, increased agonism during the birthing season is argued to occur due to the presence of infants, specifically in the context of parents guarding their young from potential threats of infanticide (van Schaik & Kappeler, 1997). In ring-tailed lemurs and Verreaux's sifaka, birth season-related increases in agonism have also been attributed to dispersals and transfers of immigrant individuals between subgroups (Brockman et al., 2001; Pereira & Weiss, 1991).

Feeding Competition, Fission-Fusion Dynamics, and Agonism in Primates

In addition to increased fitness benefits, some dominant individuals are given feeding priority, where high-ranking individuals have greater access to food resources relative to their subordinates (Overdorff et al., 2005; White & Wood, 2007). The fission-fusion behavioural strategy, first described by Hans Kummer (1971), may reduce food-related agonism due to contest competition in species that consume high-quality, spatially clumped resources (e.g., fruit) (Kummer, 1971; Riveros et al., 2017). Fission events, or the breaking apart of larger subgroups into smaller subgroups, are expected to decrease feeding competition by decreasing the number of individuals sharing a food patch (Potts et al., 2011; Riveros et al., 2017; Stevenson et al., 1998). However, fusion events, or the formation of larger subgroups from smaller ones, can result in agonism if not tempered with risk-avoidance behaviours (e.g., spider monkey embraces: Aureli & Schaffner, 2007; Riveros et al., 2017). Spider monkeys, for example, show increased agonism to members joining their subgroup in the five minutes following these fusions (Aureli & Schaffner, 2007). Despite decreased resources in the dry season, however, there was no seasonal difference in feeding-related agonism in this species (Asensio et al., 2008; Riveros et al., 2017). This was attributed to the formation of smaller subgroups during the dry season, reducing both contest competition and scramble competition at the subgroup level, though not at the community-level (Asensio et al., 2008; Riveros et al., 2017). Community-level scramble competition is thought to be mitigated via targeted agonism by resident and earlier immigrant females towards recent immigrants and subadult females (Asensio et al., 2008; Riveros et al., 2017).

Fruit, a high-quality resource, is typically spatially and temporally clumped, and therefore contestable. For example, agonism rates of mountain gorillas increased when feeding on fruit and decayed wood compared to herbaceous, lower quality vegetation (Wright & Robbins, 2014). Whereas high levels of agonism are not expected to occur with low-quality, highly dispersed and abundant resources (scramble competition), foraging-related agonism should be frequent when food patches are discrete, monopolizable, and highquality (contest competition) (Koenig, 2002). This agonism may be directed from higher-ranking to lower-ranking individuals (Koenig, 2002). High-quality food resources like fruit are often only seasonally available, especially in tropical climates where a cold-dry season is followed by a warm-wet season (Conklin-Brittain et al., 1998; Guillotin et al., 1994). Many primates, including lemurs of Madagascar, are subjected to high degrees of seasonality, and based on annual changes in photoperiod and low food availability, are able to adjust the social dynamics of their subgroups as well as their activity budgets to maximize their fitness (Baden et al., 2016; Huang et al., 2012; Lewis & Kappeler, 2005; Tecot et al., 2013).

Black-and-White Ruffed Lemurs of Madagascar

In this study, I investigated the social behaviour of black-and-white ruffed lemurs (*Varecia variegata*), a Critically Endangered primate species that exhibits a distinct suite of behaviours including female dominance, fission-fusion dynamics, reproductive synchrony, and cooperative care of offspring (Baden et al., 2016; IUCN, 2020). Ruffed lemurs are distributed throughout the eastern rainforests of Madagascar, including Kianjavato, a priority conservation site in southeastern Madagascar where this research was conducted (See Figure 1.1) (Schwitzer et al., 2013). They are the largest bodied lemurs at the site at 3.5 kilograms and subsist on a highly frugivorous diet consisting of up to 92% fruit but also rely on flowers and leaves during times of low food availability (Britt, 2000; Holmes, 2012). Much of the research relating to the social behaviour of this species has been conducted in captive settings, descriptive in nature, and/or lacks quantitative analysis (Foerg, 1982; Kerridge, 2005; Morland, 1991; Pereira et al., 1988).

Hypotheses

In this thesis, I examine predictors of agonism and affiliation in wild groups of black-and-white ruffed lemurs. My goal was to gain a better understanding of the variables that impact ruffed lemur sociality, as relationships between conspecifics likely influence the fitness outcomes of an individual.

In Chapter 2, I take a broad approach of examining agonism by calculating rates of group-wide agonism spanning a four-year study period. I test the hypothesis that fluctuating food availability will impact agonism rates in a primate species whose diet consists mostly of fruit and nectar-filled flowers. I predict that agonism rates will increase in periods of low food availability. I also assess the effects of reproductive seasons on agonism, as the mating season for ruffed lemurs is highly seasonal and restricted by reproductive synchrony exhibited by females. I predict that agonism rates will increase during the mating season due to increased competition for mates. However, I expect a decrease in agonism during the birthing season due to cooperative breeding in this species, as behaving cooperatively is likely associated with increased social tolerance. Due to the expression of fission-fusion dynamics in this species, the impact of subgroup size is also examined to account for the number of potential interactants present in the subgroup.

In Chapter 3, I investigate predictors of agonism and affiliation in individual male ruffed lemurs at a finer timescale, specifically during the mating and post-mating seasons. I assess the effect of reproductive season on agonism

and affiliation rates by comparing rates during the mating versus post-mating seasons. I predict that males will increase their agonism rates in order to compete for access to females during the mating season. Additionally, I predict that males will increase their affiliation rates in order to form bonds with females prior to mating. I also investigate dominance rank (measured by Eloratings and win/loss ratios) among males to compare the effect of rank on agonism rate. Similar to David's score, Elo-rating scores are assigned to individuals and represent their dominance ranks within their subgroups, but Eloratings are preferred for assessing rank when agonistic interactions are rare and do not occur between all members within a subgroup (Neumann et al., 2011). predict that high-ranking males will engage in higher rates of agonism in order to establish and maintain their ranks. Lastly, I analyzed female rank and agonism rate to test the prediction that females will occupy higher ranks and engage in higher rates of agonism than males during this study period, as female dominance is present in this species and females should assert female choice even in the context of male-male competition.

FIGURES



Author: Tracy Wyman Date: June 23, 2020 Sources: S.M. Holmes, ESRI Created with ArcGIS v 10.7.1

Service Layer Credits: Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 1.1. Map of the study site depicting three forest fragments in southeastern Madagascar. Black-and-white ruffed lemurs were sampled in Sangasanga (99 ha), Tsitola (954 ha), and Vatovavy (353 ha).

CHAPTER 2: Effects of reproductive season on agonism rates in black-and-white

ruffed lemurs (Varecia variegata)

ABSTRACT

Agonism within social groups of animals may fluctuate seasonally, potentially in response to food resources and access to mating opportunities, which also vary by season. As such, intraspecific agonism could play a role in accessing key resources for survival and reproduction. Ruffed lemurs exhibit female dominance and high fission-fusion dynamics (i.e., fluctuating subgroup size and composition), which are considered adaptive strategies to cope with a high degree of seasonality. I investigated whether reproductive seasons or changes in food availability had a stronger impact on rates of agonism in black-and-white ruffed lemurs, a primate species that exhibits strongly seasonal, communal breeding but does not consistently breed on an annual basis. Data were collected in two forest fragments in southeastern Madagascar across a four-year duration encompassing numerous mating and birthing seasons. Fruit and flower availability were estimated using phenological surveys, and agonism rates were calculated by summing all agonistic interactions within the focal animal's subgroup. Subgroup size was found to be the strongest predictor of agonism in ruffed lemurs, such that large subgroups were found to exhibit higher rates of agonism than small subgroups. Rates of agonism were positively associated with the mating season but only in years when mating was observed. Agonism rates also increased during the birth season in years that births were observed, though the pattern was not as clear as that seen with mating. Fruit and flower availability were not significant predictors of agonism during the four-year sampling period, suggesting that fission-fusion dynamics may function to mitigate contest competition in ruffed lemurs. Alternatively, it is also possible that ruffed lemurs are not food limited and are more so subjected to scramble competition rather than contest competition. Overall, these findings support the notion that agonism in lemur groups varies seasonally, and I suggest that this pattern is likely due to increased male-male competition for access to females during the mating season and parents protecting their offspring during the birthing season.

INTRODUCTION

The role of agonism in influencing the social structure of group-living primate species is argued to result in benefits for dominant individuals including enhanced access to food and mating partners and thus impacts an individual's reproductive success (Majolo et al., 2012). For example, high-ranking females have been found to reach sexual maturity sooner, receive priority feeding access, and experience higher lifetime fitness levels (Silk, 2007). Similar benefits are assumed for dominant males as well, particularly priority of access to conceptive females and overall higher reproductive success relative to subordinates (Kappeler & Schäffler, 2008; Majolo et al., 2012; Silk, 2007). While dominance rank has been useful in predicting the outcome and frequency of agonistic bouts (Muller & Wrangham, 2004; Ostner et al., 2002), factors such as reproductive season and food availability have also been found to impact agonism rates across many primate groups (Cowlishaw & Dunbar, 1991; Girard-Buttoz et al., 2015; Janson & Van Schaik, 1988; Sauther, 1993; Wheeler et al., 2013).

Black-and-white ruffed lemurs (*Varecia variegata*) possess a distinct suite of behaviours that allow a unique perspective on some of the factors predicted to influence agonism rates in primates and other animals. Ruffed lemurs are female-dominant (Morland, 1993), highly frugivorous (Britt, 2000), exhibit a high expression of fission-fusion dynamics (Baden et al., 2015; Holmes et al., 2016), have a polygamous mating system (Andrea L Baden et al., 2008), and show communal care of offspring (Baden et al., 2013; Vasey, 2007). They also often do not consistently give birth in sequential years but exhibit marked reproductive synchrony during mating/birthing years (Holmes et al., 2016; Ratsimbazafy, 2003). Overall, this particular set of behaviours is rare in primates. These characteristics may affect rates of agonism, which refer to displacement-causing and submissive behaviours in times of conflict and competition (Fedigan, 1992), at various points in time for this species.

Ruffed lemur diets consist of up to 92% fruit, with the remainder being made up of flowers, leaves, and fungi (Britt, 2000). During times of environmental stress, such as the transition from the warm-wet season to the cool-dry season, ruffed lemurs decrease their frequency of feeding bouts and subsist on seasonally available foods (e.g., leaves and nectar from flowers) (Britt, 2000). In general, the frequency of agonistic bouts can depend on the abundance, distribution, and/or quality of food available. Previous research involving primates has found that when resources are clustered in space, expending energy to defend a small area can lead to the monopolization of a large amount of resources (Isbell, 1991; Vogel, 2005). For example, aggression increased between Japanese macaques when feeding on fruits and seeds in smaller patches with fewer feeding sites, but there was no effect on aggression with respect to number of feeding sites when consuming low-quality foods (Hanya, 2009). Although ruffed lemurs exhibit marked frugivory and fruit abundance is highly variable across seasons, their ability to adapt to alternative

food resources may function as a way to reduce competition during durations of food scarcity (Britt, 2000).

Ruffed lemurs have shown variability in grouping patterns with regard to food availability. Ruffed lemurs in the continuous forest of Ranomafana follow the typical pattern observed in species expressing a high degree of fission-fusion dynamics: when food availability is low, smaller subgroups are observed (Baden et al., 2015). However, ruffed lemurs in disturbed forest near Kianjavato formed larger groups on average when food availability was lower (Holmes et al., 2016). Explanations for this atypical pattern are described below, but fission-fusion dynamics and other strategies such as cooperative breeding may function to mitigate agonism across seasons.

Agonism may be reduced due to communal care of offspring exhibited by kin and non-kin during the birthing season in some species (Baden, 2011; Schaffner & French, 1997). Ruffed lemurs exhibit communal care, a strategy found in a minority of species across the primate order (Baden et al., 2013; Mitani & Watts, 1997; Vasey, 2007). Cooperative care or alloparenting has been linked to reduced agonism in African lined mice (Raynaud & Schradin, 2014), laboratory mice (Curley et al., 2009), mandarin voles (Wu et al., 2013), and larids (Besnard et al., 2009). In male African lined mice, the behaviours of philopatry, alloparental care, and low levels of agonism towards pups and other males are strongly associated (Raynaud & Schradin, 2014). Studies examining the effects of birth season on patterns of agonism in a communally breeding primate have thus far been strictly limited to cotton-top tamarins in captive settings (Snowdon & Pickhard, 1999). As highly seasonal, cooperative breeders (Baden, 2011; Vasey, 2007), ruffed lemurs serve as an appropriate model species for examining the effects of reproductive season on agonism in a naturally occurring, communally breeding primate species.

Agonism may increase during the mating season in black-and-white ruffed lemurs, as has been observed in many primates including certain lemurs (Dixson, 1980; Vick & Pereira, 1989). Agonism rates were highest during the mating and birth seasons for ring-tailed, brown, and blue-eyed black lemurs (Vick & Pereira, 1989), likely as a way for individuals to exert dominance over subordinates for access to food resources and mates during these times. Both male and female ruffed lemurs are known to exhibit agonism during the mating season, though most studies have either been conducted in captive settings or descriptive in nature (Foerg, 1982; Morland, 1993). Early in conceptive phases, captive black-and-white ruffed lemur females respond agonistically to approaches and mounting attempts by other group members (Foerg, 1982). Previous research also found that females were agonistic towards males attempting to mate in a wild population of ruffed lemurs (Morland, 1993). Eventually, this agonism was tolerated and mate-guarding by males and copulation occurred, but females continued to attack and chase males attempting to mount if copulation was not female-initiated (Foerg, 1982). This has also been observed in ring-tailed lemurs and Verreaux's sifaka, which has

14

been argued to function as a way for females to assert mate choice (Morland, 1993). Appearing as mating attempts, intrasexual mounting between males and female mounting of males have also been observed in wild settings (personal observation, 2019) and is often proceeded by agonism.

Ruffed lemurs are known to exhibit a high degree of breeding synchrony (Baden et al., 2016; Foerg, 1982; Vasey, 2007), where females in a group come into estrous at the same time, which is expected to decrease mating competition between males. Males cannot easily mate-guard numerous females at the same time (according to the priority of access model: Altmann, 1962; Ostner, Nunn, et al., 2008), especially in highly arboreal (compared to terrestrial) settings. The lack of complete synchronization across females (i.e., all females are not conceptive at precisely the same time) allows for male-male competition over mates; furthermore, the relatively short breeding season should result in particularly heightened mating competition. The relative importance of agonism as a function of breeding in this female-dominated, seasonally breeding species remains unclear.

This study aims to examine how factors such as food availability and reproductive season affect agonism rates in seasonally reproducing, femaledominated black-and-white ruffed lemurs. It is important to note that ruffed lemurs do not breed every year (Holmes et al., 2016; Ratsimbazafy, 2003). This makes it possible to isolate the potential relationship between mating and/or

15

birth-related behaviours and agonism from other seasonally-varying factors, which presumably occur even in years that reproduction does not.

I hypothesized that group-wide agonism would respond to fluctuations in food availability, as the diet of ruffed lemurs is dominated by high-quality resources that can be monopolized through contest competition (Sterck et al., 1997). Specifically, I predicted increased rates of agonism during periods of low fruit and flower availability. I further hypothesized that reproductive season would have an effect on rates of black-and-white ruffed lemur agonism. I predicted an increase in the rates of group-wide agonistic behaviour during the mating season, but only in years when mating behaviours occur, as mating competition is known to induce agonism in this species. Conversely, I predicted lower rates of agonism to occur during the birth season, but only in years when births occur, as cooperative caring of offspring is expected to decrease levels of agonism.

METHODS

Study Species

The black-and-white ruffed lemur is the largest extant frugivorous lemur, sexually monomorphic, and characterized by a female-dominated social structure (A. L. Baden et al., 2008). Reproductive events are seasonal in this species with mating generally occurring between May and July and birthing occurring between September and November after a 102-day gestation period (Morland, 1990; Rasmussen, 1985). As at other sites throughout Madagascar (Baden et al., 2016; Ratsimbazafy, 2003), lemurs at this study site do not consistently mate and give birth every year.

Study Site

This study was conducted at the Kianjavato Ahmanson Field Station (KAFS) in southeastern Madagascar (see Figure 1.1). This site experienced heavy forest loss around 1950-1970, with deforestation continuing into the present (Holmes, 2017). Black-and-white ruffed lemurs have been subjects of long-term monitoring in two of the remaining forest fragments since 2010 (Holmes et al., 2019). These forest fragments are Sangasanga (99ha) and Vatovavy (353ha) (Holmes, 2017).

Data Collection

As part of the long-term monitoring efforts over the period of this study, individuals were fitted with VHS transmitter collars (Advanced Telemetry Systems model M2940). Up to six collared individuals per fragment were followed at any given time (total N = 21); new study animals were added to data collection routines when previous focal animals disappeared, dispersed, or died. Following the protocol by Louis et al. (2005), trained technicians from the Madagascar Biodiversity Partnership located, identified, immobilized, and fit ruffed lemur individuals with radio collars. Between March 2014 and December 2017 (44 months), the authors, research collaborators, technicians, and temporary field assistants collected behavioural data (N = 4,194 focal hours) using focal time sampling at five-minute intervals (Altmann, 1974; Baulu & Redmond, 1978). Focal individuals were observed for two-hour periods in rotation, such that each individual was observed for a total of eight hours per month, spread throughout the six- to eight-hour data collection per day.

Behavioural categories included feeding/foraging, resting, travelling, and socializing. If foraging was observed for a sampling point, the food species and part (e.g., ripe fruit) were recorded. Changes in subgroup membership (Holmes et al., 2016) and instances of agonistic behaviour (i.e., chasing, swatting, cuffing, biting, chatter vocalizations, and retreats) were recorded using all-occurrence sampling across all subgroup members (Altmann, 1974). Although all-occurrence sampling might overestimate the frequency of a behaviour (vs., for example, focal animal sampling), this method was utilized to capture as many agonistic bouts as possible since agonistic behaviour is considered rare in strepsirrhines (0.16 events/hr; Sussman & Garber, 2004). Agonistic interactions involving juvenile and interspecific partners were excluded from calculated rates of agonism in this study.

Throughout the study duration, research technicians collected phenological data from trees in seven 20m x 20m plots in Sangasanga and five 20m x 20m plots in Vatovavy (Holmes, 2012). Upon initial establishment of plots, diameter at breast height (DBH) was measured for all trees greater than 10 cm in diameter (Holmes, 2012). Twice per month, the availability of ripe fruit, unripe fruit, and flowers were estimated for each tree on a scale of zero to four, with

18

zero indicating an absence of the phenological stage and four indicating complete coverage of the tree crown. Interobserver reliability was improved by Dr. Sheila Holmes, who ensured quality control through recurring professional development exercises with field technicians at all sites (Holmes, 2012, 2017).

Statistical Analysis

I calculated the rate of agonism events over periods approximately two weeks in length, including one week before and one week after each phenological survey performed at a given site (hereafter phenological period). For periods of less than two weeks between phenological surveys, the interim period was divided in half. The total sampling time for each period was also calculated. For each phenological period, I calculated food availability as the basal area (m²) of fruiting or flowering food species (any species that a lemur had been observed to feed from during the study), divided by the total area of the phenology plots at a given site (m²), similar to Chapman et al. (1994). This provided an estimate of the proportion of the study area with fruit or flowers available (two separate variables; e.g., fruit availability = π ((Tree DBH/ 100)/2)²)/study site area). I then standardized the values of each food availability variable by subtracting the mean and dividing the outcome by the standard deviation of that variable. Additionally, for each phenological period, I calculated the average adult subgroup size of focal animals and noted whether a period occurred during mating or birth season months. Based on observed mating behaviours and estimated dates of birth in these populations, the mating season occurred

between May 15 and July 31 and the birth season between September 15 and November 30. For this study, since mating and birthing do not occur every year, I specified for each bi-weekly data point whether or not it occurred in a year when mating and birthing happened. For example, if mating occurred in the same calendar year as a phenological sample, that sample was considered to have occurred during a mating year; likewise, if births were observed in the same calendar year as a sample, that sample was considered to have occurred during a birth year. For a birth year to occur, mating would have had to have occurred, but there was one instance during the sampling period where mating was observed but no births resulted. Therefore, both mating year and birth year variables were used in analyses.

R Version 3.5.0 (R Core Team, 2018) was used for all statistical analyses. The "Hmisc" package was used to create a Pearson correlation matrix of the independent variables (Harrell Jr & Dupont, 2008). No variables were found to have a correlation coefficient with an absolute value larger than 0.7 (Dormann et al., 2013); therefore, all were used in further analyses. A Generalized Linear Model (GLM) with a Poisson distribution was run using the "R Stats Package" (R Core Team, 2018). Rate of agonistic behaviour was used as the dependent variable, and fruit availability, flower availability, mating season, mating year, birth season, and birth year were used as the predictor variables. An interaction term for fruit and flower availability was included to determine the impact of food availability as a whole. I also included interaction terms between mating

season and mating year and between birth season and birth year to distinguish between the impact of time of year and that of actual mating or birth-related behaviours. To account for variable observation time across phenological periods, I included the number of observation hours as an offset. Finally, average adult subgroup size and site were used as fixed variables to account for the number of potential actors of agonistic behaviour and unmeasured differences among sites. As a larger number of potential actors is likely to result in a higher rate of any given social behaviour, subgroup size was accounted for in analyses (as in Janson & Van Schaik, 1988). A two-sample Kolmogorov-Smirnov test for nonparametric data with unequal variances was conducted post hoc to evaluate differences in agonism rate between small subgroups (i.e., 3 or fewer individuals) and large subgroups (i.e., more than 3 individuals). Following calculation of the full model, the dredge function was applied from the package "MuMIn" (Barton, 2009) to compute all possible combinations of variables based on the full model. Models within two Akaike information criterion units corrected for small sample sizes (AICc) of the top model (i.e., with the lowest AICc) were considered the "best" models (Burnham & Anderson, 2004).

RESULTS

Average rates of group-wide agonism throughout the study period were as follows: 0.18 acts/hr during the mating season, 0.14 acts/hr during the birthing season, and 0.13 acts/hr during the non-breeding season (Table 2.1). Three models were within two AICc units of the top model (Table 2.2). Average subgroup size, birth year, mating season, mating year, and the mating season x mating year interaction term were included in both "best" models. Site was included in one of the "best" models. The full model was not among the "best" models. Within the full model, the significant variables were average subgroup size, birth year, mating year, and the mating season x mating year interaction term (Table 2.3). The subgroup size variable yielded the largest coefficient in the full model. A Kolmogorov-Smirnov test comparing group agonism rates between small subgroups (defined as groups with 3 or fewer individuals) and large subgroups (defined as groups with greater than 3 individuals) was found to be significant (D = 0.55682, p < 0.001) with large subgroups yielding higher rates (M= 0.71 acts/hr, SD = 0.43) of agonism than small subgroups (M = 0.33 acts/hr, SD= 0.30; Figure 2.1).

Looking only at models that incorporated average subgroup size, there were inverse relationships between agonism rates and both the mating season variable and the mating year variable when the interaction term was included (Table 2.3). However, the larger absolute value of the interaction term coefficient indicated a strong effect of the positive interaction term whereby agonism rates increased during the mating season only in years when mating occurred. When examining the coefficient values for birth season, birth year, and their interaction term in the full model, agonism appeared to increase in frequency during the birth season in birth years only, similarly but to a lesser extent than mating season in mating years. Neither fruit nor flower availability showed a significant relationship to agonism in the full model, and fruit availability was not included in any of the "best" models. Flower availability did show a negative relationship to agonism in one of the three "best" models. Finally, one of the "best" models indicated that agonism rates were higher in Vatovavy than in Sangasanga.

DISCUSSION

Lemurs exhibit low overall rates of agonism across seasons relative to other primates (Wheeler et al., 2013), but research quantitatively describing patterns of agonism in this seasonally breeding, female-dominated species has not been investigated previous to this study. The mean group-wide rate of agonism of ruffed lemurs was 0.16 acts/hr throughout the duration of this study. This value is consistent with Morland's observed rate of 0.17 acts/hr in females on Nosy Mangabe (1991) and Cowl & Schultz's observed rate of 0.14 acts/hr (2017) for ruffed lemurs. The highest agonism rates were observed during the mating season, with lower rates occurring in the birthing season and the lowest rates throughout the non-breeding season. Diet type and the degree to which specific food items can be contested has been useful in predicting agonism in nonhuman primates (Wrangham, 1980), but this study found no effect of food availability on agonism rates in ruffed lemurs. Reproductive season and subgroup size were found to have the strongest effects on agonism.

Reproductive Season and Agonism

Reproductive season was found to be a primary driver of agonistic behaviour in black-and-white ruffed lemurs. More specifically, the presence of mating and births predicted increases in agonism, which suggests that agonism plays a role in the reproductive strategies of ruffed lemurs (Cowlishaw & Dunbar, 1991; Erhart & Overdorff, 2008). The prediction that mating years would lead to increases of agonism during the mating season was supported, but the prediction suggesting a decline in agonism during the birth season was not supported.

The effect of mating season in mating years was among the strongest predictors of agonism in ruffed lemurs. Increased agonism during the mating season can be explained in part by the fact that the breeding season is condensed and breeding does not take place consistently on an annual basis, so male-male competition for female access is likely increased during this short time period (Morland, 1993). Similar results have been well documented in ringtailed lemurs (Cavigelli & Pereira, 2000; Gould & Ziegler, 2007). More specifically, rates of agonistic behaviour were higher in years that ruffed lemurs mated than years they did not, and agonism levels were lower outside of the reproductive seasons. Another possible explanation for the observed patterns of reproductive agonism could be that female-male directed agonism may function as a way for females to exert female-choice for mating partners as well as maintain dominance over males. For example, female black-and-white ruffed, ring-tailed,
and gray mouse lemurs exhibited agonism towards males when mating attempts were not female-initiated (Eberle & Kappeler, 2004; Foerg, 1982; Pereira & Weiss, 1991).

Ruffed lemurs are known to engage in cooperative caring of offspring (Baden, 2011; Vasey, 2007), which may be associated with a certain level of tolerance allowing conspecifics to perform care-related tasks for non-kin (Mitani & Watts, 1997). However, this behaviour is likely not related to declines in agonism in my study population, as a small spike in agonism was observed during the birth season. It is possible that this unexpected increase in agonism may occur due to parents guarding their infants from potentially threatening situations (Pereira et al., 1987; Vasey, 2007). Ruffed lemur males and unrelated females will guard the nest of infants when the mother is foraging and may employ agonistic behaviours to ward off unfamiliar conspecifics (Pereira et al., 1987). Additionally, following the births of ring-tailed lemurs, mothers have been observed preventing fathers from visiting nesting sites of their own infants (Pereira et al., 1987). The observed increase in agonism during the birth season may also be attributed to the fact that subgroups are larger during this season, particularly when infants are present (Holmes et al., 2016). This increase in group size equates to more helpers in this cooperative breeding primate, but larger group sizes could also mean less familiar subgroup members, towards which parents may be less tolerant (Schaffner & French, 1997).

Years in which ruffed lemurs mated and produced offspring resulted in increased agonism during the breeding seasons, whereas in years that they did not breed, agonism was actually lower during both the mating and birth seasons than outside them. These findings suggest that patterns of agonism can be predicted as a product of reproductive season, specifically when certain behaviours are exhibited. Mating-induced agonism between males in addition to the production of energy rich eggs followed by the gestation, birthing, weaning, and protection of infants by females are extremely energetically taxing (Bronson, 1989). The lack of agonism during the breeding seasons, specifically in years where mating and birthing were not observed can be explained by the fact that competition for mating resources (i.e., mating partners) is absent without actual mating behaviours. Furthermore, agonism is likely reduced due to the absence of infants needing to be protected when no births occur. This finding suggests that it is not merely reproductive season alone that influences agonism but rather specific behaviours and reproductive events that may occur within those seasons.

Seasonal Food Availability & Agonism

Fission-fusion dynamics are believed to mediate the potential for increased contest competition when food is scarce (Asensio et al., 2008; Aureli & Schaffner, 2007). At Kianjavato, the pattern of fission-fusion dynamics observed is opposite to that expected with respect to food availability: larger group sizes are observed when site-wide food availability is low (Holmes et al. 2015). The

analysis presented in this chapter indicates that despite the unusual grouping patterns, fission-fusion dynamics at this site may still function to reduce contest competition variability in response to food availability (evidenced by the lack of significant impact of food availability on agonism). Therefore, my prediction that fruit and flower availability would impact rates of agonism is not supported. Nonetheless, results of this study are consistent with past research across many primate taxa showing no significant association between seasonal food availability and intraspecific agonism (gray-cheeked mangabey: Chancellor & Isbell, 2009; Assamese macaque: Heesen et al., 2014; Verreaux's sifaka: Koch et al., 2016; blue monkey: Pazol & Cords, 2005; Wheeler et al., 2013), suggesting that there are a variety of mechanisms to mitigate contest competition among primates. Alternatively, it is also possible that ruffed lemurs exhibit scramble competition (and contest competition to a lesser extent), are not food-limited despite their highly frugivorous diet, and thus do not compete directly over food resources. The agonism observed in ruffed lemurs is likely better explained by other factors such as reproductive season and subgroup size.

Subgroup Size and Agonism

In accordance with past research investigating predictors of primate agonism, this study observed a significant association between subgroup size and agonism rate (Cowl & Shultz, 2017; Wheeler et al., 2013). Subgroup size was incorporated in order to control for its effect on agonism, and I found that subgroup size was the strongest predictor of agonism in this species. This was expected because larger groups equate to more conspecifics with which to interact and potentially behave agonistically (Eaton et al., 1981; Janson & Van Schaik, 1988). This has been observed in spider monkeys, where medium and large subgroup size corresponded with an increase in agonism compared to when subgroups were small (Asensio et al., 2008). Another study found very similar results, specifically that group size was the strongest predictor of agonism rates across all primary non-human primate taxonomic groups (Wheeler et al., 2013). The results obtained from this study – that smaller subgroups of ruffed lemurs exhibited significantly lower rates of agonism than larger subgroups – concur with these previous findings.

As indicated above, seasonal changes in subgroup size may thus impact variation in agonism across the year. One explanation for increased group size during the mating season could be that the cold-dry season coincides with an extended duration of low food availability. Adjusting activity budget and increasing group size by way of employing huddling behaviour may be necessary for maintaining optimal internal body temperatures during these cold and resource-scarce times (Kelley et al., 2016; Ostner, 2002). Although sunbathing behaviour increased in ring-tailed lemurs during the austral winter, huddling behaviour did not (Kelley et al., 2016); however, ruffed lemurs are the largest frugivorous lemur species (A. L. Baden et al., 2008; Vasey, 2007) and may therefore benefit from increased close-body contact with conspecifics. Southern bamboo lemurs received immediate thermoregulatory benefits by utilizing social huddling behaviour, and it is hypothesized that individual benefits likely increase with larger subgroups (Eppley et al., 2017). Additional explanations as to why this unusual pattern of increased group size occurs in times of scarce food availability include increased detection of food resources as well as enhanced defensive capability during intergroup encounters at food patches (Holmes et al., 2016).

CONCLUSION

Reproductive season may be the primary driver of agonistic behaviour in ruffed lemurs, as mating and birthing variables produced the strongest variation in agonism rates within and between years for the duration of this study. Despite the fact that ruffed lemurs exhibit markedly low levels of agonism compared to other primates (Sussman et al., 2005), seasonal fluctuations in agonistic behaviour suggest an important role in strategies to increase fitness. Specifically, I argue that agonism functions as a reproductive strategy, made evident by its increase in mating/birthing years and decrease in non-mating/birthing years.

Even without direct contextual data on agonistic acts (e.g., identities of actors and recipients, whether agonism was related to food or other resource), it was possible to glean potential reproductive versus ecological functions by comparing breeding and non-breeding years. I was able to determine that reproductive season predicted increased agonism in black-and-white ruffed lemurs, but site-wide food availability did not. This type of approach may be appropriate for species that are difficult to observe due to their high degree of arboreality, sexual monomorphism (i.e., cannot sex without unique identifiers), and species that breed seasonally but do not consistently mate from year to year.

To further investigate predictors of agonism in black-and-white ruffed lemurs, I suggest examining physiological factors in order to construct hormonal profiles of individuals across the breeding and birth seasons. This would be of particular importance since numerous studies investigating agonism have demonstrated that increased production of androgens in males is required to prime them for increased competition for sexual partners during the mating season (Girard-Buttoz et al., 2015; Goymann et al., 2007; Higham et al., 2013; Wingfield et al., 1990). Discerning patterns of hormones and agonism during mating versus non-mating seasons would be beneficial to better understand what drives agonism in primates and ruffed lemurs in particular. Another future course of study could be to measure the changes in fission-fusion dynamics as a predictor of agonism, which would provide additional insight to the function of this flexible grouping behaviour in this species. Particularly since agonistic behaviours are quite rare in this species, future studies might also consider investigating affiliative behaviours (e.g., grooming, huddling) in order to better understand how social bonding affects the social organization and reproductive strategies across seasons. Agonistic and affiliative behaviours function complementarily to shape the social relationships that ultimately lead to mating opportunities and differences in individual fitness (Silk, 2007; Sussman & Garber, 2004), though past research has yet to investigate the drivers of these

fundamental behaviours in ruffed lemurs.

TABLES

Table 2.1. Rates of agonism (acts/hr) across the mating, birthing, and nonbreeding seasons. The non-breeding season encapsulates the time period following the birthing and lactation season (September-December) and before the mating season (May-July).

Year	Mating Season	Birthing Season	Non-Breeding Season
2014	0.15	0.13	-
2015	0.29	0.19	0.15
2016	0.14	0.07	0.14
2017	0.14	0.18	0.16
All years	0.18	0.14	0.13

Table 2.2. Top five models based on AICc values. Models within 2 AICc units of the top model are considered the "best" models (Δ AIC values in **bold** font). All models shown share the inclusion of five variables: average subgroup size, birth year, mating season, mating year, mating season and mating year interaction term.

Variables Included		logLik	AICc	ΔΑΙϹ	weight
Subgroup Size, Birth Year,					
Mating Season, Mating Year,	6	-58/ 30	1121 12	0	0 230
Mating Season x Mating Year	0	-364.30	1101.10	Ū	0.239
Subgroup Size, Birth Year,					
Mating Season, Mating Year,					
Mating Season x Mating Year,	7	-584.05	1182.90	1.72	0.101
Site					
Subgroup Size, Birth Year,					
Mating Season, Mating Year,					
Mating Season x Mating Year,		-584.19	1183.16	1.98	0.089
Flower Availability					
Subgroup Size, Birth Year,					
Mating Season, Mating Year,					
Mating Season x Mating Year,		-584.21	1183.20	2.02	0.087
Fruit Availability					
Subgroup Size, Birth Year,					
Mating Season, Mating Year,					
Mating Season x Mating Year,		-584.27	1183.34	2.16	0.081
Birth Season					

	Estimate	Std. Error	z value	р
(Intercept)	-2.292	0.15	-15.50	< 0.001
Fruit Availability	-0.018	0.03	-0.66	0.510
Flower Availability	-0.038	0.04	-1.10	0.272
Mating Season ^a	-0.185	0.12	-1.49	0.136
Mating Year ^a	-0.384	0.09	-4.45	< 0.001
Birth Season ^a	-0.021	0.09	-0.24	0.814
Birth Year ^a	0.275	0.09	3.15	0.002
Average Subgroup Size	0.503	0.04	13.59	< 0.001
Site ^a	0.102	0.08	1.27	0.205
Fruit Availability x Flower	0.020	0.03	0.60	0.549
Availability				
Mating Season x Mating Year	0.449	0.14	3.28	0.001
Birth Season x Birth Year	0.069	0.12	0.58	0.562

Table 2.3. Results of the full GLM with Poisson distribution. The model included the number of hours of observation as an offset. ^a indicates a binary variable.

FIGURES



Figure 2.1. Boxplot comparing rates of agonism in small subgroups (subgroups with 3 or fewer individuals) versus large subgroups (subgroups with more than 3 individuals). A two-sample Kolmogorov-Smirnov test revealed that larger subgroups exhibited significantly higher rates of agonism than smaller subgroups (p < 0.05). Circles indicate outliers, whiskers indicate the range of the data, boxes indicate the interquartile range, and the bolded horizontal line indicates the median.

CHAPTER 3: Rates of male agonism and grooming in a female-dominated primate species during the mating and post-mating seasons

ABSTRACT

Agonism and affiliation are ubiquitous behavioural traits observed across all group-living primates. Social bonding through affiliative behaviours, specifically grooming, in conjunction with social dominance through agonistic behaviours, such as the displacement of a lower-ranking individual by a dominant conspecific, are fundamental factors deciding the social structure of primates. These factors are likely to ultimately affect the fitness of any given individual. This study seeks to answer the question: do males differ in their behavioural strategies in response to the mating season in a primate species that exhibits female dominance? Agonism rates are expected to increase during the mating season due to increased male-male competition and males putting themselves in "harm's way" by interacting with females more so than usual. Additionally, male grooming rates are expected to increase in the mating season, as grooming should function to establish and maintain social bonds prior to mating. Approximately 500 focal hours of data were recorded during the cool-dry austral winter in southeastern Madagascar. All occurrences of social behaviour were recorded and tallied for each individual and compared for males and females across the mating and post-mating seasons. As predicted, a trend indicating increased rates of agonism during the mating season was observed in males.

With the inclusion of females in the model, agonism rates were found to be

significantly higher in the mating season compared to the post-mating season. I found a significant positive association between individual male rank and agonism rate, but male ranks were not associated with their respective affiliative grooming rates. Additionally, male affiliation rates were significantly higher during the mating season compared to the post-mating season. The expression of agonistic behaviour was similar between the sexes, which was unexpected due to female dominance reported in this species. I expected to observe higher agonism rates in females, but males exhibited higher overall rates throughout the study period. The results of this study provide some support that agonistic and affiliative behaviours function as behavioural strategies in response to challenges associated with the mating season for ruffed lemurs. Future research should more fully investigate rates of agonism and affiliation with respect to intra- and inter-sexual dyads in order to determine these patterns of social behaviour at a finer scale across the breeding season.

INTRODUCTION

Agonistic and affiliative behaviours are fundamental traits of sociality present across primate taxa but occur at variable rates interspecifically and intersexually (Sussman et al., 2005; Wheeler et al., 2013). Agonism in the context of food and mate competition between conspecifics, as well as to construct dominance hierarchies, has been well studied in many primate species (Fedigan, 1983; Gould & Ziegler, 2007; Janson & Van Schaik, 1988; Ostner, Heistermann, et al., 2008). Affiliation through grooming behaviour is crucial to establishing bonds between individuals belonging to the same group, and therefore necessary for understanding the social structure of primate societies (Ramanankirahina et al., 2011; Sussman et al., 2005). These social behaviours are considered evolutionary strategies, as they are integral to modulating social relationships and establishing rank, which confers with it privileges within the group and ultimately fitness benefits (Fedigan, 1983; Silk, 2007; Sussman & Garber, 2004). Social rank is strongly argued and supported as a basic constituent affecting primate survival and fitness, especially in primates with long and slow life histories (Blomquist et al., 2011).

Wild primates are subjected to seasonal changes in temperature, rainfall, and photoperiod, which may affect food availability and breeding seasonality (Foerster et al., 2012; Wright, 1999). In all primates, but especially in seasonally breeding species, individuals are required to compete for limited mating opportunities; this commonly occurs in the context of males competing for receptive females, but marked competition between females has also been observed (Alberts et al., 2006; Cavigelli & Pereira, 2000; Cowlishaw & Dunbar, 1991; Huchard & Cowlishaw, 2011). Social interactions are required to maintain the benefits of group living, such as males increasing their time spent interacting and forming bonds with females prior to potential mating opportunities (Sussman et al., 2005). These benefits are modulated by an individual's ability to interact socially with conspecifics, especially during challenging times, such as periods of low food availability and breeding seasons. Previous research has found that social bonds between males and females facilitated through grooming increased the fitness levels for both sexes (Tiddi et al., 2012). Specifically in lemurs, male individuals who were more socially integrated in a group were better tolerated by females, which enabled such males to form stronger affiliative bonds (Gould, 1996).

Lemurs face severe seasonal pressures, including short-lived breeding seasons and highly variable food availability, among many other ecological stressors (Wright, 1999). Black-and-white ruffed lemurs are a female-dominant species that inhabit the forests of eastern Madagascar and exhibit high levels of fission-fusion dynamics (Holmes et al., 2016). Although the ruffed lemur mating season may span the months of May-July, they exhibit marked breeding synchrony, where females belonging to the same subgroups enter estrus within a duration lasting no longer than 72 hours (Baden et al., 2016). The highly constricted mating season is expected to increase competition for males seeking

39

access to mating partners (Baden et al., 2016). The ruffed lemur mating season, which occurs during the cold-dry season, coincides with the period of low fruit availability (Holmes et al., 2013), which was thought to further exacerbate the challenges associated with the mating season. The results from Chapter 2 of this work, however, revealed that group-wide agonism rates were strongly associated with subgroup size and reproductive season, yet there was no effect of seasonal changes in food availability on ruffed lemur agonism. The results indicated that agonism increased during the mating season in years that mating was observed. However, a finer-scale approach to determine predictors of agonistic and affiliative behaviour between individual ruffed lemurs is needed to further evaluate the specific social and ecological variables influencing this probable reproductive strategy.

The aims of this study were to examine agonism and affiliation (i.e., grooming) rates of males in a primate species that exhibits female dominance, the black-and-white ruffed lemur. Here, I tested how agonistic and affiliative behaviours are driven by reproductive and social factors, such as mating season and dominance rank, and how males and females differ in their expression of these social behaviours. I predicted that males would increase their rates of agonism during the mating season, as this is a time when social group-living and seasonally breeding species are expected to experience increased competition over access for mates (Hirschenhauser & Oliveira, 2006; Muller & Wrangham, 2004; Wingfield et al., 1990). I further predicted that males assigned higher ranks

would exhibit higher rates of agonism, as agonism should be used to maintain dominance rank. I also predicted males would increase their grooming rates during the mating season in order to form social bonds with females prior to mating. Under the priority-of-access model (Altmann, 1962), high-ranking individuals are expected to have greater access to limiting resources (e.g., mates), so high-ranking males who receive more grooming from subordinates may be afforded a greater number of grooming opportunities overall. Therefore, I predicted that high-ranking males would engage in higher rates of affiliation than low-ranking males. Lastly, due to the female dominance exhibited and reported in this lemur species, I predicted that higher dominance ranks and rates of agonism would be expressed by females compared to males across seasons.

METHODS

Study Species & Site

The study species, the black-and-white ruffed lemur, is a Critically Endangered primate close to the brink of extinction (IUCN, 2020). Ruffed lemurs are the largest extant frugivorous lemur, sexually monomorphic, and characterized by a female-dominant social structure (A. L. Baden et al., 2008; Vasey, 2007). Reproductive events are seasonal in this species with mating generally occurring between May and July (Morland, 1993). Similar to other sites throughout Madagascar (Baden et al., 2016; Ratsimbazafy, 2003), lemurs at this site do not consistently mate and give birth every year, as has been observed and investigated in Chapter 2 of this work. This study was conducted during a year when mating and births occurred.

This study took place at the Kianjavato Ahmanson Field Station (KAFS) in southeastern Madagascar, which is composed of fragmented, protected humid lowland natural forest, secondary forest, agricultural farmland (i.e., primarily used for rice production), and restored forest (Manjaribe et al., 2013). This area has high seasonal variation with a warm-wet season that takes place from December to April and a cool-dry season from May to November (Holmes et al., 2016). Ruffed lemurs were sampled in three forest fragments: Sangasanga (64 ha), Tsitola (954 ha) and Vatovavy (644 ha) (Holmes et al., 2013).

Data Collection

Focal follows on radio-collared individuals (N = 18) were conducted from May through September 2019 across the three forest fragments mentioned above. The mating season, the duration of time when pre-copulatory and mating behaviours are known to occur, was defined as taking place from May-July; the post-mating season was defined as taking place from August-September, which includes the duration of time prior to the birthing season (Morland, 1993). Behaviour was recorded at five-minute intervals using an instantaneous focal sampling technique (Altmann, 1974). Focal individuals (males, n = 9; females, n =9) were observed for two-hour periods in rotation, such that each individual was observed for a total of eight hours per month, spread throughout the six- to eight-hour workday. Three females and one male were lost and unable to be tracked for various reasons during this study period but were used in analyses when possible (see Table 3.1). Ad libitum data specific to agonistic (e.g., swatting, chasing, biting, retreats, chatter vocalizations), affiliative (e.g., grooming), and mating behaviours between ruffed lemur individuals were recorded in the subgroup being followed, regardless of whether the focal animal was involved in the social interaction or not. As part of the longstanding monitoring program, additional data including demographic information, climatic variables, and other related behavioural activity were recorded following a protocol consistent with Holmes (2017). Changes in subgroup membership (individuals within 30m of the focal animal) were also recorded (as per Holmes, 2017). Interobserver reliability was improved through biweekly professional development sessions to ensure quality control of data collection from research technicians across the three sites. Additionally, the role of a specially trained Varecia team supervisor was established in order to manage the three field teams and ensure consistent data collection protocols.

Nearest neighbour category data were recorded at five-minute intervals in order to determine time spent in the vicinity of intra- and inter-sexual conspecifics, which was used as a proxy measure for mating success during the mating season (similarly to mate-guarding: Girard-Buttoz et al., 2015; Mass et al., 2009). However, nearest neighbour was found to be a non-collared lemur (NCL hereafter) for a large number of scans; since an NCL cannot have its sex identified in this species, this measure was not included in analyses. An original objective of this study was to measure fecal testosterone levels as a predictor for agonism. Hormonal analyses took place at the University of Arizona's Laboratory for the Evolutionary Endocrinology of Primates in late Fall 2019 but were halted due to the COVID-19 pandemic. Methods used and progress made thus far are included in Appendix B and C.

Calculating Ranks, Agonism, & Grooming Rates

Agonism rates were determined for every collared individual by tallying the number of bouts they initiated divided by the total number of hours they were observed for in a given biweekly period. Observation hours included the number of hours an individual was followed for in addition to the amount of time (i.e., in 5-minute intervals) they were present in another focal individual's subgroup. I then adjusted each agonism rate by dividing by the average number of conspecifics in the subgroup for each respective biweekly period. If an individual was not followed or observed for a minimum of two hours during a biweekly period, they were excluded from the averaged dataset for that time period.

For decided bouts where there was a clear winner (i.e., individual causing the displacement) and loser (i.e., individual being displaced), wins and losses were tallied when possible in order to determine win/loss ratios and assign ranks to each individual. As an alternative to David's score, Elo-ratings are often used to assess and assign ranks to individuals in studies with small sample sizes and when social interactions are rare (Neumann et al., 2011). Individuals with an insufficient number of interactions or who were the only collared individual in a sampled subgroup could not be ranked and were therefore excluded from analyses. Elo-ratings were based on agonistic interactions where a winner may earn points and the loser lose points, and the number of points earned depends on the probability that the previously higher-ranked individual wins (Neumann et al., 2011). Individuals were plotted according to their Elo-ratings to visualize their social rank within their respective subgroups throughout the study period.

Grooming rates were calculated similarly to agonism rates, except that a grooming interaction was tallied for all focal individuals involved, whether they initiated or received the grooming. Focal-initiated grooming rates were also calculated separately for analyses examining rank effects on grooming. Agonism and grooming rates were averaged for every individual and categorized as taking place in either the mating or post-mating season. Social interaction rates were also averaged for each individual throughout the entire study period (see Table 3.1).

Statistical Analyses

Using RStudio 3.6.1 (R Core Team, 2018), a series of paired and two-sample ttests were employed to determine patterns of agonism and affiliation between male and female ruffed lemurs during and after the mating season. In the results presented here, mating season months consisted of May-July, and the postmating season consisted of data from August and September. In order to account for unequal sampling between the seasons, rates of agonism and grooming were averaged for analyses. Data were pooled across study groups and

45

forest fragments. Paired t-tests were conducted to determine differences in male agonism and affiliation rates between mating versus post-mating seasons. Wilcoxon Signed Rank tests were used when data did not meet conditions for parametric tests and had equal variances. Two-sample t-tests were conducted to compare agonism rates between males and females in both seasons. Only intraspecific interactions involving at least one identifiable collared lemur were used in my analyses (i.e., social bouts between non-collared individuals were excluded). A Kolmogorov-Smirnov test, using the "R Stats Package", was employed when the data were non-parametric and had unequal variances (R Core Team, 2018). Rates of agonism and grooming were calculated for every collared individual based on biweekly periods in order to capture as much behavioural variation as possible between individuals during the study period. More specifically, agonism and grooming rates were separated into biweekly periods within the mating and post-mating seasons for every individual. Datapoints for which an individual was either not followed or observed for a minimum of two hours during the biweekly period were excluded from analyses. Pearson correlation coefficients were calculated to assess the relationship between rank (Elo-rating scores) and agonism rates in both sexes. Elo-ratings were used in conjunction with win/loss ratios to evaluate an individual's ranking in their respective subgroup using the "Elo-rating" package in RStudio 3.6.1 (Neumann et al., 2011). Pearson correlation coefficients were also calculated to

assess the association between rank and grooming rate (i.e., of initiated bouts) for both sexes.

RESULTS

A total of 131 agonistic interactions (i.e., focal-initiated bouts; mating season, n = 100; post-mating season, n = 31) and 262 grooming interactions (mating season, n = 169; post-mating season, n = 93) were observed over approximately 503 focal hours across the three study sites (Tsitola, n = 156 hrs; Sangasanga, n = 159 hrs; Vatovavy, n = 188 hrs). There was no significant difference (t(4) = 0.66, p = 0.54) between the total number of grooming (N = 262 bouts) and agonistic (N = 226 when including interactions initiated as well as received) interactions observed in ruffed lemurs throughout the study period. Ranks based on Elorating scores were plotted for all focal individuals (Figure 3.6a, 3.6b, 3.6c) in order to construct dominance hierarchies for each site, which were found to be unstable and nonlinear overall. That is, dominance rankings were not static throughout the study duration and Elo-rating scores fluctuated markedly for most focal individuals.

Comparison of Grooming in Mating vs. Post-Mating Seasons

A significant difference (t(7) = 2.6, p = 0.03) was observed in male grooming rates, with higher rates of grooming occurring in the mating season (M = 0.23acts/hr, SD = 0.16) compared to the post-mating season (M = 0.12 acts/hr, SD = 0.08; Figure 3.3). There was no association between rank and affiliation rates in males (Figure 3.9; r = -0.186, n = 8, p > 0.05), but a significant inverse relationship was observed in females (r = -0.868, n = 6, p < 0.05).

Of the total 159 affiliative interactions, 38% included NCLs as interactants, with 22 grooming bouts directed from NCLs to focal individuals and 39 bouts directed from focal individuals to NCLs. There were 92 affiliative interactions involving only focal individuals, which I used to analyze dyadic rates of affiliation; 33% of interactions took place within male-male dyads, 30% of interactions were directed from males to females, 24% of interactions were directed from females to males, and 13% of interactions occurred within femalefemale dyads (Table 3.2). Due to the high number of NCLs in focal sub-groups, differences in dyadic rates could not be compared statistically (i.e., I could not assess whether percentages of interactions within certain dyads were high or low relative to the sex ratio within sub-groups).

Comparison of Agonism in Mating vs. Post-Mating Seasons

There was no significant difference in male agonism rates between seasons (Figure 3.1), although a trend indicating higher rates of agonism during the mating season was observed (W = 31, p = 0.08). There was no difference observed in female agonism rates between the mating and post-mating seasons (t(5) = 1.15, p = 0.25). However, agonism rates were found to be significantly higher during the mating season (M = 0.09 acts/hr, SD = 0.08) compared to the post-mating season (M = 0.04 acts/hr, SD = 0.05) when both males and females were included in the model (Figure 3.2; Z = 2.04, p = 0.04). Pearson correlation

48

coefficient analyses showed a significant positive relationship between male rank (i.e., Elo-rating scores) and agonism rate (Figure 3.7; r = 0.879, n = 8, p < 0.05), but no association was observed between rank and agonism rate in females (Figure 3.8; r = -0.246, n = 6, p > 0.05). Lastly, I compared agonism rates between males and females and found no significant difference (t(14,48) = -0.24, p = 0.81) between male and female agonism during the mating season (Figure 3.4). Likewise, a Kolmogorov-Smirnov two-sample test found no significant difference (D = 0.33, p = 0.84) in agonism rates between the sexes in the postmating season (Figure 3.5). Although there was no difference in agonism rates between males and females, females generally occupied higher ranks than males (Table 3.1; according to their Elo-ratings and win/loss ratios).

Of the total 142 agonistic interactions, 36% included NCLs as interactants, with 11 bouts directed from NCLs to focal individuals and 40 bouts directed from focal individuals to NCLs. There were 88 agonistic interactions involving only focal individuals, which I analyzed to identify dyadic rates of agonism; 33% of interactions took place within male-male dyads, 30% of interactions were directed from males to females, 24% of interactions were directed from females to males, and 13% of interactions occurred within female-female dyads (Table 3.3). As with grooming dyads, these percentages could not be compared statistically.

DISCUSSION

Grooming Rates in the Mating & Post-Mating Seasons

My results provide support for the prediction that male grooming rates would be higher in the mating season compared to the post-mating season. Male grooming behaviour was expressed at significantly higher rates during the mating season compared to the post-mating season. This may be explained by males gaining favour of females by forming bonds via grooming prior to mating. Previous research examining social behaviours in crowned lemurs observed increased male grooming rates during the mating season, specifically in weeks when females were conceptive (Kappeler, 1989). Another study investigating grooming patterns in Verreaux's sifaka found that grooming rates increased 50-100% during the mating season (Lewis, 2010).

My results indicated no effect of rank on grooming rates in males. This does not support my prediction suggesting that high-ranking males would exhibit higher affiliation rates than low-ranking males across the study period. As expected with the priority-of-access model, males occupying higher ranks should have greater access to females than low-ranking males, and should therefore have greater opportunities to affiliate and gain access to members of the opposite sex (Altmann, 1962). However, the use of the priority-of-access model may be inappropriate to apply here in a seasonally breeding species where females are scattered throughout the forest strata rather than being clumped and easily defendable (Altmann, 1962; Dubuc et al., 2011). It is also possible that the analyses used here are not fine-scale enough to identify this pattern, as I included intra- as well as inter-sexual grooming interactions in my analyses. Future analyses may consider the relationship between male rank and intersexual grooming rates specifically. Additionally, previous research has found that low-ranking individuals groom up the hierarchy, whereby low-ranking individuals exhibit more grooming towards dominants than vice versa (Cheney & Seyfarth, 1977; Tiddi et al., 2012). If this pattern is true for black-and-white ruffed lemurs, it may confound and provide some explanation for the lack of relationship between male rank and affiliation rate reported here.

Agonism Rates in the Mating & Post-Mating Seasons

The results of this study provide support for my prediction that mating season agonism would be higher than post-mating season agonism for ruffed lemurs. I observed a non-significant trend revealing that rates of agonism were higher for males during the mating season compared to the post-mating season.

Additionally, there was no difference in female agonism rates observed between the mating and post-mating seasons. However, agonism rates were found to be significantly higher during the mating season when both males and females were included in the model; it is therefore likely that the seasonal pattern observed in males alone was less pronounced due to small sample size. The seasonal differences in agonism presented here may be best explained by increased competition for mating partners. Alternative ecological causes, such as low food availability, are not supported in this population (see Chapter 2). These results are consistent with studies of colobus monkeys, ring-tailed lemurs, and Verreaux's sifaka showing increased rates of agonism during the mating season (Brockman et al., 1998; Gould & Ziegler, 2007; Teichroeb & Sicotte, 2010).

My prediction that male rank would be associated with agonism rate was supported, as results indicated that high-ranking males (i.e., individuals assigned higher Elo-ratings) exhibited higher rates of agonism than low-ranking males. These results may concur with past studies indicating that dominant males maintain their social standing by exhibiting agonism predominantly towards subordinate males than vice versa (Ostner et al., 2002; Teichroeb & Sicotte, 2010). Therefore, I suggest that the graphical representation of male dominance hierarchies reported in this study, although unstable and nonlinear (as has been reported previously: Erhart & Overdorff, 2008), are useful in predicting patterns of agonism in male black-and-white ruffed lemurs. However, I found that female rank was not significantly associated with agonism rate. Past research on agonism in ring-tailed lemurs found that the highest-ranking females exhibited the highest agonism rates (Cavigelli et al., 2003). Agonism among ring-tailed lemurs is intense and may function to establish stable dominance hierarchies, whereas black-and-white ruffed lemurs exhibit very little agonism between females, and female-dominance hierarchies are unstable (Erhart & Overdorff, 2008). It is possible that agonism between females does not function to gain fitness-related privileges associated with asserting dominance intrasexually. I speculate that very little competition occurs between females, as evidenced by

the smallest proportion of agonism being allocated towards female-female bouts. On the other hand, female-male directed agonism occurred nearly three times more frequently than either female-only or male-female directed bouts, suggesting that females establish and maintain dominance over males through their more frequently occurring intersexual interactions. Due to the frequent presence of non-collared individuals (where sex could not be determined), the rates of dyadic interactions must be treated with a high degree of caution. Nonetheless, females' use of agonism to enforce social hierarchy is also supported by my finding that females occupied higher ranks than males, according to their Elo-rating scores.

Lastly, my prediction that male agonism rates and ranks would be lower than in females across seasons was partially supported. As expected, females generally occupied higher ranks than males, but there was no significant difference in agonism rates between males and females. That being said, males surprisingly exhibited more agonism on average than females throughout the study period. These results are in contrast to Morland's one-year study on ruffed lemurs indicating a distinct difference between male (0.02 acts/hr) and female (0.17 acts/hr) agonism (Morland, 1991). I suspected that females would express higher rates of agonism than their male conspecifics due to the femaledominated social organization described in this as well as other lemur species (Morland, 1991; Pochron et al., 2003; Sauther, 1993). It is possible that this pattern emerged due to the short duration of this study, which took place during the mating season when increased intrasexual male agonism is expected to occur. Whereas females in a female-dominated primate species are conferred with certain fitness-related privileges, it is more likely that males should have to compete with one another for limited mating opportunities, which is supported by my findings that agonism occurred most frequently (42% of all decided bouts involving focal individuals) within male-male dyads. Although females engaged in marginally lower rates of agonism than males throughout the mating and postmating seasons, they were more successful fighters, which is likely important in asserting both female choice and dominance.

Overall, the results presented here demonstrate that agonism and affiliation may play important roles as reproductive strategies for ruffed lemurs. Increased sociality during the mating season likely functions: 1) to facilitate bond formation between males and females, 2) to establish and maintain dominance rank, especially in males, and 3) as a means for females to exert female choice and dominance over males. Similar behavioural strategies have been described in Verreaux's sifaka, ring-tailed, red-fronted, and crowned lemurs and are likely to occur in other lemur species with similar life history traits (Brockman et al., 1998; Cavigelli & Pereira, 2000; Gould, 1996; Kappeler, 1989; Lewis, 2010; Ostner et al., 2002). This was the first study to quantitatively investigate predictors of mating season agonism and affiliation in wild black-and-white ruffed lemurs, but additional research focused on hormonal correlates of agonism (see "Challenge Hypothesis" by Wingfield et al., 1990) is suggested for a more comprehensive understanding of ruffed lemur sociality. Endocrinological studies involving testosterone and cortisol have been particularly useful in better understanding the underlying hormonal mechanisms that influence agonism and social stability (or lack thereof) within primate social systems- both of which are known to affect individual fitness outcomes in numerous non-human primates (Beehner et al., 2005; Brockman et al., 2001; Cavigelli & Pereira, 2000; Girard-Buttoz et al., 2015; Gould & Ziegler, 2007; Higham et al., 2013; Lynch et al., 2002; Muller & Wrangham, 2004; Ostner et al., 2002; Raynaud & Schradin, 2014; Rose et al., 1975).

TABLES

Table 3.1. Average hourly rates of agonism, grooming, win/loss ratios, and rankings for all focal lemurs throughout the study period. Site: TT = Tsitola, SS = Sangasanga, VV = Vatovavy. A dash (-) indicates insufficient data to calculate values. Individuals are ordered by site and Elo-rating.

Sito	Individual	Sov	Flo_rating	Grooming	Agonism	Win/Loss
Site	mannauar	JEX	LIO-Tating	Rate	Rate	Ratio
SS	MFO	ę	1180	0.10	0.04	0.80
SS	MBE*	ę	1050	0.11	0.16	0.73
SS	TGK	ď	981	0.02	0.01	0.30
SS	LXI*	ę	928	0.21	0.15	0.65
SS	BOL	ď	806	0.07	0.01	0.14
SS	GRH	ę	-	0.21	0.02	0.67
VV	VAL	ę	1273	0.09	0.15	1.00
VV	SDN	ď	1048	0.23	0.15	0.63
VV	BNS	Ŷ	965	0.37	0.05	0.57
VV	DDL	ď	948	0.26	0.08	0.47
VV	APG	ď	924	0.26	0.07	0.36
VV	FRR	ď	842	0.35	0.02	0.20
тт	MAU	ď	1194	0.10	0.13	0.78
ТТ	SHL	ę	1050	0	0.01	1.00
TT	ANM	Ŷ	964	0.13	0.08	0.36
ТТ	RGS	ď	792	0.15	0.05	0.36
TT	RNN*	ę	-	0.09	0.09	-
ТТ	NSB*	ď	-	0.06	-	-

*Indicates that field teams lost contact with individual due to signal loss,

dispersal, or death.

Site	Male- Male	Male- Female	Female- Male	Female- Female	Total Interactions
SS	-	3% (<i>N</i> = 3)	3% (<i>N</i> = 3)	13% (<i>N</i> = 12)	18
TT	11% (<i>N</i> = 10)	9% (<i>N</i> = 8)	9% (N = 8)	-	26
vv	22% (<i>N</i> = 20)	18% (<i>N</i> = 17)	13% (N = 11)	-	48
All Sites	33% (<i>N</i> = 30)	30% (<i>N</i> = 28)	24% (N = 22)	13% (<i>N</i> = 12)	92

Table 3.2. Frequency of affiliative interactions observed within male-male, male-female, female-male, and female-female dyads.

Table 3.3. Frequency of agonistic interactions observed within male-male, male-female, female-male, and female-female dyads.

Site	Male-	Male-	Female-	Female-	Total	
	Male	Female	Male	Female	Interactions	
SS		3%	17%	8%	25	
	-	(<i>N</i> = 3)	(<i>N</i> = 15)	(<i>N</i> = 7)	25	
тт	27%	6%	8%	1%	27	
	(<i>N</i> = 24)	(<i>N</i> = 5)	(<i>N</i> = 7)	(<i>N</i> = 1)	57	
vv	15%	3%	11%		26	
	(<i>N</i> = 13)	(<i>N</i> = 3)	(<i>N</i> = 10)	-	20	
All Sites	42%	13%	36%	9%	00	
	(<i>N</i> = 37)	(<i>N</i> = 11)	(<i>N</i> = 32)	(<i>N</i> = 8)	00	





Figure 3.1. Comparison of agonism rates initiated by males in mating vs. postmating seasons. A Wilcoxon Signed Rank test revealed a near-significant trend (*p* = 0.08). Circles indicate outliers, whiskers indicate the range of the data, boxes indicate the interquartile range, and the bolded horizontal line indicates the median.



Figure 3.2. Agonism rates including both sexes in mating vs. post-mating seasons. A Wilcoxon Signed Rank test revealed significantly higher agonism rates during the mating season (p = 0.04). Whiskers indicate the range of the data, boxes indicate the interquartile range, and the bolded horizontal line indicates the median.



Figure 3.3. Comparison of grooming rates (initiated and received) of males in mating vs. post-mating seasons. A paired t-test revealed significantly higher agonism rates in the mating season compared to the post-mating season (p = 0.03). Whiskers indicate the range of the data, boxes indicate the interquartile range, and the bolded horizontal line indicates the median.



Figure 3.4. Comparison of mating season agonism between males and females. A t-test indicated no significant difference in agonism rates between males and females during the mating season (p = 0.81). Whiskers indicate the range of the data, boxes indicate the interquartile range, and the bolded horizontal line indicates the median.



Figure 3.5. Comparison of post-mating season agonism between males and females. A Kolmogorov-Smirnov test indicated no significant difference in agonism rates between males and females during the post-mating season (p = 0.84). Circles indicate outliers, whiskers indicate the range of the data, boxes indicate the interquartile range, and the bolded horizontal line indicates the median.



Figure 3.6a. Elo-rating plot indicating a nonlinear and unstable dominance hierarchy among lemurs in Tsitola group throughout the study period. Higher Elo-ratings represent higher dominance ranks. If an individual did not have a sufficient enough number of interactions with other members of the subgroup, they were not included in analyses (e.g., collared individuals NSB, SHL, and RNN).


Figure 3.6b. Elo-rating plot indicating a nonlinear and unstable dominance hierarchy among lemurs in Vatovavy group throughout the study period. Higher Elo-ratings represent higher dominance ranks.



Figure 3.6c. Elo-rating plot indicating a partially linear and stable dominance hierarchy among lemurs in Sangasanga group throughout the study period. Higher Elo-ratings represent higher dominance ranks. No agonistic interactions were observed between focal individuals following June 20th.



Elo-Rating Figure 3.7. Scatter plot with regression line indicating that male rank is strongly correlated with agonism rate (p < 0.05).



Elo-Rating

Figure 3.8. Scatter plot indicating that female rank is not associated with agonism rates (p > 0.05).



Figure 3.9. Scatter plot indicating no significant relationship between rank and grooming rates in males (p > 0.05)

CHAPTER 4: Discussion of ruffed lemur agonism and affiliation

Summary of Findings

The purpose of this research was to identify the social and ecological variables that drive social behaviours, specifically agonism and affiliation, in the Critically Endangered black-and-white ruffed lemur. My research aims were to identify and discern predictors of agonism and affiliation to better understand their functionality in a primate species that is not known for frequent social interaction (Sussman et al., 2005). My research demonstrated that black-andwhite ruffed lemurs engage in these social behaviours at higher rates during the reproductive seasons, and dominance rank is associated with the expression of agonism in males.

In Chapter 2, I found the observed group-wide rate of agonism in blackand-white ruffed lemurs to be 0.16 agonistic acts/hr, which is consistent with previously reported rates of 0.17 agonistic acts/hr (Morland, 1991) and 0.14 agonistic acts/hr (Cowl & Shultz, 2017). Compared to other primate taxa, lemurs sit on the lower side of the agonism spectrum, with 0.16 acts/hr, while old world monkeys and new world monkeys exhibit 0.58 acts/hr and 0.60 acts/hr, respectively (Sussman et al., 2005).

Ruffed lemurs are classified as exhibiting a dispersal-egalitarian competitive regime, because female philopatry does not regularly occur, femalefemale agonism is rare, and female dominance hierarchies are unstable (Erhart & Overdorff, 2008). I argue that agonism functions as a reproductive strategy in this Critically Endangered lemur species, made evident by its increase in mating/birthing years and decrease in non-mating/non-birthing years. Additionally, when averaging rates for each reproductive season, I observed the highest rates of agonism during the mating season (0.18 acts/hr), followed by lower rates during the birth season (0.14 acts/hr), and the lowest rates taking place during the non-breeding season (0.13 acts/hr). These results further indicate that reproductive season drives agonism in this seasonally breeding lemur species. Overall, Chapter 2 had a longer time horizon that enabled me to capture the variation of agonistic behaviour across numerous breeding seasons throughout a four-year span. Chapter 3 investigated agonism as well as affiliation at a finer-grain scale by examining individual rates of both throughout a single mating season.

In Chapter 3, I focused primarily on predictors of agonism and affiliation in males but included females to test the prediction that females would engage in higher rates of agonism as well as occupy higher dominance ranks than males. There were three key patterns of agonism and affiliation that emerged: 1) In keeping with the results from Chapter 2, agonism rates were higher during the mating season than outside of it, particularly in males. Additionally, grooming rates were significantly higher during the mating season than the post-mating season. 2) Male agonism rates were strongly associated with dominance rank. 3) In contrast to previous findings suggesting that females engage in higher rates of agonism, I observed higher rates (although not significantly higher) on average in males throughout the study period. However, females generally occupied the highest dominance rankings relative to males, which was expected in this female-dominated society. I argue that female-female directed agonism does not serve to establish female dominance hierarchies with varying rank-related benefits among females. Rather, as evidenced by the lack of association between female rank and agonism rate as well as the high proportion of agonism observed within female-male directed dyads (36% of bouts) compared to female-female dyads (9%) - I hypothesize that female-male directed agonism functions to drive female choice and female dominance in this species. In other words, all females hold comparably high ranks so agonism between them does not serve to gain additional benefits, but females directing agonism towards males likely functions to maintain dominance over them.

All in all, these preliminary results indicate some of the social behavioural changes that occur due to reproductive season, subgroup size, and rank, which demonstrate that it is possible to discern specific predictors of agonism and affiliation (and potentially other social behaviours of interest) using a fairly broad-scale approach. Examining group-wide agonism rates across a four-year period allowed me to identify the key factors driving agonism in ruffed lemurs. From this foundational knowledge, I narrowed my investigation to factors influencing individual rates of agonism during the mating season, which enabled me to validate previous results and discern more specific correlates of ruffed lemur sociality. Future studies with similar aims could combine these approaches in order to gain the benefits of both.

Limitations

I included subgroup size in order to account for the number of potential interactants in a group but recognize that individuals may experience more agonism in their subgroups even if rate per individual does not increase. Being unable to determine the direction of agonism (e.g., rank and sex of initiator and recipient) and whether it is uneven across individuals was a major limitation of this study. If it is uneven, then a low-ranking individual could experience more agonism in a larger group, even though more individuals are not engaging in more agonism than normal. Due to the broad approach and methodology applied in Chapter 2, it was not possible to inform on targeted agonism if the number of agonistic interactions did not change, just the recipient. Due to sexual monomorphism in this species, I was unable to definitively identify sex bias in actors and recipients of agonistic bouts when involving non-collared individuals. However, I was able to move beyond this by categorizing the social interactions involving only collared lemurs into sex-specific dyads. I found that roughly 36% of interactions involved NCLs but used collared-only interactions to provide support for the social behavioural patterns observed.

67

Future Directions

Future research should further investigate specific sex-based patterns of sociality. Although I was able to categorize dyadic interactions into sex-specific dyads to infer social relationships and provide some support for the social behavioural patterns indicated by my results, sample size of both agonistic and affiliative interactions remains a limitation, hindering definitive tests of hypotheses posed here. Studies interested in examining sex-based outcomes of agonism or affiliation should consider focusing on a single study site where more focal individuals are collared and identifiable. Alternatively, if it is possible to place nylon collars on study individuals, this would improve sample size while solving the issue of fitting expensive radio collars.

While my evaluation of agonistic behaviour encapsulated a wide range of behaviours, future studies should consider separating these behaviours into more descriptive categories, such as feeding versus non-feeding contexts and specifying whether the interaction is passive or active. By doing so, it would be possible to determine the intensity of the bout ("subtle displacements and submissions" vs. "overt aggression") and identify the context in which agonism occurs across short or long timescales in males, females, or both.

The same should be considered for answering more nuanced questions regarding affiliative behaviour. For example, whether individuals are passively huddling in close contact with one another versus actively grooming one another would provide another factor potentially useful in describing the patterns of ruffed lemur sociality. Additionally, my evaluation of affiliation was limited to one primary social affiliative behaviour, grooming. Although grooming behaviour alone is commonly used for calculating affiliation index scores, future studies should encapsulate more behaviours such as social play and huddling in order to better approximate rates of affiliation (Sussman et al., 2005).

Results from Chapter 2 found increased levels of agonism during the birth season. This finding was unexpected, as I predicted that communal breeding in ruffed lemurs would mitigate agonism during this period. While Chapter 3 specifically investigated social and seasonal predictors throughout the mating season, research examining this pattern during the birth season has yet to be conducted. Ruffed lemurs provide an excellent model to further examine the potential predictors of agonism during the birthing season in a primate species that exhibits cooperative rearing of offspring.

Lastly, hormonal profiles of individuals have been used to identify correlates of agonism and dominance rank in many primate species, including lemurs (Beehner et al., 2005; Brockman et al., 2001; Girard-Buttoz et al., 2015; Gould & Ziegler, 2007; Higham et al., 2013; Lynch et al., 2002; Muller & Wrangham, 2004; Ostner et al., 2002). As a necessity for priming males for reproduction, androgens mediate spermatogenesis and are known to positively influence male-typical behavior such as mounting, intromission, and agonistic interactions (Wallen, 2005). Previous studies have investigated the "challenge hypothesis", which predicts an increase in testosterone levels of dominant males to combat the challenges associated with the mating season (Wingfield et al., 1990). Furthermore, past research findings have confirmed a strong association between androgens and agonism among males of group-living primate species, particularly in the context of mating season when males actively challenge one another for access to mates (Dixson, 1980; Girard-Buttoz et al., 2015; Gould & Ziegler, 2007; Muller & Wrangham, 2004; Ostner et al., 2002). The challenge hypothesis is an evolutionary strategy that confers fitness benefits to dominant males exhibiting higher androgen levels (Goymann et al., 2007).

The results I present here provide support for two of the three major aspects of the challenge hypothesis: 1) Male rates of agonism increased during the mating season and were higher on average during the mating season compared to the post-mating season. 2) Dominant males exhibited higher rates of agonism than low-ranking males. Nonetheless, evidence of the third aspect the underlying physiological mechanisms - is needed to fully test the challenge hypothesis in black-and-white ruffed lemurs.

Although social interactions are relatively rare in lemurs compared to other primate species, their respective importance during reproductive seasons to exert dominance and female choice warrants further investigation. Black-andwhite ruffed lemurs provide irreplaceable ecological functions as seed dispersers and plant pollinators (Britt, 2000), and they are considered an indicator species, whereby protecting them promotes the persistence of sympatric flora and fauna (Baden et al., 2019). It would therefore behoove us to gain a firmer understanding of the social behaviours that may ultimately impact reproductive success in this species, which continues to face significant population decline (IUCN, 2020).

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APPENDIX A: PERMISSIONS

I, <u>Steig Johnson</u>, grant Li-Dunn Chen permission to use our co-authored work,
"Effects of reproductive season on agonism rates in black-and-white ruffed
lemurs (*Varecia variegata*)", as a chapter in his MA thesis, to be defended on July
17, 2020. This manuscript is in preparation to be submitted to the American
Journal of Primatology.

Signature (electronic)

Date: May 30, 2020

I, <u>Devin Marie Chen</u>, grant Li-Dunn Chen permission to use our co-authored work, "Effects of reproductive season on agonism rates in black-and-white ruffed lemurs (*Varecia variegata*)", as a chapter in his MA thesis, to be defended on July 17, 2020. This manuscript is in preparation to be submitted to the American Journal of Primatology.

Signature (electronic)

Date: May 30, 2020

I, <u>Sheila Holmes</u>, grant Li-Dunn Chen permission to use our co-authored work,
"Effects of reproductive season on agonism rates in black-and-white ruffed
lemurs (*Varecia variegata*)", as a chapter in his MA thesis, to be defended on July
17, 2020. This manuscript is in preparation to be submitted to the American
Journal of Primatology.

Signature (electronic)

Date: June 1, 2020

APPENDIX B: PROTOCOL FOR FECAL COLLECTION AND PROCESSING

Collection & Storage at Semi-Remote Field Station

A total of 63 samples were collected from the 8 focal males. Males who dispersed and could not be located via radio telemetry as well as males for whom samples were not successfully collected for both the mating and postmating season will be excluded from some analyses. Samples were collected opportunistically in the field and returned to the field site for immediate processing. Samples were prepared (dried and stored) for shipping and exportation prior to extraction following the method described below.

- Collect as much of the fecal as possible while wearing gloves and place it on shiny side of aluminum foil.
 - a. Wrap sample in foil and flatten the foil packet to increase surface area.
- 2. Mark tin foil using permanent marker on all sides with sample number, date, time, lemur ID, group, and M/F (for male or female).
 - a. Take GPS point at location of defecation (record elevation, accuracy, coordinates).
 - b. Record this info for each sample in data book.
- Place samples in cooler bag/container and bring back to field site (preferably within 4 hours).
- Place samples in Coleman[®] camping oven (while still in foil) in between 55-83° C, checking periodically to maintain heat level and dryness of packets.
 - a. Check for dryness, and also for mold. Remove any moldy sections.
- When totally dry (e.g., brittle), place each sample individually into a Whirl-pak[®] bag with a teaspoon of silica gel desiccant beads.
 - a. Place ~20 samples (more if possible) into a large gallon size
 Ziploc[®] bag with a few tablespoons of silica gel beads.
 - b. Label the Whirl-pak[®] with sample number.

- c. Label Ziploc[®] bag with range of sample numbers enclosed.
- Store samples in cabinet within locked room at field station headquarters.
 - a. Check samples daily to monitor moisture level of samples by observing color of silica beads (will change from orange to dark green if conditions are not adequately dry. Note: color change varies by brand and bead type).
- Transport samples to Antananarivo for shipping through Ariva Shipping & Logistics two weeks prior to departure from Madagascar.

APPENDIX C: HORMONE ANALYSIS

Hormone Analysis & Validation

Enzyme immunoassays were carried out at the University of Arizona's Laboratory for the Evolutionary Endocrinology of Primates (LEEP). The desiccated fecal samples were ground up, sifted in order to separate and remove plant matter present in feces using a mechanical flour sifter, weighed (0.092–0.108 g), and extracted using an alcohol-water extraction with 2.5 ml Nanopure[™] water and 2.5 ml of ethyl alcohol as has been described in previous lemur research (Zohdy et al., 2017). 1 ml of each sample from the alcohol-water extraction was combined with 4 ml ethyl acetate, vortexed for 8 minutes, and centrifuged for 3 minutes at 1000 rpm. The ethyl acetate layer was aspirated, evaporated, and resuspended in 1 ml of ethyl alcohol. The testosterone antibody used (AB156, acquired from Coralie Munro, University of California, Davis, CA, U.S.A.) crossreacts with DHT (92.4%), 4-androsten 3b,17b-diol (11.2%), dehydroandrosterone (5.44%), androstanediol (3.41%), androstenedione (2.12%), androsterone (0.51%), dehydroepiandrosterone (0.19%) and <0.1% with oestradiol, progesterone, desoxycorticosterone, desoxycorticosterone, 17a-hydroxylprogersterone, oestrone pregnenalone, cholesterol, hydrocortisone and cortisone.

Aliquoted samples of 50 ul were diluted with 300 ul of a testosteronehorseradish peroxidase (HRP) conjugate solution and 100 ul were plated in duplicate on a 96-well plate. Assays were run using the program, Gen5, on a Biotek Epoch plate reader. Spike-recovery tests were used to test the accuracy (described here: Brown et al., 2004) of the assay for *V. variegata* fecal extracts, which were found to be outside of the acceptable parameter of >90% recovery. Across 3 assays to validate for accuracy, my results varied greatly (range = 70-127%) and were not in line with the percentages typically reported in the literature (i.e., 100% +/- ~10%). Parallelism was determined by conducting serial dilutions on a high-valued sample, which was parallel to the standard curve for testosterone (F[11,12] = 3.12, p = 0.105). Samples were run in duplicate for each assay to determine mean intra- and interassay coefficients of variation (CV). Further validation with a more stringent protocol are required to move forward with assays for the remainder of the samples. In the near future, sample extracts will be loaded onto Solid Phase Extraction (SPE) cartridges in order to purify the sample further and pass the accuracy phase of the validation process. Once acceptable spike-recovery is attained (i.e., accuracy near 100%), assays will be conducted to determine the androgen concentration for each sample, and samples will be compared within and between males throughout the mating and post-mating seasons (mating season, n = 39; post-mating season, n = 24).