Using Fecal Hormones to Identify Reproductive State and Female Mate Choice in Wild Vervet Monkeys (*Chlorocebus pygerythrus*)

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Abstract

This study aimed to assess the feasibility of using fecal hormones to identify female reproductive state in wild vervet monkeys (*Chlorocebus pygerythrus*) in order to gain insight on changes in female mate choice. We followed a single study group with 16 adult/subadult females at Lake Nabugabo, Uganda over a 5-month field season in 2022. During this time, 248 fecal samples and 309 hours of behavioral observations were collected and used in analyses. Mean gestation length from hormonal data was 164.33 ± 18.09 days (range 134 – 187 days) based on six females that conceived during the study. However, gaps in fecal sample collection highlight that, while feasible, greater sampling effort is needed in future studies using fecal hormones. Generally, behavioral results indicate females displayed more proceptive and receptive behaviors towards males while ovulatory and appeared to prefer high-ranking males. However, there was no effect of female dominance rank on mate choice.

Keywords: female mate choice, fecal hormones, reproductive behavior, concealed ovulation, primates

Dedication

This thesis is dedicated to my fiancé, Daniel, who has been endlessly supportive of me throughout this process. None of this would have been possible for me without him by my side. I also dedicate this thesis to my family who has supported me from afar, including my Pawpaw and my dog, Gypsy, who both left this earth before I could return home. This work is also dedicated to the vervet monkeys at Nabugabo who let me witness a piece of their lives while I collected data for my thesis. Lastly, I dedicate my thesis to my rabbit, Wyn, who inspires me with his strength and resilience in spite of his smallness.

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Table of Contents

List of Tables

List of Figures

1 Introduction

1.1 Background

Early studies on sexual selection focused primarily on the role of males and did not explore the concept of female choice until Trivers (1972) highlighted the lack of research in this area. In the same paper, Trivers also introduced the idea that when intersexual conflict occurs, it is driven by dissimilarities in parental investment. With this idea, the focus shifted from males' role in sexual selection to the contributions of both sexes. Intersexual conflict may manifest via male sexual coercion of females, where males attempt to increase their chances of mating with a female, and/or deter her from mating with other males through aggression (Smuts and Smuts, 1993). It would be expected that male aggression towards females would increase during reproductive encounters and impose considerable costs to females, including injury, disruption of activity budget, inability to practice mate choice, and fertilization from undesirable males (Palombit, 2014). Another manifestation of sexual coercion is the potential occurrence of infanticide, which not only reduces the reproductive success of competitor males but also the females themselves (Hrdy, 1979).

The pressures associated with male coercion should elicit adaptations from females to mitigate these costs (Palombit, 2014). One adaptation often seen in female primates is prolonged receptivity (Hrdy, 1979; Palombit, 2012). Females that mate outside of their fertility window may mate with multiple males in order to confuse paternity (Hrdy, 1979). Confusing paternity is beneficial to females, as males are less likely to kill the infants of females they have mated, as well as provide protection to their potential offspring and their mothers (Hrdy, 1979).

Some primate species have also evolved concealed ovulation, where females lack visual cues indicating their reproductive status, which is likely an adaptation that also functions in part to increase paternity confusion and reduce the costs of male coercion (Hausfater and Hrdy, 1984). Assuming true concealed ovulation, males may consider each copulation as less valuable, as any one female is equally as likely to be fertile, thus reducing the need for male competition (Hrdy, 1979). Due to the potential decrease in female attractivity with concealed ovulation, males may have less incentive to monopolize females, thus reducing the risk of harassment. Heistermann *et al*., (2001) demonstrated this effect of concealed ovulation in Hanuman langurs (*Semnopithecus entellus*), where males put little effort into monopolizing females. When paired with prolonged receptivity, concealed ovulation allows females to gain protection from multiple males for themselves and their young, as each male a female copulates with has equal reason to believe he fathered her offspring. Another benefit of concealed ovulation paired with prolonged receptivity may be that it facilitates the expression of female mate choice (Reeder, 2003; Keil and Sachser, 1998).

1.2 Female Mate Choice

When studying sexual behavior, three aspects must be accounted for: attractivity, proceptivity, and receptivity. Perhaps the most obvious is attractivity, described by Beach (1976) as the stimulus value offered by an individual, particularly visual and olfactory stimuli. Attractivity may be considered as cues given by one sex that alerts the other sex to their reproductive status and value. This may be assessed through behavioral and nonbehavioral stimuli, such as secondary sexual characteristics (Kodric-Brown and Brown, 1984) including sexual swellings (Pagel 1993). Attractivity of an individual may be measured by the rate of proceptive behaviors (i.e., behaviors aimed to indicate willingness to copulate) received from the opposite sex (Beach, 1976). Proceptivity indicates an individual’s interest in mating and may be demonstrated by a female presenting to a male to initiate copulation, by maintaining proximity, or grooming a male. Where proceptive behaviors communicate interest in copulation, receptivity describes an individual’s willingness to accept the sexual encounter (Beach, 1976). Receptivity may be seen not only as accepting sexual advances from another individual, but more specifically as the cooperative effort in proceeding with the sexual encounter. For example, in giant pandas (*Ailuropoda melanoleuca*), female receptivity is indicated by the female’s tail being raised when mounted by a male (Kleiman, 1983). In primates, receptivity may also be observed as not only the female’s acceptance of a male’s copulation attempt, but also by her changing her position to make copulation easier for the male (Beach, 1976).

In primate species that display prolonged receptivity and concealed ovulation, female choice may be more dynamic than primarily selecting for paternity. In a computational model built to assess evolutionary patterns of female receptivity, Rooker and Gavrilets (2020) demonstrated that females should be more receptive outside of their fertility period if non-genetic benefits from males (e.g. protection, food, paternity confusion) are more important to females than genetic benefits. In such species, female choice may change throughout the cycle to prioritize males with more non-genetic benefits outside of ovulation and prioritize more genetically advantageous males when they are able to conceive. Carnegie *et al*. (2006) found that in white-faced capuchins (*Cebus capucinus*), females were more likely to mate with dominant males when they were fertile but mated almost exclusively with subordinate males after they became pregnant.

Changes in proceptivity and receptivity throughout the ovarian cycle have been recorded across multiple primate species (Dixon, 1990). Females have been found to follow a general trend of increased proceptive and receptive behaviors as estrogen peaks during the peri-ovulatory period (POP), and a decrease in these behaviors as progesterone increases in the luteal phase (*M.*

mulatta, Wallen *et al*., 1984; *C. apella*, Carosi *et al*., 1999). In a lab-based study relating hormonal and behavioral changes in female rhesus macaques, Wallen *et al*., (1984) found that females more frequently approached and maintained proximity to males in the days prior to their estradiol peak. Mating presentations and copulations also occurred more frequently in the two days before ovulation when estradiol and progesterone peaked and continued for two days after. During the luteal phase when progesterone rose and estradiol levels declined, copulations were not recorded and females threated males more frequently (Wallen *et al*., 1984). In tufted capuchins (*C. apella*), which appear to lack any external ovulatory cues, similar patterns have been recorded by Carosi *et al*. (1999). When hormone levels reached the POP, female tufted capuchins displayed more species-specific proceptive behaviors such as head cocking and raising eyebrows while vocalizing, as well as copulations, and a steep decline in proceptivity and receptivity with the onset of the luteal phase (Carosi *et al*., 1999; Carnegie *et al.,* 2005; 2006). In vervet monkeys, Rapkin *et al*. (1995) found that dominant females were generally more social during the follicular phase of their cycle but became aggressive during the luteal phase. Rapkin *et al.* (1995) also found that the subordinate females' behavior was influenced by the dominant female's cycle and subsequent behavior. Andelman (1987) found that male vervets did attempt to copulate more frequently when females were able to conceive; however, males also continued copulation attempts throughout the females' cycle and even into pregnancy. Females showed no change in receptivity throughout their cycle and may be equally as likely to accept copulation attempts regardless of reproductive status (Andelman, 1987). While Andelman's study gives insight into the receptivity of female vervets, little research has been done on proceptivity in vervets. Proceptivity in vervets is rather discrete and may only be apparent to observers through females maintaining proximity to or grooming males of interest, and possibly through mating presentations to males in which females crouch in front of males and expose their perianal area (Gartlan, 1969).

Female proceptivity and receptivity can be used as indicators of female mate preference. Females should prefer males with “good genes” to assure high-quality offspring, meaning a male’s attractivity should act as a signal to females of their genetic quality. As previously mentioned, secondary sexual characteristics can act as an indicator of mate quality. Early studies in primates found that these exaggerated characteristics do not correlate with female choice (Keddy-Hector, 1992). However, more recent studies have shown that there may be some female preference for male secondary sexual characteristics in primates. Throughout the primate order, in both sexes, coloration of facial skin and/or genitalia changes based on sexual status or social ranking (Higham and Winters, 2016). Snyder *et al*. (submitted) found that in vervet monkeys (*Chlorocebus pygerythrus*), females tend to prefer males with deeper red penis color. Bergman *et al.* (2009) showed that in geladas (*Theropithecus gelada*) males’ chest patches become increasingly redder with higher dominance rank, both of which are preferred by females.

Female preference for visual indicators of dominance leads to the question of why females should choose dominant males. Studies in some primate species have supported this idea (i.e., green monkeys *C. sabaeus,* Keddy 1986). Males of higher social status have greater access to higher quality food than subordinate males (i.e., priority-of-access model, Altmann, 1962; Guo et al., 2020; Watts and Mitani, 2000). When reviewing food sharing across primate species, Jaeggi and Van Schaik (2011) found that males in multi-male groups were more likely to share food with females than males in single-male groups because of greater intra-male competition in multimale groups; the authors hypothesized that food sharing served to influence future female mate choice. In chimpanzees (*Pan troglodytes*), females received high-quality food from males they copulate with, although this was noted to not necessarily correlate with ovulation (Watts and Mitani, 2000). Age also tends to be positively correlated with rank and can also give insight into genetic quality from the male's ability to survive longer (Small, 1989). Males' ability to provide parental care may also be assessed by females, however, it is less likely in species where parental care falls entirely on the females, as is the case in most primate species (Small, 1989). Small (1989) also suggested novelty (i.e., newly immigrated individuals) as an attractive trait in males, which has been demonstrated in Rhesus macaques (*Macaca mulatta*) (Bercovitch, 1997) and Japanese macaques (*M. fuscata*) (Lindburg, 1969). Novelty as a favorable trait is typically attributed to reducing the risks of inbreeding but also for females to gain protection for their offspring by giving novel males reason to believe they may have sired the female's future offspring (Paul, 2002; Hausfater and Blaffer Hrdy, 1984).

1.3 Hormones

While concealed ovulation serves to confuse males' ability to determine a female's likelihood to conceive, it also makes it difficult for researchers to determine the female's reproductive state and to study female choice. To overcome this challenge, hormonal analyses are required to determine female reproductive status. In field studies of wild primates, collecting blood is rarely feasible and opens the risk of injury to the animals and possibility of disease transmission between human and non-human primates. While hormones from serum give a more timely hormone level than delayed hormone levels in urine or feces, it requires more invasive methods of collection.

Hormones can also be successfully extracted from excreta (urine and feces) (Shideler *et al*., 1993), and there can be collected non-invasively from wild animals, their use to examine hormone metabolites has increased in the last few decades. In macaques (*M. fascicularis*) a brief lag in hormonal output in excreta has been observed at 0-2 days for urine, and 0-3 days for feces, with levels comparable to serum (Shideler *et al*., 1993). Zeigler *et al.* (1996), also noted that the delay in excretion from fecal hormones can be accounted for if known for the specific species and will still allow for accurate determination of reproductive states. The use of hormones from excreta presents a much more feasible method for field-based studies than invasive blood sampling. When studying female reproduction in vervet monkeys, blood has most commonly been used in lab-based studies (Seier *et al*., 2000; Hess *et al*., 1979). Urine has also been used in lab studies (Setchell *et al*., 1980), which was determined to be a viable field collection method by Andelman *et al*. (1985; see also Andelman, 1987).

To date, feces have not been used to quantify estradiol (E2) and progesterone (P) levels in vervet monkeys (*Chlorocebus pygerythrus*). While urine likely has a lower potential lag-time (as shown in other species), it may not be the most effective method of collection since urine samples must be collected immediately in a wild setting or risk being lost into soil, whereas feces allow for more collection time.

1.4 Vervet monkeys

Vervet monkeys are an interesting study species for female choice. They display concealed ovulation which, as previously discussed, has the potential to divert males' ability to monopolize fertile females and facilitate females' ability to practice mate choice (Andelman, 1987). Vervets have also been reported to display female co-dominance at some study sites, where the male and female hierarchies are intertwined (Young *et al.*, 2017; Hemelrijk *et al*., 2003). In lab studies on the closely related green monkey (*Chlorocebus sabaeus*), Keddy (1986) found that being co-dominant may grant females the potential to reject male mating attempts, and further allows their ability to practice mate choice (Keddy, 1986). Andelman (1987) found that higher ranked males copulated more with females throughout their cycle, however, males of all ranks were equally likely to copulate with a female while she was ovulating. Fane (2023) found that female vervets preferred dominant males and were more likely to retaliate against advances from subordinate males. However, over seven years of data collection on three vervet groups at Amboseli, Cheney *et al*. (1988) noted that in some years male dominance was positively correlated with mating success but negatively correlated in other years. Snyder *et al.* (submitted) also reported that higher ranking males received more mating refusals than lower ranking males in the vervets at Lake Nabugabo, Uganda. Females' preference for males based on ages has also been shown in vervets, where older males have been observed to copulate more than younger males (Snyder *et* al., submitted), although previous studies on vervets have not been able to show this correlation (i.e., Andelman 1987; Cheney *et al*., 1988). While it has not been shown directly, it has been hypothesized that vervet females will also prefer novel males given that male transfer occurs frequently (Cheney and Seyfarth, 1983), though the data available to date from the Amboseli population in Kenya does not support this hypothesis (Cheney *et al.,* 1988).

1.5 Objectives

The goals of this thesis were to:

1) Assess the feasibility of collecting fecal samples from female vervets to gain insight on their ovulatory cycling,
2) Examine how well conception times were correlated between fecal hormone samples and estimated conceptions from demographic data and gestation length, and
3) Test the predictions that:
    a. females will show proceptive behaviors towards males during the peri-ovulatory period (POP),

b. females will display more proceptive behaviors towards high-ranking males than to low-ranking males during the POP.

2.0 Methods

2.1 Study Site and Animals

This study focused on one group of vervet monkeys (*Chlorocebus pygerythus*) located on the shores of Lake Nabugabo, Uganda (0°22′−12°S and 31°54′E) that has been habituated since 2012. The range of the study group included farmland, forest patches, human settlements and swamps (Chapman *et al*., 2016). Although observed to copulate year-round, the Nabugabo vervets primarily give birth from October-December, putting peak conception in May-July (L'Allier *et al*., 2022; Schwegel *et al*., 2023). To capture peak conceptions, data collection was carried out from April 6th 2022 through August 23rd 2022. At the time of data collection, the group consisted of 44 individuals including 5 adult males, 3 sub-adult males, 12 adult females, 4 sub-adult females as well as 20 juveniles and infants. Females were classified as sub-adult when reaching sexual maturity based on behavioral observations, typically after three years of age (Turner *et al*., 1997). Females were considered adult after giving birth to their first infant or confirmed miscarriage (L'Allier *et al*., 2022). Males were classified as sub-adult based on genital development and emergence of canines at around four years of age and were considered adults upon successful emigration from their natal group (L'Allier *et al.*, 2022).

2.2 Hormonal Data Collection & Analyses

Fecal samples were collected opportunistically from study females throughout the day, as changes in excretion of progesterone (P) and estradiol (E2) have not been found to be influenced by time of day (Strier and Ziegler, 1994). After seeing a female defecate, feces were collected into plastic tubes that were then labeled with the name and group of the individual, date and time of collection, and held in a portable cooler stored in a freezer by the end of the day. Preliminary hormone extraction was done at the field site by thawing the fecal samples at room temperature, homogenizing them, and mixing 0.5g of feces with 10mL of a 1:1 ethanol and distilled water solution (L'Allier *et al.,* 2022). Once in the ethanol-water solution, the samples were vortexed for 10 minutes and centrifuged for 20 minutes to extract the hormones from the fecal pellets. A 2mL portion of the hormone extract supernatant was then passed through S-Plus Maxi-Clean C-18 Solid Phase Extraction cartridges, and rinsed with 2mL of distilled water to remove any impurities. The cartridges were then sealed with luer slip caps and stored away from light until transported to the Primate Behavioural Endocrinology Lab (PBEL) at York University's Glendon Campus, Canada.

Hormones were eluted in the PBEL by rinsing with 1mL of 5% methanol then eluted with 2mL of 100% methanol and collected into glass vials with an Alltech vacuum manifold. The eluted hormone-methanol extracts were then stored at -20 °C. Progesterone assays were done using Arbor Assay progesterone enzyme immunoassay (EIA) kits (K025-H1/H5). A 9-point serial dilution of pooled samples was completed to identify the optimal hormone-methanol aliquot amount to use, and to test for parallelism with the standard curve. A 30uL aliquot of hormone-methanol sample extract, as well as high and low pooled samples, were dried down in a fumehood overnight to allow for methanol to evaporate. Once samples were dry, 150uL of assay buffer was added to dilution tubes containing samples for a 1:5 dilution of the hormone-methanol extract. To prepare standards, 125uL of 1:2 dilution progesterone standard stock was mixed with 125uL assay buffer, and was serially diluted to create an 8-point serial dilution. Dilution concentrations started at 16,000pg/mL undiluted standard stock, and were halved for each subsequent dilution: e.g. 8000pg/mL, 4000pg/mL, and so on until reaching the lowest concentration at 62.5pg/mL standard stock to assay buffer. Progesterone analyses were done in 96-well microtiter plates by adding 50uL of samples, pools, and standards in duplicate rows. 25uL progesterone conjugate and 25uL of progesterone antibody were added to sample and standard wells and mixed on a plate shaker for 2 hours at 700rpm. Once done, the plate was washed four times with 300uL of wash buffer and the excess was removed before adding 100uL of color-changing TMB substrate. The plate was left to incubate with the substrate for ~30 minutes at room temperature and color development was assessed with a BioTek spectrophotometer at 630nm. Once color development was sufficient, 50uL HCl was added to stop development, and the plate was read again at 450nm. All hormone values are averaged across duplicates. The inter-assay coefficient of variance (CV) for P was 14.9%, and the intra-assay CV was 9.1% (n = 248). There was no significant difference between the standard curve and a 5-point serial dilution (t = 0.11, df = 6, p = 0.91).

Estradiol analysis was carried out using 17β estradiol EIA kits (K030-H1/H5). Validation determined a 1:1 sample to assay buffer for assays. 150uL of hormone-methanol sample extracts and pools were aliquoted into dilution tubes and dried over two-three nights. Once completely dried, 150uL of assay buffer was added to dilution tubes to achieve the 1:1 dilution. The standard curve was created by mixing 450uL assay buffer with 50uL estradiol standard stock, and extracting 150uL of the standard dilution to be added to the remaining 7 standard tubes for an 8-point serial dilution. Dilution concentrations followed the same pattern as for P, starting with 16,000pg/mL undiluted standard stock which was subsequently halved until reaching the lowest concentration of 125pg/mL. The assays were carried out in 96-well microtiter plates in duplicate rows following the same protocol as progesterone assays. The inter-assay CV for E2 was 18.3, and the intra-assay CV was 11.7 (n = 188). There was no significant difference between the standard curve and serial dilution (t = 0.13, df = 8, p = 0.89).

To identify female reproductive states, I used two different methods. The "conceptive" method was calculated by subtracting the species-specific length of gestation for vervets (163 ± 3 days; Bramblett et al., 1975) from infant dates of birth to determine the likely day of ovulation and conception, and included the three days before and three days after this date to account for individual variation to generate a seven-day periovulatory period (POP) (Strier *et al.,* 2003; Van Belle *et al*., 2009a; Schoof *et al.,* 2014). Every day after the end of the POP up until parturition was considered pregnancy. The "hormonal" method was identified by identifying the peak in E2 associated with ovulation (Setchell *et al*., 1980) and included the three days before and after this date to account for error and variability and behavioral shifts in the periovulatory period. Pregnancy was considered to start the day after POP, which coincided with the rise in P as pregnancy progressed and drop in P with parturition (Setchell, 1980).

2.3 Behavioral Data Collection & Analyses

Continuous focal animal follows based on the methodology described by Altmann (1974) were carried out on all adult and sub-adult females of the study group. Observations were recorded for a 15-minute period per individual using a handheld Psion computer during which all behaviors were recorded following a pre-determined ethogram (Appendix A). Behaviors of interest (sociosexual, affiliative intersexual interactions, and agonistic interactions) displayed by non-focal females within view were recorded *ad libitum* to supplement the focal follows. Focal periods were considered unusable if the focal individual was out of view for ≥2 minutes of the 15-minute sample.

To determine the proportion of time females spent on individual behaviors, the amount of time (for proximity to and grooming of males) or counts (for copulations and mating presentations) for each male-female dyad the total amount of time (in hours) each female was observed was determined for each reproductive state was then divided by the total amount of time for each female by reproductive state to determine the behavioral rate. All statistical analyses were completed in R Statistical Software (v4.2.1 R Core Team, 2022).

For male characteristics preferred by females, age could not be used in analyses as of the five males in the study group at the time of data collection, three males were the same age (approximately six years), one was estimated to be eight years of age, and one was estimated to be 11 years of age, thus lacking variation. Male tenure length also lacked in variation as four of the five males had immigrated into the study group within the same four-month period of each other. Thus, only male ordinal ranks were included in the final analyses.

Male and female dominance hierarchies were determined by generating Elo scores for each sex by using the "EloRating" package in R (Neumann and Kulik, 2020) with the default *k*-value of 100. Female Elo scores were calculated using aggressive and submissive behaviors (e.g. chases, submissive vocalizations, avoids, supplants, bites, hits, and lunges) observed between adult and subadult females during the 15-minute focal follows and supplemented with scan data collected from 2012 onward by the field assistants and *ad libitum* observations, while male Elo scores were determined from scans and *ad libitum* observations. For all dyadic interactions, an agonistic bout was defined as any agonistic behaviors occurring between the same two dyad members within a 15-minute period. For each bout, a winner and loser were determined based on who directed and received agonistic behavior. In cases of bouts where both individuals directed and received aggression toward/from the other, the interaction was considered a draw. For simplicity of analysis, Elo ratings were then converted into ordinal ranks (where one represents the highest rank and 5 represents the lowest), as Levy *et al.* (2020) noted that when looking at a single study group with little to no change to group size (as was the case during summer 2022) ordinal and proportional ranking methods would generate the same results.

To determine which sex was mostly responsible for maintaining proximity, two separate indices were calculated for each male-female dyad. For Hinde's index (Hinde and Atkinson, 1970), the number of approaches (AP) directed by one member of the dyad (for this study, the female (f)) per the number of approaches directed by both individuals (here, female and male (m)) is subtracted from the number of leaves (LE) directed by the female per leaves directed by both individuals, which is calculated as:

$$\frac{AP_f}{AP_f + AP_m} - \frac{LE_f}{LE_f + LE_m}$$

The resulting values range from -1 to +1, where a positive number indicates the female was responsible for maintaining proximity, a negative number reflects the male being responsible for maintaining proximity, and values near zero indicate that both dyad members contributed equally to maintaining proximity. Originally developed to study changes in proximity between mother-infant dyads in rhesus macaques (*M. mulatta*), Hinde's index has been used frequently within primatology (white-faced capuchins *C. capucinus*, Carnegie *et al*., 2005, 2006; owl monkeys *Aotus nancymaae*, Wolovich *et al*., 2010; mysore slender loris *Loris lydekkerianus*, Nekaris, 2006; olive baboons *P. hamadryas anubis*, Lemasson *et al*., 2008; and black howler monkeys *Alouatta pigra,* Van Belle *et al*., 2009b) and in other animals (bottlenose dolphins *Tursiops truncates*, Reid *et al.,* 1995; *T. aduncus*, Galezo *et al*., 2018; and wild horses *Equus caballus* Cameron *et al*., 2000). However, Brown (2001) highlighted that Hinde's index does not always give a clear account of which member of the dyad was making the most approaches or leaves, and can give similar results for very different scenarios. For example, a zero may result from either individual making an equal number of leaves and approaches or from one individual making all of the leaves and the other making all of the approaches. Brown (2001) suggested a new index which ranges from 0 – 100 by dividing the total number of approaches and leaves made by one member of the dyad (again, here, the female) by the total number of approaches and leaves made by both members of the dyad, calculated as:

$$\frac{AP_f + LE_f}{AP_f + LE_f + AP_m + LE_m} * 100$$

For Brown's index, high values closer to 100 indicate that the female is primarily maintaining proximity and low values closer to zero indicate the male is responsible for maintaining proximity while values close to 50 (45 - 55) indicate both members of the dyad are maintaining proximity. To gain further insight into the dynamics of proximity between the dyads of this study, both indices were applied and compared.

I used Generalized Linear Mixed Models (GLMM) in R using the "glmmTMB" package (Brooks *et al*., 2017) to examine the relationship between three predictor variables (female reproductive state, female ordinal rank, and male ordinal rank) and four female behaviors of interest (i.e., time spent in proximity to males, grooming, presentations, and copulations). Separate models were run for each method of determining reproductive state (i.e., conceptive or hormonal). All behaviors of interest were found to have a large number of zeros. When approaching zero-inflated continuous data, or semicontinuous data, few options are available. The Tweedie distribution can be used for positive continuous zero-inflated data in cases where $1 < \xi < 2$ where ξ is the *Tweedie index parameter* (Dunn & Smyth, 2018). Dunn and Smyth (2018) presented a simple method for estimating ξ by plotting the logarithm of the data's variances against the means, which I attempted for each method of identifying reproductive state (i.e., conceptive and hormonal) and outcome variable (i.e., time in proximity and grooming) combination. While some variables fit the $1 < \xi < 2$ assumption (conceptive proximity, hormonal grooming), others did not (hormonal proximity, conceptive grooming). To avoid any potential inconsistency, other methods of approaching zero-inflation were considered. Another method for handling semicontinuous data, was proposed by Duan *et al*., (1983) and consists of a two-part model which first uses a binary model to determine the likelihood of the occurrence of zero or positive non-zero values, and secondly models the non-zero occurrences using the model most appropriate for the data. When compared to other methods for handling semicontinuous data, Min & Agresti (2002) noted that the Duan *et al*., (1983) two-part model is more flexible in application than other models that assume an underlying normal distribution for the data. Time in proximity and grooming were modeled using the zero-inflated gamma distribution, which utilizes Duan *et al*.'s (1983) two-part method.

Zero-inflated count data also present a challenge. Leaving excess zero's unaccounted for by applying a regular Poisson regression leads to unreliable results (Harrison, 2014). As zero-inflation also creates overdispersion, a common method is to use negative binomial models; however, this distribution is unreliable in extreme cases of zero-inflation (Harrison, 2014; Lambert, 1992). Lambert (1992) presented specific adjustments that could be applied to Poisson or negative binomial models dealing with zero-inflation by applying a point mass at zero. I chose to use a zero-inflated Poisson model, which best fit the data, with an offset of the total amount of time each female had been observed in each reproductive state for that determination method to analyze the number of presentations and copulations observed. All models (zero-inflated Gamma or Poisson) were also log linked, and resulting coefficients were converted back into their exponential form.

Grooming rate could not be analyzed using GLMMs because the high zero-inflation could not be dealt with through statistical means. This also was the case for copulation data from hormonally determined reproductive states. To address these, I used Kruskal-Wallis to examine how grooming frequency varied by reproductive state, female ordinal rank, and male ordinal rank. Kruskal-Wallis results that were significant were further investigated with Dunn's test to determine which levels were significantly different from the others.

3.0 Results

Of the 16 females in the study group, four were omitted from data analyses because they did not conceive during the study period. Furthermore, insufficient fecal samples were collected from these four females to create a hormonal profile that could have been reliably used to identify possible conception or miscarriage. Data from the 12 remaining females were used for analyses as they had more fecal samples collected, and all had live births. Five of these females likely conceived prior to the study period, with the remaining seven conceiving during the study period. Of the seven females who conceived during the study period, insufficient fecal samples were collected to create a hormonal profile for female PLU; thus, her reproductive states were determined solely using the "conceptive" method.

3.1 Hormone Results

In total, 262 fecal samples were collected from all 16 females, 248 of which were from the 12 females included in the analyses. All 248 samples were analyzed for progesterone (P). Mean (herein reported as ± SD) P level was 65.71ng/g ± 37.51ng/g (range 6.13 – 286.35 ng/g, N

= 248). Once the P profiles for each female were completed, the samples from the six females (LNA, LTA, LTC, MLW, TRB, and TVL) whose conceptive period were most likely to have been captured in the data were then selected for estradiol (E2) analysis, as well as two of the already pregnant females (MCU and RAY). Mean E2 level was 21.15ng/g ± 25.25ng/g (range 1.27 – 103.64ng/g, N = 188).

Of females with complete hormone profiles (example shown in figure 1) for both P and E2, during POP (N = 6 females; 14 samples) the mean P level was 65.71ng/g ± 37.51 (range 31.27 – 148.82ng/g), and mean E2 level was 21.15 ± 25.25 (range 4.73 – 103.64ng/g). During pregnancy (N = 8 females; 14 samples) mean P level was 87.24ng/g ± 25.25ng/g (range 9.24 – 286.25ng/g), and mean E2 level was 15.37ng/g ± 13.05 (range 2.74 – 103.64ng/g). Mean gestation length based on hormonal data was 164.33 days ± 18.09 days (range 134 – 187 days).

Out of the six females, only one (TVL) had an overlap between hormonal POP and conceptive POP by one day. Differences in hormonal date of ovulation and conceptive date of ovulation ranged from 6 – 29 days (Table 1), with a mean difference of 16.33 ± 7.89 (N = 6 females). POP determined by hormones did not overlap for any of the six females; however, based on the conceptive method four (TVL, LNA, PLU, and MLW) out of seven females had overlapping POP's (Table 1).

Table 1 POP and parturition dates for all females POP could be determined for using either the conceptive or hormonal method.

Female	POP conceptive	POP Start (-3)	POP end (+3)	POP hormonal	POP start (-3)	POP end (+3)	Difference between methods (days)	Parturition
LTA	26-Apr-22	23-Apr-22	29-Apr-22	25-May-22	22-May-22	28-May-22	29	06-Oct-22
TVL	07-May-22	04-May-22	10-May-22	13-May-22	10-May-22	16-May-22	6	17-Oct-22
LNA	08-May-22	05-May-22	11-May-22	14-Apr-22	11-Apr-22	17-Apr-22	24	18-Oct-22
PLU	10-May-22	07-May-22	13-May-22	NA	NA	NA	NA	20-Oct-22
MLW	16-May-22	13-May-22	19-May-22	02-May-22	29-Apr-22	05-May-22	14	26-Oct-22
TRB	14-Jun-22	11-Jun-22	17-Jun-22	24-Jun-22	21-Jun-22	27-Jun-22	10	24-Nov-22
LTC	04-Aug-22	01-Aug-22	07-Aug-22	20-Jul-22	17-Jul-22	23-Jul-22	15	14-Jan-23

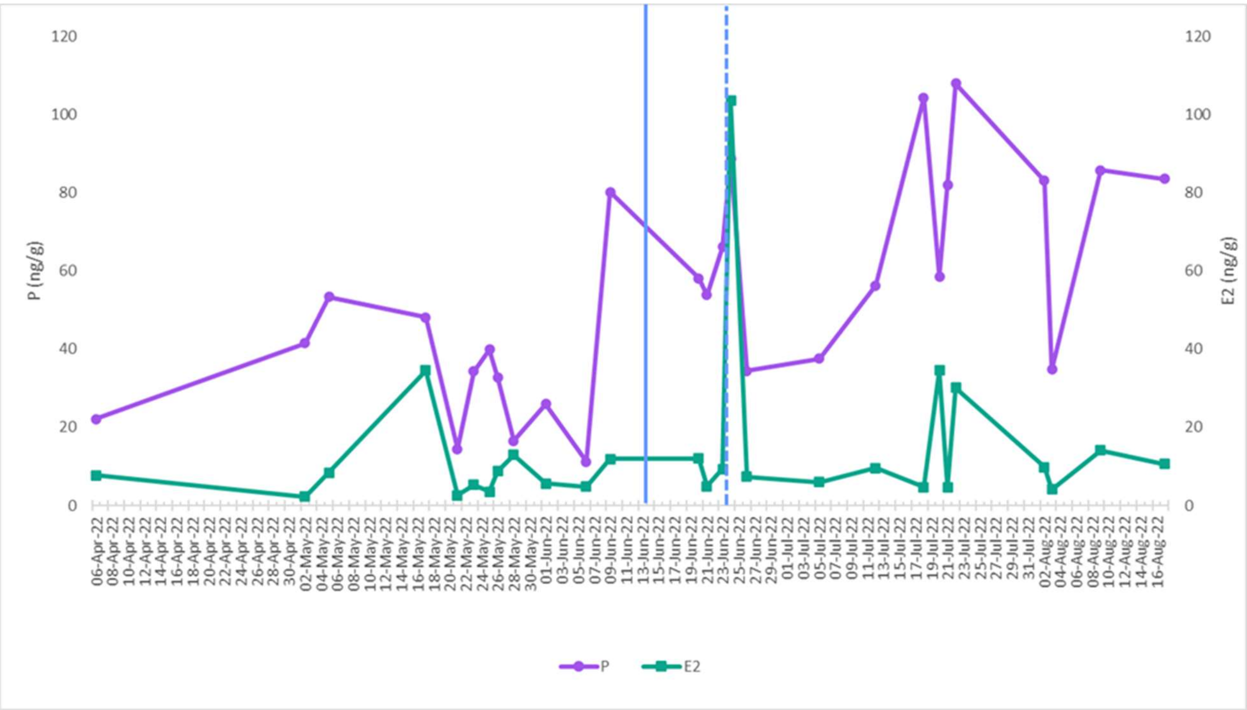

Figure 1 Graph of an individual female's (TRB) hormone profile over the study period. The solid vertical line indicates the conceptive date of ovulation, and the dashed line indicated the hormonal date of ovulation based on the spike in E2.

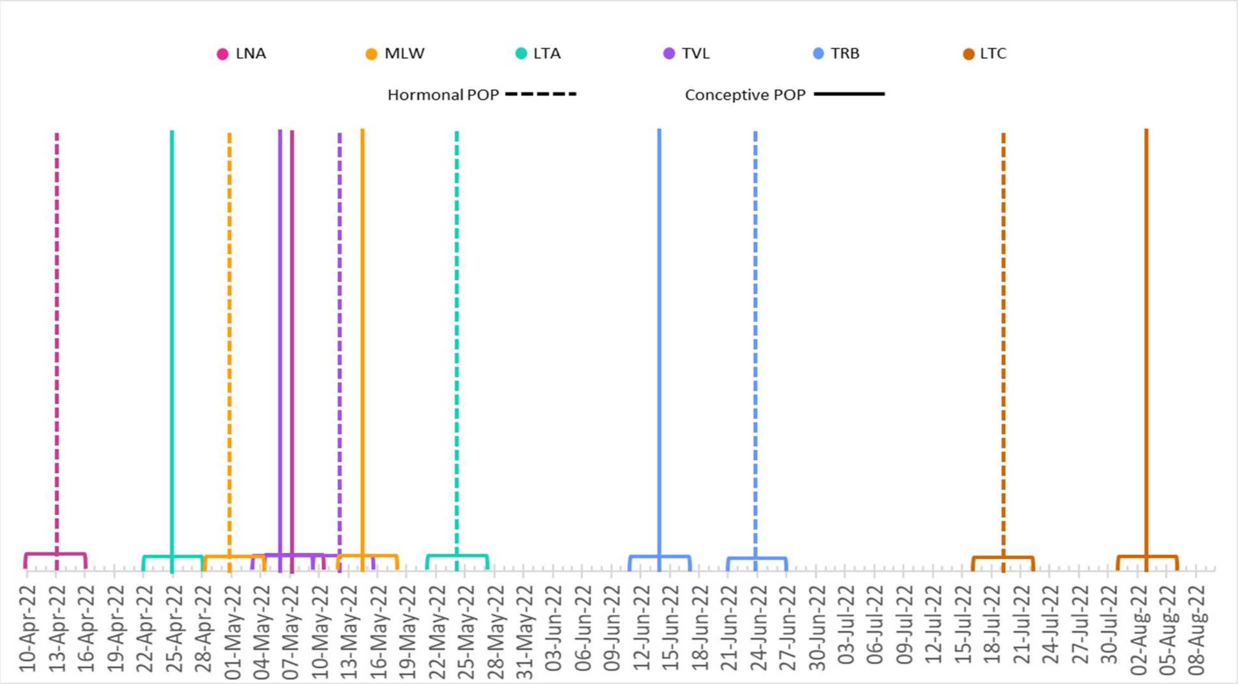

Figure 2. POP dates for all females for which both conceptive and hormonal POP could be identified.

3.2 Behavioral Results

In total 1745 focal follows (~436 hours) were collected from all 16 females, 878 of which were on the 12 females selected for analysis. A subset of 781 (~309 hours) focal samples were usable based on the amount of time the focal subject was out of view. One focal follow was completed per female per seven-day POP; the length of focal follows during the conceptive POP was 14m 39s ± 0m 43s (13m 03s – 15m 32s) per seven females; hormonal POP was 14m 52s ± 0m 33s (13m 19s – 15m 35s) per six females. Length of focal follows during conceptive pregnancy was 14m 53s ± 0m 35s (13m 01s – 18m 06s) per 12 females, and was 14m 52s ± 00m 31s (13m 06s – 17m 23s) per seven females for hormonal pregnancy.

3.3 Proximity

For amount of time spent in proximity to males relative to female reproductive state calculated from the conceptive method, GLMM results showed a positive relationship between male ordinal rank and time in proximity to females ($\beta = 0.769$, $p = 0.01$), indicating that females spent more time in proximity to high ranking rather than low rank males. There was a small but significant relationship between time spent in proximity to males based on female reproductive state ($\beta = 0.246$, $p < 0.001$), with females spending more time in proximity to males when they were in POP than when they were pregnant (Fig 3). There was no effect of female ordinal rank on time spent in proximity to males ($\beta = 0.96$, $p = 0.495$).

Results were qualitatively similar when examining reproductive state using the hormonal method, The GLMM indicates a significant difference for both male ordinal rank and female reproductive state (male ordinal rank: $\beta = 0.735$ $p = 0.006$; female state: $\beta = 0.429$, $p = 0.039$), again indicating females spent more time in proximity to high ranking rather than low ranking males, and spent more time in proximity to males during POP than when pregnant (Fig 3). Again, female ordinal rank did not show a significant difference in proximity to males ($\beta = 1.08$; $p = 0.354$).

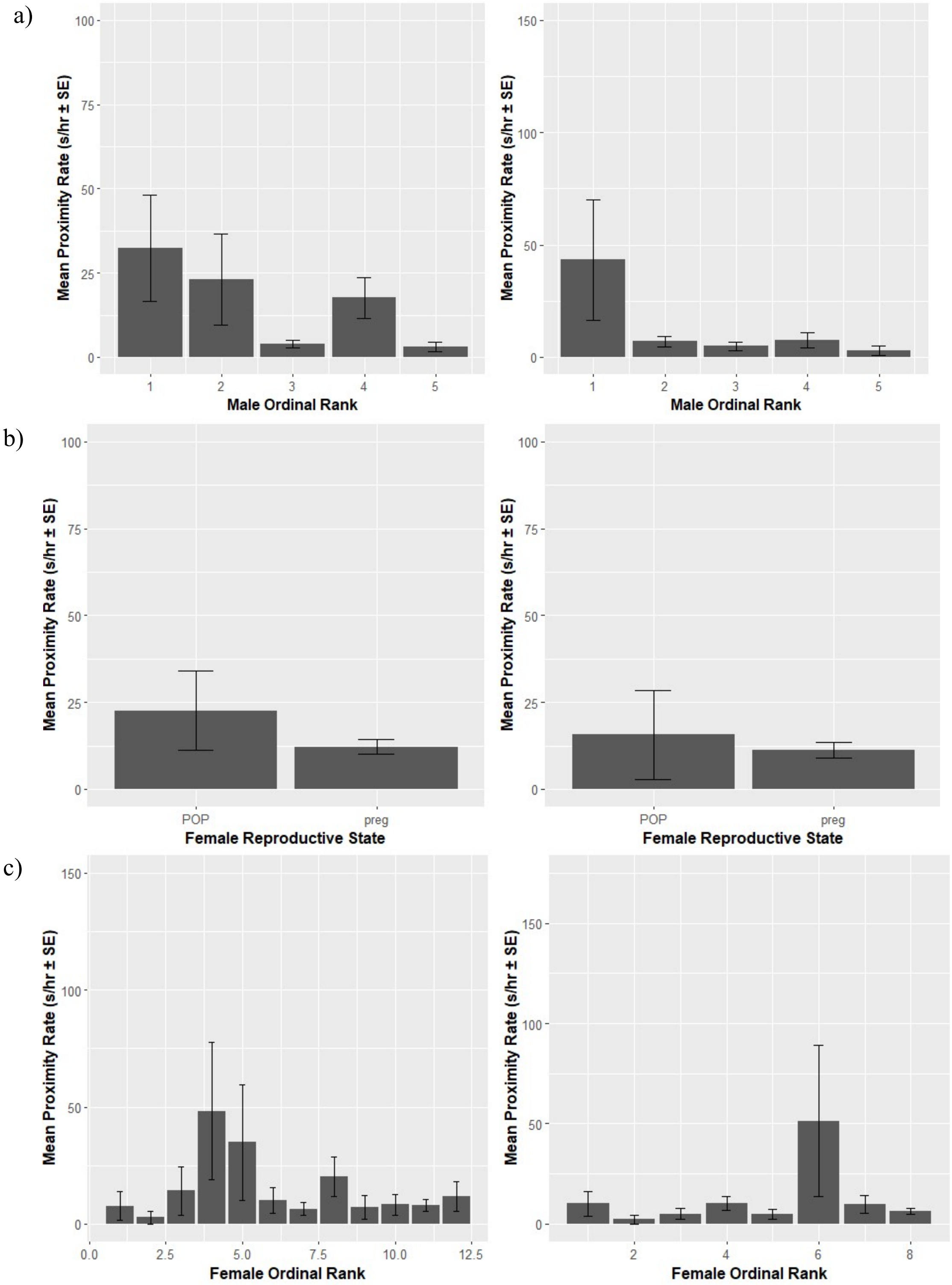

Figure 3a-c. Amount of time spent in proximity to males based on male ordinal rank (a), female reproductive state (b), and female ordinal rank (c) from date based on either the conceptive (left) or hormonal (right) method.

3.4 Proximity Maintenance

With a total of 12 females and five males, there were up to 60 possible dyads that could be examined for proximity maintenance (depending on reproductive state and determination method). However, in many cases, dyad members were not observed to approach or leave each other (especially during the brief 7-day POP) and therefore neither Hinde's nor Brown's index could be calculated. When examining POP based on the conceptive method, I had sufficient data to examine proximity for 11 of 35 possible dyads (7 females and 5 males; Table 2), three of which included the alpha male. Qualitative examination of Hinde's index indicates that proximity was mutually maintained (n = 2) or maintained by the alpha male (n = 1; i.e., negative Hinde's index). For the eight dyads containing a subordinate male, proximity was maintained by the female for two of the dyads, mutually maintained by five of the dyads, and maintained by the male for one dyad (Table 2). Unlike the Hinde's results, one of the three alpha male-female dyads had a Brown's index close to 100 (Table 2), indicating the female was responsible for maintaining proximity, and two dyads with indices closer to 0, indicating the male was responsible. Of the eight dyads including subordinate males, three of the dyads had Brown's indices close to 100 indicating the female was primarily responsible, three dyads had mutually maintained proximity, and two were close to 0 indicating the male being responsible. While slightly different, both indices indicate the alpha male making a greater effort to maintain proximity to females who were ovulating, while the effort in maintaining proximity between females and subordinate males was mutual between sexes.

For POP based on the hormonal method, I could only calculate Hinde's index for eight of the 30 possible dyads (6 females and 5 males). One of the three dyads including the alpha male had a positive Hinde's index indicating female maintained proximity, and two had mutually maintained proximity. Of the five subordinate male-female dyads four were neutral and one had a negative Hinde's index indicating male maintained proximity. One of the three alpha male-female dyads had a Brown's index close to 100 indicating it was female maintained, while the other two were close to 0, indicating it was maintained by the alpha male. Of the five dyads including a subordinate male, two had a Brown's index close to 100 (female maintained) and three were close to 0 (male maintained). Unlike Hinde's index calculated from POP based on the conceptive method, females were more mutually involved in maintaining proximity with the alpha male, and there was mutual responsibility for females and subordinate males. However, similar to the results of POP based on the conceptive method, Brown's index calculated from the hormonal method indicated more responsibility for the alpha male in maintaining proximity to females and more effort from females in maintaining proximity to subordinate males.

During pregnancy based on the conceptive method, I was able to calculate Hinde's and Brown's index for 49 of 60 possible dyads (12 females and 5 males), 11 of which included the alpha male. Three of these 11 had a positive Hinde's index indicating female maintained proximity, while eight had a negative index indicating male maintained proximity (Table 2). Of the 38 dyads including a subordinate male, eight had a positive Hinde's index indicating proximity was maintained by the female, 11 were near 0 indicating proximity was mutually maintained, and 19 had a negative index indicating male maintained proximity. For Brown's index, two of the 11 dyads including the alpha male were mutually maintained, and nine were close to 0 indicating male maintained proximity. For the 38 dyads including a subordinate male, 14 had a Brown's index close to 100 indicating female maintained proximity, 11 were mutually maintained, and 13 were close to 0 indicating male maintained proximity. The two indices yield different results for dyads containing alpha males, with Hinde's index generally indicated mutually maintained proximity and Brown’s index indicated male maintained proximity. However, both indices indicated more effort from subordinate males in maintaining proximity to pregnant females.

During pregnancy based on the hormonal method, 31 of the 40 possible dyads (8 females and 5 males) were calculated for Hinde’s and Brown’s indices, of which seven included the alpha male. Only one of the seven alpha male-female dyads was mutual, and six were negative indicating male maintained proximity. For the 24 dyads including a subordinate male, four had a positive Hinde’s value indicating female maintained proximity, seven were mutual, and 13 were negative indicating male maintained proximity. None of the seven alpha male-female dyads had a Brown’s index close to 100 (female maintained), one was mutual, and six were close to 0 indicating male maintained proximity. Of the 24 dyads including subordinate males, seven had Brown’s indices close to 100 indicating female maintained, 10 were mutual, and seven were close to 0 (male maintained). Similar to results for pregnancy based on the conceptive method, Hinde’s index generally indicated mutual maintenance of proximity in alpha male-female dyads, and male maintained proximity in subordinate male-female dyads. As with pregnancy based on the conceptive method, Brown’s index indicates that both the alpha and subordinate males had more responsibility in maintaining proximity to females.

Table 1. Comparison of Hinde's and Brown's indices for each dyad by female reproductive state separated by method of determining state (conceptive or hormonal). Note NA indicates instances where no approaches or leaves were observed between the given dyad. For Hinde's index a negative index indicates the male maintained proximity, and a positive index indicates female maintained proximity. For Brown’s index an index near or equal to zero indicates male maintained proximity and an index near or equal to 100 indicates female maintained proximity. Indices with a * indicate the dyad included the alpha male during the time of the female’s reproductive state.

Individual ID		**POP (conceptive)**		**POP (hormonal)**		**Pregnant (conceptive)**		**Pregnant (hormonal)**	
Female	**Male**	**Hinde's Index**	**Brown's Index**	**Hinde's Index**	**Brown's Index**	**Hinde's Index**	**Brown's Index**	**Hinde's Index**	**Brown's Index**
DMK	BTR	Conceived prior to study period				0.33	63.33	Hormones not available	
	TRO					-0.38*	18.75*		
	LRK					0	0		
	CHL					0	0		
	WTN					-0.5	25		
LNA	BTR	NA	NA	-0.5	75	-0.17*	42.86*	-0.17*	42.86*
	TRO	NA*	NA*	0*	0*	NA	NA	-1	50
	LRK	0	50	NA	NA	0	0	0	50
	CHL	0	100	NA	NA	0	100	0	100
	WTN	NA	NA	NA	NA	1	50	1	50
LTA	BTR	0	100	0*	100*	-0.085*	34.78*	-0.16*	37.5*
	TRO	0*	0*	NA	NA	NA	NA	NA	NA
	LRK	NA	NA	NA	NA	-0.67	50	0	50
	CHL	NA	NA	0	0	-0.33	16.67	-1	50
	WTN	NA	NA	NA	NA	0	0	NA	NA
LTC	BTR	NA	NA	NA	NA	NA	NA	-0.5	25
	TRO	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
	LRK	0	50	NA	NA	NA	NA	NA	NA
	CHL	NA	NA	NA	NA	NA	NA	NA	NA
	WTN	NA	NA	NA	NA	NA	NA	NA	NA
MCR	BTR	Conceived prior to study period				0.5*	25*	Hormones not available	
	TRO					-0.078	26.32		
	LRK					-0.1	44.44		
	CHL					-0.25	66.67		
	WTN					0	50		
MCU	BTR	Conceived prior to study period				-0.14	21.43	0.026	32
	TRO					-0.28*	33.33*	-0.28*	33.33*
	LRK					-0.5	40	0.5	40
	CHL					-0.6	50	-0.6	50
	WTN					0	100	0	100
MLT	BTR	Conceived prior to study period				0.29	68.29	Hormones not available	
	TRO					-0.3*	33.33*		
	LRK					0.06	66.07		
	CHL					0	50		
	WTN					0	100		

MLW	BTR	-0.6*	30*	NA	NA	-0.43*	36.36*	-0.51*	32*
	TRO	NA	NA	NA*	NA*	-0.29	16.67	-0.29	16.67
	LRK	-1	50	0	0	0.75	57.14	0.35	55.56
	CHL	NA	NA	NA	NA	-0.15	33.33	-0.15	33.33
	WTN	NA	NA	NA	NA	NA	NA	NA	NA
PLU	BTR	NA	NA	Hormones not available		0.38*	50*	Hormones not available	
	TRO	0*	100*			-0.33	50		
	LRK	NA	NA			-1	50		
	CHL	NA	NA			1	50		
	WTN	NA	NA			NA	NA		
RAY	BTR	Conceived prior to study period				0.1	55.56	0.1	55.56
	TRO					-0.52*	45.95*	-0.52*	45.95*
	LRK					-0.33	50	-0.33	50
	CHL					-0.08	71.43	-0.08	71.43
	WTN					NA	NA	0	100
TRB	BTR	NA*	NA*	NA*	NA*	-0.29	42.86	-0.29	42.86
	TRO	0.33	83.33	0.33	0	-0.05*	22.72*	-0.5*	25*
	LRK	NA	NA	NA	NA	NA	NA	NA	NA
	CHL	NA	NA	NA	NA	1	50	1	50
	WTN	NA	NA	NA	NA	0	100	0	100
TVL	BTR	0.5	25	0.03*	18.18*	0.026*	32*	0*	42.86*
	TRO	NA*	NA*	NA	NA	0	0	0	0
	LRK	0	0	0	100	-0.3	66.67	-0.67	40
	CHL	NA	NA	NA	NA	-0.5	75	-0.5	75
	WTN	NA	NA	NA	NA	0	100	0	100
% Female maintained		18%	36%	22%	28%	14%	29%	13%	22%
% Male maintained		27%	36%	53%	44%	14%	71%	63%	41%
% Mutually Maintained		55%	27%	24%	27%	71%	0%	22%	34%

3.5 Grooming

Over the 309 focal hours for the 12 females included in the analyses, 77.62 cumulative minutes were spent grooming with males, giving a pooled mean grooming rate of 0.031 minutes/hour ± 0.059 (range 0 – 0.427 min/hr; Table 3). Kruskal-Wallis results for intersexual grooming rate based on the conceptive method showed no significant effect of male ordinal rank ($\chi^2 = 6.576$, df = 4, p = 0.160) or female ordinal rank ($\chi^2 = 8.127$, df = 11, p = 0.702), although females only engaged in grooming with the alpha male (Fig. 4). However, female reproductive state was statistically significant ($\chi^2 = 5.029$, df = 1, p = 0.0249), with only pregnant females grooming with males (i.e., during POP females did not groom with males; Fig. 4).

When examining grooming based on hormonally determined reproductive states, none of the predictor variables were statistically significant (male ordinal rank $\chi^2 = 3.805$, df = 4, p = 0.433; female ordinal rank $\chi^2 = 5.307$, df = 7, p = 0.623; female reproductive state $\chi^2 = 2.333$, df = 1, p = 0.127). Although not significant, females were observed grooming with males during POP determined by the hormonal method (Fig 4). Unlike the conceptive method females also were observed grooming with alpha and subordinate males (Fig 4).

Table 3. Mean ± SD and range of grooming rates (minute/hour) for all study females pooled across study period.

	DMK	**LNA**	**LTA**	**LTC**	**MCR**	**MCU**	**MLT**	**MLW**	**PLU**	**RAY**	**TRB**	**TVL**	**Pooled**
Mean	**0**	**0.004**	**0.001**	**0**	**0.012**	**0**	**0.002**	**0**	**0.008**	**0.007**	**0**	**0.002**	**0.031**
SD	**0**	**0.008**	**0.03**	**0**	**0.003**	**0**	**0.004**	**0**	**0.018**	**0.013**	**0**	**0.001**	**0.059**

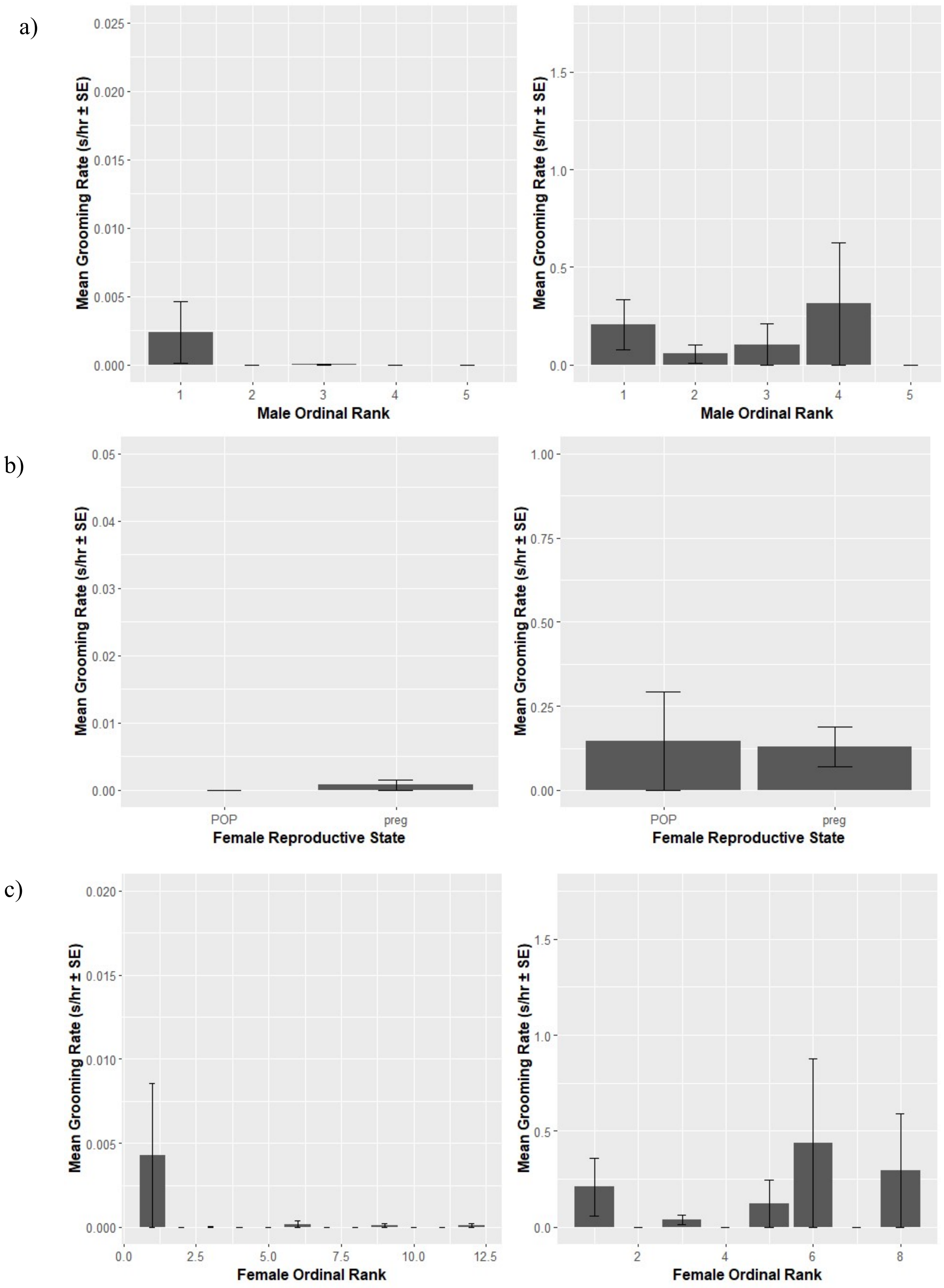

Figure 4a-c. Amount of time spent in grooming males based on male ordinal rank (a), female reproductive state (b), and female ordinal rank (c) from date based on either the conceptive (left) or hormonal (right) method.

3.6 Mating Presentations

A total of 48 mating presentations were recorded during focal follows for the 12 females used in analyses, giving a pooled mean rate of presentations of 0.031 counts/hour (± 0.059; range 0 – 0.427; Table 4). The GLMM based on the conceptive method indicated that male ordinal rank (β = 0.587, p = 0.012) and female reproductive state (β <0.001, p <0.001) were significant predictors of mating presentations, but female ordinal rank (β = 1.077, p = 0.828) was not. Similarly, the GLMM based on the hormonal method showed a significant difference in male ordinal rank (β = 0.455, p = 0.001) and female reproductive state (β <0.001, p <0.001), but not for female ordinal rank (β = 0.729, p = 0.744). Regardless of the method used to determine female reproductive state, females directed more mating presentations to higher ranking males than to lower ranking males, and females also directed more mating presentations during POP than pregnancy, although the effect sizes are negligeable (Fig. 5).

Table 4. Mating presentation mean ± SD and range (counts/hour) for all study females throughout the study period.

	DMK	**LNA**	**LTA**	**LTC**	**MCR**	**MCU**	**MLT**	**MLW**	**PLU**	**RAY**	**TRB**	**TVL**	**Pooled**
Mean	**0.008**	**0.101**	**0.015**	**0.026**	**0.009**	**0.014**	**0.023**	**0.046**	**0.045**	**0.014**	**0.038**	**0.036**	**0.031**
SD	**0.018**	**0.183**	**0.034**	**0.059**	**0.019**	**0.031**	**0.035**	**0.049**	**0.078**	**0.019**	**0.085**	**0.079**	**0.478**

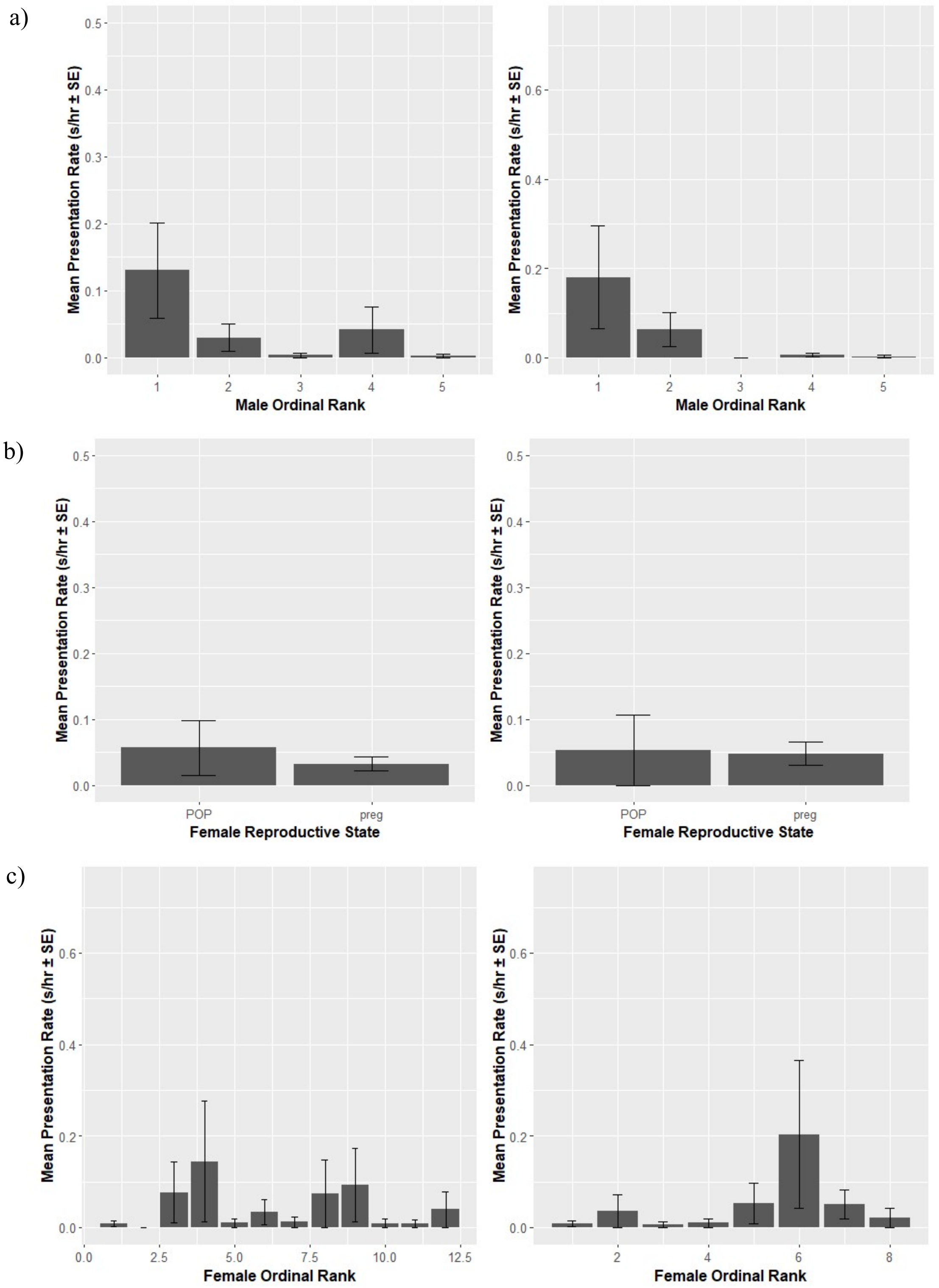

Figure 5a-c. Mating presentations towards males based on male ordinal rank (a), female reproductive state (b), and female ordinal rank (c) from date based on either the conceptive (left) or hormonal (right) method.

3.7 Copulations

Across the study period, all 12 study females had a pooled mean copulation rate of 0.035 counts/hour (± 0.065; range 0 - 0.229; Table 5). GLMM results indicate that, for the conceptive method, male ordinal rank was a statistically significant predictor of copulation rate ($\beta = 1.656$, $p = 0.036$) but female ordinal rank was not (female ordinal: $\beta = 1.459$, $p = 0.335$). There was a small but statistically significant difference for female reproductive state based on the conceptive method ($\beta < 0.001$; $p <0.001$), with females copulating more during POP than during pregnancy (Fig. 6). Although too zero-inflated for GLMM, Kruskal-Wallis results for hormonally determined reproductive states were qualitatively similar to the GLMM results for reproductive states determined by the conceptive method: male ordinal rank ($\chi^2 = 10.754$, df = 4, $p = 0.029$) and female reproductive state ($\chi^2 = 3.266$, df = 1, $p = 0.070$) were both significant predictors of copulation rate, but female ordinal rank was not ($\chi^2 = 11.66$, df = 7, $p = 0.112$). Post-hoc analysis of copulation rate and male ordinal rank using Dunn's test indicate that the alpha male (rank 1) receives more copulations than all subordinate males (1 vs 3: $p = 0.0485$; 1 vs 4: $p = 0.0103$; 1-5: $p = 0.003$), except the second highest ranked male (1 vs 2 $p = 0.145$), (Fig 6b). There were no significant differences between any of the subordinate males in receiving more copulations ($p > 0.05$). Similar to the conceptive method, during POP based on the hormonal method females copulated more than during hormonally-determined pregnancy (Fig 6a).

Table 5. Pooled mean (± SD) copulation rate and range (counts/hour) for all study females across the study period.

	DMK	**LNA**	**LTA**	**LTC**	**MCR**	**MCU**	**MLT**	**MLW**	**PLU**	**RAY**	**TRB**	**TVL**	**Pooled**
Mean	**0.041**	**0.039**	**0.023**	**0.052**	**0.017**	**0**	**0.039**	**0.084**	**0.014**	**0**	**0.061**	**0.049**	**0.035**
SD	**0.07**	**0.067**	**0.05**	**0.093**	**0.039**	**0**	**0.055**	**0.095**	**0.031**	**0**	**0.099**	**0.782**	**0.065**

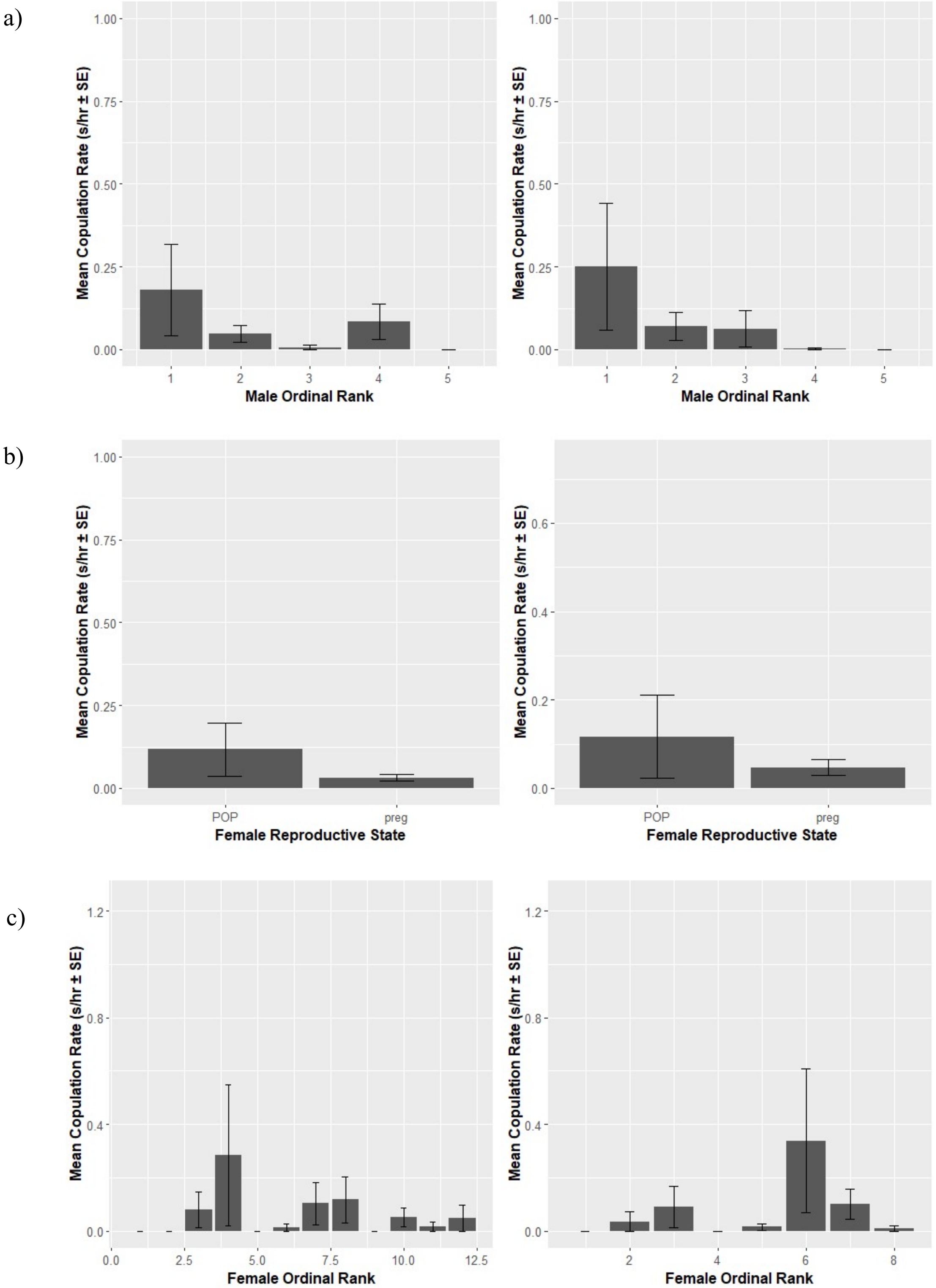

Figure 6a-c. Copulations based on male ordinal rank (a), female reproductive state (b), and female ordinal rank (c) from date based on either the conceptive (left) or hormonal (right) method.

4.0 Discussion

The use of fecal hormones to determine reproductive states in vervet monkeys appears to be feasible, albeit with greater sampling effort than could be achieved in this study. The difference between hormonally determined peri-ovulatory periods (POP) and POPs determined by date of parturition and gestation length (i.e., the conceptive method) is likely partially due to naturally occurring variability in gestation length. Female behavior varied by reproductive state, with peri-ovulatory females spending more time in proximity to males, as well as presented to and copulated with males more often than pregnant females; the only exception to this pattern was grooming. Male, but not female, dominance rank also appears to have an influence on behavioral differences across female reproductive states.

4.1 Objectives 1 & 2

The first aim of this thesis was to assess the feasibility of using fecal hormones to detect ovulation in vervet monkeys, which pairs with the second aim of comparing how closely hormonally determined dates of the POP aligned with dates of the POP identified based on parturition and gestation length. Of the seven females known to have conceived during the study period, six had sufficient fecal samples to create a hormonal profile. For all six females, the peak in estradiol associated with ovulation was identifiable from the hormone profile. However, only one female's hormonally determined POPs overlapped by one day with POP determined from gestation length, with the average time difference between the methods being about two weeks apart. Despite this discrepancy, the mean length of gestation based on the day of the rise in estradiol, is close to the 163-day length of gestation determined by Bramblett *et al.* (1975) in vervets as well as Rowell's (1969) mean gestation of 165 days, both done in lab-based settings. The average gestation length in this study is also similar to those identified for green monkeys:

164.8 ± 6.2 days (Cho *et al.,* 2002), 157 ± 3.6 days (Hess *et al.,* 1979) and 163.4 ± 6.3 (Johnson *et al.,* 1973). It is possible, especially for lower peaks in estradiol, that the actual date of conception was misidentified for some females, and/or the correct date was missing from the hormone profile (see Limitations for further discussion).

However, variation between the predicted date of ovulation based on the date of birth and published gestation length vs the hormonal date of ovulation is to be expected as gestation length is not static. For example, Hartman (1928) first determined rhesus macaques have a gestation length of 165 days, although this analysis was based on only three individuals whose gestation length ranged from 159-174 days. Further studies on gestation in rhesus macaques have noted a wider range in gestation length both within and between females (Riopelle and Hale, 1975; Silk *et al.,* 1993). In a lab-based study on vervet monkeys over two years, Rowell also noted a range in gestation of 142 – 171 days (n = 5), which is similar although slightly smaller than the range in wild vervets from this study from a single birth season (134 – 187 days; n = 6).

Several factors have been suggested to influence variation in gestation length, including maternal age, inter-gestational or inter-birth interval, and diet (Feldblum *et al*., 2022; Gesquiere *et al*., 2018; Riopelle and Hale, 1975). In chimpanzees, the inter-gestational interval length correlates with gestation length, where less time between pregnancies predicts a shorter gestation (Feldblum *et al*., 2022). Chimpanzees have also shown longer gestation periods for younger and older females compared to females in their early 30s (Feldblum *et al*., 2022). This pattern is the reverse of what has been seen in baboons where shorter gestation length is associated with older and young females (Gesquiere *et al*., 2018). In rhesus macaques, receiving a higher protein diet was shown to decrease length of gestation (Riopelle and Hale, 1975). In a previous study on the Nabugabo vervets, Schwegel *et al*. (2023) found that food availability remained constant, and did not impact interbirth intervals. Because Nabugabo is a humanized landscape, the vervets there have access to both wild and anthropogenic food sources (Chapman *et al.*, 2016). Cancelliere *et al.* (2018) found a small but insignificant increase in the protein content of crops compared to wild food sources, and noted the need for future studies to consider nutrient balance of crops compared to wild foods – which may also have implications on gestation variability. In the current study, female ages are not known for all females but likely ranged between 3.5 – 14 years. With the small sample size used in this study and lack of long-term hormonal data to compare gestation length across years, it is difficult to say what factors could explain variation in gestation length between the hormonally determined gestation length and published data. However, the difference between hormonal gestation length and the published gestation length does not appear to be outside of what could be expected based on the variation seen in other species. Use of fecal hormones for detecting ovulation in vervet monkeys does appear to be a feasible prospect. However, to be successful this would require greater human resources to be able to collect sufficient samples on a more consistent basis (i.e., once every ~3 days) than what was possible for this study.

4.2 Objective 3

The third aim of this thesis was to gain better insight into female choice in vervet monkeys based on female reproductive state. Although quantitative statistics were not always possible due to the small sample size, most notably the small number of females and the focal time during the seven-day POPs, some patterns emerged and are worth considering. As predicted, females generally did show more proceptive behaviors during POP than in pregnancy. The only exception to this was grooming, which was observed more often during pregnancy. Females spent more time in proximity to males during POP than during pregnancy regardless of the method used to calculate female reproductive state. Hinde's index indicated that during the POP, proximity was mostly mutually maintained by the male and female. In contrast, Brown's index showed fewer instances of mutually-maintained proximity between the male and female, but also indicated equal effort of male- and female-maintained proximity. During pregnancy, Hinde's index also showed mostly mutually maintained proximity, and equal male and female effort in maintaining proximity. Brown's index however revealed males had the most effort in maintaining proximity during pregnancy based on the hormonal method. Although there was a statistically significant difference indicating POP females gave more mating presentations to males than pregnant females, the effect size was so small as to likely be biologically irrelevant. However, as predicted, females copulated more during POP for both the conceptive and hormonal methods. This mirrors Andelman's (1987) findings in the population at Amboseli that female vervets also copulated more frequently around the POP. Given the near absence of overlap in POPs between the conceptive and hormonal methods of identifying female reproductive state, my results could indicate that some behavioral differences around ovulation may last longer that a seven-day POP. In Assamese macaques (*M. assamensis*), which also lack ovulatory signals and a similar pattern of behavior was found by Fürtbauer *et al*. (2011) where, while females were observed to copulate outside of the fertile period, still copulated significantly more while ovulating. Carnegie *et al.* (2005) also noted this extension of proceptive and receptive behavior in white-faced capuchins, with only a minor increase in proceptive behaviors during periovulatory periods. In a later study, Carnegie *et al.* (2006) found that the preferences of female white-faced capuchins also change between reproductive states, where females were more likely to direct proceptive and receptive behaviors toward subordinate males after becoming pregnant. This change in preference differs from female vervet mate preference reported by Andelman (1987), which appear to prefer the alpha male across reproductive states.

Females generally directed more proceptive and receptive behaviors (i.e., mating presentations and copulations, respectively) to high-ranking than low-ranking males, which is also consistent with Andelman's (1987) research on wild vervets at Amboseli, and Keddy's (1986) lab-based study of the closely related grivets. Females spent more time in proximity to high-ranking males based on both the conceptive and hormonal methods. There are an extremely limited number of alpha male-female dyads for POP, making it difficult to draw conclusions. However, Hinde's index suggests that over all alpha male-female dyads proximity is either maintained mutually or by the alpha male. In contrast, subordinate male-female dyad proximity is generally female maintained during POPs but subordinate male maintained during pregnancy. Similar results were seen for Brown's index, but with more effort from the alpha male in maintaining proximity to females. The pattern was consistent between reproductive states and methods of determination; however, with hormonally determined pregnancy, male maintained proximity occurred more often than female-maintained proximity for dyads containing a subordinate male. Females were also observed to direct more mating presentations towards higher ranked males than subordinate males regardless of method used to determine female reproductive state. This also followed for copulations, where females copulated more with higher ranking males based on both the conceptive and hormonal methods. While interactions between the predictor variables could not be tested, subordinate males rarely received mating presentations nor copulations from females, suggesting that females likely preferred dominant males across reproductive states.

A large challenge faced in this study was that the behaviors of interest (proximity to males, grooming, mating presentations and copulations) were not observed very often in the vervet study group during the study period. Previously described as the “sexless” monkey by Haddow (1952), these results are not entirely surprising for vervets. Over 2200 hours of observations, Struhsaker (1967) observed only 176 copulations, giving a mean number of copulations per hour of 0.08 for the Amboseli population. In her later study, Andelman (1987) also noted a low number of copulations per hour in her study on the Amboseli vervets (mean = 0.06 copulations per hour), especially when compared to the yellow baboons (*P. cynocephalus*) also residing at Amboseli who displayed a mean of 1.00 copulations per hour (Hausfater, 1975).

4.3 Limitations

As with any study utilizing behavioral observations, it is impossible to observe every behavior exhibited by every individual. In the current study, I was the single observer recording the behaviors of 16 females within a group of 44 monkeys. Even on a “perfect” day where all observations can be conducted back-to-back, and no observation exceeds the amount of time allowed for out-of-view, only a maximum of two focal follows per female could be completed in a day (starting at 8:00am and ending at 4:00pm), not allotting for a lunch break. Naturally, the vast majority of field days were “imperfect”. Females’ overall interactions with males are relatively rare occurrences, particularly for sociosexual behaviors. Furthermore, vervets also do not defecate very often throughout the day, and when they do, samples may be lost to impenetrable thick brush (e.g., *Lantana* camera) or the lake. Time spent searching for and collecting fecal samples also reduced the time available to conduct behavioral observations. Unlike behavioral observations, I did have some assistance in collecting fecal samples from the field assistants and other students observing the study group; however, these parties also collected data on other study groups and thus were not present with my study group on all field days. Behavioral and fecal data collection were also limited by weather conditions, especially during the first two months of data collection (April and May), which fell within the wettest season (Schwegel *et al.,* 2023). On days with heavy rainfall the monkeys would often retreat to high parts of the canopy, making the observation conditions more challenging. Likewise, on days with high winds observations could not be conducted in the forest due to the risk of tree limbs falling. On occasion the vervets would also venture into the village to forage for crops, where for ethical reasons we do not conduct observations.

Another limitation of this study is that about half of the females in the study group conceived prior to the start of the study period, and therefore were limited to an estimated date of conception based on gestation length and infant birthdate rather than through hormone analysis. Naturally, this also limited the behavioral observations around POP to females who conceived during the study period. While the timing of data collection attempted to capture the conceptive season for Lake Nabugabo vervets (May-July on average across years), neither the timing of conceptions nor the length of gestation are static and are therefore subject to variation (Schwegel *et al.,* 2023). Furthermore, not all male characteristics of interest could be included in the model of female mate choice in this study, as there was insufficient variation in male age and tenure length in the study group.

The limitations of data collection, the small number of females who conceived during the study period, and the rarity of proceptive and receptive behaviors among vervets resulted in highly zero-inflated behavioral data which limited options for statistical analysis. In the cases of particularly high zero-inflation (i.e., grooming, copulations from the hormonal method), data could only be analyzed using non-parametric tests. Even for the models with more manageable zero-inflated data (i.e., proximity, mating presentations, and copulations determined from the conceptive method), when interactions between the predictor variables were included, the results at best lacked any statistically significant difference (which could be a true result) and at worst produced no results at all. Because of this, interactions between predictor variables were not included in the final models. The issues caused by including interactions in the models were likely also caused by the high levels of zero-inflation (Bolker *et al.,* 2009). Because non-zero occurrences were already limited for individual predictors, attempting to examine interactions between the predictors likely only limited non-zero occurrences further. There are some methods to further deal with zero-inflated data, but this is a fairly “niche” subject in statistics and is often overlooked entirely (Li *et al.,* 2024). As research conducted in the wild across species is susceptible to zero-inflation, particularly when attempting to further understand rare patterns of behavior such as proceptivity and receptivity in a “sexless” monkey, further implementation of zero-inflated statistical methods is an interesting avenue for further study.

4.4 Conclusion

This study contributes to the understanding of female choice and reproduction in vervet monkeys, and assesses the validity and feasibility of using fecal samples to identify female reproductive status under the limitations of field data collection. The potential to use fecal hormones from vervet monkeys to determine their ovulatory cycle not only allows for better insight into female choice in a wild setting but also into the variability of gestation. To my knowledge, this is one of the only studies to include the variability of gestation in wild vervet monkeys. The variability of gestation length is limited throughout primatology with the exception of humans and rhesus macaques, which are commonly used in medical research (Coe *et al*., 2021); thus, a severely lacking area of study.

In this study, I identified differences in female sexual behavior across reproductive states. Consistent with previous findings, the Nabugabo females mated throughout their reproductive cycle and into pregnancy (Andelman, 1987), and they appear to prefer alpha males (Andelman, 1987; Fane, 2023). I also found that, while proceptivity and receptivity behaviors were not limited to the ovulatory period in vervets, these behaviors did increase when females are likely to conceive. Certainly, this begs the question of the potential functions of non-conceptive sex in vervets (e.g., paternity confusion, appeasement/reconciliation, male social integration).

5.0 Appendix

5.1 Ethogram

LETTER 1: Type of data to be entered

Self (S) – focal, self- or generally-directed behaviour
Contact (C) – focal in physical contact = 0m
Proximity (P) –focal within <1 body length from interactant
Direct (D) – focal gives, or directs, the behaviour
Receive (R) – focal receives the behaviour
Mutual (M) – mutual behaviour
OOO – out of sight

LETTERS 2&3: Behaviour Codes

SELF or GENERALLY DIRECTED (S)

Rest (RE), state – individual is sitting in a relaxed (i.e., non-vigilant) manner; may also be lying on side or straddling a tree branch (Struhsaker 1967a)

Scan (SC), state – individual is looking around, turning its head, in the farther visual distance >2meters; often occurs as a standing pause during a "move" (included visual foraging only during summer 2016 project); differs from "rest" because the animal is usually not sitting or lying down.

Visual Forage (VF), state – individual is slowly moving or standing still while looking around in foreground <2m (on the ground, in tree or shrub branches, grasses); differs from "Move" because "Visual Forage" is often includes locational displacement less < 1 meter.

Move (MO), state – walking or galloping, occasionally including a hop, and may be preceded or interrupted by vigilance (Struhsaker, 1967a); locational displacement greater than 1 meter and excludes positional changes/adjustments with locational displacement of less than 1 meter (Schoof).

- Move between (**MB**) – moving between trees
- Move within (**MW**) – moving within a tree
- Move on ground (**MG**) – moving on the ground

Vigilant (VG), event – standing erect on hindlimbs staring in one directing or glancing in several directions (Struhsaker, 1967a); if in tree, the actor stands quadrupedally or sits with chest forward and stares or glances (Schoof)

Self-Groom (SG), state - an individual combs through its own pelage using fingers and/or mouth, and may place foreign particles in mouth (Schoof 2016, included scratching)

Scratch (SK), event – includes scratching (*sensu* “self-cleaning” in Struhsaker 1967a).

Feed (FE), state – individual is manipulating and/or ingesting food.

- When possible, the observer should note the food species and part in the “comments”
- Food parts include: Ripe Fruit (RF), Unripe Fruit (UF), Unknown Fruit (FR), Flower (FL), Young Leaf (YL), Mature Leaf (ML), Leaf Bud (LB), Young Leaf Petiole (YLP), Mature Leaf Petiole (MLP), Bark, Dead Wood, Pine Needles, Seeds, Seed pods, Pith, Soil, Tuber/Root/Potato

Drink (DR), state – individual is ingesting water from a ground or tree source (natural or man-made), normally involves lowering head towards standing water from water holes or rain pools; individuals may also lick water from hands, fingers, tree branches, or pelage of others (Struhsaker 1967a).

Yawn (YW), event – Mouth open all the way, briefly, in the vertical rather than horizontal plane; head may go back a bit. Lips may cover teeth or roll back, exposing top and bottom canines.

Other (OT), state/event – any behaviour not described in the ethogram; use “comments” section to describe behaviour

Out of sight (OO), state – focal animal is out of sight of the observer, usually resulting from substantial visual obstruction such as a building or dense vegetation, or because the focal animal has been “lost”.

<u>SOCIOSEXUAL</u> (S,P, C, G or R; occasionally M)

Hip grab (HG), event – actor will stand behind female and grab at her hips pulling her in as if to mount but does not mount.

Inspect (IN), state – actor visually inspects, touches, licks or smells the genital region of the recipient; this may include looking from a short distance (<1ft), manipulating with hands or mouth, touching with face and includes various forms of “muzzling” as described by Struhsaker (1967a). Self licking or manipulating (males genitals) is S,P or C

Present (PR), event – female actor stands quadrupedally and orients her hindquarters towards the male recipient, sometimes glancing over shoulder; female may stop to present if followed by a male or male grabs hindquarters of a sitting female (i.e., behaviour of “estrous female” as described in Struhsaker 1967a).

- Note: a male focal animal can therefore <u>Receive a Present</u> (RPR) from a female Interactant.

Mount (MT), state – actor holds mountee hips with its hands, and legs with its feet (i.e., foot clasping); mount with <u>no thrusting</u>; may be associated with grooming and individuals may alternate between role of actor and recipient; note: mount may be incomplete, with grasping of only one body part Struhsaker 1967a)

Thrusting mount (TM), state – actor mounts (see above) recipient <u>with pelvic thrusts</u>; thrusts may be relatively <u>rapid</u> and <u>short</u> and/or relatively <u>slow</u> and <u>long</u> (Struhsaker 1967a); may be followed by a pause before dismount. (Field assistants call this copulation or mating (CO).

- In the comments" section, observer should attempt to (1) note whether the thrusts are rapid/short or slow/long, (2) count the **number of thrusts**, (3) note if the presence/absence of a "**pause**" before dismount, and (4) note the presence/absence of **ejaculate** on the male and female's genitals.

Mating refusal (MR), event –female actor who is being grabbed by male remains sitting, lies down, crouches low, or walks away; note that female may also hit, lunge, or make "anti-copulatory squeal-scream" vocalizations (i.e., behaviour of "anestrous female" as described in Struhsaker 1967a).

Penile erection (PE), event – actor has a conspicuously erect red penis; may occur during 1) grooming when recipient is male, 2) intragroup agonism, usually by the aggressor (Struhsaker 1976a)

<u>AFFILIATIVE (G or R; occasionally M)</u>

Approach (AP), state – One monkey moves into contact or within 2 meters of a second monkey (Jack 1998).

Leave (LE), state – One monkey moves out proximity (2 meters) of another monkey (Jack 1998).

Breast feed (BF) – actor suckles from one or both nipples simultaneously (Struhsaker 1967a); this is a behaviour directed by an infant (sometimes a young juvenile) to a female recipient; a.k.a., nursing or suckling.

Carry (CA) – actor has another individual, usually an infant, clinging to its ventral surface or sitting/straddling on its back while engaged in locational movement.

Entwine tails (ET; Mutual behaviour) – two individuals sitting in close proximity on a branch criss-cross their tails (Struhsaker 1967a)

Groom (GR), state – actor combs through the fur of recipient using fingers and/or mouth, and may place foreign particles in mouth; may be accompanied by lipsmacking or teeth chattering (Struhsaker 1967a).

Groom solicit (GS), event – actor presents a specific body part to recipient for grooming (Struhsaker 1967a)

Ignore (IG), event – actor presents a groom solicit that is not acted on by the recipient. (Event)

Hug (HU), event – actor wraps their arms around the recipient while sitting dorso-ventrally or ventro-ventrally (recipient-actor) and may be associated with grooming; a.k.a. embrace (Struhsaker 1967a).

Play (PL; D, R, or M behaviour; or S for rare self-play), state – Play behaviours are quite variable and encompass a large number of behaviours described elsewhere (e.g., grab, chase, wrestle, mount, groom, embrace, hop, etc…)

- in play, these behaviours – such as chase or groom – are "alternated with one another in rapid sequence" and "may be the major distinctions between play and non-play encounters, rather than uniquely different behavior patterns" (Struhsaker 1967a: 33)
- intergroup play between juveniles and juvenile males of different groups may occur (Struhsaker 1967a).

Wean (WE) – female actor refuses breast-feeding attempt, or nips and/or pushes away a suckling infant (Struhsaker 1967a); this is a behaviour directed by a female to an infant or young juvenile.

Mouth to mouth (MM), event– actor approaches recipient and the two put their mouth close together touching (or nearly).

AGONISTIC

In "comments", note the stimulus or context prior to the agonistic event(s), such as copulation/mount, male, infant, grooming, food, space, or unclear; do not leave blank (i.e., if you didn't notice anything, write "unclear").

Note: **Copulatory harassment** of a male or a mating pair has been observed, but the behaviours comprised in copulatory harassment include other behaviours described herein (e.g., biting, lunging, grabbing, etc…). As such, if copulatory harassment is observed, this should be noted in the "comments" of the associated behaviour.

Aggressive/Dominance Behaviours (G or R; occasionally M)

Bite (BI), event – as described, usually accompanied by grabbing (Struhsaker 1967a)

Bob (BB), event – Jerking or bobbing of head or entire body in up and down motion (on sagittal plane); for body includes torso bobbing from quadrupedal to bipedal position – often associated with eyelid flash (*sensu* "jerking" in Struhsaker 1967a)

Branch-shaking (BS), event – Actor deliberately bounces once or twice on a branch, sometimes associated with racing through trees creating noticeable noise, normally associated with intergroup encounters (Struhsaker 1967b, Henzi 1982)

Broadside (BD), event – Actor stands perpendicular to receiver, pauses, and then moves on; actor's tail may be raised. Note: Receiver is usually sitting (Henzi 1982; *sensu* "sideward-display" in Struhsaker 1967a)

Chase (CH), **state**– Actor moves rapidly toward the recipient, who runs away (*sensu* "chase-and-attack" in Struhsaker 1967a); recipient frequently looks back at actor while running away, and often emits submissive vocalizations

- note: some chases may be "false" (sensu "false-chase" in Struhsaker 1967a), wherein the actor gallops slowly and hesitatingly towards the recipient that is never caught → actor generally subordinate to the recipient

Coalitionary Display (CD) – any agonistic interaction involving three or more monkeys; the initial aggressor(s) is the "actor(s)", while the initial victim(s) is the "recipient(s)"; additional details on the behaviours involved should be noted in the "comment" section.

Eyelid flash (EF), event – actor exposes lighter colored eyelids by retracting brow while maintaining eye contact or staring at the recipient (Struhsaker 1967a); may also be submissive ("defensive") in nature if actor is crouching (Struhsaker 1967a)

Hit (HI), event – actor hits, or slaps, the recipient with its hand (Struhsaker 1967a)

Lunge (LU), event – Actor leaps or jumps towards the recipient. Forward lurching of the chest.

Penile Display (PD), event– Male actor displays red and erect penis while standing bipedally or sitting upright with his hands on the recipient, whose face is directed towards the actor's genitals (Struhsaker 1967a).

Red-white-and-blue display (RB), state – Male actor displays the red perianus, white pelage between anus and scrotum, and blue scrotum to the recipient; male encircles or paces next to recipient with tail lifted, or briefly stands on his hindlimbs while oriented towards the recipient, thereby exposing genitals; actors include only adult, subadult, and older juvenile males (Struhsaker 1967a; Henzi 1982). Note: Receiver is usually sitting and vocalizing. Like a male presenting/ may involved movement around receiving individual.

Solicit Assistance (SA) – aggressor or victim of aggressive behaviour solicits assistance from others by "head flagging", glancing back and forth between opponent and individual assistance is being solicited from, or vocalizing (e.g., chuttering)

Supplant (SU), event –the actor moves towards the recipient and occupies the space, eats the food, takes copulatory position, or assumes the grooming position of the recipient; the recipient or "supplantee" generally moves away, sometimes engaging in submissive behaviours such as lip-smacking or submissive vocalizations (Struhsaker, 1967a); a.k.a. displacement.

- Note: indicate in "comments" what the actor removed: food, grooming, or space (note that because "space" is generally taken when removing food or grooming from the recipient, "space" should only be used if neither food nor grooming were removed from the supplantee)

Submissive (G or R; occasionally M)

Avoid (AV), event– at the approach of another individual (i.e., the recipient of the avoidance behaviour), the actor spontaneously vacates and moves away without any threat or aggression from the other individual; the recipient may just be passing by and does not occupy the space vacated by the actor (i.e., the individual doing the "avoiding")

- note: if the space (or food/grooming partner) is then occupied by the approaching individual, this behaviour should be coded as a supplant directed by the approaching individual.

Cowering (CW), event – Actor lowers head/body by crouching towards the ground and avoids eye contact with recipient; actor may be moving away from recipient; usually associated with submissive vocalizations/lipsmacking (Henzi 1982; sensu "crouching" in Struhsaker 1967a)

Fear grimace (FG), event – actor opens mouth about halfway and exposes teeth by retracting lips on horizontal plane (resembles a "smile); often accompanied by submissive vocalizations and staring at the recipient (*sensu* "grimacing" in Struhsaker 1967a).

VOCALIZATIONS (G or R; occasionally M)

Alarm call (AC), event – Alarm calls are often multi-syllabic barks can be emitted in response to a real or perceived threat, most notably for "other vervet group", snakes, eagles (i.e., *rraup*), leopards (i.e., *chirp*), & dogs and humans at Nabugabo (http://www.psych.upenn.edu/~seyfarth/Baboon%20research/vervet%20vox.htm)

Bark (BK), event – low-pitched and gruff uni-syllabic exhaled vocalization emitted by males during intergroup encounters and occasionally during intragroup agonism; given towards other vervet monkeys who are fighting, it is emitted to stop the fighting (Struhsaker, 1967b).

Chutter (CT), event – low-pitched, monotonal and staccato vocalization emitted by females and juveniles to express aggression and solicit assistance; mouth is closed and the teeth are covered (Struhsaker, 1967b).

Submissive vocalizations (SV), event – lipsmacking, teeth-chattering, purring (Struhsaker 1967b, Henzi 1982), and also includes:

- ***Woof woof (WW)***: This call is non-tonal, deep, and has a guttural sound (Struhsaker, 1967) emitted with closed or slightly-open mouth to indicate submission (Struhsaker, 1967b) emitted by males.
- ***Wa (WA)***: This call is a continuous tonal exhalation that occurs with a grimace and indicates submission (Struhsaker, 1967b); may be combined with as "Woof-wa" vocalization emitted by males.
- ***Rraugh (RR)***: For this call the mouth is closed or partially opened and the teeth are covered (Struhsaker, 1967b). This call is emitted by yearlings when they approach older members of the group, and is a signal of nonaggression (Struhsaker, 1967b); includes both the long and short rraugh, and the aarr-rraugh.

- ***Lipsmack (LS):*** Moving the lips together quickly, opening and closing the mouth repeatedly.

Squeal-Scream (SS): high-pitched, piercing calls usually are emitted by females and juveniles that are seeking help from threats by an aggressor, and may be anti-copulatory (Struhsaker, 1967b).

INFANT INTERACTIONS (C:Contact; R:Receive or D:Direct)

*When applicable, instantaneous samples of focal animal's infant should be recorded at 0, 5 10, and 15 mintues of focal follow.

Ventral contact (VC), event –actor initiates contact between torso of infant with the ventrum of mother (Bardi et al. 2004).

End ventral contact (EC), event –Actor separates torso of infant from the ventum of mother (Bardi et al. 2004)

Breast Feed (BF), event – infant's mouth is in contact with one or both nipples simultaneously (Struhsaker 1967a)

End Breastfeed (EB), event – infant that was in nipple contact stops voluntarily (i.e., not "weaning")

Restrain (RS), event – Focal keeps infant from breaking contact (Bardi et al. 2004)

Reject (RJ), event – Focal prevents attempted contact by infant (Bardi et al. 2001)

INTERACTANT(S)

For identified monkeys, write their name or code in the Interactants (or NN) column;
For unidentified monkeys, use the following two or three letter codes; if there are multiple individuals of the same age/sex category, add number identifiers. For example, AM1, AM2
AM# – Adult male
AF# – Adult female without infant
AD# – Adult, unsexed
SM# – Subadult male
SF# – Subadult female
SB# – Subadult, unsexed
JM# – Juvenile Male
JF# – Juvenile Female
JV# – Juvenile, unsexed
IN# – infant (always unsexed)
XX# – Unidentified vervet
ZZ – other species (if known, indicate the species in the "comments"; e.g., snake, bird, dog, cow)
OBS – observer

HUM – other human

- If there are multiple interactants, use a comma to separate their IDs

ZRT- Red tailed monkey
BWC – Black and white colobus monkey
BBN - Baboon
DOG – Dog (indicate how many in the comments, and their behaviour)
COW – Cow
PIG – Pig
GOT – Goat
CKN – Chicken

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