



Original Article

Assessment of Male Reproductive Skew via Highly Polymorphic STR Markers in Wild Vervet Monkeys, *Chlorocebus pygerythrus*

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Abstract

Male reproductive strategies have been well studied in primate species where the ability of males to monopolize reproductive access is high. Less is known about species where males cannot monopolize mating access. Vervet monkeys (*Chlorocebus pygerythrus*) are interesting in this regard as female codominance reduces the potential for male monopolization. Under this condition, we assessed whether male dominance rank still influences male mating and reproductive success, by assigning paternities to infants in a population of wild vervets in the Eastern Cape, South Africa. To determine paternity, we established microsatellite markers from noninvasive fecal samples via cross-species amplification. In addition, we evaluated male mating and reproductive success for 3 groups over 4 mating seasons. We identified 21 highly polymorphic microsatellites (number of alleles = 7.5 ± 3.1 [mean \pm SD], observed heterozygosity = 0.691 ± 0.138 [mean \pm SD]) and assigned paternity to 94 of 97 sampled infants (96.9%) with high confidence. Matings pooled over 4 seasons were significantly skewed across 3 groups, although skew indices were low (B index = 0.023–0.030) and mating success did not correlate with male dominance. Paternities pooled over 4 seasons were not consistently significantly skewed (B index = 0.005–0.062), with high-ranking males siring more offspring than subordinates only in some seasons. We detected 6 cases of extra-group paternity (6.4%) and 4 cases of natal breeding (4.3%). Our results suggest

that alternative reproductive strategies besides priority of access for dominant males are likely to affect paternity success, warranting further investigation into the determinants of paternity among species with limited male monopolization potential.

Subject area: Reproductive strategies and kinship analysis

Keywords: extra-group paternity, mating skew, microsatellites, natal breeding, noninvasive sampling

Early work in evolutionary biology argued that males and females had evolved different reproductive strategies due to differential investment in their gametes (i.e., sperm and egg cells, respectively). Females typically invest more time and resources into large egg cells, produced in small quantities, whereas males invest relatively little in their smaller sperm cells, but produce these in large quantities (Trivers 1972). This sex difference was suggested to be particularly pronounced in mammals where gestation and lactation place a substantial energetic burden on females, whereas males usually contribute little to offspring care (Trivers 1972; Kleiman and Malcolm 1981). As a consequence, females were thought to be mainly limited by access to food (Trivers 1972), whereas males were limited by access to fertile females (Kappeler and van Schaik 2004). More recent theoretical developments, however, suggest that males and females can be both choosy and indiscriminate with respect to mating partners (Gowaty 2003), emphasizing that both sexes are under selection with respect to offspring viability (Gowaty 2004). Given the varying investment in offspring, males and females have developed differential reproductive strategies to maximize their reproductive output.

Among social mammals living in multimale, multifemale groups (Clutton-Brock 1989), as seen in many primates (Alberts et al. 2006), a common male strategy is to monopolize reproductive access to females around the time of likely conception. Although this strategy is mainly restricted to more dominant males who can guard females from subordinate males, other males can also increase their mating and reproductive success via opportunistic copulations (e.g., Coltman et al. 1999; Gibson 2010), coalition formation (e.g., Feh 1999; Young et al. 2013), or investment in friendships with females (e.g., Kulik et al. 2012).

Given that the majority of mammal and particularly primate species are characterized by promiscuous mating systems, genetic analyses are required to assess male paternity success in order to study male reproductive success. In mammals, male reproductive success mostly depends on male monopolization potential, that is, where males are dominant over females and fight other males to obtain high dominance status, which regulates access to fertile females (e.g., Soay sheep, *Ovis aries*, Coltman et al. 1999; Preston et al. 2005; eastern gray kangaroo, *Macropus giganteus*, Miller et al. 2010; reindeer, *Rangifer tarandus*, Røed et al. 2002). Within the primate taxon, the majority of studies on male reproductive success have focused on species with high male monopolization potential, where mate-guarding is mainly used to monopolize fertile females (savannah baboons, *Papio cynocephalus*, Alberts et al. 2006; chimpanzees, *Pan troglodytes*, Boesch et al. 2006; crested macaques, *Macaca nigra*, Engelhardt et al. 2017). Most of these studies have shown that male reproductive output is biased toward more dominant males (Ellis 1995; Alberts et al. 2006; Engelhardt et al. 2006). However, there is wide interspecific variation in alpha male paternity (Ostner et al. 2008), with dominance rank being a poor predictor of male reproductive success in some primate species

(Dewsbury 1982; Ellis 1995). One explanation for this could be that females developed strategies to reduce male monopolization potential, confuse paternity, and/or reduce the risk of infanticide. Females may therefore mate with as many males as possible, as well as concealing ovulation and synchronizing or extending their receptive period, such that monopolization becomes prohibitively costly for males with respect to both time and energy (reviewed in Zinner et al. 2004). For example, in rhesus macaques (*Macaca mulatta*), a species with limited male monopolization potential, males have restricted information about the exact time of female ovulation (Dubuc et al. 2012). As a consequence, mate-guarding by top-ranking males accounted for only 30–40% of all fertilizations (Dubuc et al. 2012), suggesting that male dominance has a limited influence on male reproductive success and that females may pursue their own reproductive interests (Dubuc et al. 2011). In a mammal species with limited male monopolization potential, the spotted hyena, *Crocuta crocuta*, male reproductive success is not linked to dominance (Engh et al. 2002); however, in primate species with limited male monopolization potential, the link can be surprisingly strong (e.g., bonobos, *Pan paniscus*, Gerloff et al. 1999; sifakas, *Propithecus verreauxi*, Kappeler and Schaffler 2008). This is particularly interesting, as males are not dominant over females in these species (females are codominant to males in bonobos, Vervaecke et al. 2000, whereas females dominate males in sifakas, Richard and Nicoll 1987). These studies question any notion of a general influence of male dominance on reproductive success and call for more studies that can help unravel the factors that determine male reproductive success, especially when females are not dominated by males.

The successful monopolization of fertile females by one or a few males is a general characteristic of many primate species (Kutsukake and Nunn 2009). Accordingly, the majority of offspring are produced by one or a few males, whereas most males sire few or no offspring (reviewed in Kutsukake and Nunn 2009; Widdig 2013), even across their lifetime (Dubuc, Ruiz-Lambides, et al. 2014). However, the degree of reproductive skew, commonly assessed by the Nonacs' binomial skew index (hereafter *B* index, Nonacs 2000), varies widely across species and can even be independent of male monopolization potential (high potential: chimpanzees, Surbeck et al. 2017; crested macaques, Engelhardt et al. 2017; limited potential: rhesus macaques, Widdig et al. 2004; ring-tailed lemurs, *Lemur catta*, Parga et al. 2016). In fact, the highest level of reproductive skew is found in species with either male or female dominance (Parga et al. 2016; Engelhardt et al. 2017).

Another important consequence when male monopolization potential is limited is that females are better able to mate with males outside their group (van Noordwijk and van Schaik 2004), such that a proportion of offspring can result from extra-group paternities (hereafter EGPs). Previous studies on primate species with high male monopolization potential revealed lower incidences of observed EGPs (0–7%, Vigilant et al. 2001; Alberts et al. 2006; Engelhardt

et al. 2017) than in species with limited male monopolization potential (16–40%, Lawler et al. 2003; Parga et al. 2016; Ruiz-Lambides et al. 2017).

Similarly, a delay in natal dispersal should provide opportunities for natal breeding, particularly with limited male monopolization potential, although mechanisms of inbreeding avoidance would be expected to reduce the probability of this occurring (cf. Widdig et al. 2017). Natal breeding is generally rare or seems to be absent in primate species with high male monopolization potential (Engelhardt et al. 2017; Wikberg et al. 2017), whereas evidence on natal breeding is mixed in species with limited male monopolization potential (0–16%, Kappeler and Schaffler 2008; Widdig et al. 2017). Consequently, studies to date have presented mixed results with respect to whether natal breeding represents a potential male strategy to improve reproductive success.

Chlorocebus spp. live in multimale, multifemale groups, where males disperse from their natal group at the time of sexual maturation and subsequently change groups on average every 2 years (Henzi and Lucas 1980). After immigration, males achieve and maintain their rank through frequent agonistic interactions (Struhsaker 1967a). Females can win encounters against males, both at the dyadic level or in coalitions (Cheney et al. 1988), and their consequent ability to reject male mating attempts underpins male mating access (Keddy 1986; Freeman 2012). *Chlorocebus* spp. and especially vervet monkeys (*Chlorocebus pygerythrus*) are therefore an ideal genus and species to investigate male reproductive success, being characterized by female codominance (Young et al. 2017), limited male monopolization potential (Cheney et al. 1988; Weingrill et al. 2011), breeding seasonality (Cheney et al. 1988), moderate sexual dimorphism (Cheney et al. 1988), and the possibility of concealed ovulation (Andelman 1987).

The first aim of the present study was to develop a sufficient number of polymorphic microsatellite markers to allow genetic paternity analysis from noninvasive fecal samples of wild vervet monkeys. Our second aim was to assign paternities across our 3 study groups over 4 breeding seasons, to assess the degree of both mating and reproductive skew, along with the relationship between male dominance and skew. Our final aim is to describe the extent of EGP and natal breeding.

Materials and Methods

Study Population and Study Period

Data were collected from 3 groups of wild vervet monkeys living on the Samara Private Game Reserve, Eastern Cape, South Africa (32°22'S, 24°52'E). The 3 study groups (RST, RBM, PT) are habituated to close range observation, and all animals are individually identifiable (Pasternak et al. 2013). Between 2013 and 2016, the number of adult males (m) and females (f) differed across groups, RST: m = 12 ± 2, f = 16 ± 2, RBM: m = 11 ± 3, f = 11 ± 1 and PT: m = 7 ± 1, f = 7 ± 1 (mean ± SD).

Vervet monkeys are seasonal breeders, and the mating season at the study site extends from April to July (Freeman et al. 2012). Genetic and behavioral data for this study were collected between April 2013 and July 2017. Individual records of the date of birth and death, days of group residency, date of male migration, identity of behavioral mother (based on nursing and association), and sex of individuals were noted immediately or within 2 days of the event. Dates of birth and the identities of behavioral mothers were not available for some individuals born before 2012.

Sample Collection and DNA Extraction

For the development of a microsatellite panel, we used 18 blood samples previously collected during a thermoregulation study for which animals were immobilized via darts containing an anesthetic (mixture of midazolam, 2.5 mg, and ketamine, 50 mg, for on average weight of 4.4 kg per monkey, McFarland et al. 2013, 2015). From each animal, 2 mL blood samples were taken, which were immediately frozen as EDTA blood (anticoagulant). Additionally, for noninvasive paternity analyses, we collected 620 fecal samples from a total of 197 individuals. Samples were collected immediately after defecation of known individuals. Six hundred and five fecal samples were stored with the “two-step” storage procedure (Nsubuga et al. 2004), and additional 15 fecal samples were frozen (see [Supplementary Material](#) online for “Fecal sample storing procedures”). In total, we collected up to 3 independent fecal samples (2.93 ± 1.20, mean ± SD) for each individual. Finally, we collected small tissue samples (1 × 1 cm, N = 12) from miscarriages and deceased individuals, which were frozen immediately after collection and stored at –20 °C until extraction. Of 111 infants born during our study period, we lack genetic samples for 14 infants, as they died before potential sample collection, leading to an infant sampling success of 87.4% (2013: 96.7% with 1 infant dying, 2014: 94.3% with 2 infants dying, 2015: 88.0% with 3 infants dying and 2016: 61.9% with 8 infants dying during an extreme drought). For the 2013 season, it was not possible to sample 3 potential sires in PT and 2 potential sires in RST. For all other seasons, we were able to sample all potential sires across the 3 groups. In total, we sampled 51 of 56 potential sires across groups and seasons considered (i.e., 91.1% male sampling success over all cohorts). This included all natal males and immigrants, together with a few males from unhabituated neighboring groups that were opportunistically sampled.

DNA isolation for blood and tissue samples was performed with the *QIAamp® DNeasy® Blood & Tissue Kit* (Qiagen, Hilden, Germany) and both dried and frozen fecal samples with the *QIAamp® Fast DNA Stool Mini Kit* (Qiagen, Hilden, Germany). We modified the provided protocol by incubating the samples in 1.2 mL of InhibitEX buffer overnight and adding 0.5 mL of InhibitEX buffer after 24 h. Isolated DNA was diluted and stored at 8 °C.

Establishment of a Microsatellite Panel and Genotyping

Using DNA extracted from 18 blood samples, we investigated 45 microsatellite loci that have been found to be highly polymorphic in other primate species (for details on investigated markers, see [Supplementary Table S1](#) online). We followed the two-step multiplex approach by running 2 successive polymerase chain reactions (PCRs) (Arandjelovic et al. 2009) to increase the amount of the PCR amplification. The first PCR (multiplex PCR) comprised up to 15 primer pairs simultaneously (15 µL approach) followed by a singleplex PCR (i.e., PCR for each locus) containing diluted multiplex products as amplification templates (10 µL approach) (see [Supplementary Material](#) online for “PCR protocol and sequencing”). All reactions were performed in a *Mastercycler® Pro* thermal cycler (Eppendorf, Hamburg, Germany). Following Engelhardt et al. (2017), the protocols were later optimized for fecal samples to work noninvasively (see [Supplementary Material](#) online for “PCR protocol and sequencing”). For fragment analysis, PCR products of different allele ranges and/or different fluorescent labels were combined for cost efficiency. The sequencing was performed by using an *ABI 3730* sequencer (see [Supplementary Material](#) online for “PCR

protocol and sequencing"). For determination of the allele sizes, the sequencing output was imported into PeakScanner software (Applied Biosystems®).

Selection of Markers

We selected a total of 21 markers for genotyping (see Table 1). First, we chose 15 core markers genotyped for all 197 individuals with an average of 14.56 ± 0.79 (mean \pm SD) marker typed per individual (see Supplementary Table S2a and b online) and tested for marker suitability (see the following marker selection criteria). Later, we added 6 additional markers for 59 of 197 individuals, to improve a given paternity exclusion rule or confirm unsolved paternities (see Supplementary Material online for "Additional six marker"). We used the following criteria for marker selection based on blood samples: preference was given to 1) tetra-nucleotide repeat markers, 2) markers with at least 4 unique alleles, 3) markers with reliable allele size scoring (no or few stutters/multiple peaks), and 4) highly polymorphic markers based on standard population genetic parameters. To test the latter, we calculated the probability that an individual would be heterozygous at a given locus (expected heterozygosity, H_{exp}), determined the actual observed heterozygosity (H_{obs}), determined the polymorphic information content (PIC), tested for any deviation from Hardy-Weinberg equilibrium (HWE), and estimated the frequency of null alleles ($F(\text{Null})$) (see Supplementary Material online for "Criteria thresholds"). All parameters were calculated from allele frequencies in *Cervus 3.0* (Kalinowski et al. 2007). For a heterozygous genotype to be approved, we aimed to confirm both alleles via 2 amplifications of 2 independent samples (mean number \pm SD of confirmation = 4.09 ± 2.21 , mean number of amplification = 4.76), whereas for a homozygous genotype, we aimed to confirm the one allele on average 4 times using 2 independent samples (mean number \pm SD of confirmation = 4.40 ± 2.26 ,

mean number of amplification = 4.43). This approach was shown to produce reliable genotypes without DNA quantification (Bellemain et al. 2005; Piggott et al. 2006).

We also tested the marker suitability by confirming the Mendelian inheritance of the markers in mother-offspring pairs. Behavioral mothers were identified from long-term observations (nursing and association). To confirm them as genetic mothers, we tested Mendelian inheritance of 97 mother-offspring pairs by matching their genotypes using the 15 core markers.

Paternity Assignment

We typed our 197 individuals at an average of 15.59 ± 1.92 loci (mean \pm SD) when including all 21 markers. However, in order to be included in a paternity analysis, mother-offspring-putative father trios required genotypes at 12 common markers. We assigned paternity using a combination of an exclusion and likelihood approach. We used the program *FindSire* (<http://www.uni-kiel.de/medinfo/mitarbeiter/krawczak/download/>, accessed September 28, 2018) to exclude potential sires mismatching a given mother-offspring pair at a particular locus. Although spermatogenesis starts at 3.5 years of age (Whitten and Turner 2009), males tend to disperse from their natal group at around 4–5 years (Henzi and Lucas 1980). However, given the ability to reproduce, we considered males older than 1250 days at the beginning of the mating season in question as potential sires. We usually knew the age of natal males from demographic records, except for 7 subjects emigrating into our study groups before the start of the systematic data collection, which we therefore also included as potential sires. Our paternity assignment followed a conservative approach by considering paternity as established only if 1) paternity exclusion of all other potential sires was based at least on the "best match" criteria and 2) paternity likelihood for a given parent-offspring trio was at the 95% confidence

Table 1. Overview of microsatellites (core markers 1–15, additional markers 16–21)

Number	Locus	k	N	H_{obs}	H_{exp}	PIC	$F(\text{Null})$	Repeat
1	D1S518	8	195	0.821	0.799	0.770	-0.020	Tetra
2	D1S548	4	197	0.680	0.671	0.605	-0.010	Tetra
3	D4S2408	8	186	0.672	0.706	0.655	0.025	Tetra
4	D4S243	9	193	0.845	0.815	0.788	-0.019	Tetra
5	D5S1457	4	197	0.736	0.684	0.627	-0.037	Tetra
6	D5S1467	5	197	0.604	0.580	0.491	-0.023	Tetra
7	D6S474	15	191	0.859	0.854	0.836	-0.006	Tetra
8	D6S501	6	194	0.768	0.696	0.660	-0.066	Tetra
9	D7S2204	7	195	0.667	0.692	0.629	0.016	Tetra
10	D8S1106	4	196	0.735	0.664	0.590	-0.053	Tetra
11	D10S1432	9	186	0.812	0.790	0.758	-0.015	Tetra
12	D10S611	4	197	0.807	0.713	0.655	-0.065	Tetra
13	D13S765	12	163	0.699	0.737	0.695	0.023	Tetra
14	D17S1304	7	194	0.789	0.792	0.760	-0.001	Tetra
15	D19S245	11	188	0.787	0.817	0.793	0.020	Tetra
16	D2S1333	8	26	0.462	0.725	0.677	0.225	Tetra
17	D6S1017	4	33	0.273	0.291	0.260	0.082	Tetra
18	D6S493nigra	5	38	0.632	0.711	0.646	0.049	Tetra
19	D11S2002	12	43	0.581	0.708	0.673	0.098	Tetra
20	D12S67nigra	9	47	0.638	0.735	0.689	0.057	Tetra
21	D18S536	7	17	0.647	0.688	0.625	0.029	Tetra
Mean		7.5	146.3	0.691	0.708	0.661	0.015	
SD		3.1	73.4	0.138	0.115	0.122	0.065	

Name of locus with indication if modified for *Macaca nigra* (locus), number of alleles at locus (k), number of individuals typed at locus (N), observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}), polymorphic information content (PIC), estimated null allele frequency ($F(\text{Null})$), and nucleotide motif repeats for each locus (repeat). Note that the last 6 markers were used on a limited number of specific trios only to enhance paternity exclusion, leading to different genetic parameters.

level, as calculated in *Cervus 3.0* (for details, see [Supplementary Material](#) online for “Paternity criteria” and [Engelhardt et al. 2017](#); [Widdig et al. 2017](#)).

Behavioral Observations

During our study period (2013–2017), we collected mating interactions (i.e., genital sniff, grab, female refusal, mount, ejaculation) and dominance interactions (i.e., submission, displace, supplant, facial threat, vocal threat, lunge, physical contact) (cf. [Freeman 2012](#)). Up to 2 observers on each group collected data for 10 h on a near daily basis (see [Supplementary Material](#) online for “Field site”). Given the relative low frequency of mating and dominance interactions known for this species ([Struhsaker 1967b](#)), we applied the method of ad libitum sampling ([Altmann 1974](#)) with a resulting mean \pm SD of 2.24 ± 1.46 mating events and 14.18 ± 12 dominance interactions per day and group over 4 mating seasons. Although we cannot exclude that we missed some interactions, it is likely that we recorded most or all of them, as the habitat of our vervet population has a good visibility and the group spread is small.

Establishing Male Dominance Hierarchy

To establish a male dominance hierarchy per group and season, we used decided dyadic agonistic interactions of males older than approximately 3.5 years. Dominance ranks were calculated in R version 3.2.3 ([R Core Team 2013](#)) with the package “EloRating” ([Neumann et al. 2011](#)) using standardized Elo-ratings averaged over each 4-month mating season to allow for the comparison of ratings between groups of different sizes. For 12 potential sires in 2013 and for 2 each in 2014, 2015, and 2016, it was not possible to calculate a rank position, as they resided only a few days within the groups and no agonistic interactions were observed. Similarly, we did not observe agonistic interactions for extra-group males mating or reproducing in one of our study groups. Therefore, these males had to be excluded from investigations of the relationship between mating/paternity success and dominance rank.

Mating and Reproductive Skew

To determine the mating and reproductive skew for each group and season, we calculated the Nonacs’ *B* index ([Nonacs 2000, 2003](#)) using the Skew Calculator 2013 (<https://www.eeb.ucla.edu/Faculty/Nonacs/pi.html>, accessed September 28, 2018). The *B* index specifies whether a given distribution is significantly different from random, with positive values up to 1 indicating a higher skew than expected, negative values down to -1 indicating a more equal distribution of reproduction, and values around 0 indicating a random distribution. In addition, the program calculates the lower and upper confidence intervals (CIs, 95%), the minimum *B* value (equal*B*), and the maximum *B* value (monopol*B*) via simulations, which further help to interpret the results. When the CIs include 0, the distribution is not significantly different from random. In cases where the lower CI is smaller than the minimum *B* value, we cannot reject the possibility of an equal distribution. In cases where the upper CI is larger than the maximum *B* value, then a complete monopolization by one individual cannot be excluded (cf. [Strier et al. 2011](#)). As the *B* index accounts for the time spent within a group, we included information of male group residency based on existing demographic data of our study population (S.P.H., L.B., unpublished data). To calculate the mating skew, we used the number of successful matings per male observed relative to his tenure for the respective season in a given group (April–July between 2013 and 2016, respectively). We defined

male reproductive success as the number of genetically assigned born or miscarried offspring per male. To compute reproductive skew, we used the number of offspring sired per male relative to his tenure for the respective season in the birth group of the respective offspring. For certain groups and seasons, it was not reasonable to calculate the reproductive skew as the number of infants or potential sires was too low to produce a meaningful *B* index.

To investigate the influence of dominance rank on mating and paternity success, respectively, we ran Spearman rank correlations per group and season in R ([R Core Team 2013](#)). As some males were included in multiple groups and seasons, skew data were not entirely independent; hence, we adjusted the α -levels (α') for the mating and reproductive skew as well as for the correlations between dominance rank versus mating or paternity success, respectively, based on the number of carried out tests and the number of significant tests ([Cross and Chaffin 1982](#)), and only considered *P* values as significant when they were smaller than the adjusted α -level.

EGP and Natal Breeding

Although we have no data on group compositions before 2008, we assessed group membership and immigration on a near daily basis from collected demographic data (2008–2017) with young males (less than 3.5 years of age) generally considered as natal males unless we observed a migratory event.

To assess the degree of EGPs, we confirmed group membership of the assigned sires around the time of conception of the infant under consideration. In the case of a migratory event, we considered males as immigrated when they constantly resided for at least 14 days in a new group and noted the first day seen on the new group as immigration date. A conception window was estimated by subtracting 161 and 165 days, respectively, from the date of birth of a given infant (163 ± 2 days, *Chlorocebus* gestation length according to [Bramblett et al. 1975](#)), resulting in a 5-day conception window. To avoid assigning an EGP to a possible migratory event, we set a window of 14 days prior and 14 days after the conception window, in total a 33-day window for the assignment of EGPs (adapted from [Ruiz-Lambides et al. 2017](#)). Infants sired by males who were members of a different social group than the infants’ birth group outside of the previously defined 33-day window were assigned as EGPs.

Based on demographic data, we were also able to estimate whether sires were natal to the infants’ birth group or not. Breeding events in which infants were sired by males still residing within their natal group (i.e., prior to natal dispersal) were defined as natal breeding.

Results

Marker Characteristics

Table 1 presents the 15 best markers (core markers) selected by confirming all criteria described earlier together with the 6 additional markers we used to enhance paternity exclusion for some trios. Based on the 197 individuals genotyped, alleles per marker ranged from 4 to 15 and the observed mean heterozygosity was similar to the expected mean heterozygosity (0.691 vs. 0.708, see **Table 1**). The mean PIC of 0.661 indicated a high power for discrimination of our microsatellites; we also found no signs of null alleles (mean $F(\text{Null}) = 0.015$). There was no significant deviation from HWE, except for 1 marker (D6S501). However, we can exclude the possibility of a segregating null allele for this locus (see **Table 1**,

$F(\text{Null}) = -0.066$) and a failure to distinguish alleles; therefore, we still included this locus in our parental assignment.

We further tested marker suitability by confirming Mendelian inheritance of markers for putative mother–offspring pairs based on observations. We confirmed 95 of the 97 behavioral mothers by matching genotypes of mother–infant pairs. The 2 remaining infants were adopted for unknown reasons by different females, although the genetic mother was still alive in the same group. All genetically determined maternities were used for the paternity analysis.

Paternity Analysis

From a total of 51 potential sampled sires, 29 males were assigned as fathers to at least 1 offspring born during our study period. Over 3 groups and 4 birth seasons, we determined paternities for 94 of 97 infants (96.91%) with high confidence using both exclusion and likelihood methods (for details, see [Supplementary Material](#) online for “Paternity results” and [Supplementary Table S3](#) online). The remaining 3 infants, typed on 15–19 markers, could not be assigned to any of the males we sampled indicating that we probably lack a sample of the actual sire. For the 2013 cohort, we have 2 unsolved paternities, with missing samples from 2 within-group males. For the 2015 cohort, we collected samples of all potential sires within the groups, hence the unsolved paternity is most likely due to an EGP.

Mating and Reproductive Skew versus Dominance

When we pooled the observed matings over the 4 seasons, all 3 groups showed a significantly mating skew, although B indices were consistently small (mating skew per group, considering males present in several seasons only once, $\alpha' = 0.05$, B index: PT = 0.023, $P < 0.001$, $N_{\text{males}} = 22$; RBM = 0.030, $P < 0.001$, $N_{\text{males}} = 30$; RST = 0.030, $P < 0.001$, $N_{\text{males}} = 31$). A similar picture emerged when looking at groups and seasons separately: mating was significantly skewed for the groups PT and RBM in all seasons and for RST in

2 of 4 seasons after α -level adjustment (see [Table 2](#)). However, the interpretation of these results needs to be treated with caution, as for all groups and seasons (except for PT in 2014), the B indices were close to 0, suggesting a random distribution. In addition, for RBM 2014–2016 and RST 2013–2014, the CIs included 0, and the lower CIs were equal to the equal B values, indicating that, in some seasons and groups, mating either occurred at random or was equally distributed ([Table 2](#)).

Furthermore, over all groups and all seasons, male mating success was not significantly correlated with male dominance rank after α -level adjustment (see [Table 2](#)). Across all groups and mating seasons, the mating success of the alpha male ranged from 0% to 57.8% ($20.3 \pm 18.5\%$, mean \pm SD), which confirms the limited potential of male monopolization in vervet monkeys, at least in some seasons and groups.

Paternity was not consistently skewed across groups when pooled across all 4 seasons, and all groups showed B indices close to 0 (reproductive skew per group, considering males present in several seasons only once, $\alpha' = 0.025$, B index: PT = 0.062, $P < 0.007$, $N_{\text{males}} = 23$; RBM = 0.061, $P < 0.001$, $N_{\text{males}} = 30$; RST = 0.005, $P = 0.248$, $N_{\text{males}} = 30$). When we considered groups and seasons separately, paternity was not skewed for 8 of a total of 9 groups and seasons. In 5 of those, CIs indicated no significant deviation from a random distribution, and the lower CIs were the same as the equal B s, indicating that an equal distribution of paternities cannot be excluded. After α -level adjustment, only the paternity distribution of PT in 2013 remained significantly skewed (see [Table 3](#)).

In contrast to mating success, however, paternity success was at least partly determined by male dominance. For RBM and RST in mating season 2014, we found a significant positive correlation between dominance rank and paternity success, which remained after α -level adjustment (see [Table 3](#)). This suggests that males of higher dominance rank produced more offspring than males of lower dominance rank, which seems not to be the case for other

Table 2. Mating skew over 3 groups and 4 mating seasons

Group-season	N_p	N_b	% α -mat	% α -tenure	B index	P (B index)	equal B	monopol B	ICI	uCI	Rho	P (rho)
						$\alpha' = 0.017$						$\alpha' = 0.0045$
PT-13	13 (10)	9	20.6	69.7	0.040	<0.001	-0.012	0.932	0.019	0.078	-0.103	0.776
PT-14	6 (6)	6	50	97.6	0.130	<0.001	-0.023	0.808	0.053	0.248	0.174	0.742
PT-15	4 (4)	4	57.8	98.6	0.073	<0.001	-0.010	0.607	0.019	0.168	^a	
PT-16	10 (10)	9	24.5	70.5	0.032	<0.001	-0.009	0.879	0.012	0.064	0.300	0.400
RBM-13	24 (19)	13	12.9	100.0	0.055	<0.001	-0.013	1.036	0.031	0.769	0.052	0.832
RBM-14	21 (20)	15	6.4	100.0	0.017	0.016	-0.020	0.921	-0.020	0.046	0.189	0.425
RBM-15	14 (12)	10	16.2	98.7	0.031	<0.001	-0.006	0.878	-0.006	0.055	0.348	0.268
RBM-16	8 (6)	4	34.4	100.0	0.048	0.002	-0.012	1.090	-0.012	0.147	0.580	0.228
RST-13	20 (14)	6	0	95.2	0.026	0.242	-0.132	0.922	-0.132	0.657	-0.162	0.581
RST-14	14 (13)	10	5	97.3	0.016	0.186	-0.046	0.875	-0.046	0.085	-0.573	0.041 ^b
RST-15	17 (17)	13	4.2	100.0	0.020	<0.001	-0.007	0.913	0.010	0.037	-0.124	0.634
RST-16	19 (17)	15	11.6	100.0	0.047	<0.001	-0.006	1.066	0.030	0.109	0.160	0.538
Mean	14.2 (12.3)	9.5	20.3	94.0	0.045							
SD	6.3 (5.3)	3.9	18.5	11.3	0.032							

Number of potential males (troop + extra-group males) for skew calculation and number of males used for Spearman rank correlation (in parenthesis) (N_p), number of observed mating males (N_b), alpha male share of mating (% α -mat), alpha male tenure share of 4 month mating season (% α -tenure), the observed B value (B index), corresponding P value of the B index (P (B index)), B index for an equal sharing of mating (equal B), B index for complete monopolization of mating (monopol B), lower confidence interval (ICI: 95%), upper confidence interval (uCI: 95%), correlation coefficient for Spearman rank correlation (rho) between dominance rank and mating success, corresponding P value of rho (P (rho)), P values (for B index and rho) are in bold when remaining significant (i.e., when smaller than the adjusted α -level [α']) and in gray shading when ICI and uCI included 0 and when equal B and ICI are equal.

^aSpearman rank test for groups and seasons with less than 5 potential mating males are not determined.

^b P value considered significant before comparison with adjusted α -level.

Table 3. Reproductive skew over 3 groups and 4 mating seasons

Group-season	N_p	N_b	InfS	InfnS	%	B index	P (B index)	equalB	monopolB	ICI	uCI	Rho	P (rho)
PT-13	13 (10)	3	8	0	0.0	0.181	0.004	-0.111	0.766	0.035	0.497	0.291	0.415
PT-14	7 (6)	6	11	0	27.3	-0.008	0.476	-0.075	1.082	-0.075	0.910	0.883	0.020 ^b
PT-15	4 (4)	2	2	0	50.0	^a	^a	^a	^a	^a	^a	^a	^a
PT-16	10 (10)	1	1	1	0.0	^a	^a	^a	^a	^a	^a	^a	^a
RBM-13	23 (19)	5	9	1	22.2	0.091	0.023 ^b	-0.105	0.873	0.012	0.272	0.474	0.040 ^b
RBM-14	21 (20)	6	12	1	33.3	0.087	0.011 ^b	-0.079	0.860	0.015	0.234	0.727	< 0.001
RBM-15	16 (12)	6	10	1	33.3	0.040	0.173	-0.099	0.999	-0.099	0.321	0.313	0.322
RBM-16	8 (6)	2	3	6	66.7	^a	^a	^a	^a	^a	^a	^a	^a
RST-13	19 (14)	6	12	0	10.0	0.088	0.014 ^b	-0.093	0.837	0.012	0.254	0.183	0.531
RST-14	15 (13)	7	10	1	20.0	0.018	0.247	-0.092	0.981	-0.092	0.931	0.733	0.004
RST-15	17 (17)	7	10	2	10.0	0.012	0.308	-0.092	0.825	-0.092	0.128	-0.183	0.483
RST-16	17 (17)	8	9	1	11.1	-0.051	0.947	-0.101	0.935	-0.101	0.188	0.299	0.244
Mean	14.17	4.92			23.7	0.051							
SD	5.88	2.31			20.0	0.069							

Number of potential sires (troop + extra-group sires) for skew calculation and number of potential sires used for Spearman rank correlation (in parenthesis (N_p), number of observed sires (N_b), number infants sampled (InfS), number infants not sampled (InfnS), alpha male share of paternity (% α -pat), the observed B value (B index), corresponding P value of the B index (P (B index)), B index for an equal sharing of paternity (equalB), B index for complete monopolization of paternity (monopolB), lower confidence interval (ICI: 95%), upper confidence interval (uCI: 95%), correlation coefficient for Spearman rank correlation (rho) between dominance rank and paternity success, corresponding P value of rho (P (rho)), P values (for B index and rho) are in bold when remaining significant (i.e., when smaller than the adjusted α -level [α'] and in gray shading when ICI and uCI included 0 and when equalB and ICI are equal.

^aFor groups and seasons with less than 5 infants sampled, there is not enough power to calculate the B index. Spearman rank test for groups and seasons with less than 5 potential sires or with less than 5 infants sampled are not determined.

^b P value considered significant before comparison with adjusted α -level.

groups and seasons. Across all groups and seasons, alpha share of paternity success ranged from 0% to 66.7% ($23.7 \pm 20.0\%$, mean \pm SD), indicating that, on average, alpha males had a limited ability to monopolize paternities across seasons and groups.

EGP and Natal Breeding

Among our 94 infants with assigned paternities, we detected 6 offspring assigned as EGPs (6.4%) and 4 offspring sired by natal males (4.3%). However, due to the lack of DNA samples for some potential sires, we could have actually missed one additional EGP (see results section "Paternity Analysis"). Note that the degree of EGP partly depends on how it was defined; if we apply a more conservative EGP assignment rule with a 30-day window before and after the conception window (data not shown), the number of EGPs would have been reduced to 4 EGPs (4.3%). For 2 of our 6 EGP offspring, the sires never resided in the group of their offspring, 2 sires had emigrated from the infant's group before conception, and 2 sires moved afterwards into the group (19 and 50 days after end of conception window, respectively). For the 3 natal breeders, the date of birth was unknown; however, genetic analysis revealed that 2 males were still residents in the group of their mother, confirming genetically that 3 infants were sired via natal breeding.

Discussion

After successful establishment of our marker panel, we were able to determine the paternity of 94 of 97 sampled infants sired between 2013 and 2016 in our study population. Our study confirmed previous observations (Struhsaker 1967b; Cramer 2012) that the mating frequency in vervet monkeys is relatively low. Furthermore, matings in our study population were consistently skewed over 4 seasons and 3 groups, but always of low magnitude. Male mating success was

not significantly influenced by male dominance rank, with males of different ranks mating equally often (over 4 mating seasons: mean \pm SE: PT: 8.3 ± 1.4 ; RBM: 7.2 ± 1.1 ; RST: 7.2 ± 1.1 successful matings per male observed). However, inspection of the data revealed that for most seasons and groups, the relationship between male dominance and mating success was positive (i.e., more matings by higher-ranking males), with only RST in 3 of 4 seasons deviating from this pattern. RST was the largest group and showed a slightly female-biased sex ratio, which might have enabled females to better exert mate choice in this group. The alpha males' share of matings was restricted to a mean of 20.3% over 4 seasons and 3 groups, which is rather low when compared with other primate species. For example, in bonobos, a species with limited male monopolization potential, the highest-ranking male in a party accounted for 40.8% of matings (Surbeck et al. 2011). However, when compared with another mammal, the spotted hyena (5% alpha male share of matings, Engh et al. 2002) in which males also have a limited male monopolization potential, the vervet alpha males' share of matings is 4-fold greater. Over our study period, matings were predominately initiated by males, but females resisted male mating attempts in approximately 28.6% of all sexual interactions observed (M.M.I.M., unpublished data). This implies the possibility that mating success, but also paternity output, can be affected by female mate choice, including cryptic choice (Kappeler and van Schaik 2004). In vervet monkeys, the equal distribution of matings across the male hierarchy, together with the potentially concealed ovulation (Andelman 1987), could therefore be due to females trying to confuse paternity (reviewed in Kappeler and van Schaik 2004). Interestingly, refusals of mating attempts by females may even be more frequent around ovulation (Andelman 1987; C.Y., unpublished data). Hence, females may be able to use different strategies to select the best sires for their offspring. In contrast to our results, mating seems to be skewed in favor of high-ranking and central males in other species with female codominance

or female dominance (Sauther 1991; Surbeck et al. 2011). This difference could be due to bonobo females advertising ovulation via sexual swellings and ring-tailed lemur females behaviorally advertising ovulation, both of which facilitate male monopolization potential. In addition, recent work revealed that male vervets being well integrated in the female networks tend to achieve higher dominance ranks (Young et al. 2017), which suggests males could gain mating access to females through positive associations rather than monopolization. This is also supported by the fact that we found no relationship between male dominance and mating success.

With respect to paternity success, our data revealed that paternity is not consistently skewed. This is in contrast with previous studies in species with limited male monopolization potential, where high-ranking males tend to gain a higher reproductive success than low-ranking males (Kappeler and Schaffler 2008; Surbeck et al. 2017; see the following discussion). Over 4 cohorts and 3 groups, reproductive skew was generally low and varied across seasons and groups. In comparison to mating success, we found a consistent positive relationship between male dominance and reproductive success, although this only reached statistical significance in 2 groups for one season. However, similarly to the monopolization potential, reproductive skew may be affected by several key variables, such as the stability of male hierarchy (Alberts et al. 2006), the degree of female synchrony, the number of within-group competitors (Ostner et al. 2008), and the number of females within a group (Kappeler and Port 2008) as discussed later.

Our paternity findings contrast with results from the closely related green monkey (*Chlorocebus sabaeus*), a species in which females can also reject male mating attempts (Keddy 1986). In a study of captive green monkeys, Weingrill et al. (2011) demonstrated that the alpha male was able to sire 76% of all infants within the group. However, the captive setting and artificial group composition (3 young males, aged 3–5 years and a 9-year-old alpha male) may explain this difference. In captivity, the male hierarchy is likely to be more stable compared with wild populations, where male emigration and immigration is frequent. In fact, in wild Hanuman langurs (*Semnopithecus entellus*), 93% of rank changes were due to immigration of males (Borries 2000). Hierarchy instability might explain the low alpha male share of paternity in the group PT in 2013, as the alpha male received 2 injuries during this mating season and died soon after the last injury. Similarly, just before the mating season 2016, the alpha male in PT dispersed and 8 new males immigrated. The subsequent instability in the group hierarchy most likely served to reduce the reproductive skew.

Overall, the average reproductive skew was rather low ($B = 0.051$), compared with other studies on species with limited male monopolization potential. For example, in bonobos, a species with female codominance, the average reproductive skew varied (over 7 years: $B = 0.22$, over 12 years: $B = 0.08$, Surbeck et al. 2017), with high-ranking males siring more offspring than low-ranking males. Ring-tailed lemurs, which perform female dominance and behaviorally advertise ovulation, showed a rather high reproductive skew ($B = 0.423$, mean calculated from B indices of Parga et al. 2016), whereas Assamese macaques (*Macaca assamensis*), which have a concealed ovulation, had a low paternity skew ($B = 0.087$, Sukmak et al. 2014). Interestingly, there were only 2 studies with a comparable low reproductive skew ($B = 0.012$, Strier et al. 2011; $B = 0.014$, Dubuc, Ruiz-Lambides, et al. 2014). The first one was conducted on a group of egalitarian, male philopatric northern muriquis (*Brachyteles hypoxanthus*). This study suggested that the low skew was caused by maternal kin networks between males, reducing

individual reproductive success, while related males benefited from shared paternities (Strier et al. 2011). The second study was conducted on rhesus macaques, where low reproductive skew was suggested to be due to the reduced male–male competition levels typical of this species (Dubuc, Ruiz-Lambides, et al. 2014). As male vervets disperse from their natal group, networks between related males are less likely to explain our findings unless males emigrate with relatives of similar age. In addition, low male–male competition is an unlikely explanation for the low skew found in our population, as vervet males tend to be involved in severe fights during the mating season (Freeman 2012). Although a severe drought reduced the number of infants in RBM and PT in 2016, it is unlikely that this event explains the pattern we have found, as we find consistent results for RST over all 4 seasons including the year of the drought.

Furthermore, the number of EGPs in our vervet population was low (6.4%) compared with other studies. For example, in ring-tailed lemurs, a species with a similar sex ratio and limited male monopolization potential, the number of EGPs was high (21–33% EGPs, Parga et al. 2016). However, the high number of EGPs in the study of Parga et al. (2016) might be misleading, as their sample size was relatively small ($N = 14$ and 9 offspring, respectively) and residency data of some males were missing. The low number of EGPs in our vervet population could be due to the fact that males change groups frequently (every 2 years, C.Y., unpublished data) making outbreeding less advantageous for females, given that seeking matings outside the group exposes females to both predation risk (Kappeler 1999) and intergroup aggression (Nichols et al. 2015). In addition, primate groups with a female-biased sex ratio show higher numbers of EGPs (Lawler et al. 2003; Ruiz-Lambides et al. 2017), suggesting that in groups with a balanced (our population) to male-biased sex ratio, the number of EGPs is likely to be smaller as males should be able to exclude extra-group males from mating. Furthermore, group instability is linked to higher numbers of EGPs (Isvaran and Clutton-Brock 2007), which may explain 4 of 6 EGP offspring in our population, which were sired during changeovers of alpha males.

Finally, the level of natal breeding in our population was also low (4 infants or 4.3%). Of these 4 offspring, 3 were still alive in July 2018 (969–1354 days old). It has been suggested that mortality due to inbreeding depression is still possible until maturation (Widdig et al. 2017), which suggests that these individuals are unlikely to be subject of close inbreeding as they reach reproductive age (1250 days). Hence, mating partners of our natal breeding males seem to be descended from differing maternal families. In addition, all 4 mothers were multiparous, rejecting the possibility that females, breeding with a natal male, might have been inexperienced with reproduction. In other mammal populations, natal breeding was rare (spotted hyenas, Engh et al. 2002; Japanese macaques, Inoue and Takenaka 2008; capuchin monkeys, Wikberg et al. 2017), and the scarcity was most likely due to inbreeding avoidance. Male vervet monkeys tend to disperse for the first time around the age of maturation (Henzi and Lucas 1980). We rarely observed matings involving natal males or even observed aggressive refusals of natal males' mating attempts in our population (M.M.I.M., personal observations), which is in line with the theory of inbreeding avoidance. In rhesus macaques, late dispersing males had higher lifetime reproductive success probably because they started reproducing in their natal groups (Weiß et al. 2016). Consequently, late dispersal and natal breeding could be a reproductive strategy to accomplish high lifetime reproductive success. However, more data are needed to assess whether this is indeed the case.

Overall, although male dominance seems to be an important factor influencing mating access and paternity success in other primates with limited male monopolization potential, male dominance in our vervet population seems to be a poor predictor of the variation in mating and reproductive skew. This suggests that other factors might influence male fitness in vervet monkeys. To better understand male reproductive strategies in species with limited male monopolization potential, future studies should investigate other male characteristics such as social abilities (male care, Langos et al. 2013; Ostner et al. 2013; male–female friendship, Smuts 1985; Kulik et al. 2012; male–male coalitions, Young et al. 2013) and proxies of health (e.g., secondary sexual traits in form of ornaments, Dixon et al. 2005; Dubuc, Winters, et al. 2014) and their impact on male reproductive success.

Conclusions

The genetic analysis based on 21 highly polymorphic markers in our population of vervet monkeys exceeded that of previous studies (Newman et al. 2002; Weingrill et al. 2011) and revealed new insights into reproductive strategies of a species with female codominance. Overall, mating and paternity skew was generally of low magnitude. Furthermore, although the mating success was not related to male dominance, paternity success was partly predicted by male dominance, suggesting that female choice may play a role in male vervet reproductive success. The successful cross-species amplification for the vervet monkey suggests that the proposed markers could be helpful for further investigations of other *Chlorocebus* species, for example, to benefit conservation efforts in closely related species (e.g., vulnerable bale monkey, *Chlorocebus djamdjamensis*, Butynski et al. 2008) or to investigate determinants of male reproductive success in the light of female choice, as well as the interplay between female and male reproductive strategies in vervet monkeys.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

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Data Availability

Details on tested markers, individual genotypes, and paternities is available in the [Supplementary Material](#) online.

References

- Alberts SC, Buchan JC, Altmann J. 2006. Sexual selection in wild baboons: from mating opportunities to paternity success. *Anim Behav.* 72:1177–1196.
- Altmann J. 1974. Observational study of behavior: sampling methods. *Behaviour.* 49:227–267.
- Andelman SJ. 1987. Evolution of concealed ovulation in vervet monkeys (*Cercopithecus aethiops*). *Am Nat.* 129:785–799.
- Arandjelovic M, Guschanski K, Schubert G, Harris TR, Thalmann O, Siedel H, Vigilant L. 2009. Two-step multiplex polymerase chain reaction improves the speed and accuracy of genotyping using DNA from noninvasive and museum samples. *Mol Ecol Resour.* 9:28–36.
- Bellemain E, Swenson JE, Tallmon D, Brunberg S, Taberlet P. 2005. Estimating population size of elusive animals with DNA from hunter-collected feces: four methods for brown bears. *Conserv Biol.* 19:150–161.
- Boesch C, Kohou G, Néné H, Vigilant L. 2006. Male competition and paternity in wild chimpanzees of the Taï forest. *Am J Phys Anthropol.* 130:103–115.
- Borries C. 2000. Male dispersal and mating season influxes in Hanuman langurs living in multi-male groups. In: Kappeler PM, editor. *Primate males: causes and consequences of variation in group composition*. Cambridge (UK): Cambridge University Press. p. 146–158.
- Bramblett CA, Pejaver LD, Drickman DJ. 1975. Reproduction in captive vervet and Sykes' monkeys. *J Mamm.* 56:940–946.
- Butynski TM, Gippoliti S, Kingdon J, De Jong Y. 2008. *Chlorocebus djamdjamensis*. The IUCN Red List of Threatened Species 2008. p. e.T4240A10699069. Available from: <http://www.iucnredlist.org/details/4240/0>. Accessed September 27, 2018.
- Cheney DL, Seyfarth RM, Andelman SJ, Lee PC. 1988. Reproductive success in vervet monkeys. In: Clutton-Brock T, editor. *Reproductive success: studies of individual variation in contrasting breeding systems*. Chicago: University of Chicago Press. p. 384–403.
- Clutton-Brock TH. 1989. Mammalian mating systems. *Proc Biol Sci.* 236:339–372.
- Coltman DW, Bancroft DR, Robertson A, Smith JA, Clutton-Brock TH, Pemberton JM. 1999. Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. *Mol Ecol.* 8:1199–1209.
- Cramer JD. 2012. *Scrotal color and signal content among South African vervet monkeys (Chlorocebus aethiops pygerythrus)* [dissertation]. Milwaukee (WI): University of Wisconsin–Milwaukee.
- Cross EM, Chaffin WW. 1982. Use of the binomial theorem in interpreting results of multiple tests of significance. *Educ Psychol Meas.* 42:25–34.
- Dewsbury DA. 1982. Dominance rank, copulatory behavior, and differential reproduction. *Q Rev Biol.* 57:135–159.
- Dixon A, Dixon B, Anderson M. 2005. Sexual selection and the evolution of visually conspicuous sexually dimorphic traits in male monkeys, apes, and human beings. *Annu Rev Sex Res.* 16:1–19.

- Dubuc C, Muniz L, Heistermann M, Engelhardt A, Widdig A. 2011. Testing the priority-of-access model in a seasonally breeding primate species. *Behav Ecol Sociobiol.* 65:1615–1627.
- Dubuc C, Muniz L, Heistermann M, Widdig A, Engelhardt A. 2012. Do males time their mate-guarding effort with the fertile phase in order to secure fertilisation in Cayo Santiago rhesus macaques? *Horm Behav.* 61:696–705.
- Dubuc C, Ruiz-Lambides A, Widdig A. 2014. Variance in male lifetime reproductive success and estimation of the degree of polygyny in a primate. *Behav Ecol.* 25:878–889.
- Dubuc C, Winters S, Allen WL, Brent LJ, Cascio J, Maestripieri D, Ruiz-Lambides AV, Widdig A, Higham JP. 2014. Sexually selected skin colour is heritable and related to fecundity in a non-human primate. *Proc Biol Sci.* 281:20141602.
- Ellis L. 1995. Dominance and reproductive success among nonhuman animals—a cross-species comparison. *Ethol Sociobiol.* 16:257–333.
- Engelhardt A, Heistermann M, Hodges JK, Nuernberg P, Niemitz C. 2006. Determinants of male reproductive success in wild long-tailed macaques (*Macaca fascicularis*)—male monopolisation, female mate choice or post-copulatory mechanisms? *Behav Ecol Sociobiol.* 59:740–752.
- Engelhardt A, Muniz L, Perwitasari-Farajallah D, Widdig A. 2017. Highly polymorphic microsatellite markers for the assessment of male reproductive skew and genetic variation in critically endangered crested macaques (*Macaca nigra*). *Int J Primatol.* 38:672–691.
- Engh AL, Funk SM, van Horn RC, Scribner KT, Bruford MW, Libants S, Szykman M, Smale L, Holekamp KE. 2002. Reproductive skew among males in a female-dominated mammalian society. *Behav Ecol.* 13:193–200.
- Feh C. 1999. Alliances and reproductive success in Camargue stallions. *Anim Behav.* 57:705–713.
- Freeman NJ. 2012. *Some aspects of male vervet monkey behaviour* [master's thesis]. [Lethbridge (AB)]: University of Lethbridge.
- Freeman NJ, Pasternak GM, Rubi TL, Barrett L, Henzi SP. 2012. Evidence for scent marking in vervet monkeys? *Primates.* 53:311–315.
- Gerloff U, Hartung B, Fruth B, Hohmann G, Tautz D. 1999. Intra-community relationships, dispersal pattern and paternity success in a wild living community of Bonobos (*Pan paniscus*) determined from DNA analysis of faecal samples. *Proc Biol Sci.* 266:1189–1195.
- Gibson KN. 2010. Male mating tactics in spider monkeys: sneaking to compete. *Am J Primatol.* 72:794–804.
- Gowaty PA. 2003. Power asymmetries between the sexes mate preferences and components of fitness. In: Travis CB, editor. *Evolution, gender, and rape*. Cambridge, MA: MIT Press. p. 61–86.
- Gowaty PA. 2004. Sex roles, contests for the control of reproduction, and sexual selection. In: Kappeler PM, van Schaik CP, editors. *Sexual selection in primates—new and comparative perspectives*. Cambridge (UK): Cambridge University Press. p. 37–54.
- Henzi SP, Lucas JW. 1980. Observations on the inter-troop movement of adult vervet monkeys (*Cercopithecus aethiops*). *Folia Primatol (Basel).* 33:220–235.
- Inoue E, Takenaka O. 2008. The effect of male tenure and female mate choice on paternity in free-ranging Japanese macaques. *Am J Primatol.* 70:62–68.
- Isvaran K, Clutton-Brock T. 2007. Ecological correlates of extra-group paternity in mammals. *Proc Biol Sci.* 274:219–224.
- Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol.* 16:1099–1106.
- Kappeler PM. 1999. Primate socioecology: new insights from males. *Naturwissenschaften.* 86:18–29.
- Kappeler P, Port M. 2008. Mutual tolerance or reproductive competition? Patterns of reproductive skew among male redfronted lemurs (*Eulemur fulvus rufus*). *Behav Ecol Sociobiol.* 62:1477–1488.
- Kappeler P, Schäffler L. 2008. The lemur syndrome unresolved: extreme male reproductive skew in sifakas (*Propithecus verreauxi*), a sexually monomorphic primate with female dominance. *Behav Ecol Sociobiol.* 62:1007–1015.
- Kappeler PM, van Schaik CP. 2004. Sexual selection in primates: review and selective preview. In: Kappeler PM, van Schaik CP, editors. *Sexual selection in primates—new and comparative perspectives*. Cambridge (UK): Cambridge University Press. p. 3–23.
- Keddy AC. 1986. Female mate choice in vervet monkeys (*Cercopithecus aethiops sabaesus*). *Am J Primatol.* 10:125–134.
- Kleiman DG, Malcolm JR. 1981. The evolution of male parental investment in mammals. In: Gubernick DJ, Klopfer PH, editors. *Parental care in mammals*. New York: Plenum Publishing Corporation. p. 347–387.
- Kulik L, Muniz L, Mundry R, Widdig A. 2012. Patterns of interventions and the effect of coalitions and sociality on male fitness. *Mol Ecol.* 21:699–714.
- Kutsukake N, Nunn CL. 2009. Causes and consequences of reproductive skew in male primates. In: Hager R, Jones CB, editors. *Reproductive skew in vertebrates*. Cambridge (UK): Cambridge University Press. p. 165–195.
- Langos D, Kulik L, Mundry R, Widdig A. 2013. The impact of paternity on male-infant association in a primate with low paternity certainty. *Mol Ecol.* 22:3638–3651.
- Lawler RR, Richard AF, Riley MA. 2003. Genetic population structure of the white sifaka (*Propithecus verreauxi verreauxi*) at Beza Mahafaly Special Reserve, southwest Madagascar (1992–2001). *Mol Ecol.* 12:2307–2317.
- McFarland R, Fuller A, Hetem RS, Mitchell D, Maloney SK, Henzi SP, Barrett L. 2015. Social integration confers thermal benefits in a gregarious primate. *J Anim Ecol.* 84:871–878.
- McFarland R, Hetem RS, Fuller A, Mitchell D, Henzi SP, Barrett L. 2013. Assessing the reliability of bilogger techniques to measure activity in a free-ranging primate. *Anim Behav.* 85:861–866.
- Miller EJ, Eldridge MDB, Cooper DW, Herbert CA. 2010. Dominance, body size and internal relatedness influence male reproductive success in eastern grey kangaroos (*Macropus giganteus*). *Reprod Fertil Dev.* 22:539–549.
- Neumann C, Duboscq J, Dubuc C, Ginting A, Irwan AM, Agil M, Widdig A, Engelhardt A. 2011. Assessing dominance hierarchies: validation and advantages of progressive evaluation with Elo-rating. *Anim Behav.* 82:911–921.
- Newman TK, Fairbanks LA, Pollack D, Rogers J. 2002. Effectiveness of human microsatellite loci for assessing paternity in a captive colony of vervets (*Chlorocebus aethiops sabaesus*). *Am J Primatol.* 56:237–243.
- Nichols HJ, Cant MA, Sanderson JL. 2015. Adjustment of costly extra-group paternity according to inbreeding risk in a cooperative mammal. *Behav Ecol.* 26:1486–1494.
- Nonacs P. 2000. Measuring and using skew in the study of social behavior and evolution. *Am Nat.* 156:577–589.
- Nonacs P. 2003. Measuring the reliability of skew indices: is there one best index? *Anim Behav.* 65:615–627.
- Nsubuga AM, Robbins MM, Roeder AD, Morin PA, Boesch C, Vigilant L. 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Mol Ecol.* 13:2089–2094.
- Ostner J, Nunn CL, Schülke O. 2008. Female reproductive synchrony predicts skewed paternity across primates. *Behav Ecol.* 19:1150–1158.
- Ostner J, Vigilant L, Bhagavatula J, Franz M, Schülke O. 2013. Stable heterosexual associations in a promiscuous primate. *Anim Behav.* 86:623–631.
- Parga JA, Sauther ML, Cuzzo FP, Youssouf Jacky IA, Lawler RR, Sussman RW, Gould L, Pastorini J. 2016. Paternity in wild ring-tailed lemurs (*Lemur catta*): implications for male mating strategies. *Am J Primatol.* 78:1316–1325.
- Pasternak G, Brown LR, Kienzle S, Fuller A, Barrett L, Henzi SP. 2013. Population ecology of vervet monkeys in a high latitude, semi-arid riparian woodland. *Koedoe.* 55:1–9.
- Piggott MP, Banks SC, Stone N, Banffy C, Taylor AC. 2006. Estimating population size of endangered brush-tailed rock-wallaby (*Petrogale penicillata*) colonies using faecal DNA. *Mol Ecol.* 15:81–91.
- Preston BT, Stevenson IR, Pemberton JM, Coltman DW, Wilson K. 2005. Male mate choice influences female promiscuity in Soay sheep. *Proc Biol Sci.* 272:365–373.
- R Core Team. 2013. *R: a language and environment for statistical computing*. Vienna (Austria): R Foundation for Statistical Computing.
- Richard AF, Nicoll ME. 1987. Female social dominance and basal metabolism in a Malagasy primate, *Propithecus verreauxi*. *Am J Primatol.* 12:309–314.

- Røed KH, Holand Ø, Smith ME, Gjøstein H, Kumpula J, Nieminen M. 2002. Reproductive success in reindeer males in a herd with varying sex ratio. *Mol Ecol*. 11:1239–1243.
- Ruiz-Lambides AV, Weiß BM, Kulik L, Stephens C, Mundry R, Widdig A. 2017. Long-term analysis on the variance of extra-group paternities in rhesus macaques. *Behav Ecol Sociobiol*. 71:67.
- Sauther ML. 1991. Reproductive behavior of free-ranging *Lemur catta* at Beza Mahafaly Special Reserve, Madagascar. *Am J Phys Anthropol*. 84:463–477.
- Smuts BB. 1985. *Sex and friendship in baboons*. New York: Aldine.
- Strier KB, Chaves PB, Mendes SL, Fagundes V, Di Fiore A. 2011. Low paternity skew and the influence of maternal kin in an egalitarian, patrilocal primate. *Proc Natl Acad Sci USA*. 108:18915–18919.
- Struhsaker TT. 1967a. Social structure among vervet monkeys (*Cercopithecus aethiops*). *Behaviour*. 29:6–121.
- Struhsaker TT. 1967b. Behavior of vervet monkeys (*Cercopithecus aethiops*). *Univ Calif Publ Zool*. 82:1–64.
- Sukmak M, Wajjwalku W, Ostner J, Schülke O. 2014. Dominance rank, female reproductive synchrony, and male reproductive skew in wild Assamese macaques. *Behav Ecol Sociobiol*. 68:1097–1108.
- Surbeck M, Langergraber KE, Fruth B, Vigilant L, Hohmann G. 2017. Male reproductive skew is higher in bonobos than chimpanzees. *Curr Biol*. 27:R640–R641.
- Surbeck M, Mundry R, Hohmann G. 2011. Mothers matter! Maternal support, dominance status and mating success in male bonobos (*Pan paniscus*). *Proc Biol Sci*. 278:590–598.
- Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B, editor. *Sexual selection and the descent of man*. Chicago: Aldine. p. 139–179.
- van Noordwijk MA, van Schaik CP. 2004. Sexual selection and the careers of primate males: paternity concentration, dominance-acquisition tactics and transfer decisions. In: Kappeler PM, van Schaik CP, editors. *Sexual selection in primates*. Cambridge (UK): Cambridge University Press. p. 208–229.
- Vervaecke H, de Vries H, van Elsacker L. 2000. Dominance and its behavioural measures in a captive group of bonobos (*Pan paniscus*). *Int J Primatol*. 21:47–68.
- Vigilant L, Hofreiter M, Siedel H, Boesch C. 2001. Paternity and relatedness in wild chimpanzee communities. *Proc Natl Acad Sci USA*. 98:12890–12895.
- Weiß BM, Kulik L, Ruiz-Lambides AV, Widdig A. 2016. Individual dispersal decisions affect fitness via maternal rank effects in male rhesus macaques. *Sci Rep*. 6:32212.
- Weingrill T, Willems EP, Krützen M, Noë R. 2011. Determinants of paternity success in a group of captive vervet monkeys (*Chlorocebus aethiops sabaeus*). *Int J Primatol*. 32:415–429.
- Whitten PL, Turner TR. 2009. Endocrine mechanisms of primate life history trade-offs: growth and reproductive maturation in vervet monkeys. *Am J Hum Biol*. 21:754–761.
- Widdig A. 2013. The impact of male reproductive skew on kin structure and sociality in multi-male groups. *Evol Anthropol*. 22:239–250.
- Widdig A, Bercovitch FB, Streich WJ, Saueremann U, Nürnberg P, Krawczak M. 2004. A longitudinal analysis of reproductive skew in male rhesus macaques. *Proc Biol Sci*. 271:819–826.
- Widdig A, Muniz L, Minkner M, Barth Y, Bley S, Ruiz-Lambides A, Junge O, Mundry R, Kulik L. 2017. Low incidence of inbreeding in a long-lived primate population isolated for 75 years. *Behav Ecol Sociobiol*. 71:18.
- Wikberg EC, Jack KM, Fedigan LM, Campos FA, Yashima AS, Bergstrom ML, Hiwatashi T, Kawamura S. 2017. Inbreeding avoidance and female mate choice shape reproductive skew in capuchin monkeys (*Cebus capucinus imitator*). *Mol Ecol*. 26:653–667.
- Young C, Hähndel S, Majolo B, Schülke O, Ostner J. 2013. Male coalitions and female behaviour affect male mating success independent of dominance rank and female receptive synchrony in wild Barbary macaques. *Behav Ecol Sociobiol*. 67:1665–1677.
- Young C, McFarland R, Barrett L, Henzi SP. 2017. Formidable females and the power trajectories of socially integrated male vervet monkeys. *Anim Behav*. 125:61–67.
- Zinner D, Nunn CL, van Schaik CP, Kappeler PM. 2004. Sexual selection and exaggerated sexual swellings of female primates. In: Kappeler PM, van Schaik CP, editors. *Sexual selection in primates*. Cambridge (UK): Cambridge University Press. p. 71–89.