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Patterns of Paternity and Group Fission in Wild Multimale Mountain Gorilla Groups

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ABSTRACT To understand variation among social systems, it is essential to know the relative reproductive success of individuals in group-living species. Particularly interesting for such studies are taxa such as mountain gorillas in which both one-male and multimale groups are common, because of the opportunity to estimate the costs and benefits to males of pursuing different reproductive strategies. We genotyped 68 individuals from two groups of multimale mountain gorilla groups in Bwindi Impenetrable National Park, Uganda to determine the distribution of paternity among the males. In both groups, the dominant male sired the majority of offspring. One group underwent a fission, and we found that the eight offspring assigned to the dominant silverback (and their mothers) remained with their father, while the two offspring of unknown paternity ended up in the small group headed by the formerly subordinate silverback. This is consistent with the proposal that the outcome of group fission in primates is not only influenced by maternal relationships among individuals, but also by patrilineal relationships. Results of this study show that subordinate males may gain reproductive benefits even while queuing for dominance status. Despite ecological differences between Bwindi and the Virunga Volcanoes, male mountain gorillas living in both populations benefit from remaining in multimale groups. Am J Phys Anthropol 135:263–274, 2008. ©2007 Wiley-Liss, Inc.

A fundamental goal of behavioral ecology is to understand how resource distribution, particularly of food and mates, influences individual behavioral strategies and their associated social systems (Emlen and Oring, 1977; Clutton-Brock, 1989b; Kappeler and van Schaik, 2002). Inter- and intra-specific variation in social systems may be due to localized variation in ecological conditions influencing how females distribute themselves, which in turn determine the distribution and reproductive strategies of males. Such variability has been observed in a wide range of taxa (e.g., birds, Davies and Hartley, 1996; equids, Linklater, 2000; primates, Kappeler and van Schaik, 2002; Lindenfors et al., 2004; Old World fruit bats, Campbell et al., 2006).

Primate social groups vary in the number of males they contain. This is thought to reflect how easily females can be monopolized (Mitani et al., 1996; Sterck et al., 1997; Nunn, 1999; Kappeler, 2000; Lindenfors et al., 2004). In combination with observational data from wild animals, the use of molecular markers to infer genetic relationships among individuals has provided insights into mating systems, reproductive strategies, dispersal patterns, genetic relatedness, and the influence of kinship on social behavior (Goossens et al., 1998; Fietz et al., 2000; Garnier et al., 2001; Griffin et al., 2002; Wimmer and Kappeler, 2002; Di Fiore, 2003; Smith et al., 2003; Möller and Beheregaray, 2004; Langergraber et al., 2007). In particular, analysis of paternity and relatedness patterns provides a better understanding of the evolutionary causes and consequences of varied social organization in animal populations (reviewed in: Strier, 2004; Lukas et al., 2005).

In groups containing multiple males, the distribution of paternity depends on several factors including the ability of individual males to monopolize females, alternative male mating strategies, and female choice (van Noordwijk and Van Schaik, 2004). Genetic studies have generally provided strong evidence for a positive relationship between male dominance rank and reproductive success (*Eulemur fulvus rufus*, Wimmer and Kappeler, 2002; *Papio cynocephalus*, Alberts et al., 2003; *Macaca mulatta*, Widdig et al., 2004; *Mandrillus sphinx*, Setchell et al., 2005; Alberts et al., 2006; *Pan troglodytes*, Boesch et al., 2006; *Macaca fascicularis*, Engelhardt et al., 2006). The distribution of paternities in multimale primate groups has sometimes been interpreted in the context of reproductive skew theory. Such studies tend to suggest that dominant males lose some paternity opportunities, because they are unable to competitively monopolize reproduction in the group, and not because they concede potentially controllable reproduction to subordinates (Engh et al., 2002; Alberts et al., 2003; Bradley et al., 2004; Widdig et al., 2004; Setchell et al., 2005). This inference is supported by one of the few studies in primates examining the success of alternative male mating strategies in an intragroup context. Constable et al. showed that within one community of chimpanzees, high-ranking males successfully employed possessive mating strategies, but that lower-ranking males were nonetheless successful in gaining paternities through opportunistic matings or through absenting themselves with a female from the community ("consortships")

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(Constable et al., 2001). Female choice, in the forms of both overt individual preferences and cryptic choice via sperm competition (Baker and Bellis, 1993; Birkhead, 1998; Eberhard, 1998; Paul, 2002; Kokko et al., 2003), is difficult to study in wild primates although some evidence of subtle female mating preferences contrary to the male dominance hierarchy is emerging (Stumpf and Boesch, 2005, 2006). Male dominance tenure is typically shorter than the time females require for maturation (Clutton-Brock, 1989a). However, a study of exceptionally stable capuchin monkey groups revealed that dominant males do not sire offspring with their grown daughters, a pattern tentatively ascribed to female-mediated inbreeding avoidance (Muniz et al., 2006).

Examining the distribution of paternities among males in species that exhibit both one-male and multimale groups can contribute to our understanding of the costs and benefits to males pursuing different reproductive strategies. Gorillas are particularly interesting for this purpose, because essentially all described breeding groups of western gorillas (*Gorilla gorilla gorilla*) in west-central Africa appear to contain only one adult male (Parnell, 2002; Gatti et al., 2004; Robbins et al., 2004), while groups containing multiple adult males are common in mountain gorillas (*Gorilla beringei beringei*, Kalpers et al., 2003; McNeilage et al., 2006). The proximate cause of this difference is variation in male dispersal patterns. Nearly all male western gorillas disperse from their natal groups (Stokes et al., 2003; Robbins et al., 2004), while male mountain gorillas either remain in their natal group or disperse and attempt to form new social groups (Harcourt, 1978; Robbins, 1995, 2001; Watts, 2000). These differences in dispersal patterns and male reproductive strategies between the two species are likely to be ultimately due to differing ecological constraints on the upper limits of group size (Stokes et al., 2003; Robbins et al., 2004), but further study of western gorillas is necessary to confirm this.

It is generally assumed that the single adult male in otherwise all female groups monopolize breeding in those groups, and this expectation was confirmed for western gorillas in a genetic analysis of 20 offspring from nine groups (Bradley et al., 2004). A long-standing question has been the distribution of reproduction in gorilla groups containing multiple adult males. Such groups typically contain only two males, but may contain more. Coresiding males may have stable dominance relationships for many years, with subordinate males obtaining a small proportion of matings while queuing for dominance status (Watts, 1991, 2000; Robbins, 1995, 1999, 2001). A recent genetic analysis of four multimale mountain gorilla groups in the Virunga Volcanoes region revealed that both dominant and subordinate males enjoy reproductive success, with the dominant siring an average of 85% of group offspring (Bradley et al., 2005). These results were interpreted as reflecting primarily the inability of the dominant male to monopolize reproduction, with perhaps some influence of female mate choice. Even though subordinate males produce only a small share of the offspring, a strategy of queuing to become dominant will likely provide greater lifetime reproductive success than dispersing and attempting to start a new group (Watts, 2000; Robbins and Robbins, 2005).

Another way males can acquire dominance status is through the fission of multimale groups (Melnick and Kidd, 1983; Ménard and Vallet, 1993; Robbins, 2001; Kalpers et al., 2003). In species with female philopatry, group fission is an important event as it may be a way for females to disperse and it often occurs along matrilines (e.g., Lefebvre et al., 2003; Archie et al., 2006; Widdig et al., 2006b), but little is known about how groups fission in species in which females disperse and do not frequently reside with kin. In the one case in mountain gorillas in which the identity of group members was known both before and after a group fission, females remained with the male with whom they had strong social relationships (Robbins, 2001; Watts, 2003), but nothing is known about whether offspring remain with their fathers or not.

Recent research indicates that relationships among paternal kin are important in primates, even in Old World monkeys in which maternal relationships play a central role (Alberts and Altmann, 1995; Alberts, 1999; Widdig et al., 2001; Buchan et al., 2003; Smith et al., 2003; Silk et al., 2006a,b; Widdig et al., 2006a,b). In particular, fathers have been shown to invest in offspring in wild baboons (Alberts, 1999; Buchan et al., 2003) and possibly to a small extent in wild chimpanzees (Lehmann et al., 2006). This suggests that in taxa with a slow maturation process such as gorillas, it could be beneficial for offspring to associate preferentially with their fathers, particularly if the group undergoes a fission.

The aim of this study is to assess the patterns of genetic structure within mountain gorilla social groups by determining paternity of offspring in two groups from Bwindi Impenetrable National Park, Uganda. Results thus far on this topic are based only upon gorillas monitored by the Karisoke Research Center in the Virunga Volcanoes (Bradley et al., 2005). These animals live at a high altitude ecological extreme for gorillas and can occur in exceedingly large groups of more than 30 individuals, which may make them unrepresentative of patterns of sociality in the taxa as a whole [the average group size is 10; (Kalpers et al., 2003; McNeilage et al., 2006)]. To best understand the roles that both ecological conditions and reproductive strategies play in determining the social system exhibited by a species, it is important to compare results from several populations. While the variation in ecological conditions, diet, and ranging patterns in gorillas is receiving an increasing amount of attention (Ganas et al., 2004; Ganas and Robbins, 2005; Robbins, 2006), variation in reproductive strategies warrants study in more populations (but see Bradley et al., 2004). Here, we present results from genetic analysis of two groups of wild mountain gorillas, one of which underwent a fission event. We describe the distribution of paternity between dominant and subordinate males, and examine whether offspring ended up in the same group as their father following a group fission. We also describe the detection of a germ cell mutation in a silverback male that produces a genotypic "mismatch" at one locus between himself and some of his progeny, and we discuss the implications of this for similar genotyping studies.

MATERIALS AND METHODS

Study site and study groups

Bwindi Impenetrable National Park (BINP) is a montane forest (331 km^2) in southwestern Uganda (0°53′–1°08′ N; 29°35′–29°50′ E) with an altitude between 1160 and 2607 m above sea level. We sampled three study groups. The Kyagurilo group has been closely monitored since August 1998, and identities of all gorillas are well known. Between 1998 and 2003, this group contained three adult males (silverbacks), one of whom emigrated in 1999. The rest of the group consisted of six adult females and seven offspring (born 1993–2002). The Habinyanja group has been habituated for tourism since 1998 and originally contained thirty members. Fission of the group in February 2002 produced Habinyanja A (HA) with one silverback, two blackbacks, ten adult females, and eight offspring and Habinyanja B (HB) with one silverback, six adult females, and two offspring.

Noninvasive sampling

Fecal samples from Kyagurilo group individuals were collected opportunistically during observation. In contrast, because the Habinyanja groups are used for tourism, and the Uganda Wildlife Authority strives to minimize disturbance by restricting nontourist visitations to the groups, direct observation of individuals was not possible and so samples were collected from the night nests of the groups and individuals were differentiated based on dung size and genotyping results, including molecular sexing analysis. Samples were also collected opportunistically from night nests of unhabituated groups or solitary males encountered within or near the range of the Kyagurilo group.

As previously described in detail (Nsubuga et al., 2004), 3–5 g of fecal samples were stored in tubes containing either desiccating silica gel beads or RNAlater^{TM} solution (AMBION, Austin, TX). Multiple samples were collected on different days from each individual.

Genetic analysis

Genomic DNA was extracted from 0.1 g of dried or RNAlater-preserved fecal material using a modified version (Nsubuga et al., 2004) of the protocol provided with the QIAamp$^{®}$ DNA Stool Kit (Qiagen). Ten to sixteen samples were extracted at one time, along with two negative controls. To estimate the amount of DNA needed in each polymerase chain reaction (PCR) to avoid "allelic dropout" (the nondetection of one of two different alleles at a heterozygous locus), the concentration of amplifiable genomic DNA in each extract was estimated using quantitative real-time PCR (Morin et al., 2001). All samples were genotyped at up to 14 tetra-nucleotide repeat microsatellite loci originally characterized in humans. The loci [D2s1326, D1s550, D5s1470, D4s1627, D10s1432, D7s817, D7s2204, D16s2624, D6s1106, D5s1457, D6s1056, D1s2130, D3s2459, vWF (von Willebrand factor), and one X–Y homologous locus for sex identification] had been tested and used in other gorilla projects (Clifford et al., 1999; Bradley et al., 2000, 2004, 2005). The 20-μl reaction mixes contained 1.0–4.0 μl DNA extract, 2.0 μl 10 × PCR buffer, 1.6 μl $MgCl_2$ (25 mM stock), 0.4 μl F primer (10 μM), 0.4 μl R primer (10 μM), 0.8 μl dNTPs (10 mM), 0.5 μl Taq Gold (Perkin–Elmer Applied Biosystems) (5 U/μl); 0.8 μl bovine serum albumin (BSA) (10 mg/μl) and ultrapure H_2O. Amplification conditions on a PTC-200 thermocycler (MJ Research) were as follows: initial denaturation at 95°C for 3 min; 45 cycles of 30 s at 95°C, 30 s at either 55 or 60°C, 30 s at 72°C, and a final extension of 30 min at 72°C.

The 5′-end of the forward primer was fluorescently labeled, and the amplified PCR products were separated through capillary electrophoresis using ABI 310 and 3100 genetic analyzers and alleles were sized relative to the internal size standard (HD400 with ROX label) using GENESCAN 2.0 (Perkin–Elmer Applied Biosystems) software. To avoid genotyping errors such as allelic dropout and "false alleles" (amplification of artifacts), genotypes were confirmed following stringent criteria recommended by Morin et al. (2001) and Taberlet et al. (1996). Specifically, heterozygotes were confirmed after each allele had been observed at least twice in two or more independent reactions, while homozygotes were confirmed after two to seven independent replications depending on the DNA template amount used (see Morin et al., 2001) criteria. Only individuals typed at a minimum of six loci were included in the analysis. Identities of samples were confirmed by typing individuals from at least two independent samples and by comparison of genotypes from known mother–offspring for the expected pattern of allele sharing.

Statistical analyses

To assess the informativeness of the 14 loci employed in generating the genotype data, we used allele frequency analysis as implemented in CERVUS (Marshall et al., 1998) to measure the expected heterozygosity, the exclusionary power of the loci, and to examine any deviations from Hardy–Weinberg equilibrium (HWE) for each locus. Furthermore, the genotype data were checked for departures from HWE, and linkage disequilibrium (LD) between all pairs of loci, using exact tests as implemented in GENEPOP version 3.4 (Raymond and Rousset, 1995).

The number of loci needed to ensure that no two individuals will share a genetic profile depends on the number and the variability of the loci analyzed. We used the population allelic frequencies (based on all individuals sampled) to estimate the "probability of identity" (P_{ID}), which is defined as the probability that any two individuals picked at random from the population would share the same multilocus genotype by chance (Paetkau and Strobeck, 1994). In addition, we used CERVUS (Marshall et al., 1998) to perform pairwise comparisons of all multilocus genotypes. For this analysis (Identity Check), we allowed two mismatches in the output options to spot potential mismatches due to genotyping error. Because not all samples used were from identified individuals, all genotypes were compared to find pairs of samples that had yielded the same microsatellite genotypes across all screened loci, and could thus be attributed to multiple sampling of the same individual.

Paternity analyses

We primarily relied upon the exclusion method for assignment of fathers to offspring (as in Vigilant et al., 2001; Bradley et al., 2004, 2005), where if the identity of the mother is known, a male is assigned as father if he has the paternal alleles of the offspring at all loci analyzed. Genotypes of potential sires within as well as from outside the group were compared with that of the offspring. To allow for potential mismatches arising out of error or mutation, males were excluded as sires if they did not share an allele with the offspring at two or more loci. The individual probability of exclusion (Pe) was calculated according to Chakraborty et al. (1988) using allele frequencies from a subset of the data consisting of all adults to minimize the presence of highly related individuals. The exclusion method was preferred because it is considered the most powerful approach when using highly polymorphic genetic makers and testing few candidate parents (Jones and Ardren, 2003). However, we also used the computer program CERVUS to evaluate potential sires using a likelihood-based approach (Marshall et al., 1998) that chooses a most likely sire regardless of the number of allelic mismatches. In the first CERVUS analysis, we used resident adults as potential sires for each offspring in their respective groups. To assess the possible occurrence of extragroup paternity, in the second CERVUS analysis run we considered all sampled adult males ($n = 23$) as potential sires for each offspring, irrespective of the offspring's social group. The settings used in the paternity simulations were as follows: 1% error rate, 10 candidate males (adults and subadults), 83.9% of loci typed, and 10,000 simulations cycles. The proportion of candidate sires sampled was set at 90.2% to simulate the chance that an unknown or nongroup male might be the sire. The level of confidence for all CERVUS analyses was set at 95%.

RESULTS

Genotypes

A total of 68 individuals were genetically sexed and typed at a minimum of six and up to fourteen microsatellite loci (Table 1). Overall, the mean proportion of completed genotypes was 81.7% for all individuals across the 14 loci. The locus D8s1106 was reported by Bradley and Vigilant (2002) to sporadically amplify nontarget DNA in fecal extracts from another population of mountain gorillas; however, this was not observed during the current study. The mean number of alleles per locus was 5.57. The mean expected heterozygosity (He) over the 14 loci used was 0.663 and ranged from 0.427 to 0.834, while the observed heterozygosities per locus ranged from 0.407 to 0.886 with an average of 0.679 (Table 2). None of the loci showed significant departures from HWE using either CERVUS or GENEPOP. Tests for LD using genotypes from only adults or nonoffspring showed that only two of the 91 pairwise associations between loci (vWF and D3s2459, $P = 0.001$, D10s1432 and D3s2459, $P = 0.001$) had significant linkage. However, after a Bonferroni correction for multiple comparisons, these linkages were no longer significant, suggesting that genotypes at one locus are independent from genotypes at another locus. The estimated average total exclusionary power or probability (Marshall et al., 1998) for the 14 loci to exclude a random individual from parentage was 0.988 when neither of the two parents is known, and 0.999 when one parent is known. Thus, the number of loci and the level of variation at the loci chosen for the current study are sufficient to resolve parentage with a high level of confidence.

We calculated the probability of identity (P_{ID}), which is the chance of finding two randomly sampled individuals with identical multilocus genotypes. For this calculation, the loci D8s1106 and D5s1470 were not included because after testing them in Kyagurilo group and a few neighboring males, they were found to be not very useful due to their low polymorphism and hence, were dropped from any further analysis. The P_{ID} estimated for 12 loci was very low (8.40×10^{-8}), i.e. <1 in 10 million. Similarly, the more conservative P_{ID} among siblings, $P_{(\mathrm{ID\text{-}sibs})}$, was 5.5×10^{-5}, implying that it is also highly unlikely that a sibling pair will display the same genotype profile across all 12 loci. Even using just the least variable loci, the P_{ID} and $P_{(\mathrm{ID\text{-}sibs})}$ estimated for six "worst" loci were 2.00×10^{-4} and 1.29×10^{-2}, respectively. Nonetheless, none of the individuals were genotyped exclusively at only these loci, and only one individual was genotyped at six loci and the rest were genotyped at seven or more loci. Hence, the chance of two different individuals having a perfect matching genotype at all loci is extremely small.

Parentage analysis

We confirmed six mother–offspring relationships that were suspected from behavioral observations of the Kyagurilo group (Table 3, Fig. 2). A previously unrecognized mother–offspring relationship between two adults (Siatu and Ntegenisa) was also identified. Mothers were assigned to ten offspring in the Habinyanja group. The male known to be the dominant male of the Kyagurilo group from 1998 to 2004, Zeus, sired five of seven (71%) assayed offspring born from 1993 to 2002 (Table 3, Fig. 2). One offspring was assigned to each of the subordinate males (Ntegenisa and Rukina). Males ($n = 18$) ranging in the neighborhood of Kyagurilo group were tested and all were excluded at multiple loci as potential sires of offspring in this group. The single siring by Ntegenisa occurred when he was only about 8 or 9 years old, and although the dominance ranks of the males at this time (1995) are not known, it is extremely unlikely that he was the dominant male given the presence of the older male Zeus. Zeus was excluded by multiple mismatches as the father of subordinate adult males Ntegenisa and Rukina.

Although Zeus was assigned as the sire of five offspring, two of these offspring (Marembo and Mukiza) had one mismatch each at the same locus (D7s2204), where both offspring must have inherited an allele of length 255 from their father. The 255 allele was independently replicated seven and nine times in Marembo and Mukiza, respectively, using different samples and extracts for each individual. The sequences from cloned DNA of the parents and offspring at this locus showed that the PCR products were not artifacts but possessed the expected allelic sequences. Zeus' genotype showed the alleles 247 and 259. The paternal allele (255) at locus D7s2204 observed in Marembo and Mukiza is four base-pairs shorter than an allele (259) present in the putative father Zeus. We suggest that this discrepancy is most readily explained as a mutation in a primordial germ cell of Zeus' that produced a deletion of four base-pairs (removal of one tetranucleotide-motif unit) from the 259 allele, so that Zeus can transmit either a 247 or 255 allele to his offspring (see Fig. 1). In the CERVUS paternity analysis, where our input parameters included all adult males and subadults ($n = 23$) as potential sires irrespective of group membership, assignments were made with high confidence to the same males identified using the exclusion method assignments. Thus, Zeus was assigned as the sire to five offspring (Table 3).

In the Habinyanja group, the dominant male, HASB, had no mismatches to eight of the ten offspring, was supported as the father by CERVUS analysis, and was accordingly assigned as sire (Table 4, Fig. 2). The other potential sire, HBSB, had one or two mismatches in each case. Both the dominant and subordinate silverbacks were excluded by multiple mismatches as the sire

TABLE 1. Genotypes of 68 gorillas at fourteen microsatellite loci

ID	Gp	sex	Age	vWF	D2s1326	D1s550	D5s1470	D4s1627	D10s1432	D7s817	D7s2204	D16s2624	D8s1106	D5s1457	D6s1056	D1s2130	D3s2459
ZS	Ky	M	SB	135/139	258/274	190/202	191/191	238/242	172/180	144/136	259/247	136/136	147/147	115/123	250/258	253/261	196/204
NT	Ky	M	BB	159/139	258/258	190/190	183/191	238/238	160/176	148/132	247/251	128/136	147/147	123/123	250/254	257/261	208/248
RC	Ky	M	BB	139/139	274/258	182/190	179/191	234/238	160/176	136/140	251/259	136/136	147/143	123/115	250/250	261/261	204/232
MT	Ky	F	AF	139/147	258/258	190/182	191/191	238/238	168/176	140/136	251/259	140/136	147/151	115/127	254/250	253/257	232/232
SK	Ky	M	Jv	139/167	242/258	182/190	191/191	238/246	168/172	136/136	251/259	128/136	147/147	115/127	258/258	261/265	196/204
BY	Ky	F	AF	135/139	258/274	202/202	191/191	242/238	164/172	140/152	239/247	132/136	147/147	111/115	250/258	253/253	204/232
BZ	Ky	M	Inf	135/139	258/274	190/202	191/191	238/238	172/172	140/144	239/247	136/136	147/147	111/123	250/258	253/261	204/232
ST	Ky	F	AF	155/159	258/274	190/182	183/183	242/238	164/176	132/140	247/259	128/128	147/147	115/123	250/254	257/257	200/248
BU	Ky	F	Inf	155/139	258/258	182/202	183/191	242/242	180/176	144/140	247/247	128/136	147/147	115/115	250/250	253/257	196/200
MR	Ky	M	Jv	139/159	274/274	190/190	191/183	242/238	164/172	136/132	247/255	128/136	147/147	115/123	250/250	253/257	196/200
MG	Ky	F	AF	167/139	274/242	178/182	191/191	238/246	172/176	136/140	251/251	136/128	147/147	127/131	258/250	261/265	204/204
MK	Ky	M	Inf	135/167	242/258	178/190	191/191	238/246	172/172	136/136	251/255	128/136	147/147	115/127	258/258	265/261	204/204
KM	Ky	F	AF	159/139	258/258	190/182	191/187	246/238	160/168	148/140	251/239	136/136	·/·	119/123	·/·	253/261	200/208
KZ	Ky	M	Jv	159/159	258/258	190/190	183/187	246/238	160/176	148/140	251/247	136/136	147/147	119/123	254/254	253/261	200/248
KK	Ky	F	AF	159/147	274/258	190/190	183/187	246/234	168/172	140/136	239/239	136/128	147/147	119/123	250/254	253/253	200/244
TN	Ky	F	AF	139/155	258/258	190/190	191/191	238/246	164/180	148/148	255/259	128/136	147/151	111/115	250/254	257/261	204/232
KB	Ky	M	Inf	139/155	258/258	190/190	191/191	238/246	164/176	140/148	259/259	136/136	·/·	111/115	·/·	257/261	204/232
93	Rj	M	SB	135/147	274/242	182/182	183/191	238/242	172/176	144/144	251/251	128/136	147/147	115/115	250/250	253/261	196/244
95	Rj	M	Med	139/135	242/258	182/182	191/191	238/242	168/172	144/148	251/259	136/136	139/147	115/115	250/250	253/253	196/244
96	Rj	M	SB	135/139	274/258	182/202	191/191	238/242	176/176	140/140	251/251	128/128	147/147	115/131	250/250	253/265	244/200
99	Rj	M	SB	139/139	242/274	190/202	191/191	238/242	172/176	136/140	251/259	128/136	147/151	127/131	250/254	261/265	200/244
97	Rj	M	Med	139/155	262/274	186/190	191/179	238/242	176/176	136/148	251/259	128/128	·/·	111/131	·/·	257/261	204/244
100	Rj	M	Med	135/139	242/258	182/182	191/183	238/246	176/176	140/148	251/259	128/136	·/·	·/·	·/·	249/253	196/244
295	Rj	M	SB	139/135	242/274	202/182	·/·	·/·	176/172	140/140	·/·	·/·	·/·	131/115	·/·	253/253	200/244
94	Rj	F	sad	135/139	258/274	·/·	191/191	238/238	176/176	144/144	·/·	136/136	·/·	115/135	250/250	·/·	196/196
120	Ro	M	Med	147/147	242/274	182/190	183/191	238/246	168/176	132/144	247/251	136/136	·/·	·/·	·/·	·/·	196/200
121	Ro	F	AF	139/155	·/·	182/182	191/191	238/238	172/176	140/148	247/255	132/136	·/·	·/·	·/·	·/·	204/236
118	Ro	F	sad	155/155	258/258	170/190	·/·	·/·	172/176	140/148	·/·	136/136	·/·	·/·	·/·	·/·	204/236
119	Ro	M	SB	147/139	242/274	190/202	191/191	238/246	176/176	144/140	251/259	128/136	143/151	·/·	·/·	·/·	·/·
z123	Rz	M	SB	139/155	258/258	194/202	183/191	246/246	168/172	148/148	251/243	136/136	143/147	115/115	250/258	·/·	236/244
124	Rz	F	Med	147/139	·/·	182/202	·/·	234/242	168/176	136/144	255/259	136/128	·/·	115/115	·/·	·/·	196/204
125	Rz	M	SB	147/155	258/274	190/202	191/183	238/246	168/172	148/148	251/251	128/136	139/147	115/115	254/258	253/261	200/244
250	WA	F	Med	139/147	242/258	182/198	183/183	242/246	180/160	148/152	251/259	128/128	·/·	115/115	250/250	253/257	232/244
251	WA	M	SB	139/167	274/274	182/202	·/·	242/238	172/176	148/152	251/251	128/136	·/·	127/135	·/·	·/·	204/204
165	L	M	Med	155/155	242/258	182/190	191/191	234/234	168/168	132/140	251/251	128/136	147/147	115/115	·/·	·/·	236/244
168	L	M	Med	147/147	258/258	194/190	183/191	238/242	168/176	144/136	259/259	136/136	139/147	115/115	250/250	257/257	204/232
91	L	M	SB	139/159	274/262	190/202	191/191	234/246	168/176	148/136	247/251	128/136	147/147	115/111	250/254	253/253	200/196
92	L	M	SB	147/155	258/242	182/190	183/183	242/234	172/176	140/136	251/259	128/136	147/147	115/115	250/250	253/257	204/244
267	WB	M	SB	139/139	258/258	·/·	·/·	·/·	176/180	136/136	·/·	128/136	·/·	131/123	·/·	·/·	·/·
269	WB	F	Med	139/159	258/274	182/182	183/183	234/242	168/176	132/140	247/259	136/136	·/·	115/123	·/·	253/261	196/200
270	WB	M	Med	139/155	258/258	·/·	·/·	238/246	180/176	136/136	·/·	128/136	·/·	131/127	·/·	261/261	204/200
HASB	H	M	SB	139/139	258/258	202/182	·/·	242/238	160/168	136/148	251/251	128/128	·/·	123/111	250/250	261/261	244/244
M1	H	F	AF	147/139	242/258	·/·	·/·	·/·	172/180	·/·	·/·	128/136	·/·	127/115	·/·	·/·	196/236
B1	H	F	Inf	139/139	258/258	202/190	·/·	242/246	160/172	140/148	251/251	128/136	·/·	123/127	·/·	261/265	236/244
M2	H	F	AF	139/159	262/270	182/190	·/·	234/246	168/176	140/148	251/247	128/136	·/·	115/123	250/254	253/253	196/200
B2	H	M	Inf	139/139	258/270	182/202	·/·	234/238	168/176	136/148	251/251	128/136	·/·	123/123	250/250	253/261	244/200
M3	H	F	AF	139/159	262/270	182/190	·/·	250/246	168/176	148/136	251/251	128/136	·/·	115/123	250/250	253/253	196/200
B3	H	F	Inf	·/·	·/·	·/·	·/·	238/246	168/168	·/·	251/251	128/136	·/·	115/123	·/·	·/·	244/196
M4	H	F	AF	139/147	258/258	190/190	·/·	238/246	160/168	132/148	251/259	128/136	·/·	115/127	250/254	253/253	244/244
B4	H	F	Inf	139/139	258/258	190/202	·/·	242/246	160/160	132/148	251/259	128/136	·/·	123/127	250/254	253/261	244/244

(continued)

TABLE 1. (Continued)

ID	Gp	sex	Age	vWF	D2s1326	D1s550	D5s1470	D4s1627	D10s1432	D7s817	D7s2204	D16s2624	D8s1106	D5s1457	D6s1056	D1s2130	D3s2459
M5	H	F	AF	147/139	258/258	·/·	·/·	·/·	168/176	·/·	·/·	128/136	·/·	115/127	·/·	·/·	·/·
B5	H	F	Inf	139/139	258/258	202/190	·/·	·/·	176/160	132/148	251/259	128/136	·/·	123/127	254/250	·/·	244/244
M6	H	F	AF	155/139	258/274	182/202	·/·	238/246	168/172	140/136	251/259	136/136	·/·	115/115	250/258	253/265	196/232
B6	H	F	Inf	139/139	258/258	202/202	·/·	238/246	160/168	140/136	251/259	128/136	·/·	115/123	·/·	253/261	196/244
M7	H	F	AF	155/139	242/262	190/202	·/·	246/246	172/168	132/140	·/·	·/·	·/·	111/115	250/250	261/261	200/232
B7	H	F	Inf	139/139	258/262	202/202	·/·	·/·	160/168	136/140	251/251	136/128	·/·	111/115	250/250	261/261	200/244
MX	H	F	AF	·/·	258/262	·/·	·/·	250/238	168/176	136/148	·/·	128/136	·/·	115/123	·/·	·/·	·/·
BX	H	M	Inf	163/139	258/258	202/202	·/·	238/250	160/168	136/148	251/251	128/136	·/·	115/123	250/254	261/265	244/244
Md2	H	F	Med	139/139	258/270	202/182	·/·	·/·	168/176	·/·	251/251	·/·	·/·	·/·	·/·	·/·	244/196
gW	H	F	Med	139/139	258/258	182/190	·/·	238/238	168/168	144/148	251/251	128/136	·/·	115/119	250/250	265/269	232/244
HBSB	H	M	SB	147/139	258/274	182/182	·/·	242/238	160/168	136/148	251/251	128/136	·/·	119/115	250/250	253/261	244/232
BM1	H	F	AF	147/139	242/274	190/190	·/·	246/246	168/172	132/148	251/251	136/136	·/·	115/127	254/254	261/261	244/244
Bb1	H	F	Inf	139/139	274/274	190/182	·/·	246/242	172/172	148/132	243/251	136/136	·/·	115/115	254/254	261/261	244/244
BM2	H	F	AF	163/163	258/258	202/190	·/·	246/250	168/180	132/136	251/251	136/136	·/·	115/115	254/254	261/265	200/244
Bb2	H	M	Inf	139/163	258/258	190/202	·/·	250/238	168/168	132/148	251/255	128/136	·/·	115/123	250/254	261/261	244/244
Bmd1	H	F	Med	147/139	·/·	182/182	·/·	·/·	160/168	148/148	·/·	·/·	·/·	119/115	·/·	·/·	232/244
Bmd3	H	F	Med	159/139	·/·	182/190	·/·	·/·	172/172	148/136	243/243	·/·	·/·	123/115	·/·	·/·	244/244
Bmd7	H	F	Med	159/139	·/·	190/182	·/·	·/·	168/172	140/140	251/255	·/·	·/·	127/115	·/·	·/·	244/244

ID = individuals identity; Gp = group; M = male; F = female; SB = silverback; BB = blackback; AF = adult female; Jv = juvenile; Inf = infant; sad = subadult; Med = adult male or adult female with no infant; ·/· = untyped.

TABLE 2. Summary statistics for the fourteen loci used in this study

Locus	Temp. (°C)	No. of Alleles	*N*	Hets	Homs	He	Ho
VWF	60	7	43	35	8	0.738	0.814
D2s1326	60	5	42	29	13	0.674	0.690
D1s550	60	7	39	27	12	0.699	0.692
D5s1470	60	4	27	11	16	0.523	0.407
D4s1627	60	5	40	33	7	0.739	0.825
D10s1432	55	6	44	39	5	0.767	0.886
D7s817	60	6	42	34	8	0.794	0.810
D7s2204	55	6	37	24	13	0.652	0.649
D16s2624	55	4	42	26	16	0.525	0.619
D8s1106	55	4	20	9	11	0.427	0.450
D5s1457	55	7	40	28	12	0.692	0.700
D6s1056	55	3	28	15	13	0.522	0.536
D1s2130	55	6	33	19	14	0.700	0.576
D3s2459	55	8	40	34	7	0.834	0.850

N indicates number of individuals; Hets indicates number of heterozygotes; Homs indicates number of homozygotes; Ho and He are the observed and expected heterozygosity under Hardy–Weinberg equilibrium, respectively.

of offspring Bb1. It is possible that the true father(s) of offspring Bb1 and Bb2 was not sampled either because he had left the group before this study began or the mother immigrated into the group when she was already pregnant or with the infant. For these two offspring, likelihood analysis by CERVUS supported assignment of Bb2 to HASB despite the single mismatch and suggested silverback no. 251 as the sire of offspring Bb1 despite mismatches at three loci. It is highly improbable that this silverback is the sire because he was not a resident male, and in addition ranged at the opposite end of Bwindi Impenetrable National Park. We consider the paternity of both Bb1 and Bb2 to be undetermined.

Subordinate silverback HBSB is hence not the sire of either of the two offspring (Bb1 and Bb2) in his splinter group (HB), while all of the offspring assigned to HASB remained in his group after the split. The two silverbacks HASB and HBSB mismatched at one locus suggesting that they do not represent a father–son pair, although it is difficult to estimate their relationship with confidence due to the lack of a maternal genotype for the presumably younger HBSB.

DISCUSSION

Paternity assignments and parent-offspring mismatches

Here we first discuss our unexpected finding of an apparent mutation producing consistent mismatches between a father and some of his offspring, and subsequently highlight the insights into gorilla social structure gained from the paternity assignments.

Parentage analysis using microsatellite analysis relies upon the assumption that at any given locus, the genotype of each true parent will exhibit one of the offspring's two alleles. Deviations from this pattern can result from two causes, laboratory errors or mutations. Means of minimizing errors in genotyping, particularly when noninvasive sources of DNA are used, have been the subject of much attention (Taberlet et al., 1999; Goossens et al., 2000; Morin et al., 2001; Fernando et al., 2003; Piggott, 2004; Roon et al., 2005). The work reported here meets or exceeds current recommendations

TABLE 3. Parentage assignments for the Kyagurilo group

								Cervus confidence	
Offspring	Loci[1]	Mother	ZS	NT	RC	Pe	Father	Delta	95%
Ntegenisa	14	Siatu	3	nd	nd	0.9999	nd	nd	nd
Rukina	14	unknown	2	nd	nd	nd	nd	nd	nd
Sikio	14	unknown	0	5	2	0.9927	ZS	2.88 E −01	*
Marembo	14	Siatu	1	6	4	0.9998	ZS	4.32 E +00	*
Kafuzi	14	Kaf-mom	6	0	4	0.9993	NT	7.67 E +00	*
Byiza	14	Binyindo	0	3	3	0.9997	ZS	5.93 E +00	*
Mukiza	14	Magwere	1	7	4	0.9999	ZS	4.66 E +00	*
Bizibu	14	Siatu	0	7	7	0.9999	ZS	7.05 E +00	*
Kabandiize	12	TN	3	4	0	0.9927	RC	7.67 E +00	*

[1] Number of loci typed.

Columns 4, 5, and 6 contain number of loci at which potential sires Zeus (ZS), Ntegenisa (NT), and Rukina (RC) are excluded. Pe indicates paternity exclusion probability calculated using allele frequencies from adult individuals. An asterisk (*) indicates that a given Cervus delta value met the strict 95% confidence level.

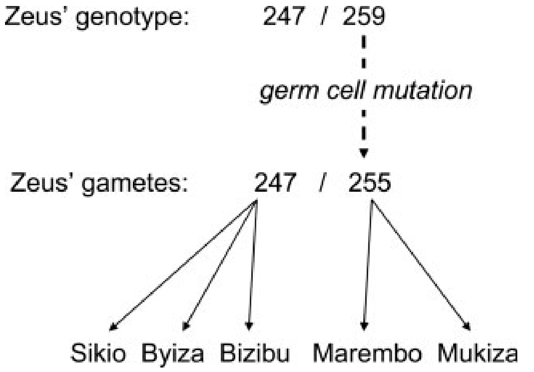


Fig. 1. Scheme showing how a premeotic mutation in the paternal (Zeus) germline could arise and be distributed into parent's (Zeus) gametes and eventually inherited by approximately half of his progeny.

for quantification of DNA and verification of results, including sequencing of the putative alleles.

Most, but not all, microsatellite mutations in humans as well as other taxa appear to involve a single-step mutation rather than a multistep change or saltatory changes in the motif unit (repeat count) (Brinkmann et al., 1998; Sajantila et al., 1999). In addition, mutations in the male germ line are at least five times as common as those transmitted through the mother (Brinkmann et al., 1998; Sajantila et al., 1999).

Two types of mutations are known to occur from puberty onwards in the gonads during different stages of the gametic formation. Early premeiotic mutations occur before primordial germ cell differentiation, while postmeiotic mutations occur during meiosis (Roosen-Runge, 1977; Woodruff and Thompson, 1992; Ibarguchi et al., 2004). The former can give rise to a cluster of identical mutant gametes or successive clusters of identical mutant gametes over the reproductive life of an individual (e.g., pipefish and shoehorses: Jones and Avise, 2001; dollar sunfish: MacKiewicz et al., 2002; Ibarguchi et al., 2004), while the latter produces a single mutant gamete.

In the work reported here, the presence in two of five offspring of a paternally-derived allele differing by one repeat unit from an allele found in the genotype of the apparent father strongly suggests the presence of a primoridal germ cell mutation in the male Zeus. Zeus' case cannot be assumed to have arisen as a single mutation event during late meiosis in a single gametic cell, because the mutant allele (255) has been observed in two offspring born some 5 years apart. Hence, this mutation would have arisen premeiotically in the paternal germline (primordial cell) and then distributed to several of the parent's (Zeus') gametes and to roughly half of his progeny (see Fig. 1).

In humans, tetranucleotide-repeat microsatellite loci have an average mutation rate of 0.6–1.3 $\times$ 10^{-3} per locus per meiosis per generation (Brinkmann et al., 1998; Sajantila et al., 1999). Assuming a similar mutation rate for gorillas, the 441 meioses observed here would yield an expected frequency of observed mutations of 0.57, which is similar to the two transmissions of mutations actually observed. The possible presence of mutations in large collections of genotypes is one reason favoring the use of likelihood-based methods of paternity assignment in which mismatches do not automatically result in exclusion of potential fathers. Alternatively, conservative use of exclusion-based paternity assignment procedure requires two mutations for exclusion of an individual, an approach we used here and in similar studies (Vigilant et al., 2001; Bradley et al., 2004, 2005; Boesch et al., 2006). Mutations have been invoked by other workers to explain single mismatches between progeny and apparent fathers (Jones et al., 1999; Constable et al., 2001).

Implications for the social system of Bwindi mountain gorillas

In sum, our paternity results show that the dominant male does not sire all offspring found in his group. In the Kyagurilo group, five of seven (71%) offspring were assigned to the male known to be dominant from 1998 to 2004. One offspring from the prior period when dominance relationships were unknown was assigned to Ntegenisa, but as he was only a young maturing silverback at that time it is highly unlikely that he held the dominant rank. The other instance of nonpaternity by the dominant silverback occurred near the end of Zeus' tenure, and was attributed to the subordinate (Rukina) who eventually assumed the dominant role. This subordinate siring was not a case of inbreeding avoidance by the dominant male, because the mother, a primiparous female, emigrated into the group in 2000. In the Habinyanja group, eight of the ten offspring tested were attributed to the dominant silverback (HASB) while the subordinate

TABLE 4. Parentage assignments for the Habinyanja group

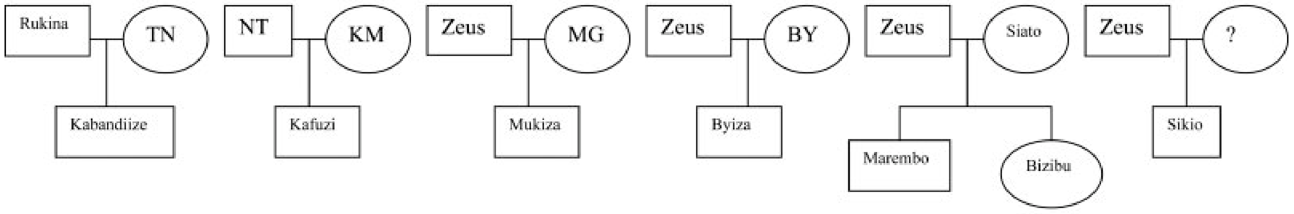
							Cervus confidence	
Offspring	Loci[a]	Mother	HASB	HBSB[b]	Pe	Father	Delta	95%
b1	11	M1	0	2	0.9983	HASB	4.41 E + 00	*
b2	12	M2	0	2	0.9977	HASB	3.60 E + 00	*
b3	11	M3	0	1	0.9327	HASB	2.02 E − 00	*
b4	12	M4	0	2	0.9994	HASB	7.17 E + 00	*
b5	12	M5	0	2	0.9966	HASB	1.53 E − 00	*
b6	12	M6	0	2	0.9989	HASB	5.40 E + 00	*
b7	12	M7	0	2	0.9992	HASB	5.48 E + 00	*
bX	12	MX	0	1	0.9954	HASB	3.86 E + 00	*
Bb1[b]	12	BM1[b]	6	3	0.9999	unknown	9.90 E + 00	*
Bb2[b]	12	BM2[b]	1	3	0.9999	unknown	3.76 E + 00	*

[a] Number of loci typed.

[b] Member of HB splinter group.

Columns 4 and 5 indicate number of loci at which potential sires HASB (dominant) and HBSB (subordinate) are excluded. Pe indicates paternity exclusion probability calculated using allele frequencies from adult individuals. An asterisk (*) indicates that a given Cervus delta value met the strict 95% confidence level.

a) *Kyagurilo group*



b) *Habinyanja group*

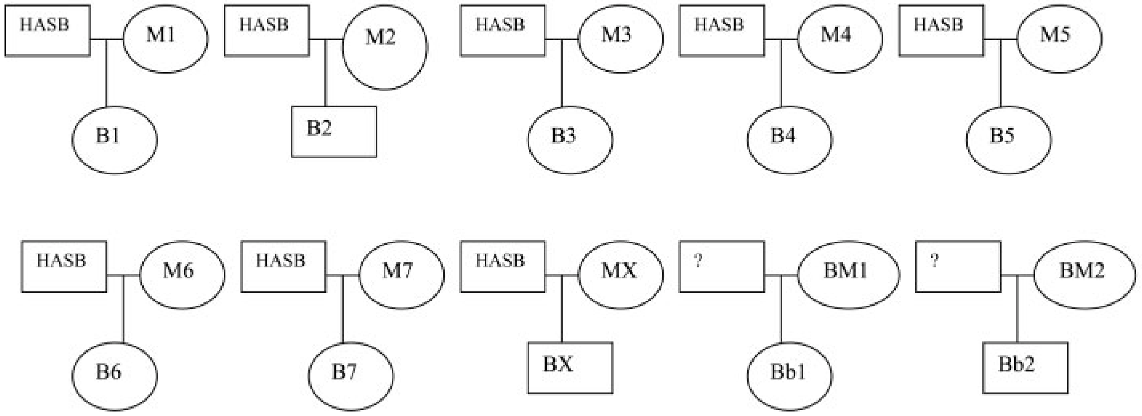


Fig. 2. Pedigree diagram showing parentage assignments in Kyagurilo and Habinyanja groups. Boxes represent male gorillas, and circles represent female gorillas. (**a**) In Kyagurilo group: Zeus (ZS), Ntegenisa (NT), and Rukina (RC) are assigned sires; TN, Kaf-mom (KM), Magwere (MG), Binyindo (BY), and ST (Siatu) are the mothers; Kabandiize, Kafuzi, Mukiza, Byiza, Marembo, Bizibu and Sikio are the offspring. (**b**) In Habinyanja group: the dominant male HASB is assigned as the sire of offspring B1, B2, B3, B4, B5, B6, B7, and BX; M1, M2, M3, M4, M5, M6, M7, MX, BM1, and BM2 are the mothers "?" indicates unsampled or unknown parent.

silverback did not sire any. At least one of the unassigned offspring was likely sired by a male no longer present in the group at the time of sampling. The results of this study are consistent with results on the Virunga mountain gorillas, where 85% of the 48 offspring analyzed had dominant males as fathers (Bradley et al., 2005).

The dominant and subordinate silverbacks were not father–son pairs in one group studied and the relationship could not be resolved in the second group. Estimates of dyadic relatedness values (A.M.N., M.M.R., and L.V., unpublished data) suggest that of the adult male within-group pairs, only the two Habinyanja silverbacks might be siblings. These findings are consistent with results from research on multimale group of Virunga mountain gorillas, where in four groups the dominant and oldest subordinate male pairs were not father and son and only a minority of pairs might be related as half siblings (Bradley et al., 2005). Thus, this provides further evidence that the social system of mountain gorillas does not always follow an age-graded structure in which subordinate adult males within groups are offspring of the dominant male.

Results on paternity distribution from the more extensive dataset on Virungas mountain gorillas were interpreted as favoring an "incomplete control" model of reproductive skew in which neither the dominant nor subordinate adult male has full control over his reproductive share (Bradley et al., 2005). The results presented here are consistent with this interpretation, and as in the previous study also suffer from limitations in the ability to rigorously test various reproductive skew models. Additional data from the groups studied here provides direct evidence in favor of the idea that subordinate males do best by staying and waiting for the opportunity to achieve dominance in a group even in the absence of interim reproductive opportunities. Rukina, who sired one offspring while subordinate, eventually deposed Zeus and achieved the dominant role in the Kyagurilo group and is the putative father of three subsequent group offspring (Robbins, unpublished data). Similarly, although not the father of any group offspring tested here, the subordinate silverback in the Habinyanja group became the dominant male of one of the two groups resulting from the group fission.

The fission of the Habinyanja group resulted in the formation of two groups, Habinyanja A and B. The eight offspring (and their mothers) assigned to the dominant silverback remained in Habinyanja A with their father, while the two offspring of unknown paternity ended up in the small fission group headed by the formerly subordinate silverback. This is consistent with the proposal that the outcome of group fission in primates is not only influenced by maternal relationships among individuals, but also by patrilineal relationships (Melnick and Kidd, 1983). Paternal kinship has been suggested to influence patterns of fission in savannah baboons (Smith, 2000) and rhesus macaques (Widdig et al., 2006b). Evidence of biased behavior toward paternal siblings compared with nonkin has been suggested in various mammalian taxa such as baboons (Alberts, 1999; Buchan et al., 2003; Silk et al., 2006a), rhesus macaques (Widdig et al., 2001, 2006b), and spotted hyenas (Wahaj et al., 2004). In a study of lowland gorillas, Bradley et al. (2004) suggested that high male reproductive skew coupled with long social development time, delayed dispersal, creates a potential for individual recognition and kin-biased behavior among adult paternal siblings. The presence of offspring in their father's group after fission as described here provides an opportunity for paternal investment, as has been described in baboons (Alberts, 1999; Buchan et al., 2003) and suggested in chimpanzees (Lehmann et al., 2006). Male gorillas are known to show high levels affiliation with their offspring, presumably due to high levels of paternity certainty associated with long-term male–female associations and very high male reproductive skew. Males protect their putative infants from predators and infanticidal males. Infanticide (or the killing of unweaned young by conspecifics), often done by males other than the father (Watts, 1989; van Schaik and Kappeler, 1997), is associated with the disappearance or disabling of protective males and/or during dominance reversals in the group. In contrast, the long-term male–female relationship is a paternal investment strategy that can prevent infanticide, and benefits the male by increasing the chances of survival of his putative young, consequently increasing his reproductive success (van Schaik and Kappeler, 1997). Hence, the distribution of the offspring and their mothers following group fission of Habinyanja is not surprising. Obviously, to elucidate the role played by paternal kin during group fission in mountain gorillas, one cannot generalize from this one case; we need more fission events for comparison. The results of our study stress the importance of knowing the demographic history of social groups, and integrating it with genetic data, to avoid incorrectly assuming paternal kin associations or extra-group paternities. For mountain gorillas as well as other primate taxa, the significance of paternal kin relationships is both highly intriguing and highly speculative, and the topic clearly requires an improved understanding of the means and extent of kin recognition systems in these various taxa.

CONCLUSIONS

Despite ecological differences between the Virunga Volcanoes and Bwindi (Robbins, 2007), the results of this study suggest that male gorillas from Bwindi as well as the Virungas gain greater reproductive benefits following the strategy of queuing for dominance rank rather than emigrating. Nonetheless, in both populations not all groups are multimale and some males do disperse, resulting in similar variation in the social system (one-male and multimale groups; formation of social groups via group fission and solitary males acquiring females) and are likely to have similar genetic structure within and between social groups. Further studies of the relationship among ecology, demography, and reproductive strategies in several gorilla populations will help explain the causes and consequences of observed variation in individual strategies, the social system, and the genetic structure of western gorillas and mountain gorillas.

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