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Matrilineal Kin Relationship and Social Behavior of Wild Bonobos (*Pan paniscus*): Sequencing the D-loop Region of Mitochondrial DNA

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ABSTRACT. Matrilineal kin-relations among wild bonobos (*Pan paniscus*) were studied by DNA analysis. Subject individuals were the members of E1 group, living at Wamba, Zaire, which has been studied since 1974. DNA samples were extracted from wadges that bonobos spat out when feeding on sugar cane. The D-loop region of mitochondrial DNA was amplified by the PCR method, and a nucleotide sequence of 350 base pairs was determined for 17 individuals. Nucleotide variations were found at 44 positions of the sequence. Based on these variations, 13 matrilineal units were divided into seven groups, and the mother of an orphan male was determined among several females. These genetic analyses, together with behavioral observation to date, revealed the following facts. High sequence variation in the target region indicated that females transfer between groups of bonobos, which is in agreement with supposition from long-term field studies. For females, there was no relationship between genetic closeness and social closeness that is represented by frequencies of proximity or grooming. After immigration into a new group, females form social associations with senior females without regard to kin relationship.

Key Words: *Pan paniscus*; D-loop region; Mitochondrial DNA; PCR direct sequencing; Kin relationship; Social behavior.

INTRODUCTION

Long-term studies on the behaviors of wild bonobos (*Pan paniscus*), living at Wamba, Zaire, have elucidated their social structure and their unique social behaviors (KANO, 1992). Bonobos form male-philopatric unit groups as chimpanzees do, i.e. females tend to transfer between groups around the age of their sexual maturation (KANO, 1982; GOODALL, 1986; FURUICHI, 1989; IDANI, 1991). However, their within- and between-group social relationships are different from those of chimpanzees in some important respects.

While female chimpanzees tend to range alone except for during estrus period, female bonobos tend to stay within a mixed party and keep close association with one another (FURUICHI, 1987; KANO, 1992). Relationship between bonobo groups is more peaceful than that between chimpanzee groups, and even mating between individuals from different groups has been observed during inter-group encounters (IDANI, 1990). Given these characteristics, genetic relations within and between groups may be different between chimpanzees and bonobos.

Due to the long life span of bonobos, however, field observations of behavior alone can provide very limited information on kin relations among group members. Furthermore, it is almost impossible to know kin relations of females who migrate between different groups.

Recent advances in molecular biology have enabled us to access easily the genetic background underlying social structures and behaviors of non-human primates and other animals. In addition to hypervariable tandem repeat regions such as minisatellite

(JEFFEREYS et al., 1985; INOUE et al., 1990) and microsatellite (WEBER & MAY, 1989; LITT & LUTY, 1989; TAKENAKA et al., 1993; INOUE & TAKENAKA, 1993) in nuclear DNA, mitochondrial DNA (mtDNA) is also a useful tool for animal genetics, because of its high rate of mutation and its unique mode of inheritance, maternal inheritance. Due to high copy number of mtDNA in each cell, analyses using minute amounts of biological samples are possible (HIGUCHI et al., 1988; TAKASAKI & TAKENAKA, 1991).

The D-loop region of mtDNA is thought to be involved in its replication. It does not code for proteins or ribonucleic acid like other parts of the mtDNA. This region ranges from position 16,026 to position 576 of the circular DNA, and its size is 1,122 bp in the case of humans (ANDERSON et al., 1981). KOCHER and WILSON (1991) examined the variability of this region in humans and chimpanzees and found that nucleotide sequences near the 5′ and 3′ ends and around the mid point of the D-loop region are rather stable, but two regions flanked by these three areas show very high rates of mutation.

The present study aims to elucidate the maternal lineage affiliation among wild bonobos of E1 group at Wamba, Zaire, by PCR-direct sequencing of part of the mitochondrial D-loop region.

MATERIALS AND METHODS

STUDY GROUP

Subject individuals were members of a wild group, called E1, living at Wamba, Zaire. This group split from E group in 1982–1983, which had been studied since 1974 (KURODA, 1980; FURUICHI, 1987; KANO, 1992). In 1990, E1 group overlapped its home range with three neighboring groups, i.e. E2 group (50–60 individuals), P group (50–60 individuals), and B group (50–80 individuals). Several other groups inhabited areas with overlapping home ranges to each of these groups.

All the members of E group were first identified in 1976. Information on kin relations between subject individuals was derived from studies by various researchers (e.g. KURODA, 1980; KANO, 1982, 1992; KITAMURA, 1983; FURUICHI, 1989).

WADGE COLLECTION

Wadge samples, the residue of sugar cane spat out by bonobos after chewing, were collected from most members of E1 group, from October 1990 to February 1991, and from February 1994 to June 1994.

The wadge samples were collected at artificial provisioning sites set in the forest. To avoid contamination between subject individuals, we cleared plant debris in the feeding sites before collecting the samples. We observed the feeding behavior of bonobos to record the name of each individual and the exact place where they spat out the wadge. After they left the feeding sites, the wadge sample was packed in a 50-ml polypropylene tube using a plastic disposable glove. Wet weight of samples was 20–35g. Each tube was filled with 90% ethanol containing 1 mM Na_3EDTA.

DNA EXTRACTION

All heat-stable laboratory wares were heated at 160°C for 4 hrs prior to use. Disposable plastic tubes, tips, purified water and physiological saline containing 1 mM Na_3EDTA were autoclaved for 15 to 20 min.

All contents of each sample tube were transferred to 200 ml of physiological saline in

a 300-ml beaker. The wadge samples were stirred in the solution and filtered through double sheets of gauze. The cells were then pelleted by centrifugation and filtered again through a nylon mesh, "cell strainer" of Beckton-Dickinson (code No. 2350) and transferred to a 1.5-ml tube. The sediments were collected by centrifugation at 13.5 k rpm 8,000*g* for 15 min.

We examined three kinds of buffer solution for extraction of DNA, CTAB buffer using 2% cetyltrimethylammonium bromide as detergent (MURRAY & THOMPSON, 1980), a cell lysis buffer consisting of 0.32 M sucrose and 1% Triton X-100 as detergent, and STE buffer containing 0.1 M NaCl, 10 mM Tris (trishydroxymethylaminomethane chloride, pH 8.0), and 1 mM Na_3EDTA with 1% SDS. Because there were no differences in the yields of DNA among them, we adopted CTAB buffer for routine extraction.

The sediments were suspended in 200 μl of CTAB buffer and digested with 10 μl of 5 mg/ml proteinase K (Boehringer) at 60°C for 2 hrs, and then extracted with CIAA and TE-saturated phenol each at half volume. DNA in the upper aqueous layer after centrifugation was precipitated with two volumes of ethanol. The precipitates were then dissolved with 200 μl TE and treated with 2 μl of 10 mg/ml ribonuclease A at 37°C for 30 min and again with 10 μl of 5 mg/ml proteinase K at 60°C for 2 hrs. After being extracted with phenol and CIAA, DNA was precipitated with ethanol as above. The samples were finally dissolved in 200 μl of diluted TE (5 mM Tris-HCL and 0.1 mM Na_3EDTA).

PCR PRIMERS FOR AMPLIFICATION AND SEQUENCE DETERMINATION

Primers used for PCR amplification and sequencing are shown in Figure 1, and the sequences are listed in Table 1. The nucleotide positions follow the numbering of the human mitochondrial sequence (ANDERSON et al., 1981). Primer 5 and the 5′ modified primers, biotinylated 1, FITC labeled 2, 3, and 4 were custom-made by Japan Bioservice. The sequence of primer 3 was fixed comparing several sequences of bonobos at Wamba determined using primer 4. Therefore, this primer may be effective only for bonobos at Wamba because the nucleotide sequences of this region are highly variable.

The thermal cycle for amplification was denaturation at 94°C for 1 min, annealing at 58°C for 2 min, and extension at 72°C for 3 min. The number of cycles was 40. The main cycle was preceded by heating at 94°C for 5 min before the addition of Taq DNA polymer-

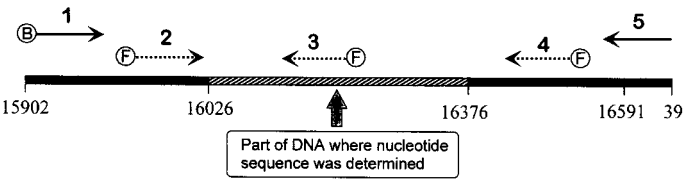

Fig. 1. The D-loop region of mtDNA and primers used for PCR amplification and sequence determination. The DNA segment was amplified using primers 5 and biotinylated 1. Nucleotide sequence was determined using FITC-labeled 4, 3, and 2. B and F indicate the positions of the labeling with Biotin (B) and FITC (F). The direction of arrows are from 5′ to 3′.

Table 1. Primer sequences and position.

	Locus code	Primer position*	Primer sequence
1	385	15902 – 15925	5′ – AACTAATACACCAGTCTTGTAAACC – 3′
2	396	15983 – 16007	5′ – TTAGCACCCAAAGCTAAGATTCTA – 3′
3	391	16208 – 16230	5′ – CAGTTGGAGGTTGATTATTGTTC – 3′
4	386	16407 – 16428	5′ – CGGGATATTGATTTCACGGAGG – 3′
5	98	19 – 39	5′ – GCTCCCGTGAGTGGTTAATAG – 3′

*Following the nucleotide number for human (ANDERSON et al., 1981).

ase. We followed the manufacturer's manual for constructing sample solution such as buffer solution, dNTP and $MgCl_2$, using 2 – 10 μl of above sample solution as template. PCR amplification was examined by polyacrylamide (5%) gel electrophoresis and visualized by silver staining (TEGELSTRÖM, 1986). Most amplification was performed with primers, biotynilated 1 and an antisense primer 5.

PCR for sequence determination was carried out using a cycle sequencing kit by Perkin-Elmer Cetus and the manufacturer's recommendation were followed with exception of thermal cycling. Cycling was preceded by incubation at 94°C for 3 min. Each of 20 cycles consisted of 30 sec at 94°C, 30 sec at 60°C, and 80 sec at 72°C. This was followed by a second set of 20 cycles, 94°C, 30 sec and at 72°C, 80 sec. The final step was incubation at 72°C for 10 min.

The nucleotide sequence was determined from both strands, with primers 5 and 6 from the 3′ terminal using the biotinylated and beads-bound strand as template, and with primer 3 from 5′ to 3′, using dissociated single strand DNA as the template. Sequences determined with a Shimadzu fluorescent automatic sequencer DSQ-1 were analyzed by GENETYX (Software Co.). The phylogenetic tree was constructed by UPGMA method (SOKAL & MICHENER, 1985) using the computer software, PHYLIP (FELSENSTEIN, 1993).

PREPARATION OF SINGLE STRAND DNA

In the sequence determination using a fluorescent primer, preparation of single strand DNA is preferable. We prepared single strand DNA using the magnetic beads (Dyna beads, DYNAL®) in combination with a biotinylated primer. The manufacturer's recommendation was modified as follows. PCR products produced with a biotinylated primer still contain a considerable amount of biotinylated primer which has not been incorporated into the amplified products. This oligonucleotide has a higher affinity to avidin-coated magnetic beads than the amplified DNA segments, so it binds with the beads competitively. The biotinylated primer was removed by centrifugation through a porous membrane (Microcon 100, Amicon Inc.) which allows molecules less than 100 k dalton to pass through but retains molecules over 100 k dalton. This step also removes the reagents used in PCR, oligonucleotide primers, dNTP's, DMSO, and $MgCl_2$.

A typical procedure was as follows. PCR amplification was carried out in 50 μl scale. After checking the amplification by PAGE, the PCR products were transferred to the upper chamber of the centrifuge tube, to which was added 400 μl of water. Centrifugation at 3,000 rpm (500*g*) for 10 to 13 min reduces the volume to less than 50 μl. After the addition of 400 μl of water, the tube was again centrifuged for 7 to 8 min to reduce the volume to 20 – 50 μl. The amplified DNA segment was absorbed by 100 μg of magnetic beads which bind 4 p moles of double strand DNA. Subsequent steps followed the manufacturer's recommendations.

PROXIMITY AND GROOMING INDICES

Proximity and grooming indices between individuals were derived from a study by FURUICHI (1989) on the same study group (E1) in 1985 – 1986. All the adult and adolescent individuals were observed using a focal animal sampling method (500 min for each male and 800 min for each female). During a sample session, individuals found within 3 m of the focal animal, and social grooming involving the focal animal, were recorded as a point sample with a 1-min interval. The proximity index was calculated as

$$\frac{f_A\,(B) + f_B\,(A)}{F\,(A) + F\,(B)},$$

where F (A) is the total number of samples for individual A, and f_A (B) is the number of samples in which individual B was found within 3 m proximity of A while A was the focal animal. The grooming index was calculated by the same formula, where f_A (B) is the number of samples in which B was grooming or groomed by A.

RESULTS

PCR Amplification and Direct Sequencing

Wadge samples were analyzed for 29 of 30 members of E1 group who were present in 1990–1991. For 21 individuals, samples were collected in 1990–1991. Supplementary samples for the other eight individuals were collected in 1994 (Table 2). For 17 individuals, 350 base pairs from 16,026 to 16,375 of positions following the nucleotide number of human sequence (Anderson et al., 1981) could be sequenced.

Individuals whose sequence was successfully determined included two mother-offspring pairs (*Sr* and *HK, Ki* and *KU*), and one pair of brothers from the same mother (*ST* and *TN*). The sequence for individuals of each of these pairs was completely identical. This fact suggests that nucleotide substitution rarely occurred within one generation.

Table 2. Wadge samples and results of sequencing.

Name of matrilineal units	Name of individuals	Birth year	Samples used for this study: Collected in 1990-1991	Samples used for this study: Collected in 1994	Successful PCR amplification	Successful sequencing
Km	MN	1967*	○		No	
	TW	1974*	○		Yes	Yes
	Ts	1984	○		No	
Sn	Sn	1945-1950*	○		Yes	No
	TN	1970*	○		Yes	Yes
	ST	1980	○		Yes	Yes
Mt	Mt	1950-1955*	○		No***	
	MO	1975*	○		Yes	Yes
Hl	Hl	1957*	○		Yes	Yes
	HY	1986		○	No	
	HZ	1990		○	No	
Sr	Sr	1957*	○		Yes	Yes
	HK	1982	○		Yes	Yes
	SJ	1988		○**	Yes	Yes
Bh	Bh	1964*	○		Yes	Yes
	Bb	1986	○		No	
	BO	1990		○	No	
My	My	1966*	○		Yes	Yes
	MA	1986		○	Yes	No****
	MR	1990		○	No	
No	No	1971*	○		Yes	Yes
	Ns	1987		○	Yes	No****
Ms	Ms	1974*	○		Yes	Yes
	Mi	1986	○		No	
	Mh	1990				
Ki	Ki	1974*	○		Yes	Yes
	KU	1988		○	Yes	Yes
KR	KR	1950-1955*	○		Yes	Yes
HT	HT	1955-1960*	○		Yes	Yes
IK	IK	1960-1962*	○		Yes	Yes

*Estimated age; **this sample appeared to be belonging to SJ by the analysis of this study; ***sample was colored; ****contamination with human.

TYPING THE SEQUENCE OF THE TARGET REGION

In this study, "matrilineal unit" was defined as a mother and her offspring(s). In the study period, there were nine mothers and their offspring(s) in E1 group (Fig. 2). Another mother (Km) who had died just before the study period left two sons and one daughter (MN, TW, and Ts). These three siblings were considered to be another matrilineal unit. Furthermore, there were three adult males whose mothers were unknown. Since they were older than 28 yrs, their mothers were assumed to have died. Assuming that they had different mothers, they formed other three matrilineal units. Thus, in total, there were as many as 13 behaviorally-identified matrilineal units in E1 group. Each matrilineal unit was designated by the mother's name or by the name of males whose mothers were unknown. As shown in Figure 2, sequence information was available for at least one individual in each matrilineal unit.

In 13 matrilineal units, seven haplotypes of sequences were identified in the target region in the D-loop region of mtDNA. Figure 3 shows a phylogenetic tree of matrilineal units constructed by the UPGMA method. Within each of four groups of matrilineal units, i.e. 1) *Km, Sr,* and *KR* units; 2) *Sn, My,* and *Ms* units; 3) *IK* and *HT* units; and 4) *Ki* and *No* units, the nucleotide sequences were completely identical. Although *IK* and *HT* units were treated as independent matrilineal units, there is a possibility that IK and HT were brothers

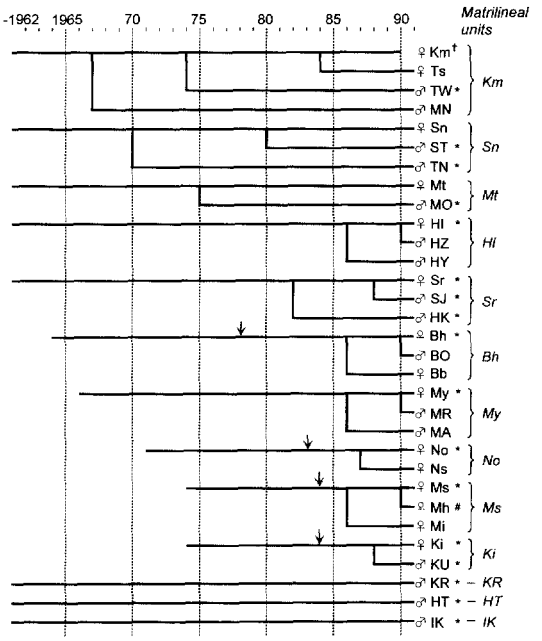

Fig. 2. Kin relationship of members of E1 group in 1991. Left end of a horizontal line of each individual indicates its birth year. Birth years before 1976 are those estimated. Abbreviations of male's name are shown by two capital letters, and those of female's name are shown by capital and small letters. *Km* marked with † died in 1990 but is shown in this figure to clarify the kin relationships between her offspring. All other individuals shown in this figure are those who were alive in 1991. ↓ indicates year of immigration for females who entered E1 group after 1976. Nucleotide sequence was determined for individuals marked with *. We could not collect wadge samples from an individual marked with #.

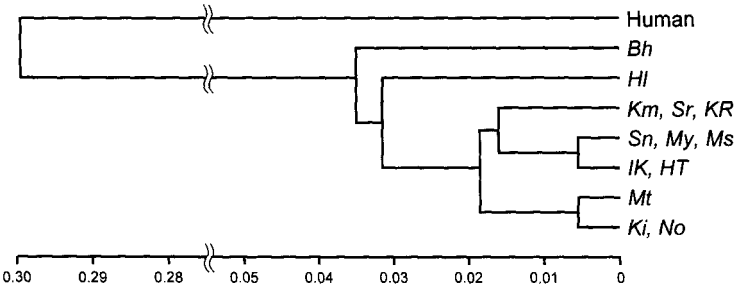

Fig. 3. Phylogenetic tree of matrilineal units drawn by the UPGMA method. A human sample (ANDERSON et al., 1981) was used as an outer group. Matrilineal units shown in the same line shared the same sequence in 350 bp region of D-loop.

born from the same mother. Another lone male unit, *KR*, had the same sequence as *Km* and *Sr* units. However, it is reasonable to assume that KR formed an independent matrilineal unit, as he was too old to be a son of Km or Sr. Mothers included in each group might be sisters or close relatives, though there were no means to confirm these relationships. The other three matrilineal units (*Mt* unit, *Hl* unit, and *Bh* unit) had their own unique sequence.

ESTIMATION OF THE MOTHER OF AN ORPHAN

In February 1994, we found a juvenile male ranging alone without his mother. His age was estimated to be 5 to 6 yrs old. We paid attention to him because there was no record of survival of infant orphans of bonobo.

In order to identify him, we compared the nucleotide sequence of the orphan with those of other members of E1 group. The orphan's sequence completely matched with the sequence of three matrilineal units. There were two adult females in these units, Sr and Km. However, Km was dead without an infant offspring in 1990. Sr was confirmed to have a son of 1.5 to 2 yrs old in March 1991 and disappeared (probably died) before September 1992. Therefore, Sr was the orphan's most likely mother in E1 group and the orphan was estimated to be SJ, Sr's son born between February and August 1988.

Thus SJ lost his mother before he grew to 4 to 4.5 yrs old, and was confirmed to survive when he was 5.5 to 6 yrs old. There is no record of him between these two periods because no research was carried out in 1993. This was the youngest record of bonobo orphans to survive long after their mother's disappearance. Although SJ's elder brother, HK, was not taking care of SJ, other adult males, IK and TN, were observed to take care of SJ in 1994. They frequently moved, fed, and exchanged grooming with SJ just as mothers do with their offspring. Moreover, other group members showed no aggressive behavior toward SJ. These might have helped SJ's survival in his critical age of weaning.

DIVERGENCE OF THE TARGET REGION AND PHYLOGENETIC RELATIONS

Table 3 shows the number of nucleotide substitutions between matrilineal units. A total of 44 positions on the 350 base pairs were variable in the study group. Most of the substitutions were transitions. However, matrilineal units of *Hl* and *Bh* showed some transversions with other units.

Table 3. Number of nucleotide differences among 13 matrilineal units.

	Sn, My, Ms	*IK, HT*	*Mt*	*Km, Sr, KR*	*Ki, No*	*Hl*
IK, HT	4 (0)*					
Mt	2 (0)	6 (0)				
Km, Sr, KR	12 (0)	10 (0)	14 (0)			
Ki, No	3 (0)	1 (0)	5 (0)	9 (0)		
Hl	21 (3)	19 (3)	23 (3)	25 (3)	18 (3)	
Bh	24 (1)	22 (1)	24 (1)	26 (1)	21 (1)	25 (2)

*Figures show the total numbers of substitutions (transitions + transversions), and the figures in parentheses show the number of transversions.

Figure 4 shows sequences of bonobos, chimpanzees, and humans, and the variable positions among the 350 base pairs. Similarity between individuals was measured using the "Homology Score" by GENETYX (LIPMAN & PEARSON, 1985). Homology scores between individuals (or matrilineal units) of this study were 92.9 – 99.7%. Homology scores between bonobos of this study and three bonobos whose sequences were determined by other studies (KOCHER & WILSON, 1991; MORIN et al., 1994) were 86.3 – 90.5%. On the other hand, homology scores between bonobos of this study and individuals of each subspecies of chimpanzee (MORIN et al., 1994) were 80.2 – 83.7% with *Pan troglodytes troglodytes* in Gabon; 79.6 – 83.7% with *P. t. schweinfurthii* in the Mahale Mountains; 80.2 – 82.3% with *P. t. schweinfurthii* in Gombe Stream; and 81.1 – 83.7% with *P. t. verus* in Ivory Coast. Homology scores between bonobos of this study and humans (ANDERSON et al., 1981) were between 81.0 and 82.4%.

These scores show that the distance between bonobos and the three subspecies of chimpanzees is nearly identical to the distance between bonobos and humans. This was probably because the variability of the target area of the D-loop was so high that variation was almost saturated in the comparison of different species. This method is sensitive for inter-individual or inter-group variation within a species, but not as sensitive for inter-species variation.

RELATIONSHIP BETWEEN KIN RELATION and SOCIAL CLOSENESS

Because unit groups of bonobos are male-philopatric, it is easy to analyze relationships between kinship and social closeness of males (e.g. KANO, 1992; IHOBE, 1992; FURUICHI & IHOBE, 1995). However, such analysis is difficult for females due to the lack of knowledge of kin relations. Although all the adult females are immigrants from other groups, there is a possibility that female-female bonds are maintained by matrilineal kin relations if related females tend to emigrate into the same group and choose each other as association partners.

We investigated whether or not females prefer to immigrate into a group which includes elder sisters whom they knew in their natal group. The average interbirth interval of females in the study group is 4.5 years (FURUICHI et al., 1995). Therefore, even if we allow for a margin of error, dyads of females whose estimated ages are different for less than 3 yrs can be excluded from possible sisters. Dyads of females who join the same group at an interval of more than ten years cannot have known each other because females leave their natal group at around 7 to 9 yrs old (FURUICHI, 1989; KANO, 1992). We examined four females (Bh, No, Ms, and Ki) who jointed the study group since 1976 when all its members were first identified (Fig. 2). Although a dyad of Ms and Ki was excluded because they were

Position			16039	16042	16047	16048	16051	16061	16063	16066	16068	16069	16071	16072	16074	16075	16076	16078	16079	16111	16125	16126	16128	16129	16134	16136	16139	16140	16142	16143	16145	16147	16148	16153	16156	16157	16162	16166	16169	16171	16172	16173	16174	16175	16180	16182	16184	16186	16187	16188	16189	16190	16193	16199	16202	16208	16210
Consensus			G	A	A	G	T	T	G	T	C	T	T	A	C	T	-	C	A	T	T	A	T	T	C	T	A	C	C	T	A	C	A	C	A	A	T	C	A	T	C	C	T	A	A	-	C	T	T	A	C	T	C	A
Species	Location	Sample name																																																						
Pan paniscus	Wamba	*IK, HT*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
		Mt	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
		Sn, My, Ms	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
		Na, Ki	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
		Km, Sr, KR	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
		Bh	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	C	.	.	.	.	.	.	.
		Hl	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	C	T	.	.	.	.
	Others	Bosandjo1 [1]	#	#	#	G	A	.	?	A	?	?	?	C	?	T	C	A	.	.	G	.	C	.	.	A	.	.	G	.	.	G	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Bosandjo2 [1]	#	#	#	G	A	.	.	A	.	?	.	C	.	T	C	A	.	.	G	.	C	.	.	A	.	T	.	G	.	.	G	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	C	C	.	.	.
		MIPPP1 [2]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	G	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	C	A	T	.	.
P. t. schweinfurtii	Gombe	MIPTSTT3 [1]	#	#	#	G	A	.	?	A	?	?	?	C	.	T	C	A	.	.	G	.	C	.	.	A	.	T	.	G	.	T	G	.	T	C	C	C	.	C	C	-	-	C	.	C	C	.	.	.	.	.	.	.	C
	Mahale	MIPTS2 [1]	#	#	#	G	A	.	?	A	?	?	.	C	.	T	C	A	.	.	G	.	C	.	.	A	.	.	G	.	T	G	.	T	C	C	C	.	C	C	-	-	C	G	C	.	C	C	.	.	.	.	.	.	.	C
P. t. troglodytes	Gabon	MIPTTMOP [1]	#	#	#	G	A	.	.	A	.	T	.	C	.	T	C	A	.	.	G	.	C	.	.	A	.	.	G	.	T	G	.	T	C	C	C	.	C	C	-	-	C	.	G	.	C	C	C	.	.	.	.	.	.	T
P. t. verus	Ivory Coast	MIPTV4 [1]	#	#	#	G	A	.	C	A	.	.	.	C	.	T	C	A	.	.	G	.	C	G	C	A	T	.	C	G	.	T	G	.	.	C	C	C	-	-	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	C
Homo s. sapiens		HUMAN [3]	.	G	G	G	A	.	.	A	.	.	C	C	.	T	C	A	.	.	G	.	C	G	C	A	T	.	.	G	.	G	G	T	.	C	A	T	C	.	-	C	.	C	.	C	C	T	.	.	.	A	T	.

(continued)

(continued)

Sample name	16214	16215	16219	16220	16224	16225	16226	16231	16234	16235	16240	16242	16243	16244	16245	16247	16248	16249	16250	16254	16255	16257	16261	16262	16264	16266	16272	16273	16274	16279	16290	16291	16292	16293	16294	16295	16296	16300	16302	16304	16305	16310	16312	16318	16320	16321	16322	16323	16324	16325	16329	16330	16331	16336	16345	16355	16356	16358	16363	16369	16375	16376
	A	T	C	A	T	C	C	G	A	A	A	C	A	C	A	A	A	A	C	T	A	A	A	T	C	T	C	C	C	C	C	G	A	C	A	A	T	C	G	C	G	T	A	T	C	G	T	C	A	T	A	C	G	T	A	C	C	A	T	C	C	C	C
IK, HT	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Mt	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
Sn, My, Ms	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
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Km, Sr, KR	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.
Bh	.	.	.	G	.	.	.	.	G	G	.	.	G	.	.	.	.	.	C	G	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	.	A	.	.	.	.	C	.	A	.	T	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	.
Hl	.	.	.	.	.	.	.	.	G	.	.	.	.	G	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	T	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.	.
Bosandjo1	G	C	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	A	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.
Bosandjo2	G	C	.	.	.	.	.	.	G	.	C	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.	.	C	G	.	.	A	.	.	.	.	C	A	.	.	.	.	.	T	.	.	.	.	.	C	.	T	.	.	.
MIPPP1	.	.	.	G	C	.	.	.	G	.	-	A	.	.	.	C	C	T	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	G	.	.	A	.	.	.	.	C	.	A	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
MIPTSTT3	G	C	.	.	C	T	.	.	.	C	C	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	A	T	.	A	T	A	C	C	C	T	A	.	.	.	C	A	.	C	.	G	.	T	.	A	.	.	.	.	C	.	T	.	.	.
MIPTS2	G	C	.	.	C	T	.	.	.	C	C	A	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	A	.	.	A	T	A	C	T	C	T	A	.	.	.	C	A	.	C	.	G	.	T	.	A	.	.	.	.	C	.	T	.	.	.
MIPTTMOP	G	C	.	.	C	T	.	.	.	C	C	A	C	.	G	.	.	.	.	.	.	.	.	.	.	A	.	A	.	.	A	C	A	C	T	C	.	A	.	.	.	C	A	.	C	.	G	C	T	.	A	G	G	.	.	.	.	T	.	.	.
MIPTV4	G	C	.	.	.	.	T	?	.	.	C	A	C	.	.	.	.	.	C	.	.	T	.	.	.	A	.	A	.	.	A	C	.	C	C	C	.	.	.	A	A	.	A	.	.	.	.	C	T	.	.	.	.	.	C	.	T	A	T	.
HUMAN	G	C	.	.	C	T	.	A	.	C	C	A	C	T	G	.	.	.	.	C	C	.	.	.	.	A	T	A	G	.	A	C	.	C	.	C	.	A	.	.	.	.	A	.	C	.	.	.	T	.	.	.	.	.	C	.	.	T	A	.

Fig. 4. Nucleotide substitutions in 350bp region of D-loop among bonobos, three subspecies of chimpanzees, and humans. Only variable positions are shown in this figure, and dotted positions had the same nucleotide with consensus. The positions with the symbols, #, ?, and - are those of no available data, the nucleotide could not be specified, and the positions of nucleotide deletion. [1] MORIN et al., 1994; [2] KOCHER & WILSON, 1991; [3] ANDERSON et al., 1981.

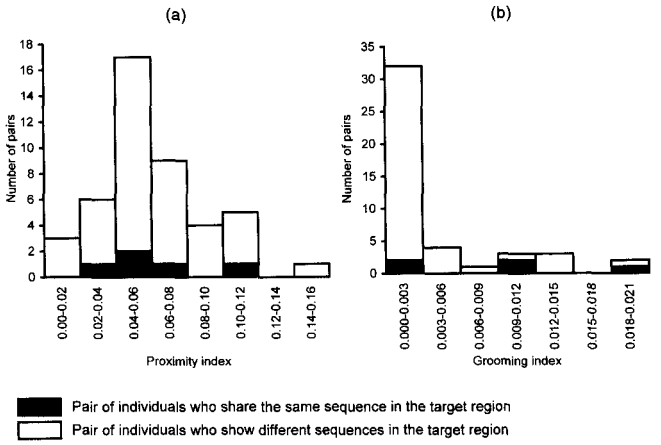

Fig. 5. Frequency distributions of (a) proximity index and (b) grooming index of adult and adolescent females of E1 group.

too close in estimated age, all the other five dyads (Bh-No, Bh-Ms, Bh-Ki, No-Ms, and No-Ki) remained as possible sisters who know each other. Among these dyads, however, only one dyad (No-Ki) shared the same nucleotide sequence. Although the small sample of this study precludes statistical analysis, the present results do not support a tendency that female bonobos choose groups that include known elder sisters.

A comparison between genetic and social closeness does not suggest that females tend to choose matrilineally related females more than non-related ones as association partners. Figure 5 illustrates the relationship between genetic closeness and indices of proximity and grooming of females. There was no significant difference in proximity or grooming index between the pairs of females that shared the same sequence in the target region and the pairs of females that showed different sequences (proximity: $n1=5$, $n2=40$, $U=98.0$, $p=0.94$; grooming: $n1=5$, $n2=40$, $U=66.0$, $p=0.17$).

DISCUSSION

In studies of primate species with long life spans, long-term observations are needed to know matrilineal relationships. In this study, we could estimate the mother of an orphan and fill a blank of observations with analysis of matrilineal linkage by sequencing mtDNA. Application of this technique to studies of new groups may greatly help clarify kin relationships, and facilitate studies of social structure and reproductive strategy.

In a matrilineal group, most females should share the same sequence of mtDNA. Therefore, if many different sequences are found among females as in this study, we could infer that female transfer is frequently occurring in the species. The results of this genetic study agree with previous behavioral observations at Wamba, where dispersal of adolescent females occurs with no records of male immigration or emigration (FURUICHI, 1989; KANO, 1992).

Even if direct observations of inter-group transfer of individuals are difficult, studies of variations in mtDNA can determine whether or not females transfer between groups. Simi-

larly, studies of Y chromosomal variations will determine whether or not males transfer between groups. Thus the analysis of nucleotide sequences may be used to clarify the extent of dispersal of each sex.

Both chimpanzees and bonobos form male-philopatric unit groups. Unlike chimpanzees, however, bonobos form large mixed parties in which females maintain close associations (e.g. FURUICHI, 1987; WHITE, 1988; KANO, 1992; ELSACKER, 1995). In a species which forms female-philopatric groups, females tend to associate with closely related females. Thus, it is an interesting problem how female bonobos can form or maintain close associations with one another.

Present study suggests that females do not selectively immigrate into a group which includes their elder sisters, nor choose matrilineally related females they find in the new group as association partners. FURUICHI (1989) and IDANI (1991) reported that newly immigrated females tend to seek one or two special partners among senior females. With these specific senior females, immigrant females have affinitive social interactions, such as following, grooming, and genital contact, much more frequently than with other females. In 1984, IDANI found four pairs of immigrant and senior females: No-Km, Ms-Bh, Ki-Bh, and Ki-Hl. This study revealed, however, that these pairs were not related closely genetically. Selected senior females tend to be adult or old adult females of high social status. It seems that immigrant females chose such females by their high social status, not by kin relatedness.

Thus the close aggregation of female bonobos seems to be maintained by newly formed social association, not by kin relatedness. Female bonobos frequently use sexual behavior to regulate inter-individual relationships (e.g. KANO, 1992; WRANGHAM, 1993; HASHIMOTO & FURUICHI, 1994; DE WAAL, 1995). They appear to employ sexual behavior as a novel means to develop close social relationships, instead of kinship.

Abbreviations: CIAA: 24:1 mixture of chloroform and isoamylalcohol; PAGE: Polyacrylamide gel electrophoresis; TE: 10 mM trishydrosyamino-methane hydrochloride and 1 mM Na_3EDTA; EDTA: ethylenediaminetetraceticacid; FITC: fluoresceinisothiocyanate.

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REFERENCES

ANDERSON, S.; BANKIER, A. T.; BARRELL, B. G.; DE BRUIJN, M. H. L.; COULSON, A. R.; DROUIN, J.; EPERON, I. C.; NIERLICH, D. P.; ROE, B. A.; SANGER, F.; SCHREIER, P. H.; SMITH, A. J. H.; STADEN, R.; YOUNG, I. G. 1981. Sequence and organization of the human mitochondrial genome. *Nature*, 290: 457–465.

ELSACKER, L. V. 1995. A review of terminology on aggregation patterns in bonobos (*Pan paniscus*). *Int. J. Primatol.*, 16: 37–52.

FELSENSTEIN, J. 1993. PHYLIP (phylogeny inference package) Version 3.5c. Distributed by the author. Dept. Genet., Univ. of Washington, Seattle.

FURUICHI, T. 1987. Sexual swelling, receptivity, and grouping of wild pygmy chimpanzee females at Wamba, Zaire. *Primates*, 28: 309–318.

FURUICHI, T. 1989. Social interactions and the life history of female *Pan paniscus* in Wamba, Zaire. *Int. J. Primatol.*, 10: 173–197.

FURUICHI, T.; IDANI, G.; IHOBE, H.; KURODA, S.; KITAMURA, K.; MORI, A.; ENOMOTO, T.; OKAYASU, N.; HASHIMOTO, C.; KANO, T. 1995. Population dynamics of wild groups of bonobos at Wamba, Zaire. Paper presented at the 11th Congress of the Primate Society of Japan, Aichi, Japan.

FURUICHI, T.; IHOBE, H. 1995. Variation in male relationships in bonobos and chimpanzees. *Behaviour*, 130: 212–228.

GOODALL, J. 1986. *The Chimpanzees of Gombe: Patterns of Behavior.* Harvard Univ. Press, Cambridge, Massachusetts.

HASHIMOTO, C.; FURUICHI, T. 1994. Social role and development of noncopulatory sexual behavior of wild bonobos. In: *Chimpanzee Cultures*, WRANGHAM, R. W.; MCGREW, W. C.; DE WAAL, B. M.; HELTNE, P. G. (eds.), Harvard Univ. Press, Cambridge, Massachusetts & London, pp. 155–168.

HIGUCHI, R.; VON BEROLDINGEN, C. H.; SENSABAUGH, G. F.; ERLICH, H. A. 1988. DNA typing from single hairs. *Nature*, 332: 543–546.

IDANI, G. 1990. Relations between unit-groups of bonobos at Wamba, Zaire: encounters and temporary fusions. *Afr. St. Monogr.*, 11: 153–186.

IDANI, G. 1991. Social relationships between immigrant and resident bonobo (*Pan paniscus*) females at Wamba. *Folia Primatol.*, 57: 83–95.

IHOBE, H. 1992. Male-male relationships among wild bonobos (*Pan paniscus*) at Wamba, Republic of Zaire. *Primates*, 33: 163–179.

INOUE, M.; TAKENAKA, A.; TANAKA, S.; KOMINAMI, R.; TAKENAKA, O. 1990. Paternity discrimination in a Japanese macaque group by DNA fingerprinting. *Primates*, 31: 563–570.

INOUE, M.; TAKENAKA, O. 1993. Japanese macaque microsatellite PCR primers for paternity testing. *Primates*, 34: 37–45.

JEFFEREYS, A. J.; WILSON, V.; THEIN, S. L. 1985. Hypervariable "microsatellite" regions in human DNA. *Nature*, 314: 67–73.

KANO, T. 1982. The social group of pygmy chimpanzees (*Pan paniscus*) of Wamba. *Primates*, 23: 171–188.

KANO, T. 1992. *The Last Ape: Pygmy Chimpanzee Behavior and Ecology.* Stanford Univ. Press, Stanford.

KITAMURA, K. 1983. Pygmy chimpanzee association patterns in ranging. *Primates*, 24: 1–12.

KOCHER, T. D.; WILSON, A. G. 1991. Sequence evolution of mitochondrial DNA in humans and chimpanzees: control region and a protein-coding region. In: *Evolution of Life: Fossils, Molecules, and Culture*, OSAWA, S.; HONJO, T. (eds.), Springer-Verlag, Tokyo, pp. 391–413.

KURODA, S. 1980. Social behavior of the pygmy chimpanzees. *Primates*, 21: 181–197.

LIPMAN, D. J.; PEARSON, W. R. 1985. Rapid and sensitive protein similarity searches. *Science*, 227: 1435–1441.

LITT, M.; LUTY, J. A. 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Amer. J. Hum. Genet.*, 44: 397–401.

MORIN, P. A.; MOORE, J. J.; CHAKRABORTY, R. C.; JIN, L.; GOODALL, J.; WOODRUFF, D. S. 1994. Kin selection, social structure, gene flow, and the evolution of chimpanzees. *Science*, 265: 1193–1201.

MURRAY, M. G.; THOMPSON, W. F. 1980. Rapid isolation of high-molecular-weight plant DNA. *Nucl. Acids. Res.*, 8: 4321–4325.

SOKAL, R. R.; MICHENER, C. D. 1985. A statistical method for evaluating systematic relationships. *Univ. of Kansas Sci. Bull.*, 28: 1409–1438.

TAKASAKI, H.; TAKENAKA, O. 1991. Paternity testing in chimpanzees with DNA amplification from hairs and buccal cells in wadges: a preliminary note. In: *Primatology Today*, EHARA, A.; KIMURA, T.; TAKENAKA, O.; IWAMOTO, M. (eds.), Elsevier, Amsterdam, pp. 612–616.

TAKENAKA, O.; TAKASAKI, H.; KAWAMOTO, S.; ARAKAWA, M.; TAKENAKA, A. 1993. Polymorphic microsatellite DNA amplification customized for chimpanzee paternity testing. *Primates*, 34: 27–35.

TEGELSTRÖM, H. 1986. Mitochondrial DNA in natural populations: an improved routine for the screening of genetic variation based on sensitive silver staining. *Electrophoresis*, 7: 226–229.

DE WAAL, F. B. M. 1995. Bonobo sex and society. *Sci. Amer.*, 272: 82–88.

WEBER, J. L.; MAY, P. E. 1989. Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. *Amer. J. Human Genet.*, 44: 388–396.

WHITE, F. J. 1988. Party composition and dynamics in *Pan paniscus*. *Int. J. Primatol.*, 9: 179–193.

WRANGHAM, R. W. 1993. The evolution of sexuality in chimpanzees and bonobos. *Human Nature*, 4: 47–79.

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