

Skewed Matrilineal Genetic Composition in a Small Wild Chimpanzee Community

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Key Words

Demography · Genetic diversity · Habitat fragmentation · Mitochondrial DNA · *Pan troglodytes verus* · Emigration · Immigration · Small population

Abstract

Maternal kinship is important in primate societies because it affects individual behaviour as well as the sustainability of populations. All members of the Bossou chimpanzee community are descended from 8 individuals (herein referred to as original adults) who were already adults or subadults when field observations were initiated in 1976 and whose genetic relationships were unknown. Sequencing of the control region on the maternally inherited mtDNA revealed that 4 (1 male and 3 females) of the 8 original adults shared an identical haplotype. We investigated the effects of the skewed distribution of mtDNA haplotypes on the following two outcomes. First, we demonstrated that the probability of mtDNA haplotype extinction would be increased under such a skewed composition in a small community. Second, the ratio of potential mating candidates to competitors is likely to decrease if chimpanzees become aware of maternal kinship and avoid incest. We estimated that the magnitude of the decrease in the ratio is 10 times greater in males than in females. Here we demonstrate a scenario in which this matrilineal skewness in a small community accelerates extinction of mtDNA haplotype, which will make it more difficult to find a suitable mate within the community.

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Introduction

In recent years, the habitats of great apes have become degraded and fragmented due to human encroachment [Cowlshaw and Dunbar, 2000; Walsh et al., 2003]. Data from several long-term chimpanzee (*Pan troglodytes*) field studies indicate that

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community sizes typically range between 40 and 150 individuals [Goodall, 1986; Boesch and Boesch-Achermann, 2000; Wrangham, 2000; Nishida et al., 2003; Mitani, 2006]. The wild chimpanzee (*P. t. verus*) community in Bossou, Republic of Guinea, whose core area is restricted to approximately 6 km² by human activity, has been observed since 1976 [Sugiyama, 1984]. With a size of approximately 20 members since 1976, Bossou is one of the smallest chimpanzee communities known [Sugiyama, 1999, 2004]. Male chimpanzees usually remain in their natal group for life and form intermale bonds to defend the resources within their territory, while most females leave their natal group and join neighbouring communities [Goodall, 1986; Nishida, 1990]. According to the demographic record of the Bossou community, however, female immigration from outside has never been observed, but the disappearance of both males and females from this community has regularly been observed [Sugiyama, 1999, 2004]. While none of these disappeared individuals has been observed in the adjacent chimpanzee communities of the Nimba mountains to date, it is likely that they emigrated from Bossou since they were healthy at the time of their disappearance [Sugiyama, 2004]. In this study, we assumed that all healthy adolescents who disappeared from the Bossou community had emigrated. In spite of the frequent disappearance of individuals and no immigration from outside, the size of the Bossou community has remained stable for nearly three decades due to its relatively high reproductive rate [Sugiyama, 2004].

When our research team started field observations in 1976, we identified 7 females, consisting of 6 full adults and 1 subadult, and their offspring, in addition to 3 adult males, 2 of which did not remain in the community. We therefore refer to these 8 individuals (7 females and 1 male) as the 'original adults'. Based on long-term mother-offspring records (fig. 1), we defined 7 'recorded matriline' (RMs), derived from the 7 original adult females. Generally, primates such as chimpanzees avoid mating with maternally related individuals [for reviews, see Goodall, 1986; Smuts et al., 1986; Pusey, 2004]. Conversely, the effect of paternal kinship on behaviour is not as clear as maternal kinship; for example, the incidence of mating between females and their fathers varies depending on individuals [Pusey, 2004]. In other behaviours such as male-male cooperation, a lower correlation has been observed for paternal kinship than maternal kinship, prompting the hypothesis that chimpanzees are not capable of recognizing paternal kinship [Langergraber et al., 2007]. If Bossou chimpanzees are capable of recognizing maternal kinship or genetic matrilineage (GM) beyond our mother-offspring record, we might expect maternal genetic composition to affect mate choice.

The genetic diversity of the Bossou community is becoming increasingly important as female immigration has not been recorded and most of the adult females have either ceased, or are close to ceasing, menstruation. In addition, the Bossou community lost a quarter of its members due to a flu-like epidemic at the end of 2003, resulting in 2 of the 7 RMs becoming extinct within 4 months [Matsuzawa, 2006]. This marked decrease in numbers requires urgent attention, and comparisons need to be made with previous demographic records to evaluate the threat of loss of diversity.

Although Sugiyama et al. [1993] analysed faecal and food wadge (remains of eaten fruit) samples collected from the Bossou community to establish paternity using a microsatellite locus, these authors did not establish the relationships among the RMs. In addition, despite Shimada et al. [2004] reporting the existence of 5 mitochondrial DNA haplotypes (mt types) in the Bossou community, the relationships between these mt types and the RMs were not explored. Our primary goal in this

study was therefore to investigate the maternal genetic relationship between the RMs (i.e. GMs) and skewness in matrilineal genetic composition, using information based on these mt types. Since mtDNA is maternally inherited and the mothers of the original adults were unknown, the 1 original adult male and 7 RMs were targeted for mtDNA typing. Based on the resulting information regarding the relationships between RMs, we also investigated the impact of demographic changes on GM diversity within this small chimpanzee community.

Materials and Methods

Mother-Offspring Record

Long-term observations of the Bossou chimpanzee community in Guinea started in 1976. All individuals have since been individually recognized and monitored. Seven RMs were recorded based on mother and offspring relationships gathered since 1976. Besides these RMs, 1 original adult male has remained in the community since the beginning of our observation (fig. 1).

Sample Collection

We collected non-invasive samples including hair, urine and faeces from the chimpanzees of Bossou. During the sampling periods, there were 21 individuals in the community. We genotyped 13 of the 21 individuals encompassing all 7 RMs and the 1 original adult male of the community. Names of the 13 chimpanzees from which we took samples are the following (with their abbreviations in parentheses): Fana (Fn), Jire (Jr), Juru (Ju), Kai (Ka), Nina (Nn), Nto (Nt), Pama (Pm), Pili (Pl), Tua (TA), Velu (Vl), Vuavua (Vv), Yo (Yo) and Yolo (YL) (table 1). The 8 original adults are indicated with an asterisk in table 1. The first character of the individual's name indicates its RM, indicated in italic capital letters in figure 1. The sampling period was from January to March 1999, and samples were collected by M.K.S. and S.H. Additional sampling was also conducted from December 2001 to March 2002 by S.F. The 13 individuals genotyped are represented in bold circles and rectangles in figure 1.

To collect hair samples, we tracked a known individual until it slept in a bed it built in a tree about 10 m above ground. The next morning, we climbed the tree and took hairs from the bed using forceps after the chimpanzee had left. We did not collect samples from the bed when we found dried leaves in it, as this is a sign of bed reuse. We stored hairs collected from a single bed in a sterile plastic disposable tube after adding absolute ethanol. Some hairs had hair bulbs. We collected urine if we observed a known individual dropping urine on the leaves of a tree or bush. Urine was collected and deposited in a sterile tube using sterile, disposable plastic syringes, and 2 vol of 70% ethanol was added. This method is the same as that of Hayakawa and Takenaka [1999]. We collected faeces deposited by known individuals by wiping the surface of the faeces with a sterile cotton swab soaked in saline + 1 mM EDTA solution. The faecal samples attached to the wet cotton swab were collected by shaking in the tubes containing the 2 ml of saline + 1 mM EDTA solution, and then 10 ml of absolute ethanol was added to the tube. This method is the same as that used by Sugiyama et al. [1993].

DNA Extraction

The method of DNA extraction was as follows. We extracted DNA from single hairs using Isohair (Nippon Gene Co. Ltd.) according to the manufacturer's instructions. For urine samples, we followed the method described by Hayakawa and Takenaka [1999]. For faecal samples, DNA was extracted using the QIAmp® DNA Stool Kit (Qiagen) according to Morin et al. [2001]. After centrifugation (800 g, 10 min at room temperature), the precipitate was suspended in 1.6 ml ASL buffer and incubated at room temperature for 30–60 min. The final elution of DNA was incubated in AE buffer for 20–30 min. To avoid cross-contamination during DNA extraction, we wore disposable caps and gloves, and used pipettes with filtered tips.

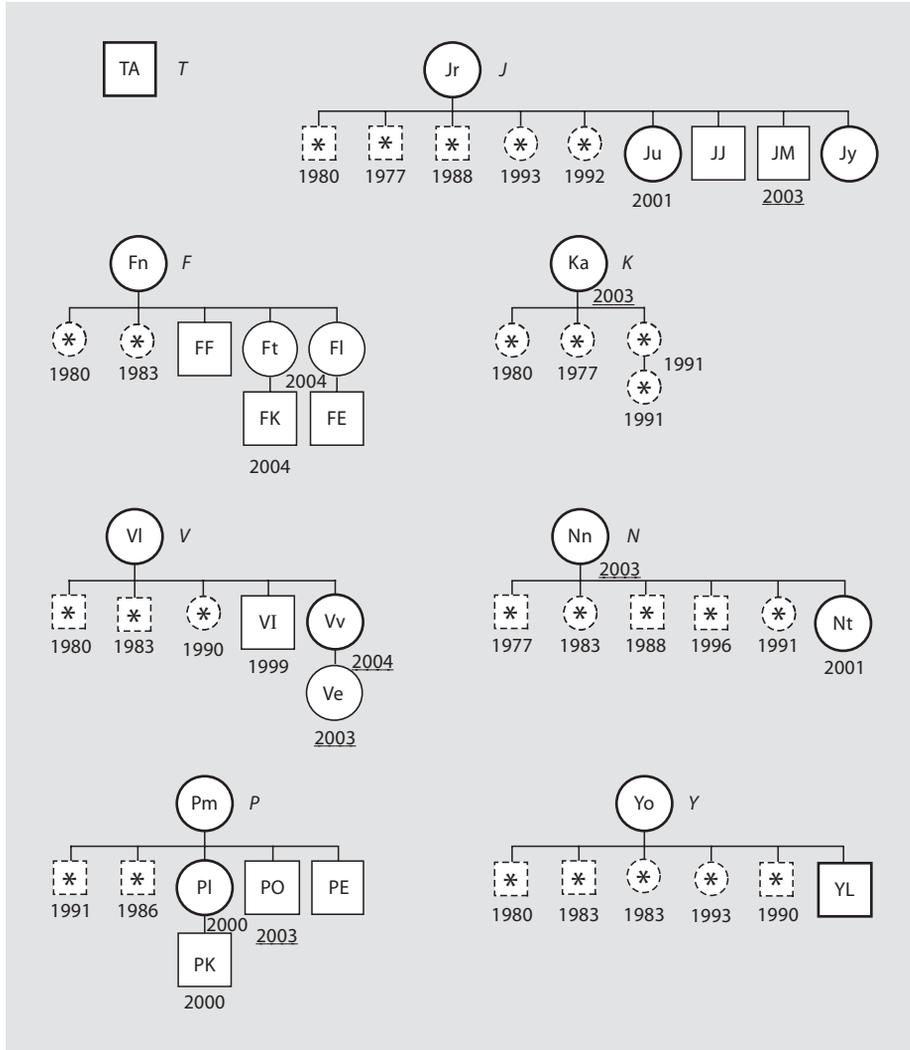


Fig. 1. The mother-offspring relationships for the RMs and the original adult male of the Bossou chimpanzee population. Genealogies were constructed based on observations from 1976 to 2007. Circles and rectangles indicate females and males, respectively. The three kinds of lines – dotted, bold and solid – represent individuals that disappeared before sampling began, individuals that were used for mtDNA genotyping and those that were not used, respectively. Four-digit numbers below figures show the year of disappearance, and figures without a year indicate present members. Individuals with the year underlined disappeared during the period of the flu-like epidemic. Although the year for the individual Vv is underlined, she is presumed to have emigrated and not died due to illness. Italicized characters show the names for the RMs. Individual names are abbreviated here and are described in the text.

Table 1. Summary of observed haplotypes in each sample

RM ¹	Individual name ²	Sample type	Sample ³	Majority ⁴	Inconsistency ⁵	RM haplotype ⁶	Consistent/total ⁷
<i>F</i>	Fana (Fn) +*	hair	1	1	0	<u>Bs-TJFK</u>	2/2
		faeces	1	1	0		
<i>J</i>	Jire (Jr) +* Juru (Ju)	hair	2	2	0	<u>Bs-TJFK</u>	3/3
		faeces	1	1	0		
<i>K</i>	Kai (Ka)*	hair	1	0	1	<u>Bs-TJFK</u>	10/12
		urine	11	10	1		
<i>N</i>	Nina (Nn)* Nto (Nt)	hair	2	2	0	Bs-N	3/3
		faeces	1	1	0		
<i>P</i>	Pama (Pm)* Pili (Pl) +	faeces	1	1	0	Bs-P	2/2
		hair	1	1	0		
<i>T</i>	TUA (TA)*	urine	3	3	0	<u>Bs-TJFK</u>	3/3
<i>V</i>	Velu (Vl)* Vuavua (Vv)	urine	5	5	0	Bs-V	16/16
		urine	11	11	0		
<i>Y</i>	Yo (Yo)* YOLO (YL)	hair	3	3	0	Bs-Y	8/8
		faeces	1	1	0		
		urine	4	4	0		

¹ RMs and 1 original male by field observation are depicted in figure 1.

² Names of males are represented by capital letters. Plus signs represent females with infants; asterisks indicate original adults (see text).

³ Number of samples whose mtDNA sequences were obtained.

⁴ Number of samples showing RM haplotype, which is determined by majority-rule when more than 2 haplotypes were detected for each RM, assuming that the majority was an authentic haplotype.

⁵ Number of samples not showing RM haplotype.

⁶ The most prevailing haplotype (i.e. major mt type, Bs-TJFK) was underlined.

⁷ Number of samples showing RM haplotype per number of samples yielding mtDNA sequence.

mtDNA Sequence Determination and Analyses

We determined about 605 bp of the hypervariable region I of the mtDNA control region. We designed primer sets, L15926 (5'-TAC ACT GGT CTT GTA AAC C-3'), corresponding to positions 15326–15344 of the complete chimpanzee mtDNA sequence of Horai et al. [1995], DDBJ/EMBL/GenBank accession No. D38113) and H16555 (5'-TGA TCC ATC GTG ATG TCT TA-3', corresponding to positions 15971–15990 of D38113). We performed PCR under the following conditions: each 50 µl reaction contained 1–5 ng template DNA, 1 × EX Taq amplification buffer, 1.25 units EX Taq enzyme (Takara), 0.5 µM each primer and 200 µM each dNTP. Amplification conditions were: denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 60 s, and 20 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 60 s, and a final extension at 72°C for 3 min. To avoid cross-contamination, we used pipettes dedicated for the preparation of PCR with filtered tips. The PCR products were purified by using Microspin™ S-400 HR columns (Amersham Pharmacia), or gel excision was followed by

ethanol precipitation if more than one PCR band appeared on a 0.9% agarose gel. The purified PCR products were labelled directly using the ABI Prism™ Big Dye Terminator Cycle Sequencing kit (Perkin Elmer). Sequencing with automated sequencers (ABI377 or ABI310, Perkin Elmer) was done from both ends using the same primers as used in the PCR. We multiplied aligned nucleotide sequences with the use of Clustal W [Thompson et al., 1994]. We sequenced at least 2 samples per RM regardless of the individual (table 1). If several multitypes were found in a RM, the most common type was regarded as the authentic one. We excluded the data if the quality was low in the chromatogram and/or the sequences diverged from corresponding regions of published chimpanzee mtDNA sequences.

Probability of mt Type Extinction

The probability that any mt type in the community would become extinct if 1 or 2 females disappeared was calculated on an annual basis. We restricted the examination to females because males do not contribute to the inheritance of mtDNA. We then compared these annual probabilities using the observed composition of mt types (P_{obs}) with those calculated under the assumption that mt types (P_{exp}) were uniformly distributed. These distributions were calculated every year as follows:

$$\begin{aligned}
 P &= n_1 C n_d / n C n_d, \text{ if } n_d = 1 \\
 P &= 0, \text{ if } n_1 = 0 \\
 P &= [(n - n_1) n_1 + n_2 + n_1 C n_d] / n C n_d, \text{ if } n_d = 2 \text{ and } n_1 \geq n_d \\
 P &= [(n - n_1) n_1 + n_2] / n P n_d, \text{ if } n_d = 2 \text{ and } n_1 < n_d
 \end{aligned}$$

where n , n_d , n_1 and n_2 are the total number of females, the number of disappeared females, number of mt types observed in 1 female and number of mt types observed in 2 females, respectively.

For comparison, we generated a hypothetical population consisting of the same number of members as Bossou with an even mt type distribution as follows:

$$\begin{aligned}
 &\text{In case } (x - 1)n_s \leq n < xn_s \\
 n_x &= \text{MOD}(n/n_s), \\
 n_{(x-1)} &= n_s - n_x, \\
 n_{(x-2)} &= 0,
 \end{aligned}$$

where n_s , n_x and MOD() are the number of mt types, the number of mt types observed in x females and the remainder after division in parentheses. Note that $(x|x \leq 3)$, $(n|n < 10)$, $(n_s|n_s < 5)$ for the Bossou community (see online suppl. appendix for an example, www.karger.com/doi/10.1159/000181187).

Analysis of Demography

Previous studies [Sugiyama, 1999, 2004] classified individuals by age into 4 categories – infant (0–3 years old), juvenile (4–7 years), adolescent (8–11 years) and adult (12 years and more) – and indicated that the disappearance of individuals was most apparent in the adolescent and adult categories. A field record suggests that sexual maturation of females in this community is earlier than that of other communities: age at first swelling and age at first parturition are 7.6 and 9.9 years, respectively, on average [Fujita, 2003]. We therefore referred to individuals over 8 years old as ‘independent’ and analysed their demographic records. At the time of the disappearance of independent individuals, we counted the number of available mating candidates (i.e. individuals of the opposite sex) and mating competitors (i.e. individuals of the same sex), and calculated the ratio between them (i.e. sex ratio for mate, or SRM), based on either condition: (1) the ‘regardless of mt type’ (MIX) or (2) the ‘mt type distinction’ (DTN). Under the MIX condition, we defined [SRM]_{MIX} for each emigration event by an individual e as:

$$\begin{aligned}
 [\text{SRM}]_{\text{MIX}}(e) &= [n_o]_{\text{MIX}}(e) / [n_s]_{\text{MIX}}(e), \\
 [\text{SRM}]_{\text{MIX}}(e) &= \frac{[n_o]_{\text{MIX}}(e)}{[n_s]_{\text{MIX}}(e)}
 \end{aligned}$$

where $[n_o]_{\text{MIX}}(e)$ is the number of independent individuals of the opposite sex to e regardless of mt type, and $[n_s]_{\text{MIX}}(e)$ is the number of independent individuals of the same sex as e regardless of mt type, including e . While we also defined $[\text{SRM}]_{\text{DTN}}$ for each emigration event by an individual e :

$$[\text{SRM}]_{\text{DTN}}(e) = \frac{1}{[n_o]_{\text{DTN}}(e)} \sum_{[o]_{\text{DTN}}(e)}^{[n_o]_{\text{DTN}}(e)} \frac{1}{[n_s]_{\text{DTN}}(o)},$$

where $[o]_{\text{DTN}}(e)$ is an independent individual of the opposite sex to e with an mt type different from e , $[n_o]_{\text{DTN}}(e)$ is the number of $[o]_{\text{DTN}}(e)$, and $[n_s]_{\text{DTN}}(e)$ is the number of independent individuals of the same sex as e , including e , with an mt type different from $[o]_{\text{DTN}}(e)$ (see online suppl. appendix for example, www.karger.com/doi/10.1159/000181187).

Results

We successfully obtained mtDNA sequences from 10 of 17 (59%) hair samples collected from night beds, 34 of 45 (76%) urine samples, and 5 of 6 (83%) faecal samples (table 1). We assigned the 5 mt types, Bs-TJFK, Bs-N, Bs-P, Bs-V and Bs-Y, to the 8 original adults, i.e. all of the RMs in Bossou, *T, J, F, K, N, P, V* and *Y* (table 1). Four of these individuals (*T, J, F* and *K*) shared an identical haplotype (mt type Bs-TJFK), while the other 4 RMs (*N, P, V* and *Y*) had haplotypes that differed from each other. The observed sequences diverged sufficiently from one another to facilitate clear identification of haplotype, and sequence data were deposited in the DDBJ/EMBL/GenBank International Nucleotide Sequence Database under Accession No. AB189231–AB189235. The details of sequence alignment and generation of phylogenetic trees for these sequences are reported in a supplementary figure together with other wild chimpanzee sequences in Shimada et al. [2004]. Since we genotyped the mt type in all of the RMs, it is impossible that other mt types will be found in this community given the absence of female immigration from the outside and maternal inheritance of mtDNA.

Figure 2a shows the frequency of mt types within individuals in the Bossou community. Since the 4 RMs (*T, J, F* and *K*) share the identical mt type, Bs-TJFK, the Bs-TJFK has accounted for nearly 50% in this community since 1976 (fig. 2a). We define the most prevailing mt type, Bs-TJFK, as ‘major type’ and the others as ‘minor types.’ In the same way, we define the GM bearing the major type as the ‘major GM’ and the others as ‘minor GMs.’ A similar finding was made for the major mt type when only independent females were considered (fig. 2c). Given the small number of independent males in the community, only one mt type, Bs-TJFK, was observed in independent males for approximately half of the field observation period (fig. 2b).

Figure 3 shows the probability of mt type extinction in females of the community if 1 or more females disappeared over time. In the event that 1 female disappeared, the average probability of extinction was 0.48 (max.: 0.6, min.: 0.2, solid triangle in fig. 3). In the event that 2 females disappeared, the average extinction probability was 0.77 (max.: 1.0, min.: 0.42, solid square in fig. 3). In both cases, these probabilities are higher than those obtained assuming even mt type distribution within the community, increased in average by 61 and 22% if 1 or 2 females disappeared, respectively. In all cases, extinction probabilities were highest in 2005 and lowest in 2000, which correspond to the maximum and minimum total number of females, respectively (fig. 2c).

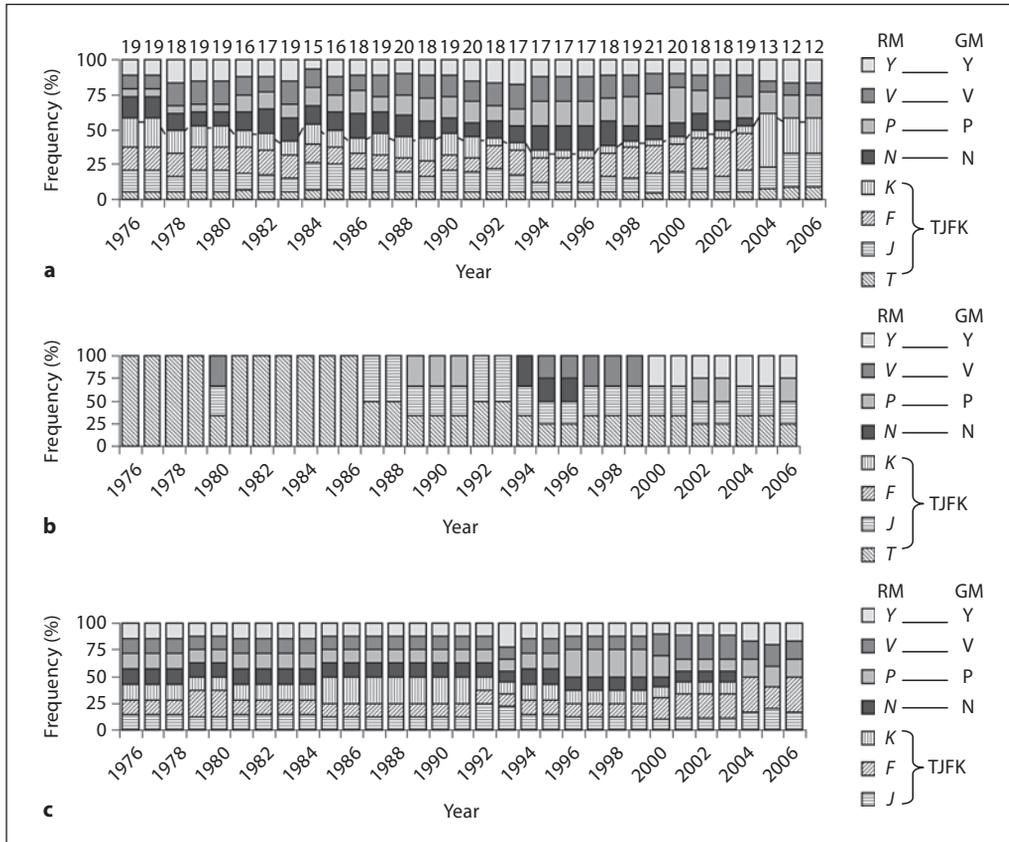


Fig. 2. Proportion of RM and GM in the Bossou community (1976–2006). The proportion of RM and GM in the total Bossou population (a), the independent (≥ 8 years old) males (b) and the independent females (c) are shown. The total numbers are indicated above each bar. The four RMs that shared the same mt type (Bs-TJFK) are crosshatched. The youngest individual (FE) born in 2007 was not included.

We have not observed any differences in the emigration rate among GMs within the Bossou community to date (number of major haplotypes per total are 6/10, 2/7, 3/4, 2/4, for emigrated females, males, remaining females, and males, respectively; fig. 1).

Table 2 shows the averaged SRM in the Bossou community at the time of the disappearance events of independent individuals (see Methods section and online suppl. appendix, with its suppl. table 1, www.karger.com/doi/10.1159/000181187). In the DTN condition, since we did not consider members of the opposite sex in the same GM as mating candidates, SRM diminished by 52–88% for all disappearance events compared to the MIX condition. Within these disappearance events, the disappearance of males was associated with a greater decrease (i.e. MIX – DTN) in SRM than when females disappeared. In both sexes, SRM of the major mt type decreased more than those of minor mt types did.

Table 2. Averaged sex ratio between opposite to same sex in the Bossou community when young adults disappeared

Sex	mt type ¹	n	SRM		Difference	
			DTN ²	MIX ³	MIX – DTN	% ⁴
Females	Major	6	0.136 (0.001)	0.373 (0.004)	0.237 (0.001)	63.7 ⁵ (19.7)
	Minors	4	0.170 (0.002)	0.356 (0.003)	0.187 (0.001)	52.4 ⁵ (54.9)
	Subtotal	10	0.150 (0.001)	0.366 (0.003)	0.217 (0.002)	59.2 ⁶ (63.0)
Males	Major	2	0.438 (0.008)	3.500 (0.500)	3.063 (0.383)	87.5 ⁷ (0.0)
	Minors	5	0.601 (0.042)	2.833 (0.556)	2.232 (0.355)	78.9 ⁷ (22.2)
	Subtotal	7	0.554 (0.036)	3.024 (0.560)	2.470 (0.464)	81.4 ⁶ (32.3)

Figures in parentheses indicate variances. SRM = Sex ratio for mate, opposite/same-sex ratio.

¹ The major mt type is Bs-TJFK, and others, i.e. Bs-N, Bs-P, Bs-V and Bs-Y, are minors.

² The number was counted under mt type distinction, i.e. opposite-sex individuals in the same mt type were not mating candidates; see text for calculation method.

³ The number was counted regardless of mt type, i.e. opposite-sex individuals in the same mt type were counted as mating candidates; see text for calculation method.

⁴ $100 \times (\text{MIX} - \text{DTN})/\text{DTN}$. ⁵ $t = 2.7$, d.f. = 4, $p = 0.05$.

⁶ $t = 6.7$, d.f. = 15, $p < 0.00001$. ⁷ $t = 3.2$, d.f. = 5, $p = 0.02$.

Figure 4 shows the observed decrease in SRM from MIX to DTN per disappearance event and the expected decrease in SRM assuming an even distribution of GM. This indicates that a skewed mt type composition has a greater effect on minor GMs compared to the major GM.

Discussion

Relationship among Maternal Lineages

Since the infinite site model in molecular evolution assumes that each mutation occurs at a new site, two haplotypes showing identical sequence always come from the same origin [Kimura, 1969]. Accordingly, the observation that identical mt types in chimpanzees have arisen in geographically distinct areas can be attributed to the low level of population structure within chimpanzee subspecies [Morin et al., 1994; Goldberg and Ruvolo, 1997]. This suggests that ancestral populations of chimpanzee subspecies were panmictic and that establishment of the current geographic distribution pattern was so recent that mutations in mtDNA do not accumulate to differentiate among current local populations [Gagneux et al., 2001; Shimada et al., 2004]. Although mt types with identical sequence are regarded as maternal kin, there are

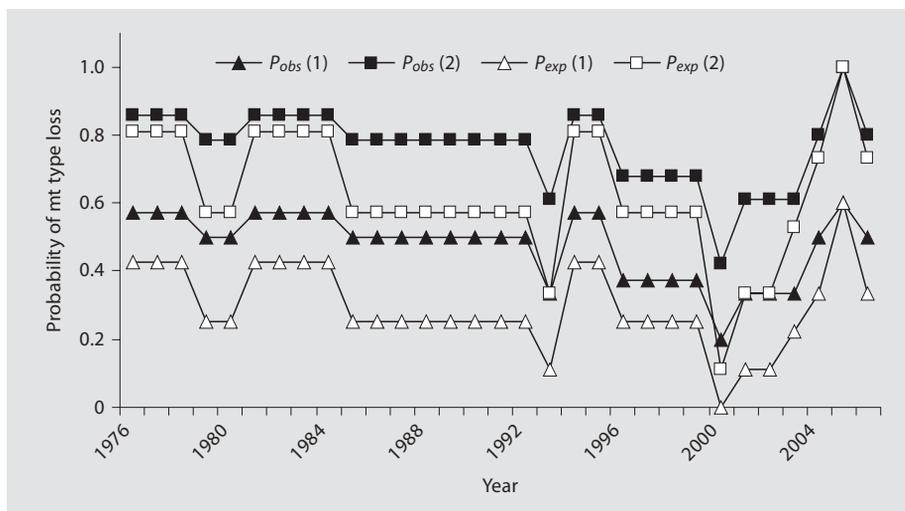


Fig. 3. Chronological change of the probability of mt type loss in females of the Bossou community from 1976 to 2007. The probability that mt types are extinct from females in Bossou community if 1 (triangles) or 2 (squares) females disappear per year is calculated. Solid and open symbols indicate the annual probabilities calculated using the observed mt type composition (P_{obs}) and those expected for an even distribution of mt type (P_{exp}), respectively.

two kinds of maternal kin: maternal relationship sufficiently close for chimpanzees to recognize kinship, and maternal relationship not sufficiently close for kin recognition. Although an additional genetic marker is necessary to estimate the time elapsed from the common maternal ancestor of the 3 RMs sharing the identical mt type (Bs-TJFK), we hypothesized that the chimpanzees belonging to the 3 RMs recognize maternal kinship. This provides a new clue to explain field observation data. Interestingly, based on our field observations, individuals that are related to the original adult male and the 3 RMs (T , J , F and K) tend to stay together significantly more often than the other RMs [Sugiyama, 1988; Sakura, 1994]. This may corroborate previous studies on apes in which it was proposed that individuals sharing identical mt types within a community are likely to be closely related maternal kin [Hashimoto et al., 1996; Goldberg and Wrangham, 1997; Mitani et al., 2000].

Skewed mt Type Distribution

Based on our genotyping results, we observed a skewed distribution of mt types within the Bossou community. Figure 3 shows that this skewness in mt type enhances the probability of mt type extinction. Consequently, because of this skewness, Bossou is more susceptible to mt type extinction compared to a population of equal size with even distribution of mt type. This skewed distribution of GM may affect chimpanzee behaviours such as migration patterns and mate choice to avoid incest. To estimate the effect of the skewed GM distribution on the migration behaviour of this chimpanzee community, we evaluated the difficulty associated with finding a mating candidate among individuals within the community by introducing SRM,

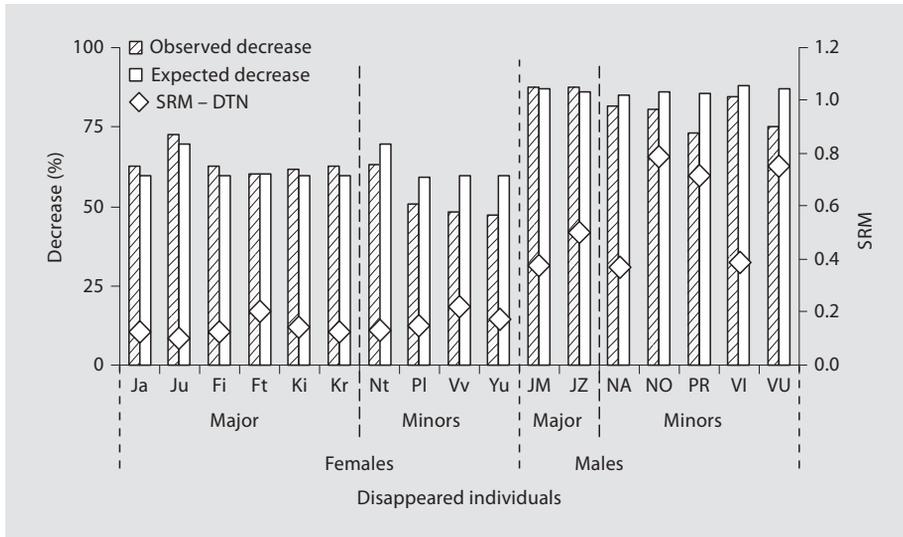


Fig. 4. Decrease in SRM per disappeared individual when GMs are considered. Hatched bars indicate the observed decrease in SRM from the MIX condition (counted independently of mt type) to the DTN condition (estimated using distinct mt type). Open bars indicate the expected decrease in SRM assuming an even distribution of GMs.

which represents the ratio of potential mating candidates (i.e. the opposite sex) to potential competitors (i.e. the same sex) for the disappeared individuals (table 2; on-line suppl. appendix, suppl. table 1, www.karger.com/doi/10.1159/000181187).

Migration Behaviour and Kinship within the Bossou Community

If we assume that individuals with the same mt type are sufficiently closely related to recognize their kin, the decrease in the SRM between the sexes becomes significant (59.2% decrease in females vs. 81.4% decrease in males, $p < 10^{-6}$, $t = 6.73$) when we change the condition from MIX to DTN (table 2). Since SRM calculations are not currently available for other chimpanzee communities, primarily due to the lack of information on GMs for all individuals, we were not able to compare the SRMs between Bossou and other communities and thus to test whether the skewed distribution in GMs at Bossou indeed explains the unique migration patterns exhibited by this small community of chimpanzees. However, we can compare the average sex ratios (males/females) across chimpanzee communities for which long-term data are available. The average sex ratio for independent individuals in the Bossou community is 0.33 (table 3), which is among the lowest of any chimpanzee community for which equivalent data are available (table 3). This finding suggests that it is generally easier for males in the Bossou community to find a potential mate than it is for males in other communities, if we consider sex ratio independently of kinship. However, males in the Bossou community show a 10 times greater SRM decrease (MIX – DTN) on average than females (2.47 in males vs. 0.22 in females, table 2). This means that the consideration of mt type to calculate sex ratio affects males more

Table 3. Comparison of averaged sex ratio

Communities	Period	Sex ratio (M/F) in adults and adolescents	Average number of individuals			Reference
			total	males	females	
Bossou	1976–2007	0.33	10.91	2.69	8.22	this study
Tai	1982–1996	0.38	35.34	9.67	25.67	Boesch and Boesch-Achermann [2000]
Mahale	1980–1999	0.44	52.40	16.10	36.30	Nishida et al. [2003]
Gombe	1965–1971	0.95	38.14	18.57	19.57	Goodall [1986]
	1972–1983	0.53	32.67	11.25	21.42	Goodall [1986]
	1965–1983	0.67	34.68	13.95	20.74	Goodall [1986]

severely than females in the Bossou community. If averaged SRM decreases (MIX – DTN) become available in other chimpanzee communities, SRM will indicate how difficult it is for males in the Bossou community to mate. Moreover, competition among females is not expected to be significant because the oestrous cycles in females are not synchronous. The relationship between difficulty for males in this community in mating with maternally non-related females and male emigration at Bossou merits further research.

Chronological Changes in Genetic Diversity within the Bossou Population

Our field observations have shown that all of the females born in Bossou have left the community, and that no immigration of females into the community has occurred since 1976. As mtDNA is inherited maternally, the lack of female immigration significantly affects mtDNA diversity.

A flu-like epidemic that struck the Bossou community in December 2003 resulted in deaths of 4 chimpanzees (Ka, JM, Ve, PO) and the disappearance (presumed death) of an old female chimpanzee (Nn). Once the epidemic had abated, another female chimpanzee (Vv), whose baby (Ve) died shortly after the epidemic, disappeared in May 2004 and is suspected to have emigrated (underlined in fig. 1). During the epidemic, the RMs *K* and *N* were lost. Because RM *K* carried mt type Bs-TJFK, which is common to RMs *T*, *J* and *F*, the number of mt types decreased from 5 to 4, i.e. only mt type Bs-N was lost; the first extinction of an mt type in the Bossou community since the initiation of our observations. Furthermore, each of the RMs harbouring haplotypes Bs-V, Bs-Y and Bs-P consists of only 1 adult female, as the males' mtDNAs are not inherited by the offspring (fig. 1). These 3 females, Vl, Yo and Pm, are original adults and have not born offspring since 1991, 1991 and 1998, respectively. In the event that these 3 adult females are lost without bearing any new female offspring that remain in Bossou, only 1 mt type, Bs-TJFK, will be passed on to the next generation of the Bossou community. Currently, all fully adult females are estimated to be over 40 years old (fig. 1), which, based on hormonal assays conducted in captive chimpanzees, is the approximate age for the onset of menopause [Videan et al., 2006]. Females younger than 40 years are Jy and Fl born in 2004 and 1997, respectively, both of which carry the mt type Bs-TJFK. Thus, if no female immigration occurs and the biased proportion of GMs is maintained, only one mt type, Bs-TJFK, will remain in the Bossou community in the near future regardless of community size.

A previous study indicated that the mtDNA diversity of the chimpanzees at Bossou is similar to that recorded in the neighbouring Nimba communities and suggested that female immigration into Bossou had occurred prior to our observations [Shimada et al., 2004]. Although assays of nuclear diversity should be undertaken to accurately estimate the risk of inbreeding, the possibility of rapid GM extinction is suggestive of a relatively higher risk of inbreeding in the Bossou community than in other communities that have been studied for an extended period.

This study provides an actual example for predicting the potential risk associated with the migration of females between distinct populations of animals such as chimpanzees. Considering the skewed proportion of GMs revealed by this study, the conservation status of the Bossou community is more sensitive than can be inferred from demographic data alone. Our research team as well as local people are currently conducting an afforestation project called the 'Green Passage Plan' to promote the migration of chimpanzees between the Bossou and Nimba forests [Hirata et al., 1998; Matsuzawa, 2006]. The maintenance of mt type diversity in the Bossou community is thus highly dependent upon whether new females will immigrate through the passage.

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