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RESEARCH ARTICLE

Mitochondrial DNA Genealogy of Chimpanzees in the Nimba Mountains and Bossou, West Africa

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The chimpanzee populations of the Bossou and Nimba regions in West Africa were genetically surveyed to 1) reveal the genetic relationship between the Bossou and Nimba populations, and 2) elucidate the evolutionary relationship between the Bossou-Nimba and other West African populations. The chimpanzee group at Bossou is characterized by its small population size, no evidence of contact with neighboring populations, and no female immigration. It is believed that most females and adolescent males emigrate from this population. To reveal the genetic signature of these characteristics, we examined the genetic diversity of Bossou and two neighboring populations (Seringbara and Yealé) in the Nimba Mountains by sequencing approximately 605 bp of the mitochondrial DNA (mtDNA) control region. A total of 20 distinct mtDNA variants were observed from 56 sequences of noninvasively collected, anonymous samples. Nucleotide diversity in the Nimba Mountain populations was 0.03–0.04, and did not differ significantly from that in the Bossou population. Very few mitochondrial variants are shared among the sites sampled, which suggests that there is little gene flow involving mtDNA. Nevertheless, no clear population structures were revealed in either population. A comparison with published sequences from West African

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chimpanzees (*Pan troglodytes verus*) indicates that the variants observed in the Bossou and Nimba regions are scattered throughout the subspecies, rather than clustered according to geographic region. This suggests that the Bossou-Nimba populations derived only recently from the common ancestral population of the West African chimpanzees, and did not pass through a bottleneck. Am. J. Primatol. 64:261–275, 2004.

Key words: chimpanzee; Bossou; Nimba; mtDNA; genetic diversity; noninvasive sampling

INTRODUCTION

The wild population of *Pan troglodytes verus* at Bossou, Republic of Guinea, is the smallest wild chimpanzee community under long-term study. It has consisted of about 20 individuals since the start of observations in 1976 [Sugiyama, 1999]. Chimpanzees in Bossou mostly confine their activity to a core area of about 6 km^2. Since the core area is surrounded by savanna and a little gallery forest, the Bossou chimpanzee group has rarely been observed to encounter neighboring groups. The Nimba Mountains area, located on the border between the Republic of Guinea, Ivory Coast, and Liberia, is separated by savanna vegetation from Bossou, the closest neighboring population (Fig. 1). UNESCO designated 220 km^2 of the Nimba Mountains regions as a ''strict nature reserve,'' and the chimpanzee population is considered to be protected in this reserve [Matsuzawa & Yamakoshi, 1996].

Contrary to the commonly recorded norms of chimpanzee behavior, female immigration has not been observed in the Bossou group. However, both sexes probably emigrate [Sugiyama, 1999]. This migration pattern is clearly different from other groups under long-term study [Gagneux et al., 2001; Goodall, 1986; Nishida et al., 1990]. Sugiyama [1999] investigated the relationship between this unusual migration pattern and the habitat isolation at Bossou from a behavioral perspective. He hypothesized that the absence of neighboring competitors negates the need for the alpha male to form alliances with other within-group males for support. Therefore, the other group males take on the role of competitors for limited food resources and females. Sugiyama [1999] also suggested an alternative hypothesis that the observed difference in social structure and migration patterns may be a characteristic of this subspecies. Conclusive answers await further analysis; however, if human land use continues to encroach on the chimpanzee habitat, this valuable research population may be lost. Our investigation of the genetic relationship of the Bossou group with surrounding groups in the Nimba Mountains may provide useful data for a conservation plan for the chimpanzee populations in this area. The Bossou group data will be compared with results from several Nimba Mountain populations that have been studied already [Matsuzawa et al., 1999], including those at Goera, Yealé, and Seringbara [Humle & Matsuzawa, 2001; Matsuzawa & Yamakoshi, 1996; Shimada, 2000; Sugiyama, 1995]. Within this framework, we investigate the genetic relationship between the Bossou group and other Nimba Mountain populations, and examine the matrilineal and geographical similarities among these populations to understand the long-term migration pattern of the Bossou-Nimba region as compared to the entire *Pan troglodytes verus* subspecies.

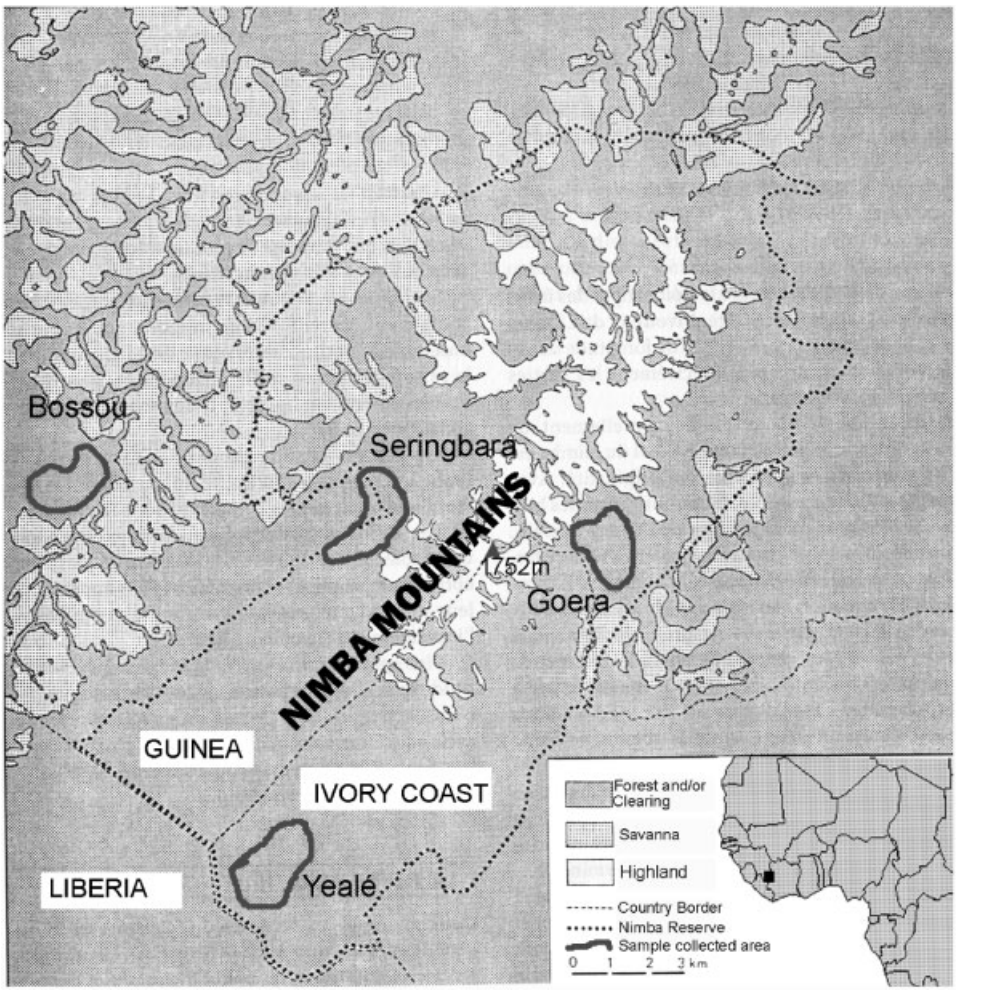

Fig. 1. Map of sampling sites. This map is based on UNESCO [1998].

MATERIALS AND METHODS

Sample Collection

Noninvasive samples, including hair, feces, and wadges (chewed fruit remnants), were collected from wild chimpanzees living at Seringbara, Yealé, and Goera in the Nimba Mountains, and neighboring Bossou (Fig. 1). We collected hairs from night beds by climbing the tree to the bed and taking hairs by forceps after the chimpanzee left. (We use the term ''bed'' because in the ecological literature, ''nest'' refers to a shelter for bearing and nursing offspring.) We stored hairs collected from one bed in a plastic disposable tube, after adding absolute ethanol to the tube. We collected the feces by wiping the surface of the feces with a cotton swab soaked in a saline/EDTA (1 mM) solution, and washing the cotton swab in 2 ml of the saline/EDTA solution. We then added 10 ml of absolute ethanol. We collected the wadges using forceps, and stored them in a plastic disposable tube after adding absolute ethanol. These samples were stored at room

temperature, avoiding sunlight in the field. Hair samples were transferred to a −20°C freezer, and feces and wadge samples were stored in a refrigerator in the laboratory. While the chimpanzees in the Nimba Mountains have not been identified individually, all of the samples from individuals in the Bossou population were from known and identifiable individuals [Sugiyama, 1999]. At the Seringbara site, local guides from Bossou village collected samples under the direction of M.K.S. during October–November 1999, except for eight samples that were collected by S.F. and S.Hi during January–February 2000. At the Yealé site, local guides collected samples under the direction of T.H. during February–December 1999. At the Goera site, M.K.S. collected samples during February–March 1999 and October 1999. Anonymous sampling was also conducted in Bossou during January–March 1999 by S.Ha and M.K.S. to compare the results with sampling from known individuals.

DNA Extraction

We used different methods of DNA extraction according to the sample type (hair, feces, and wadges), as follows:

Hair.

We extracted DNA from single hairs using Isohair (Nippon Gene Co., Ltd., Tokyo, Japan) according to the manufacturer's instructions. The hair root was visible in most samples.

Feces.

DNA was extracted with the use of the QIAmp® DNA stool kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with the following modifications [Morin et al., 2001] (Shimada et al., unpublished results): after centrifugation ($800 \times g$, 10 min at room temperature), the precipitate was suspended in 1.6 ml ASL buffer, and incubated at room temperature for 30–60 min. DNA was eluted in AE buffer for 20–30 min.

Wadges.

We followed the method of Sugiyama et al. [1993], with a slight modification. Instead of using 95% ethanol and EDTA, we used absolute ethanol to store the samples.

Mitochondrial DNA Sequence Determination

Shimada et al. (unpublished results) characterized about 605 bp of the hypervariable region I of the mtDNA control region of 14 individuals in the Bossou population. The primer sets used were L15926 (5′-TACACTGGTCTTG-TAAACC-3′ corresponding to positions 15326–15344 of the complete chimpanzee mtDNA sequence of Horai et al. [1995], DDBJ/EMBL/GenBank accession number D38113), and H16555 (5′-TGATCCATCGTGATGTCTTA-3′; corresponding to positions 15971–15990 of D38113), and inner primer set; L15933 (5′-GGTCTTGTAAACCGGAAACG-3′; 15332–15351 of D38113) and H16538; 5′-TCTTATTTAAGGGGAACGTGTG-3′, 15954–15975 of D38113).

The purified PCR products were sequenced directly from both ends with the use of the ABI PRISM™ BigDye terminator cycle sequencing kit (Perkin Elmer, Wellesley, MA), and the same primers were used for PCR. Sequences were

generated with automated sequencers (ABI 377 or ABI310; Perkin Elmer, Wellesley, MA).

Nucleotide Sequence Analyses

Published mtDNA sequences of wild chimpanzees were retrieved from the DDBJ/EMBL/GenBank International Nucleotide Sequence Database through the DDBJ website (www.ddbj.nig.ac.jp). We also obtained geographic information regarding the published *Pan troglodytes verus* sequences by contacting authors. Multiple alignment of nucleotide sequences was performed with the use of CLUSTAL W [Thompson et al., 1994].

We constructed a phylogenetic network using the reduced median network method [Bandelt et al., 1995] with NETWORK 3.0.1.2 software (URL http://www.fluxus-engineering.com). The neighbor-joining method [Saitou & Nei, 1987] was used to construct a phylogenetic tree.

Since the sample size of the Goera site was small, we did not include this population in the population structure analysis. Therefore, only Bossou, Seringbara, and Yealé were included in the analysis. Nucleotide diversity within population, net number of nucleotide difference (D_A), pairwise F_{ST}, and analysis of molecular variance (AMOVA) [Excoffier et al., 1992] analyses were conducted with the help of ARLEQUIN version 2.000 [Schneider et al., 2000]. For the calculation of D_A and F_{ST}, the following formulae were used, respectively:

$$D_A = \hat{\pi}_{12} - (\hat{\pi}_1 + \hat{\pi}_2)/2,$$

where $\hat{\pi}_1$ and $\hat{\pi}_2$ are estimates of the average number of nucleotide substitutions for a randomly chosen pair of variants in populations 1 and 2, respectively, and $\hat{\pi}_{12}$ is the estimate of the average number of nucleotide substitutions between variants from populations 1 and 2 [Nei & Li, 1979]:

$$F_{ST} = (f_0 - f_1)/(1 - f_1),$$

where f_0 is the probability of identity by descent of two different genes drawn from the same population, and f_1 is the probability of identity by descent of two genes drawn from two different populations [Slatkin, 1991].

To test the significance of the variance component and F-statistics, 1,000 random permutations were generated and compared with observed values.

RESULTS

Success Rate of Nucleotide Sequence Determination

We collected three types of samples (hair, feces, and wadge) from four sites (Seringbara, Yealé, Goera, and Bossou). When we had easy access to chimpanzee night beds, we collected hair samples from used beds. Feces were not easy to find, except at the Bossou site, where we were able to distinguish chimpanzee individuals and follow them to collect fresh feces. It was generally difficult to collect samples at the Goera site because, as suggested by the field survey, the chimpanzee group did not use this site continuously [Shimada, 2000]. The numbers of samples collected from Seringbara, Yealé, Goera, and Bossou were 26, 23, four, and 21, respectively. A total of 74 samples (58 hairs, four feces, and 12 wadges) were used.

Table I shows the success rates of DNA sequence determination for samples collected at four sites, and for three sample types. The average success rate was 76%, though it varied depending on the sample type. The success rate was the highest for hair samples (88%), followed by feces (50%) and wadges (25%). The

low success rates for feces and wadges may be attributed to the condition of the samples, which were probably highly degraded. In contrast, the hair samples were collected from fresh night beds, so they probably had better-quality DNA.

Observed mtDNA Variants

We were able to determine ca. 605-bp nucleotide sequences of the mtDNA control region from a total of 56 samples. Twenty distinct variants were identified from those sequences. The nucleotide sequence data were deposited in the DDBJ/EMBL/GenBank International Nucleotide Sequence Database under accession numbers AB189236–AB189251. Table II shows the distribution of those

TABLE I. Success Rate of Sequence Determination*

	Sample type			
Population	Hair	Feces	Wadge	Total
Seringbara	23/25	–	0/1	23/26
Yealé	7/11	2/2	2/10	11/23
Goera	1/1	0/2	1/1	2/4
Bossou	20/21	–	–	20/21
Total	51/58	2/4	3/12	56/74
Rate (%)	88	50	25	76

*Values to the left of the slash are numbers of samples from which sequences were successfully determined, and those to the right of the slash are total number of samples used. Hyphen indicates no sample.

TABLE II. Variant Distribution Among the Four Locations

	Sampling location				
Variant	Seringbara	Yealé	GoeraBossou		Total
S-1	8	0	0	0	8
S-2	5	1	0	0	6
S-3	2	0	0	0	2
S-4	1	0	1	0	2
S-5	3	0	0	0	3
S-6	1	0	0	0	1
S-7	3	0	0	0	3
Y-1	0	1	0	0	1
Y-2	0	1	0	0	1
Y-3	0	2	0	0	2
Y-4	0	1	0	0	1
Y-5	0	1	0	0	1
Y-6	0	1	0	0	1
Y-7	0	1	0	0	1
Y-8	0	1	0	0	1
Y-9	0	1	0	0	1
Bs-TJFK[a]	0	0	1	10	11
Bs-N[a]	0	0	0	1	1
Bs-P[a]	0	0	0	4	4
Bs-V[a]	0	0	0	5	5
Bs-Y[a]	0	0	0	0	0
Total	23	11	2	20	56

[a]These variants will be reported elsewhere.

20 variants among the four sampling locations. Because most of the variants were found at only one site, we named the variants after sampled sites. Variants S-1 to S-7 were mostly from Seringbara, while variants Y-1 to Y-9 were exclusively found in Yealé. Although five distinct variants were found in Bossou in our previous study (Shimada et al., unpublished results), we did not find variant Bs-Y in this study. Three of the remaining four variants were found exclusively at Bossou. The fourth, Bs-TJFK, was found at both Bossou and Goera.

Table III shows the alignment of the 21 mtDNA variants from the Nimba Mountains and Bossou. Only variant sites are presented. The variant names are the same as shown in Table II. Figure 2 shows a phylogenetic network based on the sequence data shown in Table III. We identified six major mitochondrial lineages out of 21 variants, all but one of which are known in the Bossou population (Shimada et al., unpublished results). The only one not found in the Bossou population is the Y-3 variant. There are several reticulations in this phylogenetic network. In particular, interior reticulations form two complexes: one that connects clusters 1, 5, and 6 (RC156), and one that connects clusters 2, 3, and 4 (RC234). Single site differences make both of the reticulation complexes three-dimensional (16,233 bp and 16,323 bp for RC156, and 16,233 bp and 16,399 bp for RC234). Since recombination cannot occur in mtDNA, all of the reticulations were caused by parallel substitutions.

Nucleotide Diversity Among the Three Populations

Because the number of individuals from which the samples were collected is unknown, we estimated nucleotide diversity within each population by considering two extreme possibilities: 1) minimum estimates were modeled as if each sample collected came from a unique individual, and 2) maximum estimates were constructed under the assumption that each individual bears a unique mitochondrial variant. Table IV shows these estimates, as well as the actual values for the Bossou population, in which samples were collected from known individuals (Shimada et al., unpublished results). The minimum estimate (0.034) was not far from the true value (0.038) for the Bossou population (insignificant in z statistics: $z=0.138$. As for the Seringbara and Yealé populations, the differences between the minimum and maximum estimates were insignificant $z=0.242$ and 0.006, respectively. The true value of the nucleotide diversity must lie between the minimum and maximum estimates. The estimated values for the Seringbara and Yealé populations are between 0.029 and 0.043. Those values are not significantly different from the true value (0.038) for the Bossou population, and both maximum and minimum estimates are not significantly different from the true value (0.038) for the Bossou population (minimum estimate of Seringbara vs. Bossou; $z=0.366$, maximum estimate of Seringbara vs. Bossou; $z=0.102$, minimum estimate of Yealé vs. Bossou; $z=0.190$ d.f.$=7.6$, maximum estimate of Yealé vs. Bossou; $z=0.182$. Therefore, the three chimpanzee populations are considered to have the same nucleotide diversities.

Goldberg [1998] characterized mtDNA HV I region sequences (ca. 370 bp) from anonymous hair samples collected from 19 Eastern chimpanzee (*P. troglodytes schweinfurthi*) populations, and calculated nucleotide diversity (minimum estimates) within each population. The nucleotide diversities of these 19 populations ranged from 0.009 to 0.030, with an average of 0.021. This estimate is somewhat lower than our estimate (0.03–0.04) for the Nimba and

TABLE III. Nucleotide Sequences of mtDNA Variants Found in This Study

Name	Nucleotide position corresponding to Anderson Sequence (J01415)
	111111111111111111111111111111111111111111111111111111111111111111
	555566666666666666666666666666666666666666666666666666666666666666
	999911111111111111111111222222222222222222223333333333333333333333444444455 55
	337712233444556777778899022233446677788999900111222455556677788899034689113 3
	690214947038676013892713723503890134848023634017037825570245901759085777265 8
S-1	atacctgcacctgcc-ccttatccccttaactctcgcatccc-aagtctccctcactcatcctacacgttggcgtc
S-2	...t..a....c..t-.t.......cc.g..t.ta.....tc..a.................g...a..aat...
S-3	.ctt.cat.....ttct..cg.....ccggt..c...g..t.cg....ct..c.c.c.c......gt...aa..a.
S-4	...t.....t..a..-...............t.......c............c..................
S-5	..............-.......................-.......t.......................
S-6	...t.....t..a..-...............tc......c.g..........c..................
S-7	.ctt.cat.....ttct..cg....tccggt..c...g..t.cg....ct..c.c.c.c......gt...aa..a.
Y-1	...........a..-.t......t...............c.......t......................
Y-2	.ctt.ca......ttct..cg.t...ccggt..c...g.tt.cg...tct..c.c.ctc.....t.t...aa..at
Y-3	...t.......cat.-...........c.g.ctc....c...c.g....tt......t.c..cg...ac.a.t...
Y-4	.ctt.ca......ttct..cg....tccggt..c...g..t.cg....ct..c.c.c.c......gt...aa..a.
Y-5	..............-..........c............-..........t...................
Y-6	...t.....t..a..-..............ct........c...........c..................
Y-7	...t.......a..-...............t.......c...........c..................
Y-8	tcttt.a.c...attct.cc....t..cg....g...g....-g.....t..ctt.ctcc-....gt..caa.aa.
Y-9	.ctt.ca......ttct..cg....tccggt..c...g..t.cg....ct..c.c.c.c......gt...aa..at
Bs-TJFK	...t.c...t..a..-...............t.......c...c.........c..................
Bs-P	...tt.a....c..t-.t.......cc.g..t.ta.....tc..a.................g...a..aat...
Bs-V	.ctt..a...t.a.tc.t.c.c.tt.ccgg...c.atg....cg....ct....c.c.c..t...gt...aa..a.
Bs-Y	.ctt.cat.....ttct..cg....tccggt..c...g..t.cgg...ct..c.c.c.c......gt...aa..a.
Bs-N	.cttt.a.....attct.cc....t..cg....c...g....-g.....t..ctt.ctcc.....gt..caa.aa.

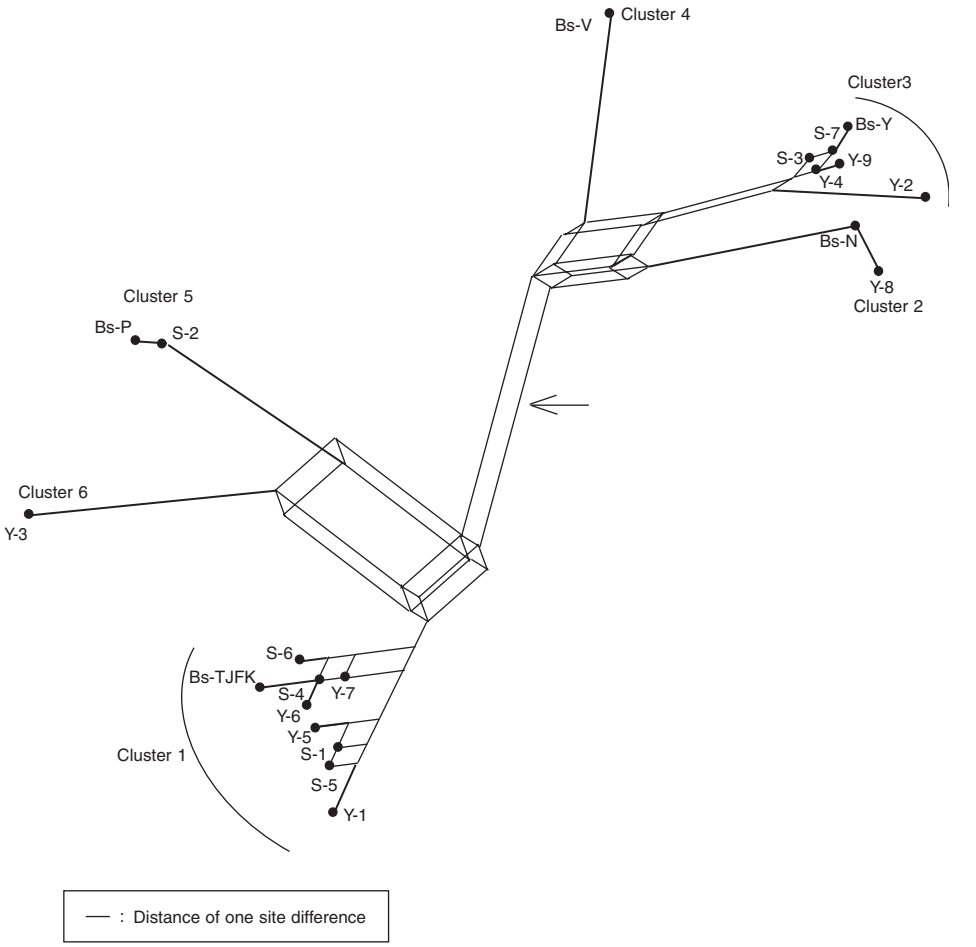

Fig. 2. A phylogenetic network of mtDNA hypervariable region I variants observed in chimpanzees at the Bossou and Nimba regions. The root is located using bonobo (*Pan paniscus*) sequence as an outgroup.

TABLE IV. Comparison of Three Types of Estimates of Nucleotide Diversity

	Estimation method		
Population	True	Minimum[a]	Maximum[b]
Bossou	0.038	0.034	0.054
Seringbara	N.D.[c]	0.029	0.036
Yealé	N.D.[c]	0.043	0.043

[a]Calculated under the assumption that all samples come from different individuals.
[b]Calculated under the assumption that each individual bears a different variant.
[c]Not determined because individual identification was not possible.

Bossou populations. However, since the sequence regions analyzed in these two studies differed, a simple comparison may not be meaningful. Gagneux et al. [1999] also reported nucleotide diversity (minimum estimates) based on a total of

292 sequences. They found that the Eastern chimpanzees had lower diversity (range = 0–9%, mode = 3%) than the Western chimpanzees (range = 0–16%, mode = 7%).

Population Structure Within the Bossou-Nimba Chimpanzee Group

Table V shows the F_{ST} and net (D_A) number of nucleotide differences among the Bossou population and the two populations of the Nimba Mountains. No population differed significantly from any other population. As in the case of nucleotide diversity, both minimum and maximum estimates were constructed for each population. In all cases in which maximum estimates were used, negative estimates of D_A and F_{ST} were obtained. This is because f_0 is less than f_1 for negative F_{ST}, and because π_{12} is less than $(\pi_1+\pi_2)/2$ for negative D_A, which means that two genes randomly chosen from two different populations are more closely related than genes from the same population. Such a negative value suggests that population differentiation is negligible. Therefore, the mtDNA analysis does not show any distinct population structure within the chimpanzee habitat that includes Bossou and the Nimba Mountains.

We conducted an AMOVA analysis for the Bossou group and the two Nimba Mountains groups to further investigate their population structures. Small variances were observed between the Bossou and Nimba Mountain groups (i.e., Bossou vs. total of two groups of Seringbara and Yealé: $\sigma_a^2 = 0.583$; 6.27%; $P = 0.341$; SE = 0.023) and between the two Nimba groups (i.e., Seringbara vs. Yealé $\sigma_b^2 = 0.251$; 2.70%, P = 0.231; SE = 0.015), while the within-group variance ($\sigma_c^2 = 8.468$; 91.03%, $P = 0.016$; SE = 0.004) constituted the majority of the total variance ($\sigma_T^2 = 9.303$). Neither of the between-group variance components were statistically significantly different from zero. Therefore, the AMOVA result also suggests that the mtDNA revealed no distinct population structure in the chimpanzee habitat of the Bossou-Nimba region.

TABLE V. Net Number of Nucleotide Differences (D_A) and Pairwise F_{ST} Among Sampling Sites*

Population pair	D_A	F_{ST}
Bossou vs. Seringbara		
Min	0.0019 (0.077)	0.06 (0.076)
Max	−0.0010 (0.574)	−0.03 (0.599)
Bossou vs. Yealé		
Min	0.0001 (0.367)	0.01 (0.334)
Max	−0.0005 (0.476)	−0.01 (0.449)
Seringbara vs. Yealé		
Min	0.0005 (0.283)	0.02 (0.234)
Max	−0.0030 (0.866)	−0.09 (0.895)

*Values in parentheses are the proportion of permutations leading to a calculated value larger or equal to that observed under the null hypothesis of random distribution [Schneider et al., 2000]. Min and Max designate values calculated using minimum and maximum estimates, respectively, for Seringbara and Yealé population. The real numbers of variants were used for the Bossou population. See text for detail about minimum and maximum estimations.

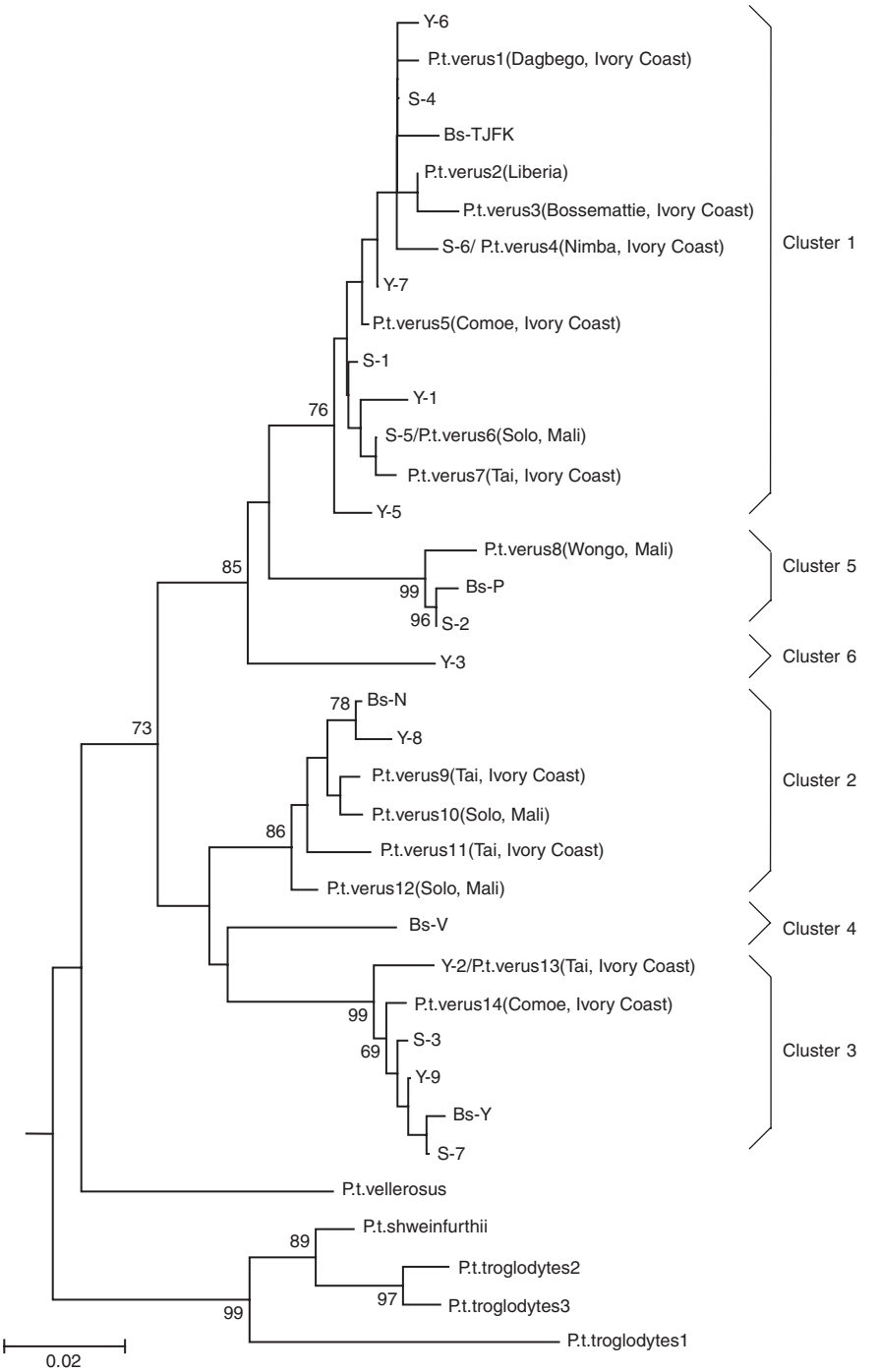

Fig. 3. A neighbor-joining tree of chimpanzee mtDNA variants including those described in this study and previously published data. The root is located using bonobo (*Pan paniscus*) sequence as an outgroup.

Phylogenetic Relationship of mtDNA Variants Within the Bossou-Nimba Region and Other West African Regions

Figure 3 shows the phylogenetic relationship of the *Pan troglodytes verus* mtDNA variants with sequences from the three other subspecies and *Pan panisucus* as outgroups. The mitochondrial variants observed in the Bossou and Nimba regions represent a variety of variants documented within this subspecies. They do not cluster according to geographic region. In fact, the six clusters found in Bossou and Nimba region represent all of the major mitochondrial clades of the *verus* subspecies. Similar distribution patterns of mtDNA variants have been reported for West African chimpanzees as well [Gagneux et al., 1999; Goldberg & Ruvolo, 1997a, b; Morin et al., 1994]. The absence of a clear population structure of mtDNA variation suggests panmixture of the ancestral population of the *verus* subspecies, and that insufficient time has passed since the separation of the current habitats for geographically distinct population structure to evolve. These results also suggest that the Bossou-Nimba groups derived from the ancestral population of the West African chimpanzee without passing through a serious bottleneck.

DISCUSSION

Relationship Between Sample Size and Number of Mitochondrial Variants

Figure 4 shows the relationship between the sample sizes and the observed numbers of variants. This graph was constructed by a random drawing from our samples. The number of variants found from the Bossou and Seringbara populations appears to plateau at roughly the number of variants we actually

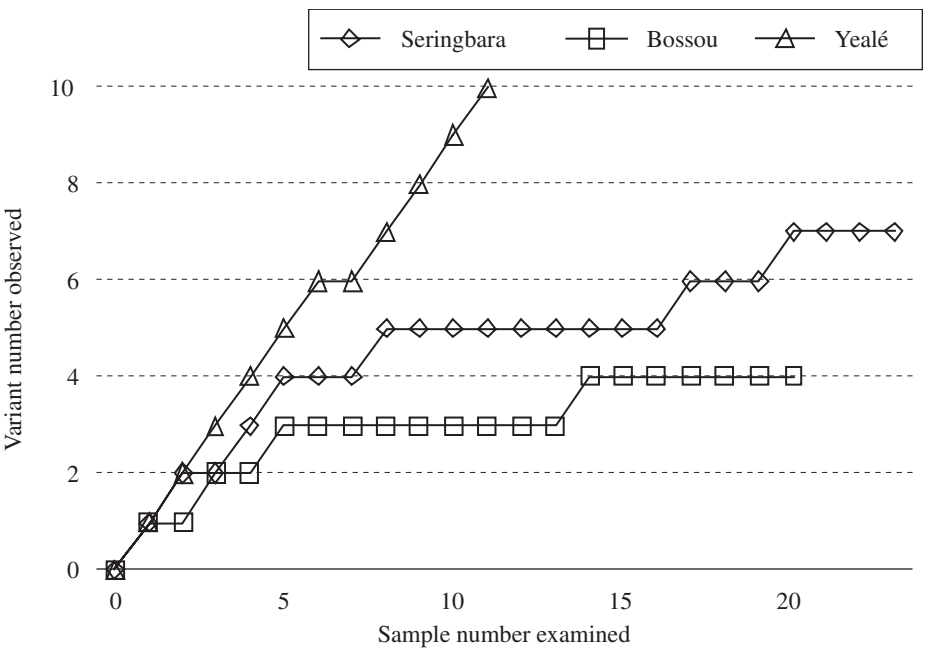

Fig. 4. Relationship between the number of sequences and the number of variants.

observed, despite the fact that one known variant from the region (Bs-Y) was not found within the population. In the Yealé population, the number of variants increased with the sample size. This suggests that the real number of variants in the Yealé population may be much larger than has been observed thus far. Limited records of direct observation at Yealé sites suggest the presence of multiple groups in the area [Humle & Matsuzawa, 2001]. Furthermore, a bed census study documented many more beds in Yealé than in Seringbara [Shimada, 2000]. This finding supports the expectation of a larger population size in Yealé.

Variant Sharing Among Bossou and Nimba Groups

Only three mtDNA variants were shared among groups in the Bossou-Nimba region (Table II). This suggests that there is a low-level gene flow via females in this region, and seems to contradict the results from the AMOVA analysis. There are at least two possible explanations for this paradox. The first possibility is missing variants. According to Fig. 4, we may be missing some mitochondrial variants in the Yealé population. This may also be true of the Seringbara and Bossou populations to some extent. If we conduct more extensive sampling in these populations, we may be able to find more variants shared among populations.

Another possibility is that the D_A and F_{ST} analyses lack sensitivity (see Table V) for recent population history. These measures may be appropriate for summarizing the relatively long-term population history, but they may not detect recent decreases in gene flow. In this case, an examination of shared variants among populations may be more appropriate for estimating recent population history. If so, the results shown in Table II suggest that there has been almost no recent exchange of individuals among the chimpanzee populations in the Bossou-Nimba region.

CONCLUSIONS

This mtDNA survey of chimpanzee populations of the Bossou-Nimba region revealed two findings: 1) very few mitochondrial variants are shared among the sites sampled, suggesting little gene flow involving mtDNA; and 2) mtDNA variant distribution does not suggest any clear population structure within the chimpanzee habitat of the Bossou-Nimba region.

We propose two explanations for these findings: 1) the survey was too limited to reveal actual gene flow, as suggested by the relationship between the number of sequences characterized in this study and the number of samples obtained at the Yealé site; and 2) the ancestral population of *Pan troglodytes verus* was panmictic, and insufficient time has passed for a clear pattern in the geographic distribution of mtDNA variants to emerge.

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