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Source: Primate Conservation, 26(1) : 67-74

Published By: Conservation International

URL: <https://doi.org/10.1896/052.026.0105>

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Gene Flow and Genetic Diversity of Chimpanzees in Tanzanian Habitats

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Abstract: Tanzania is located at the southeastern end of the eastern chimpanzee (*Pan troglodytes schweinfurthii*) distribution. Except for two national parks, their habitats have been degraded due to human activities. To clarify the gene flow and genetic diversity of chimpanzees in Tanzania, we analyzed the mitochondrial sequences of chimpanzees in six sites (Lwazi, Wansisi, Mahale, Karobwa, Ugalla-Masito, and Gombe), some of which are now isolated. The southernmost habitat (Lwazi) was about 150 km away from the nearest habitat but, considering the geographic distance, the genetic distance of the chimpanzees between Lwazi and the other habitats was not high. In contrast, the genetic distance between the chimpanzees in the northernmost habitat (Gombe), and the other habitats was relatively high considering the geographic distance. The results suggest that the Malagarasi River, which runs between Gombe and the southern habitats, limits gene flow. The genetic difference analyses also suggest that the habitats of Wansisi, Mahale, Karobwa, and Ugalla-Masito can be regarded as one population (“Greater Mahale”). The genetic distance between Lwazi and Gombe was lower than that between Gombe and the Greater Mahale habitats. This result suggests that early chimpanzees came to the Greater Mahale habitats through the southern habitats around Lwazi. The nucleotide diversity was not different from that in other countries, probably due to the sequence variety. There were unique haplotypes in several habitats where the number of chimpanzees was estimated to be small, which implies that some haplotypes are probably be at risk of disappearing. These data will be useful for conservation planning.

Keywords: Chimpanzee, gene flow, genetic diversity, Tanzania

Introduction

Environmental degradation, in this case, the loss, degradation and fragmentation of chimpanzee habitats through human activities, immigration and population growth, reduces and fragments their regional populations. Appropriate chimpanzee habitats become smaller and fragmented, making it difficult to determine the population distribution and gene flow before the advent of human activities. An understanding of the gene flow of animals between fragmented habitats is of great value for the design of conservation strategies.

The eastern chimpanzee (*Pan troglodytes schweinfurthii*), occurs in the Democratic Republic of the Congo (DRC), Uganda, Rwanda, Burundi, and the United Republic of Tanzania (Tanzania) (Inskipp 2005). The most recent estimate of the number of eastern chimpanzees was 76,000–120,000; most of them, 70,000–110,000, in DRC (Inskipp 2005). Tanzania, at the southern and eastern extremes of the distribution

of *P. t. schweinfurthii*, is estimated to have a population of just 1,500–2,500 (Inskipp 2005). All wild Tanzanian chimpanzees live along the eastern shore of Lake Tanganyika and unfortunately face the threat of extinction (Bakusa and McManus 2005). Forest loss outside Gombe National Park has been estimated at 4% per year, and the annual growth rate of the human population in the Kigoma region (Fig. 1) has increased. Furthermore, many refugees from DRC, Burundi, and Rwanda have settled in western Tanzania (Whitaker 2002), having a negative impact on the chimpanzee populations in the area (Ogawa *et al.* 2006a).

Kano (1972) first reported the distribution of chimpanzees along the eastern shore of Lake Tanganyika in detail. Thereafter, other studies have been conducted (Massawe 1992; Zamma *et al.* 2004; Ogawa *et al.* 2006b), and Ogawa *et al.* (1997) identified another chimpanzee habitat, namely Lwazi, which marks the southernmost tip of their geographic range. Although no researchers have ever observed chimpanzees in

this area, Ogawa *et al.* (1997) found 16 chimpanzee nests and two chimpanzee fecal samples; furthermore, the local people in the area have observed chimpanzees. We need to confirm whether the chimpanzees in Lwazi are in fact eastern chimpanzees and clarify their genetic backgrounds.

Yoshikawa *et al.* (2008) summarized the current distribution of chimpanzees and estimated the presence of four local populations: Gombe, Lilanshimba, Ugalla-Masito-Mukuyu-Mahale-Karobwa-Wansisi, and Lwazi. The number of chimpanzees outside the national parks of Gombe and Mahale has been estimated to be less than 700 (Yoshikawa *et al.* 2008). Today these four populations are isolated from each other, but we do not know the exact time when this happened.

Genetic studies can clarify the level of gene flow among populations. In the case of the great apes, rivers influence their distribution and their genetic structure (Eriksson *et al.* 2004; Anthony *et al.* 2007). The Ugalla River in western Tanzania is the eastern border of the chimpanzee geographic range (Fig. 1). In addition, the Malagarasi River, a large river in western Tanzania, may have limited gene flow (Fig. 1). The Malagarasi River is the second longest river in Tanzania and the largest river flowing into to Lake Tanganyika. The Rugufu River may also be a barrier to gene flow (Fig. 1). It is important to determine the genetic structure of the populations in these habitats to clarify the genetic diversity, including populations with a small number of chimpanzees (Yoshikawa *et al.* 2008).

A number of studies have examined gene flow among eastern chimpanzee habitats. Genetic divergence analysis has indicated that eastern chimpanzees expanded their range from eastern DRC in recent times; between 20,000 and 61,000 years ago (Goldberg and Ruvolo 1997a; Gagneux *et al.* 1999). Among the chimpanzees, it is the females rather than the males that typically disperse from their natal community (unit group) upon reaching maturity (Nishida 1979; Pusey 1979; Boesch and Boesch-Achermann 2000); this process influences the genetic structure within a community (Inoue *et al.* 2008). As expected from these findings, mitochondrial haplotypes were found to be shared by chimpanzees from different habitats, and they were not sorted into location-specific clusters (Morin *et al.* 1994; Goldberg and Ruvolo 1997b). Although there are some studies on the phylogenetics of the eastern chimpanzees that include samples from Tanzania (Morin *et al.* 1994; Goldberg and Ruvolo 1997b), they were taken from only a few sites.

In this study, we collected DNA samples non-invasively (feces, urine, and saliva) from eastern chimpanzees in six Tanzanian habitats. In all habitats except for the two national parks, it is difficult to observe chimpanzees and collect fresh samples, which would provide a relatively large amount of DNA compared with old samples. We analyzed mitochondrial DNA because PCR amplification of mitochondrial regions is easier than it is for nuclear DNA when dealing with degraded noninvasive samples. Using the sequence data, we examined the genetic structure and genetic diversity of chimpanzees in Tanzania in detail.

Materials and Methods

Samples

We collected noninvasive genetic samples from chimpanzees from six habitats in western Tanzania (Fig. 1). Eiji Inoue collected non-invasive samples, such as feces, urine, and saliva, of almost all chimpanzees of the M group at Mahale, which has been studied for more than 40 years (Nishida 1990; Inoue 2005; Inoue *et al.* 2008). Hideshi Ogawa collected fecal samples from around chimpanzee nests at Lwazi, Wansisi, Karobwa, Ugalla-Masito, and Gombe. The Malagarasi River runs between Gombe and the other habitats. Gombe and Lwazi are isolated from the other habitats (Yoshikawa *et al.* 2008). Yoshikawa *et al.* (2008) suggested that the chimpanzee habitats in Ugalla-Masito, Karobwa, Mahale, and Wansisi comprised one continuous population. We, therefore, defined this estimated population as the “Greater Mahale” population in this study.

DNA extraction and sequencing

DNA was extracted using either a QIAamp DNA Stool Mini Kit (Qiagen, California, USA), QIAamp DNA Mini Kit (Qiagen), QIAamp DNA Micro Kit (Qiagen), or ISOHAIR (Nippon Gene, Tokyo, Japan), depending on the sample, according to the manufacturers’ instructions.

A 331-base-pair segment of the mitochondrial hypervariable control region was analyzed. We conducted polymerase chain reaction (PCR) amplification using the primers L16041 and H16498 (Morin *et al.* 1994; Eriksson *et al.* 2004) or the primers L16031 (5'-TAAACTATTCTCTGTTCTTTCA-3') and H16405 (5'-CGGGATATTGATTCACGGAGG-3'). The PCR products were purified and then directly sequenced using the dye termination method and an ABI 3100 sequencer (Applied Biosystems, California, USA).

Analyses

We used the mitochondrial sequence data of the eastern chimpanzees (*P. t. schweinfurthii*) from Uganda (Langergraber *et al.* 2007, EU077270-EU077418), Rwanda, and DRC (Keele *et al.* 2006, DQ370332-DQ370353) to construct a phylogenetic tree. Using MEGA 4.0 (Tamura *et al.* 2007), we aligned all the determined haplotypes including the data from other countries, trimmed the reference sequences to fit the determined sequences, and then removed the duplicate sequences from the same origin. We constructed a neighbor-joining tree including one sequence of central chimpanzees from Cameroon (*P. t. troglodytes*, DQ367534) as an outgroup (Saitou and Nei 1987; Tamura *et al.* 2004). The bootstrap values were calculated using MEGA 4.0 software. Genetic differentiation (F_{ST}), gene diversity, and nucleotide diversity were calculated with Arlequin ver. 3.0 (Nei and Li 1979; Excoffier *et al.* 2005). Statistical analyses of F_{ST} values were also conducted with Arlequin.

Results

Twenty-two haplotypes were detected among the 138 sequences obtained from the six habitats (Table 1). These sequences have been deposited in DDBJ/EMBL/GenBank under accession numbers AB677454–AB677475. Three of the five haplotypes from Lwazi were also found in other

habitats. Haplotype B was found in all habitats excluding Gombe. All four haplotypes found in Gombe were unique. Ugalla-Masito, Karobwa, and Lwazi chimpanzees had nine, two, and two unique haplotypes, respectively.

Phylogenetic tree analyses revealed that mitochondrial haplotypes were shared by chimpanzees from different countries (Fig. 2). Almost all clusters included some

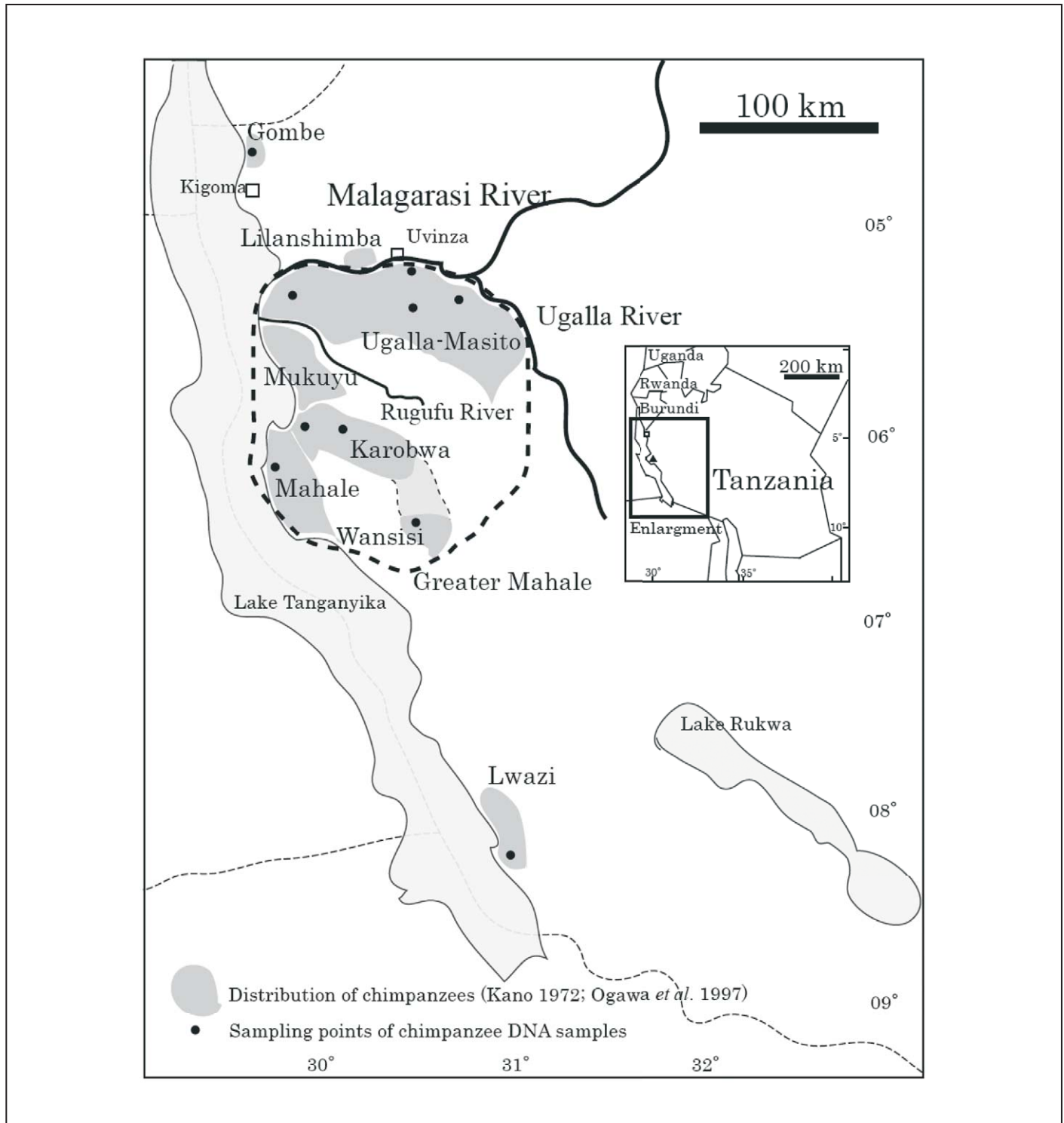


Figure 1. Distribution of chimpanzees and sampling sites in Tanzania. This figure was modified from Figure 1 in Yoshikawa *et al.* (2008). Using the data presented in their discussion, we connected the Karobwa and Wansisi habitats.

sequences from Tanzania, but the distribution of haplotypes was skewed. In total, eight of 22 haplotypes from Tanzania belonged to cluster IV, which had only one haplotype from Uganda. Among those eight, three major haplotypes (A, B, and D) were found in many samples in four or five Tanzanian habitats. The haplotypes from Gombe (S, T, U, and V) were

similar to the other haplotypes from the other countries, but not to the other haplotypes from Tanzania.

The F_{ST} values among the habitats in the Greater Mahale population were low, and they were not significantly positive between Karobwa and the other habitats (Table 2). The F_{ST} values between Lwazi and the other habitats were lower

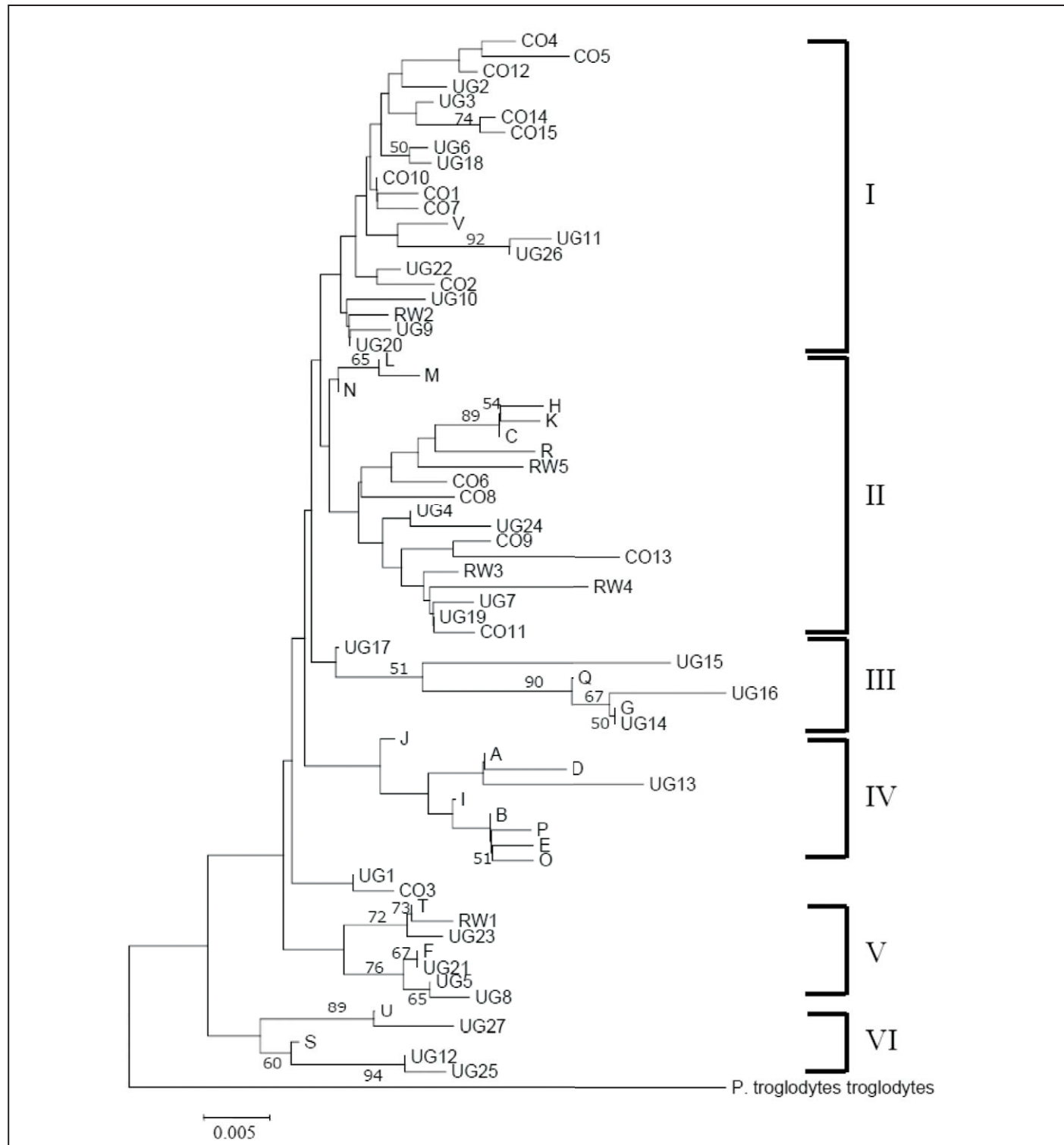


Figure 2. Phylogenetic tree using the neighbor-joining method. Bootstrap values $\geq 50\%$ are shown above the relevant branches. A-V indicates the haplotypes found in this study (Table 1). The sequences beginning with CO, RW, and UG were those from DRC, Rwanda, and Uganda, respectively.

than those between Gombe and the other habitats. Figure 3 revealed no correlation between the geographic distance and F_{ST} (Pearson correlation coefficient, $r = 0.15$, $P = 0.60$). The F_{ST} values between Gombe and the other regions were relatively high considering the geographic distance (Fig. 3).

Table 3 shows the diversity in each habitat. Although the number of haplotypes was large in Ugalla-Masito, the gene and nucleotide diversity were similar among the habitats. Gene diversity was highest in Gombe and Karobwa (0.87) and lowest in Mahale (0.68), while nucleotide diversity was highest in Gombe (0.022) and lowest in Mahale (0.013).

Discussion

Lwazi population

We confirmed that the chimpanzee samples from Lwazi belonged to eastern chimpanzees (*P. t. schweinfurthii*) because the haplotypes of samples from Lwazi were shared by other eastern chimpanzees (Fig. 2). Three of five haplotypes found in Lwazi were shared by chimpanzees in other habitats (Table 1), and all haplotypes found in Lwazi were sorted into the same cluster as that of the other sequences of eastern chimpanzees. The F_{ST} values between Lwazi and other habitats in Greater Mahale were not high considering the large geographic distances between them (Fig. 3). These results suggest that chimpanzee habitats in Lwazi and Greater Mahale had been continuous until they were isolated from each other recently.

Gene flow of chimpanzees in Tanzania

The F_{ST} value and the geographic distance were not correlated (Fig. 3). This was probably due to the large difference in F_{ST} values of Gombe and the other habitats. Langergraber *et al.* (2011) found that the F_{ST} value between Mahale and Gombe was lower than those between Gombe and the habitats in Uganda, although the distance between Gombe and Uganda habitats was greater than 400 km. The result of the phylogenetic tree also confirmed this fact (Fig. 2). The unique haplotypes in Gombe (S, U, and V) are close to the sequences from Uganda but not to those from other Tanzanian habitats. Cluster IV contained many sequences from all habitats of Tanzania excluding Gombe but did not include those from the other countries. The probable barrier between Gombe and other Tanzanian habitats is the Malagarasi River, as has been reported in studies on the effect of rivers on the gene flow in other great apes (Eriksson *et al.* 2004; Anthony *et al.* 2007).

The genetic distance between Gombe and Lwazi was smaller than those between Gombe and the other habitats (Table 2), even though the geographic distance between Gombe and Lwazi was greater than those between Gombe and the others (Fig. 3). This may suggest that chimpanzees in Greater Mahale came through the southern habitats around Lwazi. Assuming that the geographic distances between Gombe and the habitats in Greater Mahale were the distances between Lwazi and Gombe plus the distances between Lwazi and the habitats in Greater Mahale, a positive significant correlation between genetic and geographic distances was found (Fig. 4, Pearson correlation coefficient, $r=0.85$, $P<0.001$). The actual pathway between Gombe and Lwazi was longer than the geographic distance between them

Table 1. Haplotype constitutions in Tanzanian habitats.

	Mitochondrial haplotype																				Total				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T		U	V		
Lwazi	1	6	2		1	4																		14	
Wansisi	1	3		2			4																		10
Mahale	16	22	11	2																					51
Karobwa	3	1		3			1	1	1																10
Ugalla-Masito		22		5			3			1	1	3	1	2	1	1	1	1							42
Gombe																				1	2	1	2		6
	21	59	13	12	1	4	8	1	1	1	1	3	1	2	1	1	1	1	1	1	2	1	2		138

Table 2. FST values among Tanzanian habitats.

		Lwazi	Greater Mahale			Gombe	
			Wansisi	Mahale	Karobwa		Ugalla-Masito
Greater Mahale	Lwazi	-					
	Wansisi	0.16	-				
	Mahale	0.07*	0.20	-			
	Karobwa	0.10*	0.03*	0.03*	-		
	Ugalla-Masito	0.07	0.10	0.05	0.04*	-	
	Gombe	0.14	0.26	0.25	0.21	0.25	-

* No significant difference was found between two habitats (Exact test, $P > 0.05$)

because chimpanzees needed to avoid the Malagarasi River. Although it is difficult to determine the actual pathway, Figure 4 strongly suggests the path from Gombe to Greater Mahale was via Lwazi. This result indicates the importance of Lwazi habitats for understanding the expansion of chimpanzees in Tanzania. Genetic analyses of chimpanzees on the western shore of the Lake Tanganyika in DRC will provide important data on the history of eastern chimpanzees in the southern habitats.

The genetic structure within Greater Mahale was also clarified. Figure 4 shows that the genetic distance between Mahale and Wansisi was high for the geographic distance between them. Inferring from this result and the current distribution (Fig. 1), the direct gene flow between Mahale and Wansisi was limited, and Karobwa habitats connected them. A river that runs between Ugalla-Masito and Karobwa-Mahale-Wansisi, the Rugufu River, is another possible barrier to gene flow. The F_{ST} values between Ugalla-Masito and Karobwa-Mahale-Wansisi was low, and that between Ugalla-Masito and Karobwa was

Table 3. Genetic diversity among Tanzanian habitats.

Habitat	N	No. of haplotypes	Gene diversity	Nucleotide diversity
Lwazi	14	5	0.76	0.017
Greater Mahale	Wansisi	10	4	0.78
	Mahale	51	4	0.68
	Karobwa	10	6	0.87
	Ugalla-Masito	42	12	0.71
Gombe	6	4	0.87	0.022

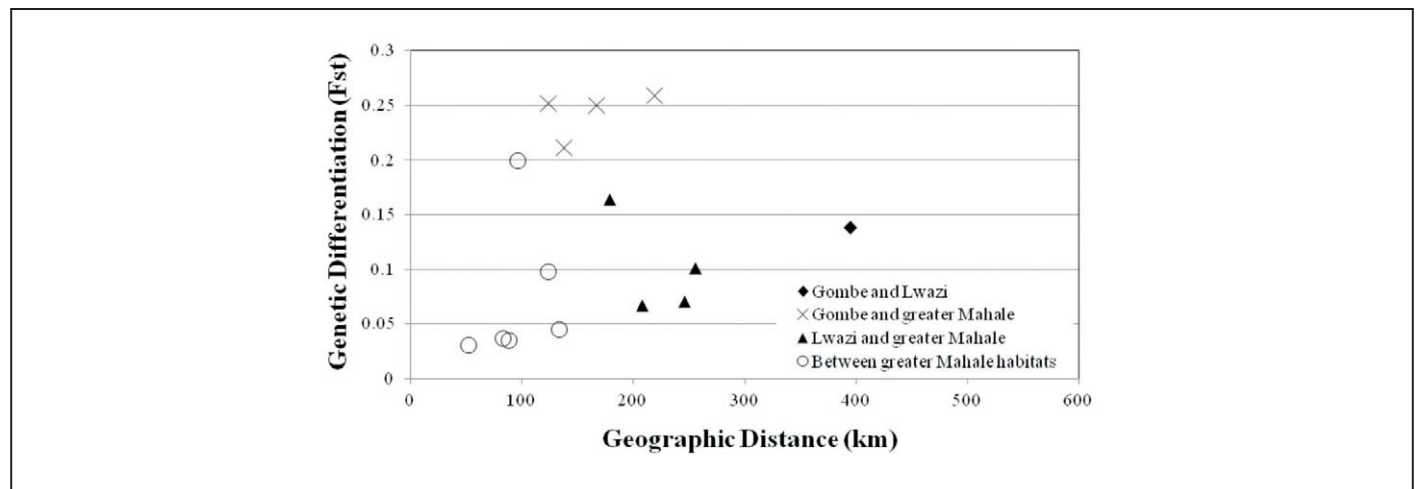


Figure 3. Relationship between the genetic and geographic distances.

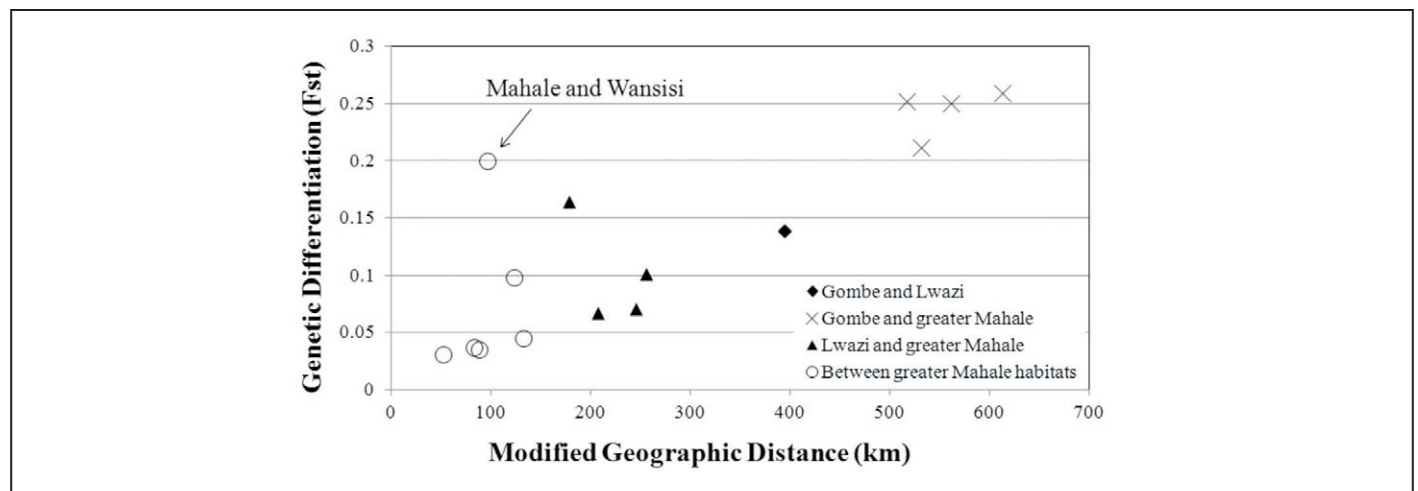


Figure 4. Relationship between the genetic and geographic distances with some modifications (see ‘Discussion’).

not significantly positive. These results suggested that gene flow has not been limited. The F_{ST} values among the habitats in Greater Mahale were low, and gene flow was not limited between Karobwa, which is located in the center of the Greater Mahale habitats, and the other habitats. These results confirmed that the Greater Mahale habitats can be regarded as one continuous population, estimated from the result of the current distribution (Yoshikawa *et al.* 2008).

Genetic diversity

Gombe exhibited the highest diversity among the habitats in Tanzania (Table 3). This result may reflect the low genetic diversity among the southern Tanzanian habitats, which mark the southeastern end of the chimpanzee's distribution, due to the limited gene flow between Gombe and these habitats. To evaluate this hypothesis, we compared the diversity in the southern Tanzanian habitats (Lwazi, Wansisi, Mahale, Karobwa, and Ugalla-Masito) to that in the 19 habitats of Uganda, DRC, and Rwanda reported by Goldberg (1998).

Although the range of gene diversity in the southern Tanzanian habitats (0.68–0.87) was similar to that in the other countries (0.64–1.02), gene diversity as such was significantly lower (Mann-Whitney U test, $P=0.04$). This significant difference was probably due to the small number of haplotypes in the southern Tanzanian habitats. We analyzed 51 individuals of the M group in Mahale but found only four haplotypes (Table 1). Langergraber *et al.* (2007) analyzed mitochondrial DNA of three chimpanzee communities in Uganda and found 14, 16, and seven haplotypes among 28, 94, and 20 individuals, respectively. Thus, there were fewer haplotypes per social community in southern Tanzania than in Uganda. In contrast, the nucleotide diversity was not different between the habitats in southern Tanzania and the other countries (Mann-Whitney U test, $P=0.21$). This probably reflected the fact that the sequences in southern Tanzania belonged to several clusters in the phylogenetic tree (Fig. 2). This also suggested that the southern Tanzanian chimpanzees have not experienced a severe bottleneck with respect to genetic diversity, resulting in the retention of sequence variety. Sequence variety is important for total genetic diversity in the southern Tanzanian habitats.

Unique haplotypes were found in Lwazi, Karobwa, Ugalla-Masito, and Gombe. The number of chimpanzees in those habitats is small (Pusey *et al.* 2007; Yoshikawa *et al.* 2008). Consequently, there are probably just a few individuals with unique haplotypes. Chimpanzees in an isolated and limited habitat are at risk of losing mitochondrial diversity, resulting in difficulty in suitable mating (Shimada *et al.* 2009). It is, therefore, important to maintain the habitats and population sizes of chimpanzees to preserve their diversity.

Acknowledgments

We are indebted to the Tanzania Commission for Science and Technology, the Tanzania Wildlife Research Institute, Tanzania National Parks, Mahale Mountains National Park, and Mahale Mountains Wildlife Research Centre for

permission to conduct research in Tanzania. We are also grateful to M. Nakamura, T. Kano, G. Idani, M. Kanamori, T. Sakamaki, J. Itani, A. Collins, S. Kamenya, J. Moore, A. Hernandez-Aguilar, A. Piel, F. Stewart, H. Y. Kayumbo, E. T. Massawe, T. Nemoto, E. K. Sehele, B. Kadyugenze, M. J. H. Mbalamwezifor, and other research colleagues and local assistants for their support in conducting the field study and collecting DNA samples. We thank J. Yamagiwa, N. Nakagawa, and other members from the department of Human Evolution Studies, Kyoto University for advice on the research. We appreciate the experimental support provided by A. Takenaka, S. Nakamura, T. Tanaka-Ueno, and other members of the Molecular Biology Section, Primate Research Institute (PRI), Kyoto University; S. Ito, and other members of the Laboratory of Animal Genetics, Faculty of Applied Biological Sciences, Gifu University; and L. Vigilant and other members of the Department of Primatology, Max Planck Institute. This study was supported financially by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) with a grant-in-aid for Science Research (#12375003, #16255007, #06061064, #09041160, #1257597, #17255005, #19107007, and #22570223), the Global Environment Research Fund (F-061), the Research Fellowships of the Japan Society for the Promotion of Science for Young Scientist (No. 82604), and the global COE program A06 to Kyoto University.

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Received for publication: 9 December 2011

Revised: 7 July 2001