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Genetic diversity among African great apes based on mitochondrial DNA sequences

ANA KARINA ZAVALA GUILLÉN*, GORDON M. BARRETT and OSAMU TAKENAKA

*Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan; *Author for correspondence: A.K. Zavala Guillén (e-mail: zavalabarrett@yahoo.com.ar; fax: 1(613)247–0592)*

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Abstract The mitochondrial DNA D-Loop region was sequenced, analyzed and used as a molecular marker for populations of chimpanzee (*Pan troglodytes*), bonobo (*P. paniscus*) and gorilla (*Gorilla gorilla* and *G. beringei*), and also compared with data previously reported for these taxa. In *Gorilla*, a main disjunction between western (*G. gorilla*) and eastern (*G. beringei*, including *G. b. graueri*) species was observed, as well as high mitochondrial diversity within the western species. The genetic distance values within *G. gorilla* (0.14) were higher than those between subspecies (eastern lowland and mountain 0.12). Likewise, values of genetic diversity within this species (0.05) were higher than those between species (western and eastern lowland gorilla 0.04). Similarly, in genus *Pan* a main differentiation between western (*P. t. verus*) and central forms (*P. t. troglodytes* and *P. t. schweinfurthii*) was observed. The obtained values of genetic distance and genetic diversity revealed that the central subspecies are closer to each other than either of them is to the western one, while bonobos composed a distinct clade that expresses a well-defined specific identity. The current distribution, phylogeny and levels of genetic diversity in African great ape populations are consistent with the hypothesis that Pleistocene climatic events led to cyclical periods of isolation in forest refugia followed by expansion and dispersal. The implications of this high level of genetic diversity for taxonomic classification, wildlife management and conservation are discussed.

Introduction

The question regarding who our nearest relations are has a long and contentious history in science. Long before the dawn of molecular analysis, pioneers like Darwin (1964) had already proposed that the African great apes were our closest relatives and, moreover, that the African continent was the cradle of mankind. Since then, various branching patterns have been proposed for the hominoids and the existence of propinquity among *Homo*, *Pan* and *Gorilla* is no longer in dispute. Nevertheless, considerable controversy remains, particularly with respect to details of the branching pattern within the Hominoidea, the exact times of divergence, the processes driving speciation and local genetic differentiation, and the taxonomic relationships at the specific, subspecific and even population level. Whereas the general consensus

is to accept a dichotomous chimpanzee–human clade with gorilla as the closest outgroup (Hacia 2001), the classification of gorillas has recently been the subject of reassessment and restructuring, due in no small part to the results of molecular investigations. Furthermore, several analyses have also revealed large genetic distances among the subspecies of *Pan*, which in some cases are greater than those between species (Morin et al. 1994; Ruvolo et al. 1994; Kaessmann et al. 1999; Jensen-Seaman et al. 2001), thus raising the question as to whether a similar reclassification is in order for this genus as well.

In addition, and as numerous researchers have repeatedly pointed out, both *Pan* and *Gorilla* contain a high degree of genetic diversity (Garner and Ryder 1996; Gagneux et al. 1999; Kaessmann et al. 1999). We know that populations of the same genus or species can be exposed to different ecological conditions and stresses that may produce interpopulational differentiation. We also know some of the historic events that are probably responsible for the current layout of the African territory and its species, including climatic changes, glaciations, and forest fragmentation. Nevertheless, owing to the complex intermingling of biological and environmental processes that has impacted African great ape populations and guided their speciation, the current genetic diversity as well as local patterns of differentiation among populations is incompletely understood.

This study aims to further contribute to the body of knowledge on great ape phylogeny and genetic diversity through the investigation, analysis and interpretation of part of their genetic composition and structure. To this end, the mtDNA hypervariable D-Loop region of *Pan* and *Gorilla* specimens was obtained, sequenced and analyzed.

Materials and methods

Sample composition

DNA samples from both captive and wild populations of the following African great ape taxa were obtained: *P. paniscus*, *P. t. verus*, *P. t. troglodytes*, *P. t. schweinfurthii*, *G. gorilla* and *G. beringei*. Samples were obtained from a total of 36 individuals. In the case of wild individuals, hair samples were collected and kindly provided by field researchers, while blood samples were taken from those individuals maintained in captivity. Finally, comparisons with data previously reported were made for both *Pan* and *Gorilla* (Garner and Ryder 1996; Goldberg and Ruvolo 1997; Gagneux et al. 1999; Deinard and Kidd, 2000; Jensen-Seaman et al. 2001). The taxonomic identification as well as the country of origin is specified for each sample in Figures 1 and 2; however, in the case of *P. t. verus*, many of which were captive subjects, the precise country of origin of the individuals was not always known.

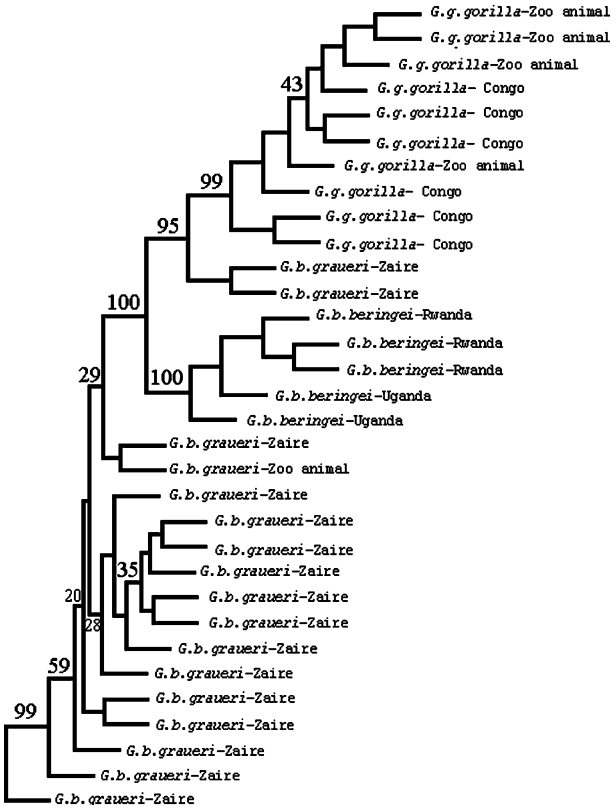

Figure 1. Unrooted phylogenetic consensus tree relating mitochondrial D-Loop sequences of western lowland gorillas (*G. g. gorilla*), eastern lowland gorillas (*G. b. graueri*), and mountain gorillas (*G. b. beringei*) from this study and other published sequences. Country of origin of the samples follows the taxonomic classification of each individual. In the case of zoo animals, individuals from the Zoological Society of San Diego, the Audubon Zoological Gardens and the Royal Zoological Society of Antwerp are included. Numbers at branchpoints represent percent bootstrap values, based on 500 replicates.

DNA extraction

Blood DNA was extracted using standard protocols (Sambrook et al. 1989). Extraction from hair samples was performed using the ISOHAIR Kit (Nippon Gene Co. Ltd., Toyama, Japan) according to the instructions supplied by the manufacturer.

DNA amplification

From the extracted DNA, a segment of the hypervariable D-Loop region of the mitochondrial DNA was amplified by the polymerase chain reaction (PCR).

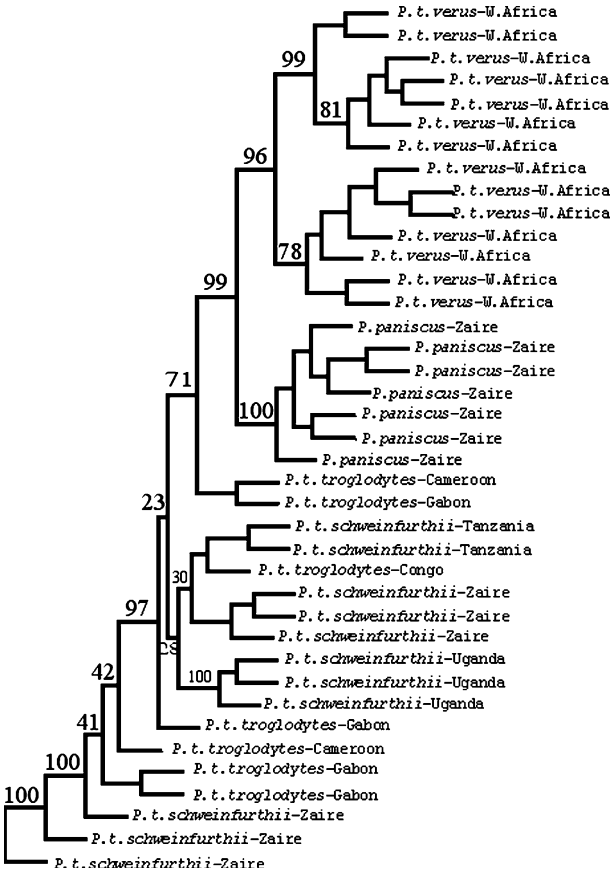

Figure 2. Unrooted phylogenetic consensus tree relating mitochondrial D-Loop sequences of chimpanzee subspecies (*P. t. troglodytes*; *P. t. verus*; and *P. t. schweinfurthii*) and bonobos (*P. paniscus*) from this study and other published sequences. Country of origin of the samples follows the taxonomic classification of each individual. Numbers at branchpoints represent percent bootstrap values, based on 500 replicates.

The following primers (custom synthesized by Japan Bioservice for O. Takenaka) were used: (5′-AACTAATACACCAGTCTTGTAAACC-3′); (5′-GAGCTAATAGAAAGGCTAGGACCAA-3′); and (5′-GCTCCCGTG-AGTGGTTAATAG-3′).

The PCR conditions applied differed according to sample requirements and the desired amplification length. Generally, the amplification of a 1.2 kb segment was carried out through one initial denaturation (94 °C for 6 min), 35 cycles of denaturation (94 °C for 1 min), annealing (50 °C for 2 min), and extension (72 °C for 2 min), followed by a final 10 min extension at 72 °C. In the case of 600 bp length fragments, the initial denaturation at 94 °C was allowed to proceed for 1 min, followed by an additional 40 cycles of

denaturation (94 °C for 1 min). Annealing and extension remained the same. AmpliTaq Gold Polymerase (Perkin Elmer, Foster City, CA, USA) was used for blood samples, while DNA from hair samples was amplified with *PfuTurbo* DNA Polymerase (Stratagene Cloning Systems, La Jolla, CA, USA).

DNA purification and cloning

The PCR product was purified using 1.25% NuSieve GTG Agarose gel (FMC BioProducts, Rockland, ME, USA) and a Millipore Ultrafree-DA centrifugal filter device (Millipore Japan, Tokyo, Japan). Purified fragments were inserted into pGEM-T Easy Vector System I (Promega Corporation, Madison, WI, USA) and transformed into competent *Escherichia coli* cells. Isolated plasmids were screened for insertions by the *Eco*RI restriction enzyme, and one cloned library was produced for sequencing.

DNA sequencing

Sequi Therm EXCEL II Long-Read DNA Sequencing Kit-LC products (Epicentre Technologies, Madison, WI, USA) were used in the PCR for sequencing. Dual wavelength LICOR equipment performed the technique using the following primers: (1) M13 forward/IRD800–(CACGACGTTG-TAAAACGAC), and (2) M13 reverse/IRD700–(GGATAACAATTTCACA-CAGG). To confirm the correct sequence, each amplified fragment was sequenced in both senses, and three clones from each individual were used to resolve any discrepancies.

Phylogenetic analysis

The obtained sequences were aligned by GENETYX (Software Co.) and the phylogenetic analysis conducted by the UPGMA method (Sokal and Michener 1985) using the software package PHYLIP (Phylogeny Inference Package) (Felsenstein 1993). A total of 500 bootstrap replications were generated from the data set and unrooted phylogenetic trees were constructed by three different methods: genetic distance matrix (principles of neighbor-joining under the (Kimura 1980) 2 parameter model and the Tamura and Nei (1993) models were applied), maximum parsimony, and maximum likelihood. Since the trees generated showed similar topologies, results were interpreted using consensus trees and phylograms. Moreover, to establish genetic structure and relationships of the populations, sequences were analyzed under the principles of similarity and dissimilarity, from the quantitative and qualitative point of view and considering not only the rate but also the pattern of nucleotide substitutions. The following genetic measures were calculated: sequence evolutionary

similarity and evolutionary distance (summarized as sequence similarity), type and frequency of nucleotide substitutions, genetic distance and nucleotide diversity within and between populations (Kimura 1980; Nei 1987; Li 1997; Page and Holmes 1998).

Results

Gorilla

In nucleotide substitutions a clear tendency was observed, with transitions (82%) outnumbering transversions (18%), and substitutions between pyrimidines outnumbering those between purines. These results are in agreement with those reported elsewhere for humans and other primates (Brown et al. 1982; Vigilant et al. 1991). Regarding sequence similarity, within subspecies the highest value was found in mountain gorillas (99%), followed by the eastern (94%) and western lowland (91%). Interpopulation analysis found the highest congruence between eastern lowland and mountain gorillas (91%), a lower value between western lowland and mountain gorillas (82%) and slightly lower still between western and eastern lowlands forms (81%). Genetic distance and genetic diversity values were calculated both within and between populations, and both measures were found to be lowest in mountain gorillas (Table 1).

Another particular feature of this genus was apparent upon intergeneric comparisons of the sequenced region. While *Pan* and *Homo* show similar organization and size, *Gorilla* sequences are shorter due to a deletion of around

Table 1. Inter- and intrapopulational values of genetic diversity and genetic distance in *Gorilla* spp.

		G.g.gorilla	*G.b.beringei*	*G.b.graueri*
		Genetic distance		
G.g.gorilla	**Genetic diversity**	**0.14** / **0.05**	**0.29**	**0.25**
G.b.beringei		**0.04**	**0.02** / **0.01**	**0.12**
G.b.graueri		**0.04**	**0.01**	**0.14** / **0.04**

Values above the diagonal represent the results of genetic distance calculations, and those below genetic diversity. Values appearing within shaded blocks are intrapopulational measures, while those in white blocks are interpopulational measures.

90 bp, located close to the origin of the D-Loop. This portion is a C/G rich hypervariable one that is unalignable with the sequences from other genera and also exhibits interindividual variation. This observation is consistent with previous reports (Foran et al. 1988; Garner and Ryder 1996).

Phylogenetic relationships

In the *Gorilla* phylogenetic tree (Figure 1), representatives of the subspecies *G. b. beringei* compose a distinct cluster in accordance with their low degree of intrapopulational genetic diversity. Although the other two taxa conserved their own identity in the tree, homogeneity is not as high as in mountain gorillas. All members of the *G. g. gorilla* subspecies clustered together, while those of *G. b. graueri* show a looser distribution in the tree, reflective of the variability existent in this subspecies as observed from the quantitative data. Similar clustering was also observed in a phylogram of this genus (data not shown), where mountain gorillas exhibit a defined subspecific identity. Eastern and western forms also composed their own cluster, although a higher within species variability is evident, particularly in the latter.

Pan

In *Pan*, nucleotide substitutions exhibited the same tendency found in *Gorilla*, with transitions outnumbering transversions within subspecies and also within the genus, as 84% of the total differences were due to the latter type. Within transitions, substitutions between pyrimidines outnumbered substitutions between purines, both within subspecies and within the genus. Regarding sequence similarity, a more conservative mitochondrial pattern is found within *P. paniscus* (98%) as compared to *P. troglodytes* (95%). Similarity in the mitochondrial sequences is highest between the subspecies that are geographically closest, *P. t. troglodytes–P. t. schweinfurthii* (95%), and lowest between those that are most distant, *P. t. verus–P. t. schweinfurthii* (88%); intermediate similarity occurs between *P. t. verus–P. t. troglodytes* (89%). Higher genetic affinities are also evident between the subspecies that are geographically more proximate. Inter- and intrapopulational genetic distance values as well as those for genetic diversity appear in Table 2.

As an overview of interspecific genetic differences and relationships, *P. paniscus* showed lower values of intraspecific genetic distance and genetic diversity compared with those obtained for *P. troglodytes* in addition to the higher value of intraspecific sequence similarity previously described for the latter species. Also, between species comparisons revealed that all subspecies of *P. troglodytes* share similar values of sequence similarity with *P. paniscus*. Sequence similarity values between *P. paniscus–P. t. troglodytes* and *P. paniscus–P. t. verus* subspecies are equivalent (86%), and only slightly lower for *P. paniscus–P. t. schweinfurthii* (85%).

Table 2. Inter- and intrapopulational values of genetic diversity and genetic distance in *Pan* spp.

		P.paniscus	*P.t.verus*	*P.t.troglodytes*	*P.t.schweinfurthii*
		Genetic distance			
P.paniscus	Genetic diversity	0.05 / 0.02	0.15	0.15	0.2
P.t.verus		0.03	0.08 / 0.04	0.13	0.17
P.t.troglodytes		0.04	0.07	0.09 / 0.06	0.07
P.t.schweinfurthii		0.05	0.03	0.01	0.08 / 0.01

Values above the diagonal represent the results of genetic distance calculations, and those below genetic diversity. Values appearing within shaded blocks are intrapopulational measures, while those in white blocks are interpopulational measures.

Phylogenetic relationships

The phylogenetic tree generated for this genus showed two distinct clusters. One groups together all members of the subspecies *P. t. verus*, while the second includes all representatives of the species *P. paniscus* and locates between the two main *P. troglodytes* western and eastern forms. The members of the subspecies *P. t. schweinfurthii* and *P. t. troglodytes* formed a third, intermingled cluster and appeared paraphyletic with respect to *P. paniscus* and *P. t. verus*. A similar pattern of relationships was observed in a generic phylogram (data not shown), with *P. paniscus* self-defined in its specific rank and still clustered intermediate between *P. troglodytes* western and eastern forms. Also, *P. t. verus* has a well-defined subspecific identity and again *P. t. troglodytes* and *P. t. schweinfurthii* present an intermixed distribution.

Discussion

The analysis of the mtDNA of our closest relatives has shown them to be genetically heterogeneous populations with a high level of variability both between and within genera. These results are in good agreement with previous works developed using different markers (e.g. Morin et al. 1994; Ruvolo et al. 1994; Garner and Ryder 1996; Xu and Arnason 1996; Gagneux et al. 1999; Kaessmann et al. 1999).

In *Gorilla* populations, the main mitochondrial disjunction observed is the one between western (*G. gorilla*) and eastern forms (*Gorilla beringei beringei*

and *G. b. graueri*), which is in accordance with their recent taxonomic revision (Groves 2001). Unfortunately, we were unable to obtain samples from individuals of the Cross River gorillas (*G. g. diehli*), a fragmented and geographically isolated population of western gorillas that has recently been elevated to subspecific status (Groves 2001). The two areas inhabited by eastern and western gorillas are currently separated by savanna grassland, and no populations occur over the gap of more than 1000 km. While evidence indicates that these two areas might previously have formed a contiguous forest, there is nothing credible to suggest the existence of gorillas in the intervening area in recent times (Hofreiter et al. 2003).

During the Pleistocene, the African continent underwent drastic climate and habitat changes, with cyclical arid/retractions and moist/expansions of large areas of forest, the main habitat of these populations. The African fauna including great apes might have survived through these times by following a pattern of arid conditions-forest retraction-population isolation in forest refugia and moist conditions-forest expansion-population dispersal, thus shaping their speciation processes and distribution (Hamilton 1981; Grubb 1982; Colyn et al. 1991). These cyclical environmental changes brought about the reduction of forest to savanna and may also have forced the separation of *Gorilla* into western and eastern populations. Then, over time and with the two groups evolving under differing ecological conditions and pressures, the western forms became restricted to lowland forest while the eastern ones were distributed both in lowland forest and mountains. Finally, the eastern territory also became fragmented, reducing the gene flow between the populations inhabiting forest and mountains. The geographic isolation and differing ecological conditions may account then for the general differentiation between eastern and western forms.

The high diversity within populations, however, particularly among those of the western species living under similar environmental conditions, warrants further analysis. In the present study we found that the genetic distance value based on mtDNA analysis within *G. gorilla* is higher than that between subspecies (e.g. eastern lowland and mountain). Likewise, values of genetic diversity within the western lowland gorilla are higher than those between species (e.g. western and eastern lowland gorilla). Therefore, and in agreement with previous studies, we emphasize the particularly high level of mitochondrial genetic variability within western lowland gorillas. One possible scenario that may account for this high degree of mtDNA diversity is division of the population into shifting demes that have diverged, diversified and then partially recombined during periods of isolation in refugia. Further and more expansive studies are needed to clarify the extent and taxonomic significance of this variability.

In *Pan*, a similar scenario results from the analysis of its mitochondrial DNA. Like *Gorilla*, a main disjunction between the western (*P. t. verus*) and central forms (*P. t. troglodytes* and *P. t. schweinfurthii*) was observed, while a third clade consisting of the bonobos (*P. paniscus*) confirms their unique

identity as a species. *Pan* populations subjected to Pleistocenic habitat changes might also have undergone processes of fragmentation, isolation and gene flow limitation. Examples of isolated populations are those of *P. paniscus*, which is separated from *P. troglodytes* by the imposed geographic barrier of the Congo River. *P. paniscus* composed a consistent mitochondrial clade with the highest value of intrapopulation homology and the lowest interindividual genetic distance. This adds to a number of differences observed between bonobos and chimpanzees in diverse areas such as social relationships and sexual behavior (Furuichi 1987; Kano 1992; Furuichi and Ihobe 1995).

Geographic distance might also contribute to the considerable divergence between *P. troglodytes* western and central forms. The genetic relationships among subspecies of *P. troglodytes* revealed that *P. t. verus* constitutes a clade that is clearly distinct from the other two subspecies. The obtained values of genetic distance and genetic diversity confirm that the subspecies *P. t. troglodytes* and *P. t. schweinfurthii* are closer to each other than either is to *P. t. verus*, which is in agreement with previous works using nuclear and mitochondrial markers (Morin et al. 1994). Also, genetic distance values between *P. t. verus* and *P. t. schweinfurthii* forms are as high as those between *P. t. verus* and *P. paniscus*. Lastly, the geographic proximity of central forms is congruent with their genetic affinities, as they compose a clade where members of both subspecies intermix.

In *Pan* as well as in *Gorilla*, the hypothesis of differentiation of populations by a process of cyclical dispersal-retraction to forest refugia-isolation during the Pleistocenic climatic changes might explain the main disjunction between western and central forms. The two major recognized Pleistocene refugia are the 'West Central' located in the current territories of Cameroon and Gabon and the 'East Central' in the eastern part of the former Zaire. It is interesting to note that their location is coincident with – at least part of – the present distribution of the great apes (Figure 3). Conceivably, populations that were already settled in the areas that constituted forest refugia might have had longer periods of adaptation and possibly pre-retraction instances of population expansion that contribute to their high genetic diversity. Thus, coincident location in the areas that made up the western refuge in Pleistocene times may account for the high genetic diversity values seen in *P. t. troglodytes* and *G. gorilla*. Furthermore, we found a general low level of genetic variability in *P. t. schweinfurthii*. This is in general agreement with previous studies, although members of the Rwenzori and Ituri forest populations, which differ in being located in probable Pleistocenic forest refugia, are reported to have higher diversity (Goldberg and Ruvolo 1997).

With each new study it becomes increasingly apparent that the combined labor of genes and environment has created over time a reservoir of molecular richness in great ape populations. But recognition of these high levels of biodiversity carries implications and responsibilities. The possibility has been raised that current classification and taxonomy may require further revision. The existing classification, which is based mainly on morphology and

Figure 3. Present-day distribution of great apes in central Africa in relation to the major Pleistocene refugia (shaded regions). Dotted areas, heavy black borders and the striped area represent the distribution of chimpanzees, gorillas and bonobos, respectively.

geographic distribution, does not always reflect the significant diversity that has been revealed by genetic markers in this and other studies. In the genus *Pan* for example, intra-subspecific diversity can be as high as that between the two species. In particular, the subspecies *P. t. verus* shows such a clear separation from the central forms that Morin et al. (1994) have speculated about the chances of this subspecies being elevated to full species rank. Given the recent precedent established in *Gorilla*, our results tend to support such an amendment, although genetic data must clearly be but one resource used to establish the true extent of the biodiversity when clarifying taxonomic relationships among our closest relatives.

Knowledge of these high levels of variability also brings the urgent necessity of promoting legislation for the conservation and management of the great ape populations and their habitats. Considering the degree of variability contained within these taxa and their relatively small population sizes, the loss of even a single group may well represent a significant and irreversible loss of genetic diversity. At the same time, better knowledge of the genetic diversity of these populations can facilitate and improve their management in captivity as well as

in the wild. Genetic data in the form of known sequences can be implemented as markers that enable researchers to assign individuals to their corresponding subspecies or even population, to determine kin relationships, to reconstruct population structure, and to census, among others (Morin et al. 1994; Hashimoto et al. 1996). It also has the potential to facilitate such targeted management efforts as subspecies interbreeding control and the relocation or reintroduction of individuals to appropriate areas (Garner and Ryder 1996).

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References

Brown W.M., Prager E.M., Wang A. and Wilson A. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. Journal of Molecular Evolution 18: 225–239.

Colyn M., Gautier-Hion A. and Verheyen W. 1991. A re-appraisal of palaeoenvironmental history in Central Africa: evidence or a major fluvial refuge in the Zaire Basin. Journal of Biogeography 18: 403–407.

Darwin C. 1964. On the Origin of Species. Harvard University Press, Cambridge, Massachusetts.

Deinard A.S. and Kidd K. 2000. Identifying conservation units within captive chimpanzee populations. American Journal of Physical Anthropology 111: 25–44.

Felsenstein J. 1993. PHYLIP (phylogeny inference package) Version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, Washington.

Foran D.R., Hixson J.E. and Brown W.M. 1988. Comparisons of ape and human sequences that regulate mitochondrial DNA transcription and D-Loop DNA synthesis. Nucleic Acids Research 16: 5841–5861.

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

Furuichi T. and Ihobe H. 1995. Variation in male relationships in bonobos and chimpanzees. Behaviour 130: 212–228.

Gagneux P., Wills C., Gerloff U., Tautz D., Morin P.A., Boesch C. et al. 1999. Mitochondrial sequences show diverse evolutionary histories of African hominoids. Proceedings of the National Academy of Sciences USA 96: 5077–5082.

Garner K.J. and Ryder O.A. 1996. Mitochondrial DNA diversity in gorillas. Molecular Phylogenetics and Evolution 6: 39–48.

Goldberg T.L. and Ruvolo M. 1997. The geographic apportionment of mitochondrial genetic diversity in east African chimpanzees, *Pan troglodytes schweinfurthii*. Molecular Biology and Evolution 14: 976–984.

Groves C.P. 2001. Primate Taxonomy. Smithsonian Institution Press, Washington, DC.

Grubb P. 1982. Refuges and dispersal in the speciation of African forest mammals. In: Prance G.T. (ed.), Biological Diversification in the Tropics. Columbia University Press, New York, pp. 537–553.

Hacia J.G. 2001. Genome of the apes. Trends in Genetics 17: 637–645.

Hamilton A.C. 1981. The quaternary history of African forests: its relevance to conservation. African Journal of Ecology 19: 1–6.

Hashimoto C., Furuichi T. and Takenaka O. 1996. Matrilineal kin relationships and social behavior of wild bonobos (*Pan paniscus*): sequencing the D-Loop region of mitochondrial DNA. Primates 37: 305–318.

Hofreiter M., Siedel H., Van Neer W. and Vigilant L. 2003. Mitochondrial DNA sequence from an enigmatic gorilla population (*Gorilla gorilla uellensis*). American Journal of Physical Anthropology 121: 361–368.

Jensen-Seaman M.I., Deinard A.S. and Kidd K.K. 2001. Modern African ape populations as genetic and demographic models of the last common ancestor of humans, chimpanzees and gorillas. The American Genetic Association 92: 475–480.

Jensen-Seaman M.I. and Kidd K.K. 2001. Mitochondrial DNA variation and biogeography of eastern gorillas. Molecular Ecology 10: 2241–2247.

Kaessmann H., Wiebe V. and Pääbo S. 1999. Extensive nuclear DNA sequence diversity among chimpanzees. Science 286: 1159–1163.

Kano T. 1992. The Last Ape: Pygmy Chimpanzee Behavior and Ecology. Stanford University Press, Stanford, California.

Kimura M.A. 1980. Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120.

Li W.H. 1997. Molecular Evolution. Sinauer Associates, Inc., Sunderland, Massachusetts.

Morin P.A., Moore J.J., Chakraborty R., Jin L., Goodall J. and Woodruff D.S. 1994. Kin selection, social structure, gene flow, and the evolution of chimpanzees. Science 265: 1193–1201.

Nei M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Page R.D.M. and Holmes E.C. 1998. Molecular Evolution. A Phylogenetic Approach. Blackwell Science, Oxford, UK.

Ruvolo M., Pan D., Zehr S., Goldberg T., Disotell T.R. and Von Dornum M. 1994. Gene trees and hominoid phylogeny. Proceedings of the National Academy Sciences USA 91: 8900–8904.

Sambrook J., Fritsch E.F. and Maniatis T. 1989. Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Tamura K. and Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.

Vigilant L., Stoneking M., Harpending H., Hawkes K. and Wilson A. 1991. African populations and the evolution of human mitochondrial DNA. Science 253: 1503–1507.

Xu X. and Arnason U. 1996. A complete sequence of the mitochondrial genome of the western lowland gorilla. Molecular Biology and Evolution 13: 691–698.