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Genetic Analysis of Putative Familial Relationships in a Captive Chimpanzee (*Pan troglodytes*) Population

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ABSTRACT

Twelve autosomal dinucleotide repeat loci were analyzed in chimpanzees genomes by DNA amplification using primers designed for analysis of human loci. The markers span the entire length of human chromosomes 21 and 22. Nine markers were polymorphic in chimpanzee as well, with a somewhat comparable level of polymorphism and allele size range. Even in the presence of very limited information and in spite of missing samples, it was possible to reconstruct a complex pedigree and to provide molecular data that corroborate family relationships that were deduced from cage history and behavioral data. The conclusions were further supported by mitochondrial DNA analysis. The data presented in this report show that the extremely abundant source of human markers may be exploited to validate, with molecular evidence, hypotheses on individual relationship or alleged pedigrees, based upon behavioral observations.

Key words: *nonhuman primates, microsatellite, genotyping, pedigree, heterozygosity*

Introduction

The importance of nonhuman primates in biomedical research, coupled with the fact that all nonhuman primates are at least listed on Appendix II of the Convention on International Trade of Endangered Species (CITES; http://www.cites.org/eng/app/index.shtml) and have thus been restricted in their exportation from their native range countries, has necessitated the establishment of breeding colonies within the United States. These breeding colonies have often been derived from a limited number of founders; therefore, monitoring genetic variation within these colonies has been of prime concern. Indeed, the loss of genetic variation would imperil both the health of the colony and the usefulness of the animals for understanding the underlying genetic contribution to phenotypic variation.

Since in many primate societies only one or a few males sire offspring, the maximization of genetic variation within the breeding colonies is facilitated by controlling matings between various males and females within the colony. Sometimes multiple males and females may be housed together and assumptions of paternity are based on behavioral observations. However, in order to validate that individuals born from a given mating are indeed the offspring of the presumed sire, it is necessary to corroborate the behavioral data with molecular evidence, like DNA test. Furthermore, biomaterials from colony animals may be distributed to the research community, and DNA testing validates the familial assumptions for any study of inherited traits. The Yerkes National Primate Research Center (YNPRC) is one of eight NIH funded primate research centers administered by the National Center for Research Resources. In an effort to make chimpanzee (*Pan troglodytes*) DNA and cell lines more widely available to the research community, YNPRC, in collaboration with the Coriell Institute for Medical Research, established a repository that would ul-

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timately include biomaterials from all individuals in the colony (http://ccr.coriell.org/Sections/Collections/YERKES/?SsId=66). Molecular analysis is routinely done as part of the quality control procedure: microsatellite genotyping is performed to ensure sample integrity throughout the process of receiving blood, establishing cell lines, and DNA extraction. Also a portion of the mitochondrial cytochrome oxidase C subunit 1 (*cox1*) is sequenced as a means of species identification by DNA barcoding[1].

The main goal of the present paper is to obtain molecular data that would confirm familial relationships of individual chimpanzees that are part of the Yerkes chimpanzee resource. To this end, microsatellite DNA loci were analyzed: the markers are CA di-nucleotide repeats that are highly polymorphic in humans and, therefore, they are likely to be informative in chimpanzees as well.

Materials and Methods

Pan troglodytes samples

Blood samples were received from the Yerkes National Primate Research Center of Emory University, and DNA was extracted using standard methodologies[2]. Table 1 shows a partial list of *Pan troglodytes* samples stored at the Coriell Institute for Medical Research. These samples are available to the research community and the data presented here increase the value of these samples for genetic studies.

TABLE 1
ID OF THE INDIVIDUALS GENOTYPED. THE FAMILY RELATIONSHIP REPORTED IS BASED ON BEHAVIORAL OBSERVATIONS

Sample ID	Relationship
S003489	Half sib of S003629
S003610	Son of S003657 and S003612
S003612	Mother of S003610
S003619	Daughter of S003657
S003628	Daughter of S003657; sister of S003654
S003629	Half sib of S003489
S003654	Daughter of S003657; sister of S003628
S003657	Father of S003610, 3619, 3628, 3654

DNA markers

Table 2 lists the twelve markers utilized in the present study. Five markers are located on human chromosome 21, while seven markers span human chromosome 22: the average distance between adjacent markers is 10 or 8 cM, respectively. All markers are part of the ABI Prism Linkage Mapping Panel, version 2 (Applied Biosystems). Markers D21S263 and D22S280 gave a very faint signal, while D22S539 was found monomorphic in *Pan troglodytes*: those three markers were not further investigated.

TABLE 2
LOCI AND FLUORESCENT DYES

Locus	Dye Label	Het	ASR HSa	ASR PTg
D21S1256	NED	0.65	101–121	105–131
D21S1914	HEX	0.86	262–284	258–280
D21S263	HEX	0.75	198–232	NA
D21S1252	NED	0.80	156–180	146–166
DS1S266	FAM	0.59	159–181	149–157
D22S420	HEX	0.77	156–172	146–158
D22S539	NED	0.58	203–221	197
D22S315	FAM	0.78	184–214	162–182
D22S280	HEX	0.82	216–228	NA
D22S283	NED	0.89	132–160	124–144
D22S423	HEX	0.82	290–312	282–294
D22S274	NED	0.77	280–302	272–282

HET – heterozygosity (in *Homo sapiens*); ASR HSA – allele size range in *Homo sapiens*; ASR PTG – allele size range in *Pan troglodytes*. NA – not available.

PCR amplification and genotyping

Markers were amplified as single reactions and PCR products were pooled for analysis. Approximately 50 ng of genomic DNA were amplified in a final PCR volume of 15 μL containing 1.5 μL 10x GeneAmp PCR buffer II, 1.5 μL 25 mM $MgCl_2$, 1.5 μL 2.5 mM dNTP mix, 1.0 μL Primer Mix, 0.12 μL AmpliTaq Gold DNA Polymerase, with an ABI GeneAmp PCR System 9700 Thermal Cycler. PCR reactions were performed as follows: a 12 min hold at 95 °C was followed by 10 cycles of 15 sec at 94 °C, 15 sec at 55 °C, 30 sec at 72 °C and 20 additional cycles of 15 sec at 89 °C, 15 sec at 55 °C, 30 sec at 72 °C, with a final extension of 10 min at 72 °C.

Amplified PCR products were pooled at a ratio of 5 μL for each FAM labeled product and 10 μL for each HEX and NED labeled product. D.I. water was added to a final volume of 100 μL. The pooled PCR product was mixed with loading buffer at a 2:3 ratio for a 5 μL final volume (loading buffer contains 4.5 μL formamide, 1.0 μL blue dextran and 1.5 μL of S400HD-ROX as an internal size standard). The mixture was heated at 95 °C for 3 minutes and cooled on ice for 3 minutes before being separated on an ABI 377 Sequencer using a 36 cm denaturing polyacrylamide gel for resolution of the dinucleotide repeat products. Separated allele fragments were analyzed using ABI GeneScan and Genotyper software and genotypes were scored.

mtDNA analysis

The segment corresponding to the human first hypervariable region of the mitochondrial control region (HVR I) was amplified in the chimpanzee samples using the same primers and PCR conditions as used for human samples[3]. PCR fragments were sequenced directly using ABI BigDYE Terminator reaction kits, separated on an

ABI 377 Sequence Analyzer. Sequenced fragments were aligned and sequences verified using Sequencher (GeneCodes).

Results

The 400 markers included in the ABI Prism Linkage Mapping Set derive from a genetic map of the human genome[4–6]. Since there is a remarkable similarity between human and chimpanzee genomes[7–9], it was expected that those primers, although designed to amplify human DNA sequences, were able to amplify chimpanzee DNA sequences as well[10–11]. The results are summarized in Table 2. All twelve markers gave a positive PCR result; however, two of them, D21S263 and D22S280, gave a very weak signal in chimpanzee genome and therefore were excluded from the analysis. Table 2 shows also that, with the exception of D22S539, all markers were polymorphic also in *Pan troglodytes*. In some cases, such as marker D21S1914, the allele size range observed in chimpanzee overlaps with the one reported for humans; in other cases, such as marker D22S274, the allele size range shows a bimodal distribution, with the chimpanzee alleles being on the lower size range compared with humans (Figure 1). Therefore, we concluded that the human markers already available in the ABI Prism Linkage Mapping Set can be utilized for pedigree analysis on chimpanzee genomes.

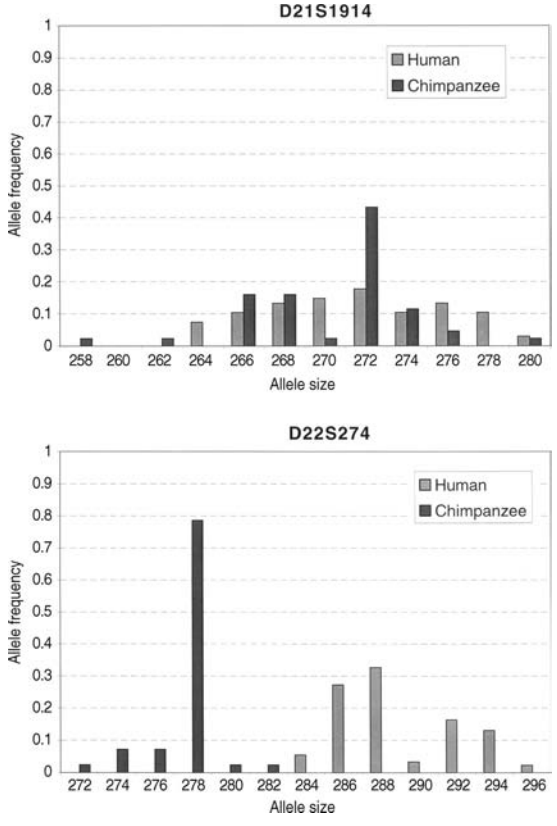

Fig. 1. Allele size and frequency for markers D21S1914 and D22 S274 in humans and chimpanzee. Allele size distribution for marker D21S1914 overlaps in the two species, while marker D22S274 shows a bimodal distribution. Allele frequency was calculated by genotyping 50 unrelated Caucasians and 25 chimpanzees.

Pedigree 1

S003657 was reported as the father of S003610, S00 3619, S003628 and S003654. Behavioral observations indicate that S003610 is the son of S003657 and S003612, while S003619 is the daughter of S003657 with a second mate, and S003628 and S003654 are daughters of S00 3657 with a third mate.

Figure 2 shows the alleged pedigree and the genotypes for four chromosome 21 loci. Both S003657 and S003612 are heterozygous at all four loci, their phase being unknown. S003610 is heterozygous at all four loci as well, but in his case the phase can be deduced: alleles 121, 268, 161 and 149 are consistent with inheritance from the father, while alleles 123, 274, 157 and 157 are consistent with inheritance from the mother. Assuming no recombination (the distance between each markers is approximately 10 cM), we suggest the most likely phase also for S003657 and S003612.

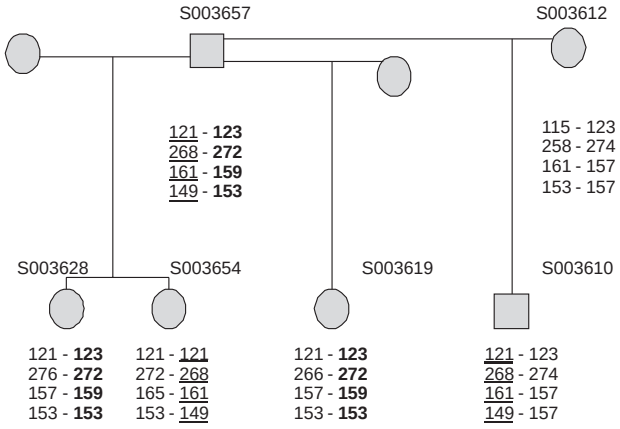

Fig. 2. Pedigree 1. Genotypes for chromosome 21 markers. Haplotypes are indicated by alleles underscored or in bold. Markers, from the top, are: D21S1256, D21S1914, D21S1252, D21S266.

At locus D21S1914, S003619 carries paternal allele 272, and allele 266 that is not present in S003612, indicating that S003619 is the offspring of S003657 with a mate different from S003612. At the same locus D21S 1914, S003628 inherited allele 276 from the mother, while the full sister S003654 inherited maternal allele 272 (or, less likely, 268). The presence of three distinct maternal alleles, 266 in S003619, 276 in S003628, and 272 (or 268) in S003654, all absent in S003612, provides the evidence of an additional mate.

Analysis of chromosome 22 markers, shown in Figure 3, shows consistent results: at locus D22S283, S003610 carries maternal allele 127. At the same locus, S003619, S003628 and S003654 carry maternal alleles that are not present in the mother of S003610, indicating the presence of, at least, a different mate.

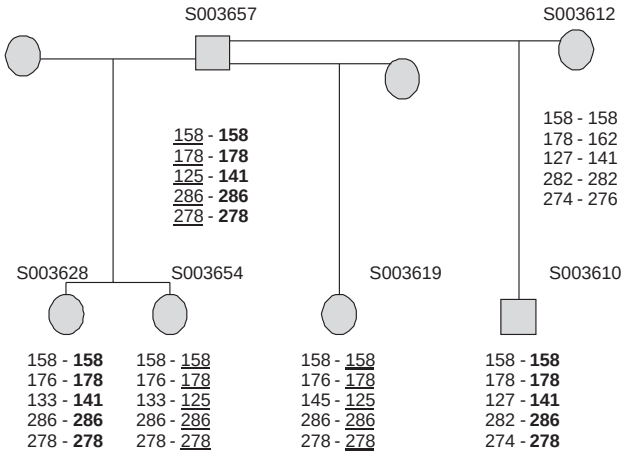

Fig. 3. Pedigree 1. Genotypes for chromosome 22 markers. Haplotypes are indicated by alleles underscored or in bold. Markers, from the top, are: D22S420, D22S315, D22S283, D22S423, D22 S274.

Consistent with the nuclear DNA data, evidence of three different mtDNA lineages in the pedigree fully support our conclusion: as shown in Table 3, S003612 and S003610 share the same mtDNA, S003619 has a different mtDNA, and S003654 shows a yet different mtDNA.

TABLE 3
MITOCHONDRIAL DNA DATA

ID	Cox1	CytB Cluster	Cyt B
S003657	III	Ivory Coast	ND
S003612	IV	Gabon	Ia
S003610	IV	Gabon	Ia
S003619	II	ND	ND
S003654	III	Ivory Coast	IIIa
S003629	I	Ivory Coast	II
S003489	I	Ivory Coast	IIa

COX1 – cytochrome oxidase I; CYT B – cytochrome b; ND – not done.

Pedigree 2

S003629 and S003489 were reported as half sibs generated by the same father with two different mates. No samples from any putative parents were available. Figure 4 reports the alleged pedigree and genotypes of S003629 and S003489 for chromosome 21 loci. The data show that the two chimpanzees share one allele at every locus, consistent with the inheritance of an identical chromosome (we surmise that it is the paternal one). Similar results were obtained for chromosome 22 loci (Figure 5), with identical alleles shared at every locus. At the same time, S003629 and S003489 show two different mtDNAs (Table 3), perfectly in agreement with the hypothesis that S003629 and S003489 are the offspring of two different, and maternally unrelated, mothers and a common father.

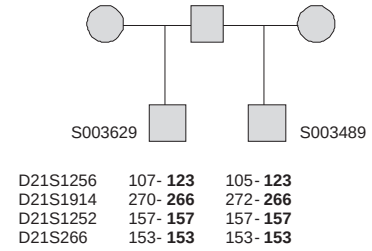

Fig. 4. Pedigree 2. Genotypes for chromosome 21 markers. Alleles shared between the two individuals are shown in bold. Markers are the same ones listed in figure 2.

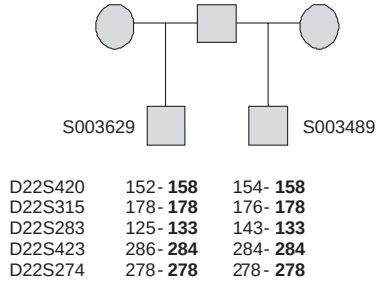

Fig. 5. Pedigree 2. Genotypes for chromosome 22 markers. Alleles shared between the two individuals are shown in bold. Markers are the same ones listed in figure 3.

Discussion and Conclusions

The ABI Prism Linkage Mapping Panel, version 2 was developed to define a 10 cM (on average) resolution human index map. The 400 markers have been selected, based on heterozygosity and chromosomal location, after extensive screening on a large number of DNA samples. Given the high degree of homology between human and chimpanzee genomes, it was expected that human primers were able to amplify chimpanzee sequences. Indeed, all twelve markers gave a positive PCR result, although two of them, D21S263 and D22S280, gave a faint signal. A likely explanation is that the primers for those two markers are not 100% identical to the chimpanzee's DNA sequences, and even a small nucleotide difference may account for the result.

The markers were polymorphic also in chimpanzees, the only exception being marker D22S539, which was monomorphic for allele 197. The rather small number of samples analyzed does not allow us to have a reliable estimate on the markers heterozygosity values, when they are utilized in chimpanzee. However, our data suggest the following trend: i) chimpanzees show a reduced number of alleles; ii) the alleles show a reduced number of CA repeats (data not shown). The results, most likely, are the consequence of a bias of ascertainment[12]: the markers were selected for their high heterozygosity values in humans, and the heterozygosity correlates with the length

of the di-nucleotide repeats. It is interesting to note that D22S539, the only marker that was monomorphic in chimpanzee, is at the same time the least informative marker in humans, with a heterozygosity value of 0.58.

The human markers proved to be effective to validate conclusions based on behavioral observations. In pedigree 1, a single individual, S003657, had offspring from three different mates, but DNA sample was available from only one mate, S003612. Locus D21S1914 gave the crucial information (Figure 2): the presence of three distinct maternal alleles, all absent in mate S003612, provides the evidence of two additional mates. These conclusions were supported by the evidence of three distinct mitochondrial lineages.

Pedigree 2 posed a different challenge: samples were available only for the two alleged half sibs S003629 and S003489, while samples were altogether missing from the father, as well as from the two mothers. Analysis of chromosome 21 and 22 loci shows the presence of identical alleles at each locus (Figures 4 and 5). Since mtDNA analysis clearly indicate the presence of two different lineages (Table 3), the shared alleles very likely derived from the common father who, by chance, transmitted to both sons the same chromosomes.

ABI panels are designed for human identification, therefore the markers were selected for their high heterozygosity values in humans and this represents an obvious limitation of the proposed approach. Ideally, chimpanzee's genomic libraries should be screened and the most informative markers should be selected for identification purposes.

At any rate, our data show that the already available human markers represent an extremely abundant source of information that can be utilized to validate, at the molecular level, hypotheses based exclusively on behavioral observations. In particular, the examples shown in this work demonstrate that it is possible to reconstruct family trees even in challenging situations, for example where only scanty information is available, or in the occurrence of missing samples.

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REFERENCES

1. LORENZ JG, JACKSON WE, BECK JC, HANNER R, Philos Trans R Soc Lond B Biol Sci, 360 (2005) 1869. DOI: 10.1098/rstb.2005.1718. — 2. SAMBROOK J, FRITSCH EF, MANIATIS T, Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor, New York, 1989). — 3. JOHNSON JR, LORENZ JG, J Calif Gt Basin Anthropol, 26 (2006) 33. — 4. WEISSENBACH J, GYAPAY G, DIB C, VIGNAL A, MORISSETTE J, MILLASSEAU P, VAYSSEIX G, LATHROP M, Nature, 359 (1992) 794. DOI: 10.1038/359794a0. — 5. GYAPAY G, MORISSETTE J, VIGNAL A, DIB C, FIZAMES C, MILLASSEAU P, MARC S, BERNARDI G, LATHROP M, WEISSENBACH J, Nat Genet, 7 (1994) 246. DOI: 10.1038/ng 0694supp-246. — 6. DIB C, FAURÉ S, FIZAMES C, SAMSON D, DROUOT N, VIGNAL A, MILLASSEAU P, MARC S, HAZAN J, SEBOUN E, LATHROP M, GABOR G, MORISSETTE J, WEISSENBACH J, Nature, 380 (1996) 152. DOI: 10.1038/380152a0. — 7. YUNIS J, PRAKASH O, Science, 215 (1982) 1525. DOI: 10.1126/science.7063861. — 8. GAGNEUX P, VARKI A, Mol Phylogenet Evol, 18 (2001) 2. DOI: 10. 1002/ajpa.1061. — 9. HACIA JG, Trends Genet, 17 (2001) 637. DOI: 10. 1016/S0168-9525. — 10. DEKA R, SHRIVER M, YU L, JIN L, ASTON C, CHAKRABORTY R, FERRELL R, Genomics, 22 (1994) 226. DOI: 10.1006/geno. 1994.1369. — 11. ROBLEDO R, BENDER P, LEONARD J, ZHU B, OSOEGAWA K, DE JONG P, XU X, YAO Z, ROE B, Genomics, 84 (2004) 678. DOI: 10.1016/j.ygeno.2004.07.001. — 12. COOPER G, RUBINSZTEIN D, AMOS W, Hum Mol Genet, 7 (1998) 1425.

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GENETSKA ANALIZA SRODSTVEBIH ODNOSA KOD POPULACIJE ČIMPANZI (*PAN TROGLODYTES*) U ZAROBLJENIŠTVU

SAŽETAK

Dvanaest autosomnih dinukleotidno repetitivnih lokusa analizirani su u genomu čimpanza pomoću DNA amplifikacije koristeći početnice dizajnirane za analizu ljudskih lokusa. Markeri su prisutni čitavom dužinom ljudskih kromosoma 21 i 22. Devet markera je također polimorfno i kod čimpanzi s određenim nivoom komparabilnosti plimorfizma i raspona alela. Čak i u prisutnosti vrlo ograničenih podataka te usprkos nedostatku uzoraka, bilo je moguće rekonstruirati

kompleksne srodstvene odnose te dati molekularne podatke koji ih podupiru, a koji su dobiveni na temelju povijesti kaveza te podataka o ponašanju. Zaključci su nadalje poduprijeti mitohondrijskom DNA analizom. Podaci koje donosimo u ovom izvještaju pokazuju kako bogat izvor ljudskih markera može biti upotrebljen u svrhu validacije pretpostavki o individualnim srodstvenim vezama.