

ANALYSIS OF THE COL2A1 POLYMORPHISM IN A SMALL Pan troglodytes POPULATION, AND ITS FORENSIC APPLICATION

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Common chimpanzees (*Pan troglodytes*) are a protected species, whose capture and trading is forbidden. In spite of international agreements, a high number of such great apes is annually reduced to captivity, then sold to private zoos and circuses. To elude controls, private owners use to declare false births in captivity (true births are rarest events) and exchange animal identities. All means enacted to control such illegal activities have been unsuccessful. We have been committed in a project aimed at developing a biological test to certify controversial animal identities, inspired to procedures for individual identification and parenthood tests used in humans.

An obliged step of this project was the development of hypervariable DNA polymorphisms (AmpFLPs; STRs) in Chimpanzee genome. Given the close relatedness between primate genomes, some of the human DNA markers may be also used in Chimpanzees. We here refer on a human gene - Col2A1 3'HVR (12q14.3) - which is in fact highly polymorphic in *Pan troglodytes*.

MATERIALS AND METHODS

Genomic DNA was obtained by hair roots using standard extraction procedures (an average of 30 nanograms per root was obtained). Twelve hypervariable loci were investigated using human oligonucleotide primers. We first focused on the 3'HVR COL2A1 locus (1), which showed a singularly high number of allelomorph fragments. Amplification conditions were similar to those used for the corresponding human gene, and separation of the amplified fragments was achieved by horizontal PAGE (8.5% T, 3%C) and silver-staining. An allelic ladder, prepared by mixing equimolar quantities of fragments, helped the typing work. To establish the degree of omology between chimpanzee and human alleles, the most common allele was run on agarose, excised and purified, subcloned in pUC18 vector and sequenced. The resulting basic repeat unit showed very high omology (98%) with man's.

RESULTS AND DISCUSSION

Alleles and genotypes detected in this study are shown in Fig. 1. In Table 1, the distribution of observed and expected genotypes is shown. Chimpanzee alleles have a lower molecular

size compared with humans. Four major and six rare alleles were identified in 40 individuals, accounting for 18 genotypes. The system has a heterozygosity is 0.74 and the commonest genotype occurs in 20% individuals. A fair agreement between observed and expected genotypes was surprisingly observed. The high level of homology in the repeat unit and the complete identity of the flanking regions suggested that the entire COL locus is probably highly conserved in these primates.

Three cases of disputed maternity - involving as many controversial cases of animal identity - were solved by DNA analysis of Col2A1 hypervariable gene. The protocol employed, besides being highly informative and more effective than other methods so far employed, had the additional advantage of avoiding blood withdrawal to animals. Characterization of several other Pan troglodytes polymorphisms is under way in our laboratory.

REFERENCES

- (1) Wu, S., Seino, S., Bell, G.I., Nucleic Acids Res. 18, 3102, 1990.

Figure 1

Some alleles and genotypes identified are here shown. At both extremities of the gel a human allelic ladder, obtained by the admixture of the most common human alleles has been run.

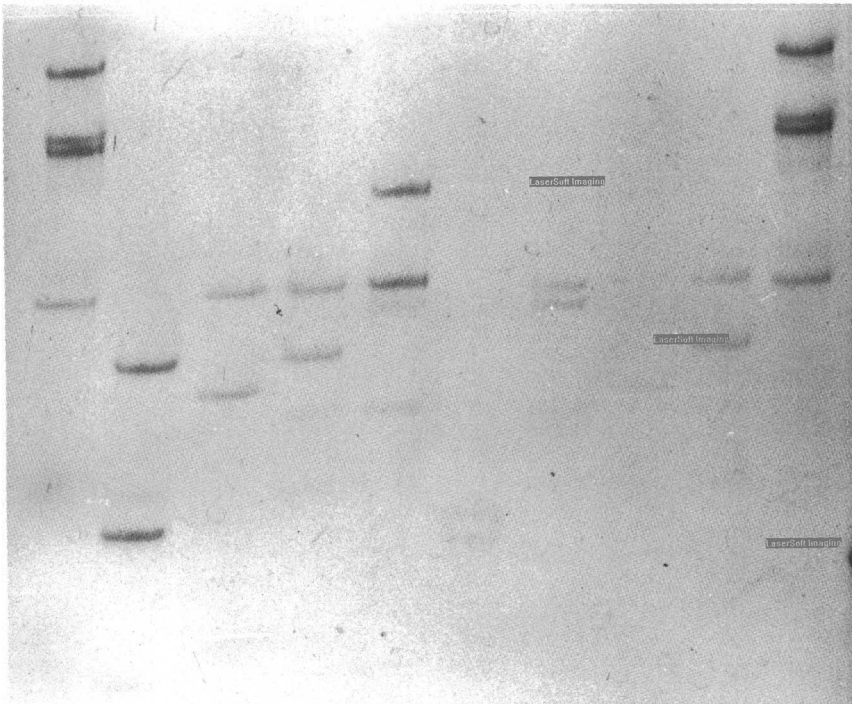


Table 1

Observed and expected COL2A1 phenotypes in a small Pan troglodytes population. A provisional nomenclature has been employed (from A to D for major alleles, from highest to lowest molecular weights).

TYPE		OBSERVED	EXPECTED	CHI SQUARE
B	B	5	4.23	0.14
C	C	1	1.88	0.41
C2	C2	1	0.18	3.62
D	D	1	0.73	0.10
D1	D1	1	0.06	13.18
A	B	2	1.41	0.25
A	B1	1	0.17	3.84
A	C	1	0.94	0.00
B	B1	1	1.05	0.00
B	C	8	5.64	0.98
B	C1	1	0.35	1.19
B	D	2	3.53	0.66
B1	C2	1	0.22	2.75
B2	C2	1	0.07	11.67
C	C2	1	1.17	0.03
C	D	4	2.35	1.15
D	D1	1	0.44	0.71
D	D2	1	0.14	4.95
sum		34		