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# A Preliminary Study on Blood Protein Variations of Wild and Domestic Camelids in Peru

Yoshi KAWAMOTO[1], Akio HONGO[2], Yuji TOUKURA[2], Tetsuya INAMURA[3], Norio YAMAMOTO[4], Yuichi SENDAI[5] and Emiko TORII[6]

[1]Primate Research Institute, Kyoto University
[2]Obihiro University of Agriculture and Veterinary Medicine
[3]Aichi Prefectural University
[4]National Museum of Ethnology
[5]The Graduate University for Advanced Studies
[6]Peru Amano Museum

## INTRODUCTION

Origin and domestication of camelids in the New World is controversial with regard to the contribution of two extant wildlife in the Andes, the guanaco (*Lama guanicoe*) and the vicuña (*Vicugna vicugna*). There are two domestic forms that are typically known as the alpaca (*Lama pacos*) and the llama (*Lama glama*). Those wild and domestic forms all share the same chromosome number (2n=74) (TAYLOR *et al.*, 1968) and any intercross of them can produce fertile offspring (GRAY, 1972). Their domestication has been discussed primarily by comparative morphology and archaeozoology. Two major hypotheses are presented for the ancestry of the domestic camelids. One is monophyly from the guanaco (for example, COOK, 1919; HERRE, 1952). The other considers multiple-ancestry of the alpaca and the llama from the vicuña and the guanaco, respectively (for example, DARWIN, 1868). An alternative hypothesis of such polyphyly considers establishment of the alpaca by crossing the vicuña and the llama after domestication of the llama from the guanaco (HEMMER, 1990).

Recent molecular studies suggested congruence with the polyphyletic ancestry hypothesis in the comparison of mitochondrial DNA sequences and microsatellite DNA typing (STANLEY *et al.*, 1994; KADWELL *et al.*, 2001). In order to extend molecular phylogenetic assessment further for testing origin and phylogenetic relationship of domestic camelids in South America, we examined blood protein variations detectable by conventional electrophoretic procedures in this study. In this preliminary study, small number of blood samples were collected from the four species then compared for 18 protein loci. Results of genotyping and quantification of genetic diversity within and between animals are reported here.

## MATERIALS AND METHODS

We compared a total of 27 blood samples collected in Peru in 2001 and 2002. Two samples, one male and one female, were obtained at Zoological Park in Lima from each of camelid species compared in this study. Additional 19 blood samples were obtained from the vicuña population at Pampa Galéras National Conservation Area by the courtesy of villagers in Lucanas, Ayacucho (Table 1, Fig. 1).

Heparinized bloods were separated into plasma and cell portions by centrifugation. We used starch gel electrophoreses in the blood protein typing by referring standard procedures used in previous study (KAWAMOTO *et al.*, 1984). Some of plasma proteins were also examined by polyacrylamide gel electrophoresis (GAHNE *et al.*, 1977; PENEDO *et al.*, 1988). The proteins

Table 1. List of samples examined in this study.

| Name of animal | No. of samples | Source |
|---|---|---|
| Guanaco (*Lama guanicoe*) | 2 | Zoological Park, Lima |
| Vicuña (*Vicugna vicugna*) | 21 | Zoological Park, Lima (2) |
| | | Pampa Galéras (19) |
| Alpaca (*Lama pacos*) | 2 | Zoological Park, Lima |
| Llama (*Lama glama*) | 2 | Zoological Park, Lima |


Fig. 1. Map showing the location of Pampa Galéras where the samples of vicuña were collected.

examined were plasma albumin, plasma transferrin, plasma $\alpha_2$-macroglobuline, plasma group specific component, plasma leucine aminopeptidase, cell glucosephosphate isomerase, cell NADH-dependent diaphorase, cell esterases (3 kinds), cell adenylate kinase, cell malate dehydrogenase, cell lactate dehydrogenase, cell isocitrate dehydrogenase, cell phosphogluconate dehydrogenase, cell superoxide dismutase, and cell acid phosphatase. They were encoded by at least 18 loci.

Gene diversity within species was measured by expected proportion of average heterozygosity assuming random mating of the population. Quantification of interspecific differentiation was made by NEI's standard genetic distance (NEI, 1972).

## RESULTS

Among 18 blood loci examined here, we could successfully resolve individual polymorphism at least for six protein loci; they were transferring (Tf), group specific component (Gc, alternatively known as vitamin D-binding protein), NADH-dependent diaphorase (Dia), one of erythrocyte esterases which has substrate specificity to 4-methyl umbelliferyl acetate (known as Esterase D (EsD) in primate species), malate dehydrogenase (MDH) and phosphogluconate dehydrogenase (PGD) (Fig. 2). Other 12 protein loci were typed as being all the same with identical allele for the four species.

*Tf* exhibited the highest degree of polymorphism where seven different phenotypes with six alleles, alphabetically denoted *a-f*, were observed (Table 2). The guanaco samples had alleles *a* and *d*, the vicuña alleles *e* and *f*. Alleles *c*, *e* and *f* were observed in llamas and *a*, *b* and *e* in alpacas.

Phenotypic variation in *Gc* was controlled by three alleles *B*, *K* and *N*. Allele *N* was not observed in the samples of guanacos and llamas and nor allele *K* in vicuñas and alpacas.

Phenotypes in the proteins Dia and EsD showed distinct difference among compared species. Neither the alleles *Dia 2* nor *EsD 2* were observed in vicuñas. Two private alleles (*Dia 1*' and *Dia 3*) were found in vicuñas at *Dia* locus. Private variation was also observed in *MDH* and *PGD* loci.

The estimates of average heterozygosity were 7.6%, 4.6%, 10.4% and 9.7% in guanacos, vicuñas, alpacas and llamas, respectively. Domestic forms showed relatively high variability than wild forms.

Quantification of NEI's standard distance revealed close relationship between guanacos and llama (0.019) and between vicuñas and alpacas (0.034). The distance between two wild camelids was 0.175 and that between two domestic forms was 0.110, suggesting three to nine times larger than guanacos/llamas and vicuñas/alpacas distances.

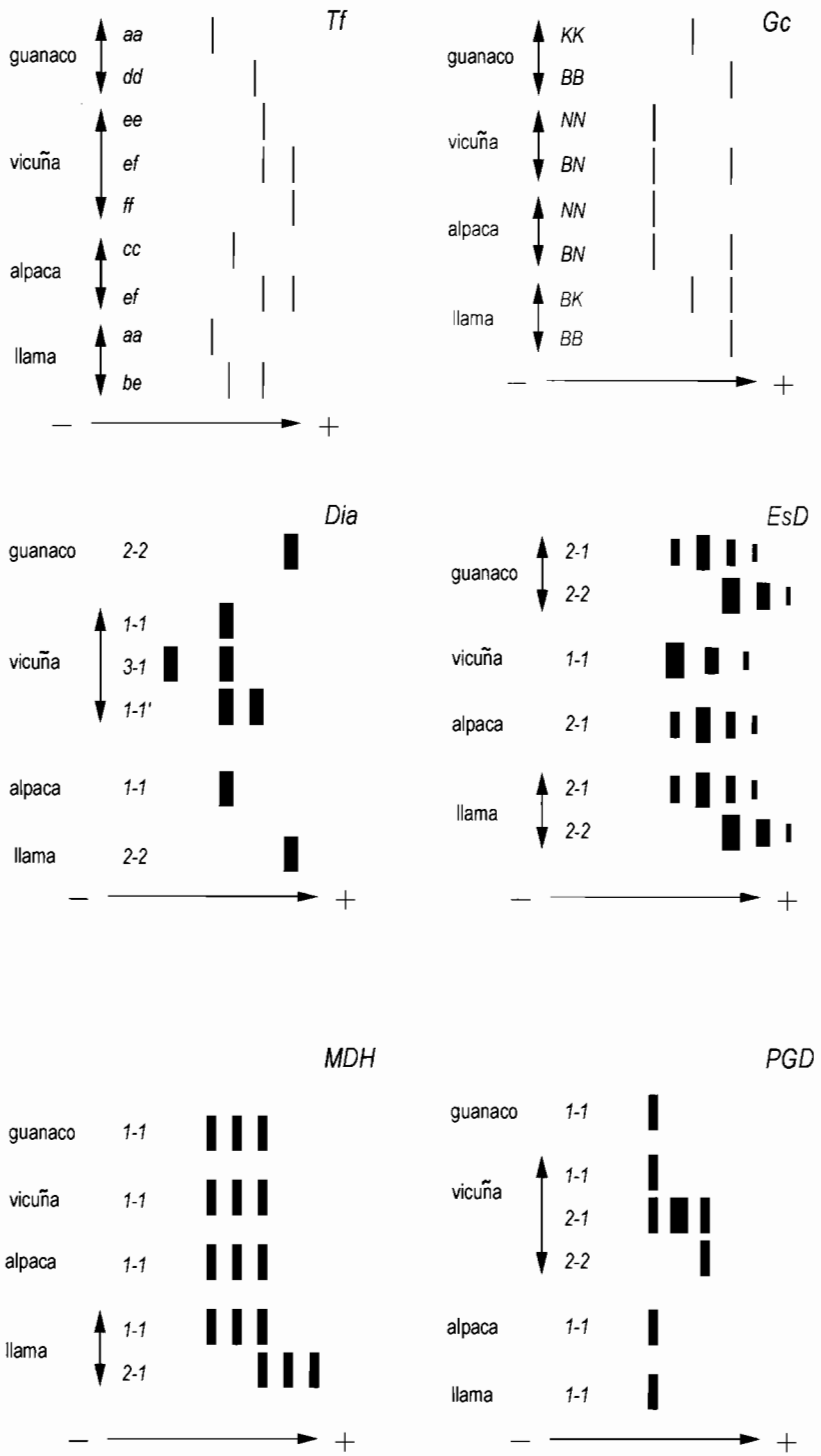


Fig. 2. Illustration of electrophoretic variations in blood proteins found in guanacos, vicuñas, alpacas and llamas in Peru.

Table 2. Estimates of allele frequencies.

| Locus | Allele | Guanaco N=2 | Vicuña N=21 | Alpaca N=2 | Llama N=2 |
|---|---|---|---|---|---|
| *Tf* | *a* | 0.50 | 0 | 0 | 0.50 |
| | *b* | 0 | 0 | 0 | 0.25 |
| | *c* | 0 | 0 | 0.50 | 0 |
| | *d* | 0.50 | 0 | 0 | 0 |
| | *e* | 0 | 0.69 | 0.25 | 0.25 |
| | *f* | 0 | 0.31 | 0.25 | 0 |
| *Dia* | *1* | 0 | 0.95 | 0.75 | 0 |
| | *1'* | 0 | 0.05 | 0 | 0 |
| | *2* | 1 | 0 | 0 | 1 |
| | *3* | 0 | 0 | 0.25 | 0 |
| *EsD* | *1* | 0.25 | 1 | 0.50 | 0.25 |
| | *2* | 0.75 | 0 | 0.50 | 0.75 |
| *Gc* | *B* | 0.50 | 0.07 | 0.25 | 0.75 |
| | *K* | 0.50 | 0 | 0 | 0.25 |
| | *N* | 0 | 0.93 | 0.75 | 0 |
| *MDH* | *1* | 1 | 1 | 1 | 0.75 |
| | *2* | 0 | 0 | 0 | 0.25 |
| *PGD* | *1* | 1 | 0.90 | 1 | 1 |
| | *2* | 0 | 0.10 | 0 | 0 |

## DISCUSSION

Previous studies reported several kinds of genetic polymorphism in blood proteins among South American camelids. They are variations in domestic forms, including catalase, phosphogluconate dehydrogenase (PGD), glucose-phosphate isomerase, transferrin (Tf) and group specific component (Gc) (PENEDO *et al.*, 1988: PENEDO and JUNEJA, 1989). Wild forms are less intensively studied for their blood protein profiles.

In this study, due to lack of samples for comparison test, we could not perform identification of the allele types for Tf and Gc. A total of 11 alleles were reported for Tf in alpacas and llamas by PENEDO *et al.* (1988). It was impossible to judge the alleles type only by referring figures and description in the previous report (PENEDO *et al.*, 1988). For the typing of Gc, we tried to make denotation of allelic names by referring electrophoretic patterns in the previous report (Fig. 2 in PENEDO *et al.*, 1988). PENEDO *et al.* (1988) also found segregation of two alleles at catalase locus in llamas, which were proved to follow codominant mode of inheritance. However, we failed in resolving the polymorphism of catalase because genotyping was difficult due to various intensity of electrophoretic bands among tested animals. Protein polymorphism of glucose-phosphate isomerase reported in the previous study was not detected in this study.

The incidence of polymorphism of PGD was observed in llamas (PENEDO *et al.*, 1988) but those of Dia, EsD and MDH were not reported so far. They show typical polymorphism in the

samples collected in Peru. In particular, Dia and EsD seem to be useful because they significantly show allele segregation between two wild camelids. It is important to investigate the distribution of those protein polymorphism in South American camelids further in future study. If they are good genetic marker, we will be able to apply those markers for the discrimination of animal populations relating to guanacos or vicuñas and for the quantification of degree of intermixture between domestic forms or between wild and domestic forms (WHEELER, 1995; KADWELL *et al.*, 2001).

Though the number of samples were very small, only two individuals for three species, we calculated genetic diversity indices of average heterozygosity and NEI's genetic distance in this study. Genetic variability was lower in the vicuñas samples than guanacos, alpacas and llamas. The degree of average heterozygosity was not dependent on sample size. This may result from recent decrease of the vicuña population by hunting in the park (WHEELER and DOMINGO, 1997).

Results of clustering based on NEI's distance depicted two clusters each of which contain one wild and one domestic species (Fig. 2). This result is likely to support the hypothesis of polyphyly, the ancestry of alpacas from vicuña and llamas from guanacos. But it should be tested further by increasing samples from different sources in future study.

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## REFERENCES

COOK, O.F. (1919) Domestication of animals in Peru. Jour. Hered., 10: 176–181.

DARWIN, C. (1868) The Variation of Animals and Plants under Domestication (publication in 1998, The Johns Hopins University Press, London).

GAHNE, B., JUNEJA, R.K. and GROLMUS, J. (1977) Horizontal polyacrylamide gradient gel electrophoresis for the simultaneous phenotyping of transferrin, post-transferrin, albumin and post-albumin in the blood plasma of cattle. Anim. Blood. Grps. Biochem. Genet., 8: 127–137.

GRAY, A.P. (1972) Mammalian Hybrids (2nd ed.). Commonwealth Agricultural Bureaux, Edinburgh.

HEMMER, H. (1990) Domestication: The Decline of Environmental Appreciation. Cambridge University Press, Caambridge.

HERRE, W. (1952) Studien über die wilden und domestizierten Tylopoden Südamerikas. Der Zoologische Garten, 19 (2–4): 70–98.

KADWELL, M., FERNANDEZ, M., STANLEY, H.F., BALDI, R., WHEELER, J.C., ROSADIO, R. and BRUFORD, M.W. (2001) Genetic analysis reveals the wild ancestors of the llama and the alpaca. Proc. R. Soc. Lond. B, 268: 2575–2584.

KAWAMOTO, Y., ISCHAK, T.M. and SUPRIATNA, J. (1984) Genetic variations within and between troops of the crab-eating macaque (Macaca fascicularis) on Sumatra, Java, Bali, Lombok and Sumbawa, Indonesia. Primates, 25: 131–159.

NEI, M. (1972) Genetic distance between populations. Amer. Nat., 106: 283–292.

PENEDO, M.C.T., FOWLER, M.E., BOWLING, A.T., ANDERSON, D.L. and GORDON, L. (1988) Genetic variation in the blood of llamas, *Llama glama*, and alpacas, *Llama pacos*. Anim. Genet., 19: 267–276.

STANLEY, H.F., KADWELL, M. and WHEELER, J.C. (1994) Molecular evolution of the family Camelidae: a mitochondrial DNA study. Proc. R. Soc. Lond. B, 256: 1–6.

TAYLOR, K.M., HUNGERFORD, D.A., SNYDER, R.L. and ULMER, F.A.JR. (1968) Uniformity of karyotypes in the Camelidae. Cytogenetics, 7: 8–15.

WHEELER, J.C. (1995) Evolution and present situation of the South American Camelidae. Biol. J. Linn. Soc. 54: 271–295.

WHEELER, J.C. and DOMINGO, H.R. (1997) Community participation, sustainable use, and vicuña conservation in Perú. Mountain Research and Development 17: 283–287.

**Abstract**

Polymorphism in blood proteins were examined for guanacos, vicuñas, alpacas, llamas in order to study genetic diversity, phylogenetic relationship, origin and domestication of camelids in South America. Samples were collected from captive animals at Zoological Park in Lima and from vicuñas populations at Pampa Galéras National Conservation Area. Electrophoretic examination for 18 loci revealed genetic polymorphisms at transferrin (*Tf*), group specific component (Gc protein) (*Gc*), NADH-dependent diaphorase (*Dia*), cell esterase (having substrate specificity to 4-methyl umbelliferyl acetate) (*EsD*), malate dehydrogenase (*MDH*) and phosphogluconate dehydrogenase (*PGD*) loci. Estimates of expected average heterozygosity were 7.6%, 4.6%, 10.4% and 9.7% in guanacos, vicuñas, alpacas and llamas, respectively. Calculation of genetic distance suggested close relationship between guanacos and llamas (D=0.019) and between vicuñas and alpacas (D=0.034).

# ペルーのラクダ科野生動物と家畜の血液タンパク多型（予報）

川本　芳[1]・本江　昭夫[2]・藤倉　雄司[2]・稲村　哲也[3]
山本　紀夫[4]・千代　勇一[5]・鳥居恵美子[6]

[1]京都大学霊長類研究所
[2]帯広畜産大学
[3]愛知県立大学
[4]国立民族学博物館
[5]総合大学院大学
[6]天野博物館

南米に生息する4種のラクダ科動物（グアナコ，ビクーニャ，アルパカ，リャマ）の遺伝的多様性，系統関係，起源と家畜化を調査するため，血液タンパク質の多型について分析した。試料はペルーのリマ市にある動物公園の飼育個体とパンパ・ガレーラス国立保護区のビクーニャ集団から得た。電気泳動法により18遺伝子座を検索した結果，トランスフェリン（Tf），Gcタンパク（Gc），ディアフォラーゼ（Dia），血球エステラーゼ（4-メチルウンベリフェリルアセテートに基質特異性を示す）（EsD），リンゴ酸脱水素酵素（MDH），グルコン酸6リン酸脱水素酵素（PGD）の各座位で多型が検出できた。遺伝子頻度から推定した平均ヘテロ接合率の期待値はグアナコが7.6%，ビクーニャが4.6%，アルパカが10.4%，リャマが9.7%となった。遺伝距離の計算結果から，グアナコとリャマ，ビクーニャとアルパカ，の近縁性が示唆された。