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# Phylogeographic Analysis of Madagascan Goats Using mtDNA Control Region and SRY Gene Sequences

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In the present study, we estimated the genetic diversity and relationships, as well as the propagation routes, of Madagascan goats using mtDNA control region and SRY gene sequences. The mtDNA sequences of 40 Madagascan goats revealed 10 haplotypes and a quite low nucleotide diversity (0.0014), suggesting a founder and/or bottleneck effect resulting from goat migration to Madagascar island. The analysis of sequences identical to Madagascan haplotypes indicated close genetic relationships between goats from Madagascar and Africa. Sequence analysis of the SRY gene in 40 male Madagascan goats revealed two haplotypes: Y1A (45%) and Y2A (55%). The paternal result indicated genetic influences from Africa, South Asia, and the Near East proximal to Madagascar. The analyses of the mtDNA control region and SRY gene sequences suggested a genetic relationship between Africa and Madagascar. Moreover, SRY sequences indicated influences from South Asia and the Near East. These phylogenetic results provide important genetic information for elucidating the propagation routes of Madagascan goats.

**Key words:** *Capra hircus*, genetic diversity, Madagascar, mitochondrial DNA, propagation route, Y-chromosome

## INTRODUCTION

Since their domestication in the Fertile Crescent, domestic goats (*Capra hircus*) have become widespread throughout the world (A Mills et al., 2017). For Africa, the introduction of goats was most likely initiated with westward movement from the Near East into Northeastern Africa via the Mediterranean maritime route (Pereira et al., 2009). The southward movement of domestic goats in Africa was linked to climatic shifts that likely occurred after maritime propagation (Pereira and Amorim, 2010). The propagation routes of goats to Madagascar can be inferred from linguistics studies. The word for goat in Malagasy, a Madagascan language, originated from the African languages Bantu and Swahili, suggesting an African propagation route (Adelaar, 2006; Blench, 2008). Although variable propagation routes for various animals have been inferred from genetic studies (Ardalan et al., 2015; Herrera et al., 2017), the propagation routes of Madagascan goats have not been investigated yet by genetic analyses.

Several phylogenetic studies on goats have been reported in recent years. Molecular analyses using mtDNA control region sequences revealed six maternal haplogroups (A, B, C, D, F, and G). Haplogroup A, observed in over 90% of domestic goats, is the most divergent (Luikart et al., 2001; Naderi et al., 2007). Most animals with haplogroup B are primarily found in Southeast Asia (Lin et al., 2012). Haplogroup C is observed in scattered locations within Asia and Europe with a low frequency. The minor haplogroups D, F, and G, are found in Asia and Northern Europe, Sicily, and the Near East and Northern Africa, respectively, at low frequencies (Naderi et al., 2007).

Genetic variations of the SRY gene on the Y-chromosome are useful for understanding paternal genetic diversity. To date, four haplotypes (Y1A, Y2A, Y1B, and Y2B) have been reported on the basis of 3'UTR sequences (Waki et al., 2015). Haplotypes Y1A and Y2A are present worldwide and are described as the primary ancestral haplotypes, whereas haplotypes Y1B and Y2B are found specifically in Europe and Southeast Asia, respectively (Waki et al., 2015; Tabata et al., 2018).

These genetic sequences, including mtDNA and SRY variants, are useful tools for the elucidation of the history of

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animal domestication (Herrera et al., 2017; Tabata et al., in press). The aim of the present study was to obtain genetic information and estimate the genetic relationships and propagation routes of Madagascan goats using mitochondrial and Y-chromosomal sequences.

## MATERIALS AND METHODS

All procedures in the present study were performed according to the Research Guidelines of Kobe University.

We collected blood samples from 40 native male goats randomly selected from 12 locations in Madagascar: Antananarivo ( $n = 4$ ), Morondava ( $n = 4$ ), Belo sur Mer ( $n = 3$ ), Manja ( $n = 4$ ), Ifaty ( $n = 4$ ), Vatolatsaka ( $n = 4$ ), Betioky ( $n = 5$ ), Ampanihy ( $n = 3$ ), Beloha ( $n = 2$ ), Faux cap ( $n = 1$ ), Berenty ( $n = 5$ ) and Anjampolo ( $n = 1$ ) (Supplementary Figure S1). Blood samples were stored at 4°C until DNA extraction. Genomic DNA was extracted from fresh blood using the standard phenol/chloroform method (Sambrook and Russell, 2001).

We sequenced the hypervariable segment I (HVI) of the mitochondrial control region from base pairs (bp) 15,707 to 16,187 (481 bp) on the basis of the goat mtDNA reference sequence (DDBJ accession no. AF533441.1) (Parma et al., 2003). Polymerase chain reaction was performed according to previously described methods (Sultana et al., 2003). Standard double-stranded DNA sequencing was performed using 20 ng of the amplified product with the BigDye® Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan), a primer with the sequence 5'-TACCCA-CACAAACGCCAACACC-3', and an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). All sequences were deposited in the DDBJ database (accession nos. LC433967–LC433976).

We also sequenced the *SRY* 3'UTR region (479 bp), covering positions 2568 to 3046, on the basis of the goat *SRY* reference sequence (DDBJ accession no. EU581862) (Ross et al., 2008). This region was amplified and sequenced, as previously reported (Waki et al., 2015). All sequences obtained in this study were deposited in the DDBJ database (accession nos. LC433977–LC433978). The variants were detected with goat mtDNA reference sequence (DDBJ accession no. AF533441.1) and were revealed in Supplementary Table S1 (Parma et al., 2003).

Sequence alignment of HVI was performed using ClustalW program (Chojnacki et al., 2017) in MEGA 7.0.14 (Kumar et al., 2016). In order to investigate the genetic relationships among mtDNA sequences, an unrooted neighbor-joining phylogenetic tree (Saitou and Nei, 1987) was constructed using the Tamura–Nei distance method (Tamura and Nei, 1993), including 22 reference sequences from six mtDNA haplogroups in goats selected by Naderi et al. (2007) (DDBJ accession nos. AY155721, EF618134, EF617779, EF618200, EF617945, EF617965, AB044303, EF617706, AJ317833, DQ121578, AY155708, AJ317838, EF618413, DQ188892, AY155952, EF617701, DQ188893, DQ241349, DQ241351, EF618084, EF618535, EF617727). Confidence in the phylogenetic tree was assessed by the bootstrap method, with 1000 replications (Felsenstein, 1985) incorporated into the MEGA 7.0.14 (Kumar et al., 2016). The nucleotide diversity was calculated using DnaSP 5.10 (Librado and Rozas, 2009). Median joining was used to construct a network using 40 Madagascan goat sequences (Table S1). All mutations were

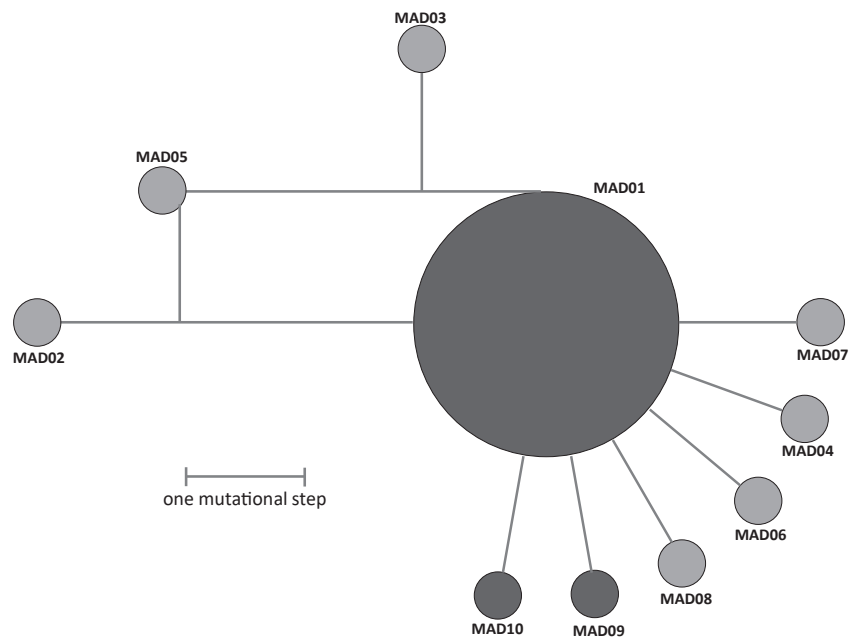
equally weighted using NETWORK 5.0.0.1 (Bandelt et al., 1999). We used default parameters for all analyses in these bioinformatic software.

In order to infer relationships among Madagascan mtDNA haplotypes and other areas, we carried out a BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to detect homologous regions within mtDNA control region of Madagascan goat sequences. Furthermore, we analyzed the distribution of paternal haplotypes in goat populations in Eurasia and Africa using previously published data of 1,086 West and East Eurasian goats (Supplementary Table S2) (Tabata et al., 2018).

## RESULTS AND DISCUSSION

We determined mtDNA sequences of the 481 bp HV1 region from 40 Madagascan goats. The sequence analysis identified 11 segregating sites (S), including nine transitions and two transversions. Based on these variations, 10 haplotypes (Hap) were observed. A neighbor-joining tree was constructed on the basis of these sequences, and all Madagascan goat haplotypes were categorized into Haplogroup A (Supplementary Figure S2). Figure 1 shows a median-joining network constructed using 10 haplotypes from Madagascan goat sequences, indicating an ancestral haplotype (MAD01) present at high frequency. The network illustrates star-like appearance with one (MAD04, 07–10), two (MAD03) and three (MAD02, 05) step mutations stemming from the most common Madagascar haplotype MAD01. Subsequently, nucleotide diversity ( $\pi$ ) and haplotype diversity ( $H_d$ ) was calculated using the 481 bp mtDNA sequence, showing extremely low values of  $0.0014 \pm 0.0005$  and  $0.404 \pm 0.099$ , respectively, in the Madagascan population.

Several previous studies revealed high mtDNA divergence in most of worldwide goat populations, including



**Fig. 1.** A median joining network of 481 bp in the mtDNA HV1 sequence. The network was constructed from 40 Madagascan goat samples. The numbers of individuals in each haplotype is proportional to the area of its circle. Line scale indicates the equivalence of a mutational step. The haplotypes (MAD01, 09 and 10) with dark gray circle were matched previously reported sequences by BLAST search (Fig. 2).

African populations (Luikart et al., 2001; Naderi et al., 2007; Awotunde et al., 2015; Kibegwa et al., 2016). On the other hand, an extremely low mtDNA diversity within Madagascan goats was revealed by comparison of nucleotide diversity (0.0014) with that of African goats (0.0257) (Kibegwa et al., 2016). This low diversity in Madagascan goats is likely the result of a founder and/or a bottleneck effect caused by migration to the island. A similar reduction in the genetic diversity resulting from founder effect was also reported for rice in Madagascar (Mather et al., 2010).

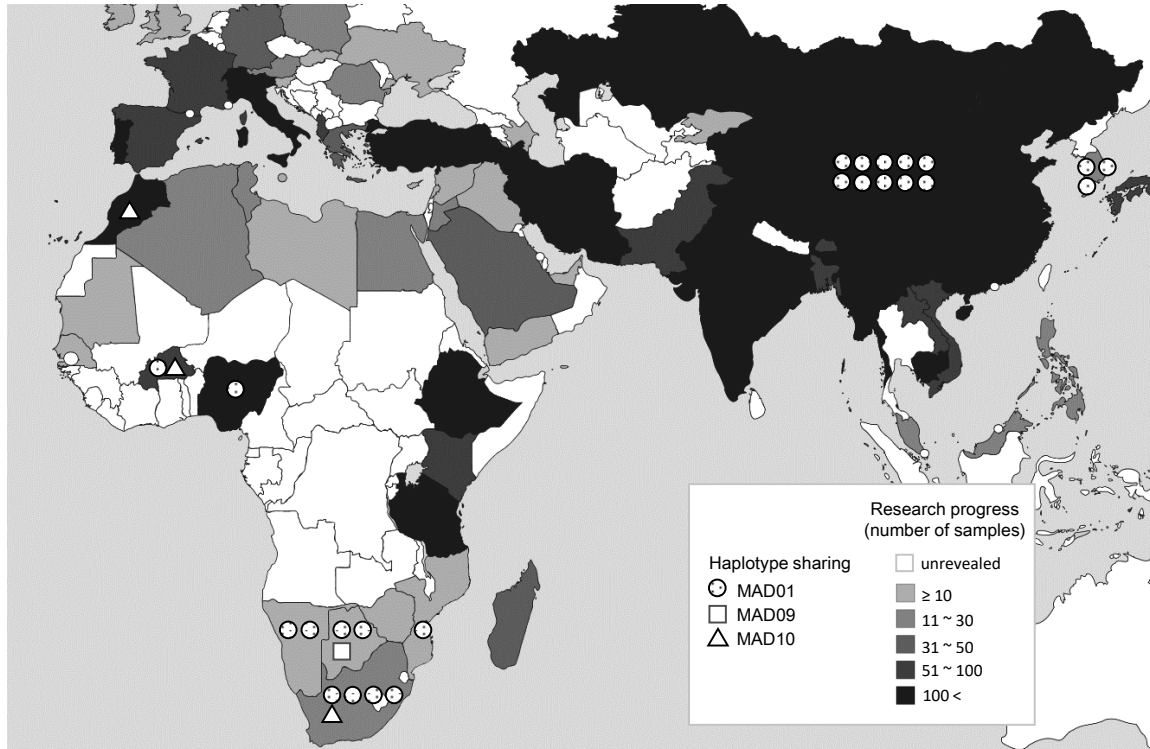
In order to infer the propagation routes of Madagascan goats, we carried out a BLAST search using Madagascan mtDNA haplotypes. Consequently, three out of 10 Madagascan haplotypes (MAD01, MAD09, and MAD10) matched previously described sequences. MAD01 matched 24 sequences, MAD10 matched three, and MAD09 matched one (Fig. 2). These haplotypes revealed close genetic similarity and were differentiated by two variant sites (Fig. 1 and Table S1). Sequences predominantly identical to MAD01 were obtained from Africa (Burkina Faso, Nigeria, Namibia, South Africa, Botswana, Zimbabwe, and Mozambique) and East Asia (China and South Korea). The MAD10 was shared with African haplotypes (Morocco, Burkina Faso, and South Africa), and MAD09 also with Africa (Zimbabwe).

Most of the sequences identical to MAD01, MAD09, and MAD10 were observed in African populations, regardless of the low number of total sequences investigated in Africa (Fig. 2). This result indicates a strong genetic similarity

between Madagascan and African populations. On the other hand, these sequences are not shared between Madagascar and India. This result is consistent with the hypothesis that Madagascan goats propagated from South and East Africa via the Mediterranean maritime route, as well as southward movement in Africa (Pereira et al., 2009; Pereira and Amorim, 2010).

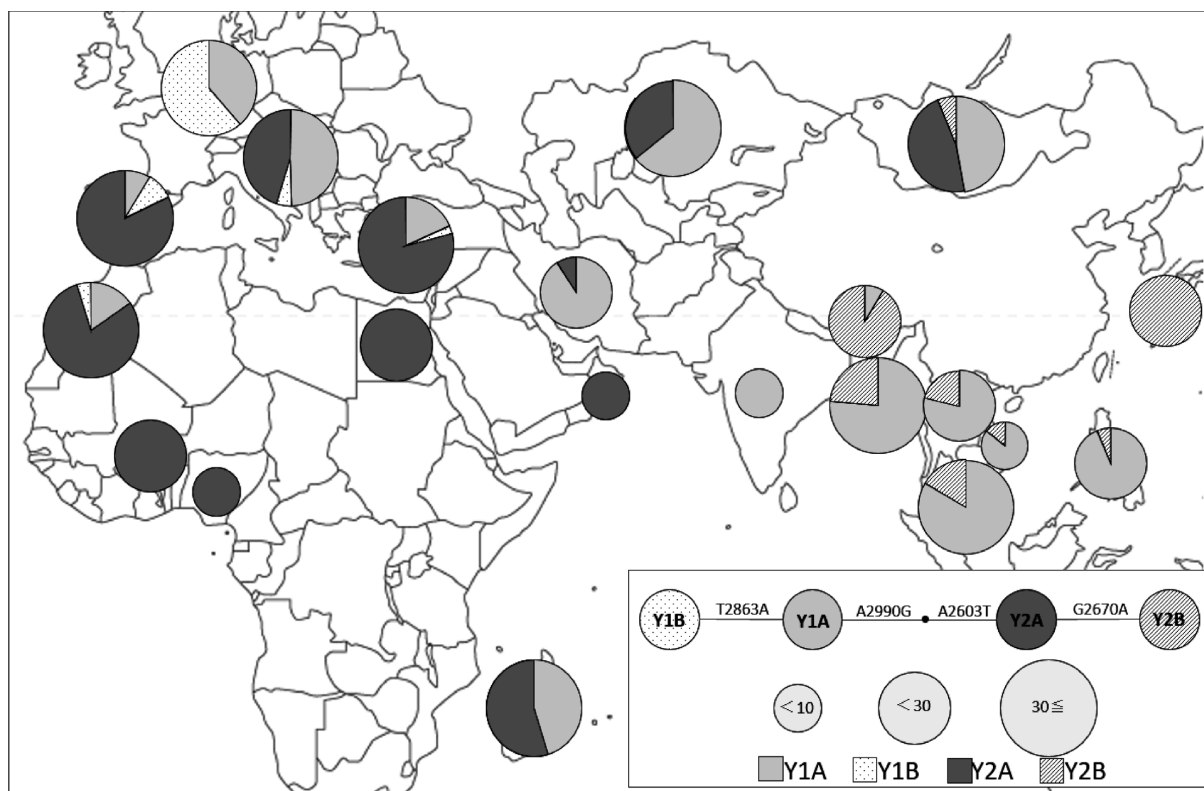
*SRY* sequences of Madagascan goats ( $n = 40$ ) revealed two haplotypes: Y1A (45%,  $n = 18$ ) and Y2A (55%,  $n = 22$ ). We analyzed these in conjunction with the previously reported data of *SRY* haplotypic structure and frequency (Tabata et al., 2018). Fig. 3 illustrates the overall geographic distribution of goat *SRY* haplotypes. The haplotypes Y1A and Y2A present in Madagascan goats are observed worldwide, and Y1B and Y2B are obtained from Europe and Southeast Asia, respectively (Waki et al., 2015; Tabata et al., 2018).

Although *SRY* analyses of African goat populations have previously been studied only in Morocco, Burkina Faso, Nigeria, and Egypt (Pereira et al., 2009; Vidal et al., 2017), Y2A seems to be the predominant haplotype in African populations, including Madagascar (55%). This suggests a close relationship among Madagascan and African populations. The Y1A haplotype is the second most common within the Madagascan population (45%), and the frequency of Y1A tends to increase eastward in Eurasia, whereas Y2B is restricted to Southeast Asia. Therefore, these results suggest that the Madagascan Y1A may have been introduced



**Fig. 2.** Distribution of sequences identical to Madagascan haplotypes. We carried out a BLAST search in order to identify mtDNA sequences identical to Madagascan haplotypes. In this map, the color shade in each country indicates the number of animals reported in the database. The accession numbers of identical sequences include KX913839, KX913841, KX913843, KX913854–5, KX913862–3, KJ420443, KJ420476, LJ420486, KC339658, EU910283, EF618545–6, EF618351–4, EF618242–3, EF618240, AY853293 and AJ317805 (MAD01), AJ317802 (MAD09), and GQ169008, EU910281, and AJ317822 (MAD10). Small open circles indicate small countries not shown on this map.





**Fig. 3.** Geographic distribution of caprine *SRY* haplotypes in the world. The size of each circle is proportional to the sample size, and each specific haplotype is represented by a different color or pattern. Data information in this figure was obtained from Tabata et al. (2018).

from South Asia or the Near East rather than from Southeast Asia (Waki et al., 2015). These paternal data indicate a genetic influence on Madagascar from Africa, South Asia, and the Near East (Waki et al., 2015).

In this study, we presented the phylogenetic structure of Madagascan goats on the basis of mtDNA and Y-chromosome sequences. Although both analyses suggested a genetic relationship between Africa and Madagascar, Y-chromosomal sequences were also influenced by South Asia and the Near East. These results could contribute to understanding for the propagation or introgression history of Madagascan goats.

#### ACKNOWLEDGMENTS

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#### COMPETING INTERESTS

The authors have no competing interests to declare.

#### AUTHOR CONTRIBUTIONS

RT and HM participated in designing the study and writing the manuscript. YY, FRT, FMR and TY corrected materials and prepared for this study. RT performed most of the experiments. RT, FK, SS, TY and HM discussed and contributed to interpretations and conclusions. All authors read, commented and approved the final manuscript.

#### SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: <https://bioone.org/journals/supplementalcontent/10.2108/>

zs180184/10.2108.zsj.36.294.s1.pdf).

**Supplementary Figure S1.** The map of Madagascan regions collected goat samples in this study.

**Supplementary Figure S2.** Neighbor-joining tree of goat mtDNA haplotypes.

**Supplementary Table S1.** The position of variants within HVI on mitochondrial control region in Madagascan goats.

**Supplementary Table S2.** Information of geographic region, country, sample size and the reference used in analyses of caprine *SRY* haplotypes.

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