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The Origin of the White Roman Goose

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Abstract In order to avoid interference from nuclear copies of mitochondrial DNA (numts), mtDNA of the white Roman goose (domestic goose) was extracted from liver mitochondria. The mtDNA control region was amplified using a long PCR strategy and then sequenced. Neighbor-joining, maximum parsimony, and maximum-likelihood approaches were implemented using the 1,177 bp mtDNA control region sequences to compute the phylogenetic relationships of the domestic goose with other geese. The resulting identity values for the white Roman geese were 99.1% (1,166/1,177) with western graylag geese and 98.8% (1,163/1,177) with

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eastern graylag geese. In molecular phylogenetic trees, the white Roman goose was grouped in the graylag lineage, indicating that the white Roman goose came from the graylag goose (*Anser anser*). Thus, the scientific name of the white Roman goose should be *Anser anser* 'White Roman.'

Keywords Mitochondrial DNA · Phylogenetic relationship · Geese

Introduction

Based on morphology, it was assumed that Chinese domestic geese were derived from the swan goose (*Anser cygnoides*), and the European and American geese were from the graylag goose (*Anser anser*). Shi et al. (2006) used four mtDNA restriction fragment length polymorphisms as genetic markers to discriminate these two types of domestic geese. Therefore, the current domestic geese are considered subspecies of both *A. anser* and *A. cygnoides*. In general, it is believed that the Roman goose originated from Europe, and the white Roman goose was derived from the Roman goose and became popular worldwide. In Taiwan, the white Roman goose is the dominant species, accounting for about 95% of the geese raised.

To date, the molecular phylogenetic origin of the white Roman goose, a domestic goose, is still undetermined. According to the study of Donne-Goussé et al. (2002), of phylogenetic relationships among Anseriformes, 45 waterfowl representing 24 genera were divided into two clades that correspond to two subfamilies, Anatinae and Anserinae. Within the Anserinae subfamily, the genera *Branta* and *Anser* were clustered together. The close phylogenetic relationships of the subspecies of *Anser* were investigated by Ruokonen et al. (2000) using the mitochondrial nucleotide sequences of tRNAglu, tRNAphe, and the control region. Accordingly, the seven *Anser* goose species were grouped into four lineages: (1) snow and Ross' goose, (2) graylag goose, (3) white-fronted goose, and (4) bean, pink-footed, and lesser white-fronted goose.

Mitochondrial DNA genes have been used to study the origin of domestic cattle (Loftus et al. 1994), swine (Giuffra et al. 2000), goats (Luikart et al. 2001), and chickens (Liu et al. 2006). In white Roman geese, the molecular phylogeny of the mitochondrial genome is still ambiguous. The purpose of this study is to evaluate the phylogenetic relationships and to determine the origin of white Roman geese.

Materials and Methods

Samples and DNA Purification

Liver tissue samples were collected from domestic geese at the Changhua Animal Propagation Station, COA-LRI. Much evidence indicates that the nuclear copy of mtDNA (numts) can be a source of contamination for results of phylogenetic analysis. Mitochondria of the white Roman goose were isolated and then used to extract mtDNA to avoid interference from numts. The mitochondrial isolation

method was modified from that of Fernandez-Vizarra et al. (2002). The fresh liver was cut into small pieces and then homogenized in a glass homogenizer on ice with a homogenization medium. Homogenate was centrifuged (1,200*g*, 4°C) for 5 min. The supernatants were collected, and the centrifugation step was repeated three times. The final supernatants were centrifuged in a high-speed centrifuge (12,000*g*, 4°C) for 10 min to pellet mitochondria. The mtDNA was extracted and purified from the pelleted mitochondria using an Easy Tissue and Cell Genomic DNA Purification Kit (GeneMark, Gmbiolab Co., Taiwan), in a solution-based approach.

Sequence Analysis

Using the extracted mtDNA as a template and the long PCR method, the mtDNA control region was amplified with the Avian mtDNA primers (Sorenson et al. 1999) L16087 (5′-tggtcttgtaarccaaaranygaag) and H3784 (5′-cggtctgaactcagatcacg) to produce a DNA fragment of more than 4 Kb.

The 50 μl long PCR reaction solution consisted of 5 μl 10× PCR buffer, 10 ng mtDNA, 5 mM each dNTP, 1 U Super Run *Taq* DNA polymerase, and 10 μM each primers. The PCR reaction used the following amplification profile: one cycle at 95°C for 10 min; one cycle at 85°C (add dNTP and the polymerase); 30 cycles of 94°C for 20 s, 62°C for 30 s, and 68°C for 6 min; and finally held at 4°C. The DNA fragments were isolated by gel extraction and then sequenced using the Avian mtDNA primers L16087, H614 (5′-ggraaratgccgcgatyacg), and L537 (5′-cctctggttc ctcggtcag).

Phylogenetic Analysis

The BlastN programs (National Center for Biotechnology Information, http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to search the sequence database of the goose mtDNA control region, and 16 geese were selected from data banks: one tundra bean (*Anser fabalis rossicus,* AF159951), three pink-footed (*Anser brachyrhynchus,* AF159952*,* AF159953, AF159954), two lesser white-fronted (*Anser erythropus,* AF159955, AF159956), two European white-fronted (*Anser albifrons albifrons*, AF159957, AF159958), two Greenland white-fronted (*Anser albifrons flavirostris*, AF159959, AF159960), two western graylag (*Anser anser ans*er, AF159961, AF159962), one eastern graylag (*Anser anser rubrirostris*, AF159963), one Ross' (*Anser rossii,* AF159964), one emperor (*Anser canagica,* AY112969), and one white roman (EU932689). The mitochondrial control region of the white Roman goose (accession no. GQ120441) and the 16 selected geese were used to construct a phylogeny and to evaluate the origin of white Roman geese. The emperor goose was used as an outgroup.

The complete mtDNA control region sequences of the 17 geese were aligned with the Emboss\emma program, and the neighbor-joining method with the F84 parameter in Phylip version 3.68 (Felsenstein 2008) was used for phylogenetic analysis. Statistical evaluation of phylogenies was based on 1,000 bootstrap replicates. A consensus tree was constructed with Consense in the Phylip software. Maximum parsimony (search option: more through search; number of tree to save:

10,000) and maximum likelihood analyses (under the HKY85 model) were also done with Phylip.

Results

Phylogenetic relationships among the white Roman and 16 selected geese were evaluated by neighbor-joining, maximum parsimony, and maximum likelihood methods using 1,177 nucleotides (mtDNA control region) to obtain three phylogenetic trees (Fig. 1). These three analyses gave similar topologies, although the white Roman goose branching order was not found in the graylag mtDNA lineage. It was not able to solve the branching order within the graylag clade. The three trees were similar, however, to those of Ruokonen et al. (2000). The monophyly of western graylag, eastern graylag, and white Roman geese was strongly supported in the three analyses.

Discussion

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The close phylogenetic relationships of the genus *Anser* were grouped into four mtDNA lineages (Ruokonen et al. 2000). The differentiation in the mtDNA region among the species is low (0.9–5.5%); however, the monophyly of graylag geese was strongly supported (100%) by the phylogenetic trees (Ruokonen et al. 2000). In this study, white Roman geese were compared with western and eastern graylag geese. The identities of the mtDNA control regions were 99.1% (1,166/1,177) and 98.8% (1,163/1,177), respectively. The white Roman goose was grouped into the graylag goose lineage supported by high bootstrap values (Fig. 1). The data strongly indicated that the white Roman goose (domestic goose) originated from graylag geese (*A. anser*). Thus, the scientific name for white Roman geese should be given as *Anser anser* 'White Roman.'

The Nuclear Copies of mtDNA (numts)

The mtDNA control region sequence of the white Roman goose (EU932689) was also selected and used in this analysis. This goose was not grouped with the graylag goose lineage. Total genomic DNA was isolated from web tissues of this goose. In the Blastn results (NCBI), the control region of this goose was 100% identical to seven other geese, in five species, including tundra bean goose (AF159965), pink-footed goose (AF159966), lesser white-fronted goose (AF159967), European white-fronted goose (AF159968), Greenland white-fronted goose (AF159969), western graylag goose (AF159970), and eastern graylag goose (AF159971). The nuclear copies of mtDNA (numts) are a major problem in studies of goose mtDNA (Quinn 1992; Sorenson and Quinn 1998). It is clear that the mtDNA control region sequence of the white Roman goose (EU932689) was from numts.

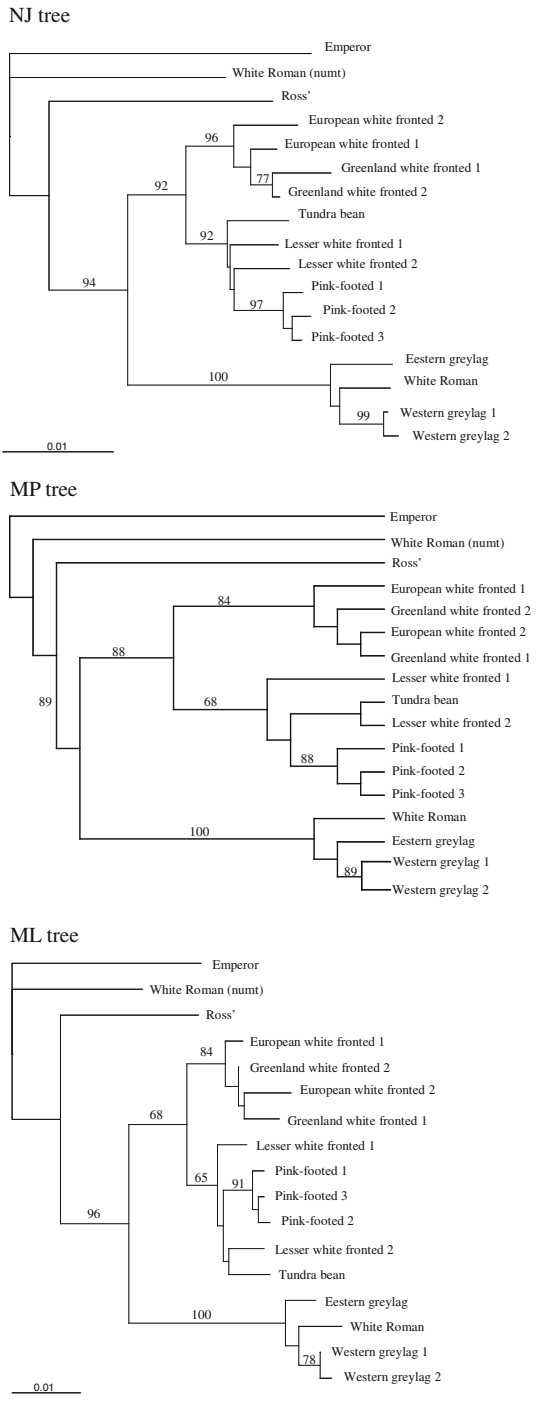

Fig. 1 Neighbor-joining (NJ), maximum parsimony (MP), and maximum-likelihood (ML) trees for the mtDNA control region sequences. The numbers above the branches show the percentage of bootstrap support from 1,000 replications. In the ML tree, transition/transversion ratios are set to 2.0. Emperor goose (AY112969) was used as the outgroup

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