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Research paper

Analysis of the complete mitochondrial genome of the Zhedong White goose and characterization of NUMTs: Reveal domestication history of goose in China and Euro

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ABSTRACT

To understand the phyletic evolution of geese, the complete mitogenome of the Zhedong goose was sequenced for the first time. It is composed of 37 genes and 1 control region, and the structure and arrangement of all genes sequenced are identical to those of other goose breeds. We confirmed the accuracy of the mitogenome sequence through RT-PCR and found numts from amplification in genomic DNA. Comparisons of the phylogenetic trees and sequences of geese that were suggested a clade of Chinese geese, except the Yili goose, were classified in the Euro clade. Several breed-specific mutations and Chinese breed-specific mutations were found. Our results suggest that Chinese geese evolved from the swan goose, splitting from their common ancestors at different times, which was consistent with studies before. Furthermore, numts in most genes of Zhedong goose clustered with European geese in the phylogenetic tree, suggesting that the haplotypes in the Euro clade might be more ancient. However, the mitogenome of the swan goose shows distinctive evolutionary positions in some genes, which suggest its unclear relationship with Chinese geese and European geese. The current study added to the understanding of the evolution of geese and provided evidence that the typing of numts is an encouraging way for the evolutionary study of geese and the mitochondrial genomes of geese deserve further investigation.

1. Introduction

The farming of geese has a long history in China. The birds are widely distributed from E102° to E122°, and N22° to N48°, in areas containing a wide variety of geographical conditions and climate (Qiu et al., 1988). Based on the natural conditions and preference for domestication, various native breeds can be found in specific regions. In 2014, the Chinese government listed 11 native goose breeds in the List of National Livestock and Poultry Genetic Resources in China (http://www.moa.gov.cn/zwllm/tzgg/gg/201402/t20140220_3791641.htm). Within the flourishing geese industry in China, geese are widely exploited and utilized, and crossbreeding is carried out. However, the conservation of purebred stocks, which is crucial for geese breeding in the future, has been ignored and gene introgression has been found in supposedly purebred stocks (Sun et al., 2014).

To help ensure that goose breeding is sustainable, an understanding of the evolutionary relationships among geese in China is needed. Chinese native goose breeds have been grouped into "Chinese goose" breeds and the Yili goose. Chinese goose breeds are thought to have originated from the swan (*Anser cygnoides*) and the Yili goose from the Graylag goose (*Anser anser*). This hypothesis is supported by several studies (Wang et al., 2005a; Li et al., 2011; Liu, 2003; Shi et al., 2006; Sun et al., 2014).Most of these studies were based on the analysis of mitochondrial DNA (mtDNA), which is considered a neutral molecular marker in studies of phylogenetic and population genetics (Xiang et al., 2014; Liu et al., 2014). MtDNA is inherited maternally and evolves ten times faster than nuclear genes (Avise, 2012). In particular, the D-loop, which is the most variable portion of mtDNA, is widely used in reconstruction of the history of species or even populations (Liu et al., 2015).

However, all of these studies on geese ignored the effect of nuclear integration of mtDNA (numts), which is a challenge for studies in birds (Sorenson and Quinn, 1998). The number of numts discovered is increasing with the development of next-generation sequencing technology (Soto-Calder et al., 2014; Li et al., 2012). Numts are numerous in genomic DNA in different taxa (Tsuji et al., 2012), but the reason for this remains poorly understood. They are amplified unconsciously and can confuse the analysis of genetic diversity and may lead to incorrect phylogenetic inferences (Peng et al., 2015; Xiang et al., 2014). In this study, we aimed to sequence the mtDNA genome of the Zhedong goose (ZD). The Zhedong goose is a native breed from Zhejiang Province, China, and is characterized by a rapid growth rate, great

Abbreviations: numts, nuclear integration of mtDNA; RT-PCR, reverse transcription PCR; mtDNA, mitochondrial DNA; ZD, Zhedong goose; SW, Swan; WR, White Roman goose; WGT, Wugangtong goose; XP, Xupu goose; YL, Yanling goose; YG, Yangiang goose; gDNA, genomic DNA; NJ, neighbor-joining.

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meat quality, early sexual maturity, and strong disease immunity. We also amplified genomic DNA to search for numts. Our comparisons of phylogenetic trees, mitogenomic sequences, and numts of Chinese geese, swan, and White Roman geese suggest an interesting phenomena and help to fill gaps in knowledge from previous studies.

2. Methods

2.1. DNA and RNA isolation

Blood and spleen samples were obtained from the Chinese geese raised in the Xiangshan Goose National Conservation Farm (Xiangshan, China) and the Tianhong Goose Farm (Shaoxing, China), both of which had conserved this native breed for several years. To exclude numts in genomic DNA, mitochondrial DNA in spleen samples was isolated using a mitochondrial DNA Isolation Kit (Biocision Inc, USA), and genomic DNA was obtained using the standard phenol–chloroform method from blood samples as described by Maniatis (1989). RNA was obtained using Trizol (Invitrogen, USA) and reverse translated using a PrimeScript 1st Strand cDNA Synthesis Kit (Takara, Dalian, China). Both DNA and cDNA were stored at −20 °C until used.

2.2. PCR amplification and sequencing

Fourteen primers were designed according to the mitogenome of the Yanling breed (KJ778677) (Table S1). The mitogenome of ZD was sequenced using the mitochondrial DNA isolated from the spleen. The same amplification was used to search for numts in genomic DNA. To verify the accuracy of the mitogenome, we carried out reverse transcription PCR (RT-PCR) for 13 genes and two ribosomal RNAs (Table S2). All the PCR products were purified using a DNA PCR Purification Kit (Axygen, Hangzhou, China), and sequenced bi-directionally. As the sequencing results of primer sets G-P6 and G-P14 were not good, the PCR products were ligated into the pMD18-T vector (Takara, Dalian, China) and transformed into DH5α cells (Tiangen, Beijing, China). Three clones for each fragment were selected for sequencing.

2.3. Sequence analysis

The sequences were aligned and assembled in Dnastar, I. (1998), and the sequence diagram was checked in Chromas Lite (Technelysium). The distribution of tRNAs was analyzed using tRNA Scan-SE1.21

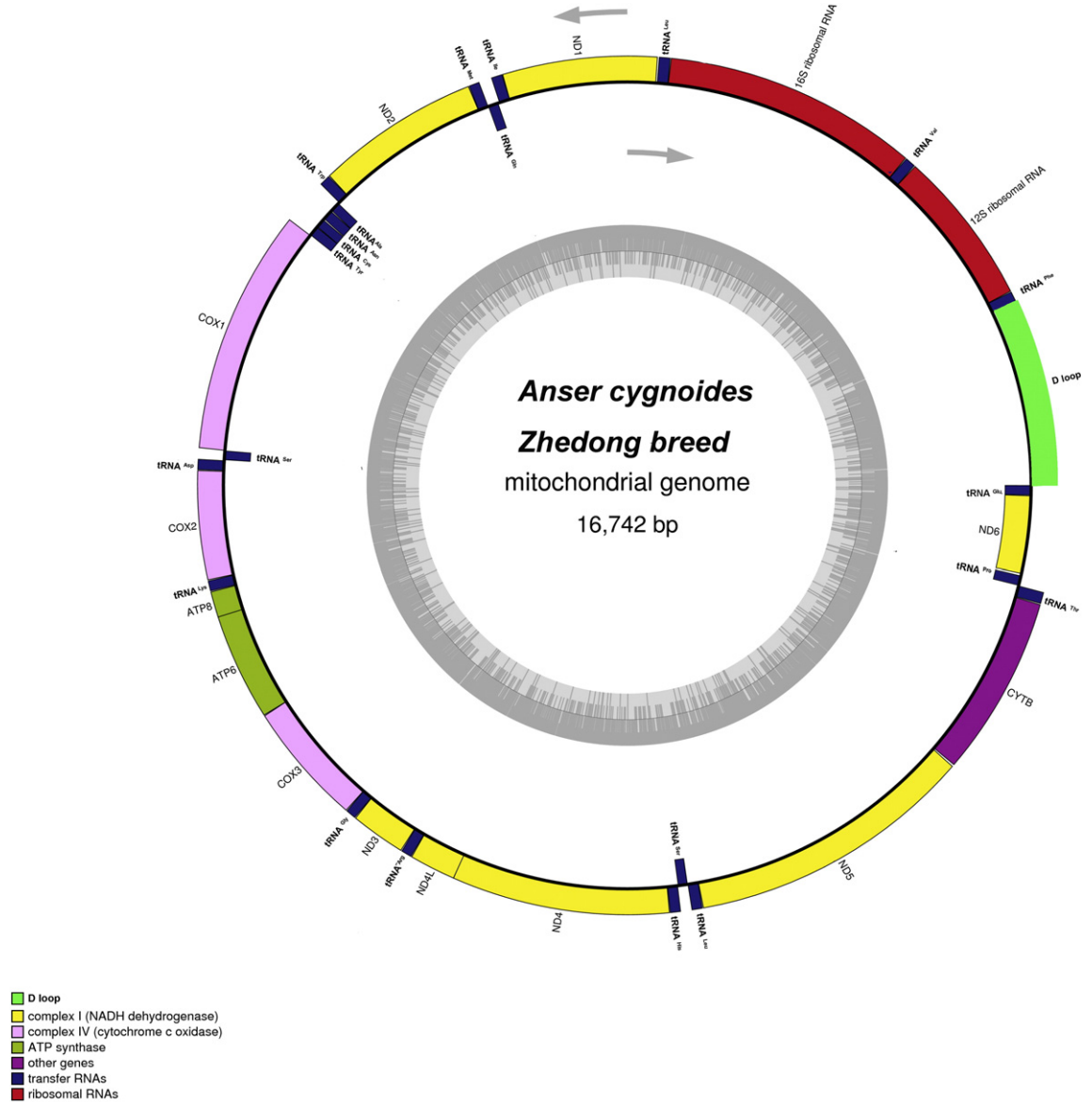

Fig 1. The organization of the mitogenome of the Zhedong goose.