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No Evidence for Paternal Leakage of mtDNA in Inter-Specific Hybrids of the Domestic Fowls (*Gallus gallus*) and Guinea Fowls (*Numida meleagris*)

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Abstract: Paternal transmission of mtDNA observed in animals will challenge the cornerstone of mitochondrial genetics-strict maternal inheritance and will affect the biohistorical and phylogenetic inferences based on mtDNA data. Thus, any studies showing paternal transmission of mtDNAz would gain much concern in this field. MtDNA lineages present in interspecific hybrids provide an ideal model to trace paternal mitochondrial leakage. In this study, we analyzed mtDNA lineages in domestic fowls (*Gallus gallus*, N=22), guinea fowls (*Numida meleagris*, N=17) and mule-chicken (an interfamilar hybrid between domestic fowls and guinea fowls, N=25) to test the transmission profile of sperm mtDNA. Length difference was found in mtDNA control region sequences of domestic fowls (539bp) and guinea fowls (498bp) by using same primer pair for amplification and sequencing, while the homologous sequences in mule-chicken were either same to those of domestic fowls or guinea fowls. No mule-chicken was found to be heteroplasmic for both mtDNAs from domestic fowls and guinea fowls. Our results failed to detect the paternal leakage of mtDNA. We speculated that the recent reported cases of mtDNA paternal transmission in a human sporadic patient and bird (great tit) were rare and it would not alter the bedrock principles of mitochondrial genetics.

Key words: mtDNA, paternal transmission, hybrid, domestic fowl,guinea fowl

INTRODUCTION

The field of mitochondrial genetics has mainly based on several bedrock principals, such as maternal inheritance, absence of recombination, high mutation rate and rich copy per cell (Birky, 1996, 2001; Giles *et al*., 1980; Hutchison *et al*., 1974). Any violation of these cornerstones would affect the biohistorical and phylo-genetic inferences based on mtDNA data and arose hot debates on this aspect (Schwartz and Vissing, 2003). Paternal transmission of mtDNA has been reported in lower organisms (Schistosoma mansoni (Jannotti-Passos *et al*., 2001)) and fungi (Yang and Griffiths, 1993), plants (Erickson and Kemble, 1990) and considered to occur very rarely in animals (mice (Gyllensten *et al*., 1991), drosophila (Kondo *et al*., 1990), mussels (Zouros *et al*., 1992)) and human mitochondrial disorders (Bromham *et al*., 2003; Schwartz and Vissing, 2003). Recently, Schwartz and Vissing (2002) re-provoked the issue of paternal transmission of mtDNA according to their observation of paternal inheritance of skeletal muscle mtDNA in a sporadic patient, although two different groups failed to detect similar mtDNA paternal inheritance in their own patient samples (Filosto *et al*., 2003; Taylor *et al*., 2003). Kvist *et al*. (2003) added more fuel to this fire by an observation of paternal leakage of mtDNA in a bird, the great tit (Parus major). Many issues about molecular anthropology and phylogeny will be revised if paternal inheritance of mtDNA occurred frequently, say, with a frequency ~1% (Schwartz and Vissing, 2003).

The interspecies hybrids provide unique opportunities for our understanding of the mtDNA genetics in that the maternal inheritance or paternal mitochondrial leakage would be more easily to identify in the hybrid zones. For example, Hutchison *et al*. (1974) first demonstrated that the mammalian mitochondrial genome was inherited maternally by using the mule, the hinny and their parents (horse and donkey) as model animals (Hutchison *et al*., 1974). Gyllensten *et al*. (1991) discovered paternal inheritance of mitochondrial DNA in hybrids between *Mus musculus* and *Mus spretus* and 2 following studies by Kaneda *et al*. (1995) and Shitara *et al*. (1998) confirmed this result in F1 hybrids. Interspecific or interfamiliar mating has been observed in birds. Up to now, several chicken hybrids, such as turkey- chicken (Olsen, 1960), pheasant-chicken

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(Shaklee *et al*., 1954), chicken-quail (Wilcox and Clark, 1961) and chicken-guinea fowl (Steklenev and Kozikove, 1989; Liu *et al*., 2000) have been reported. Considering the recently reported paternal leakage case in great tit (Kvist *et al*., 2003), we opted to select the hybrid animal model of chicken-guinea fowl to test if the introgression and paternal leakage of mitochondrial lineages existed in this animal. This hybrid, we called it mule-chicken, was an interfamiliar F1 hybrid progeny of domestic fowl (*Phasianidae: Gallus gallus*) and Guinea fowl (*Numididae: Numida meleagris*) and was created by artificial insemination between these 2 species (Liu *et al*., 2000). A primer pair was designed to specifically amplify the mtDNA control region segments of domestic chicken, guinea fowl and their F1 hybrids, respectively. If paternal leakage of mtDNA happened, we would expect a heteroplasmic status of both mtDNAs from domestic chicken and guinea fowl in the mule-chicken.

MATERIALS AND METHODS

Sample information: In total, 64 blood samples were obtained from Poultry Research Institute of Guizhou University. Among them, 17 were guinea fowls, 22 were domestic fowls and 25 were mule chicken (the F1 interfamilial hybrid progenies).

DNA typing: Genomic DNA was extracted from the blood samples by using the standard phenol-chloroform procedure. Amplification of the mitochondrial control region was performed with primer pair L16750 (5'AGGACTACGGCTTGAAAAGC3')/H547 (5' ATGTGCCTGACCGAGGAACCAG3'). The numbers in the primer names indicate the homologous positions of 3' end of the primers in the mtDNA complete sequence of *Gallus gallus domesticus*. L and H refer to light and heavy strands, respectively. PCR were performed in a 50 μL volume (about 100 ng DNA, 20 pmol primers, 200 uM dNTP, 1.5 mM $MgCl_2$, 10 × PCR Buffer 10 μL and 2 unit of Taq polymerase (S_{ABC}) following 35 cycles of 1min at 94°C, 1min at 63°C and 1min at 72 °C. PCR products were purified on spin columns (Watson BioTechnologies Inc, Shanghai) and Sequencing reactions were performed for both strands by using BigDye™ Terminator Cycle Sequence Kit (ABI Applied Biosystems) according to the manufacturer's manual and run with the ABI 377 Sequencer (ABI Applied Biosystems).

Data analysis: Sequences were edited and aligned by DNAstar package (DNASTAR Inc.) and the nucleotide variations in the analyzed segments were exported by using MEGA 2.1 (Kumar *et al*., 2001), respectively. A network profile of the haplotypes identified was constructed according to Bandelt *et al*. (2000).

RESULTS AND DISCUSSION

Figure 1 shows the amplification result of PCR products of the domestic fowls, guinea fowlsand their F1 hybrid progenies. It is obvious that there was length mutation in the same region fromthe 2 species. The mule-chicken samples, present a band with a size exclusively same to its mother. None showed hetergeneous bands of both domestic fowls and guinea fowls.

Following sequencing analyses show that there are 539bp for domestic fowls and 498bp for guinea fowls, respectively. Among the mule chicken samples, 20 are 498bp, 5 are 539bp, which all are consistent with their maternal pedigree during the hybridization. In total, 11 deletions and 4 insertions are observed between the sequences of guinea fowls and domestic fowls. The 14 haplotypes identified in whole samples are clearly separated into 2 distant clades, with one encompassing the domestic fowls and the other one containing the guinea fowls (Fig. 2). In each clades, no more than 10-mutation distances are found within one species. The haplotypes identified in the mule chicken samples are same to those of domestic fowls and guinea fowls.

Most of the mule chicken samples (N=20) fall into the guinea fowls clade, only 5 individuals cluster with the domestic fowls. This clustering pattern is consistent with the breeding history: Backcrossing domestic (*Phasianidae*) and Guinea Fowl (*Numididae*) was less successful than crossing domestic chicken (*Phasianidae*) and Guinea Fowl (*Numididae*).

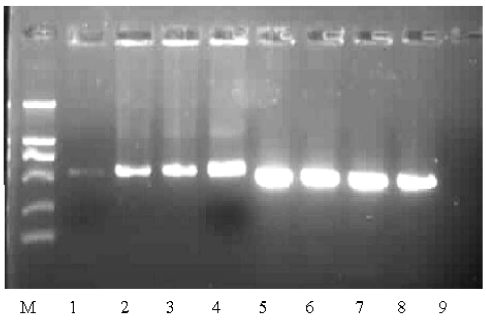

Fig. 1: PCR products of domestic fowls, guinea fowls and their F1 hybrids.M-DNA marker: Lanes 1-3 are the domestic fowl samples: Lanes 4-6 are mule chicken samples: Lanes 7-8 are guinea fowl samples: Lane 9-PCR negative control

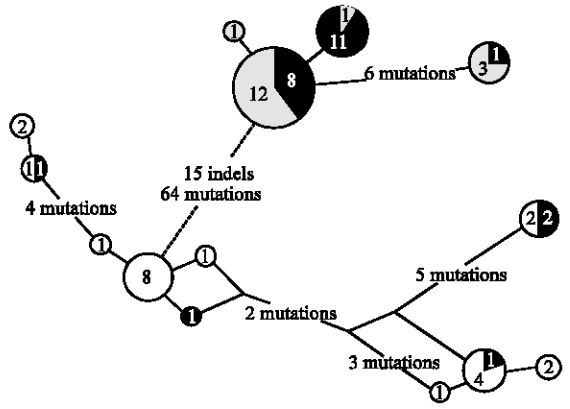

Fig. 2: Network profile of haplotypes in domestic fowl, guinea fowl and their hybrid progenies. The links between the haplotypes are specified by the number of nucleotide mutations if the number larger than 1. The exact number of individuals sharing the same haplotype is inside the circles. Circle areas are proportional to haplotype frequencies. Circle in white refers to the domestic fowls, gray refers to the guinea fowls and black represents the mule chicken samples

Shitara *et al.* (1998) reported that PCR technique could detect a few molecules of paternal mtDNA even in the presence of 10^8-fold excess of maternal mtDNA. It thus suggested that the sensitivity of the PCR technique be sufficient to detect the paternal mtDNA. However, we failed to detect heteroplasmy in all the 25 mule chicken samples. The results gave no support to the hypothesis of mtDNA paternal leakage in chicken-guinea fowl hybrids. Since, the cases of mtDNA paternal transmission were mainly found in muscle of human patient (Schwartz and Vissing, 2002) and in other tissues from kidney, spleen, pancreas and heart of the mouse F1 hybrids (Shitara *et al.*, 1998), we are forced to speculate that mtDNA from blood might not be the best candidate for detecting paternal leakage of mtDNA. However, the paternal case reported by Kvist *et al.* (2003) was also from blood samples and this would undermine our speculation.

In short, our typing of mtDNA types from mule chicken, a hybrid of two different species, did not show evidence for mtDNA paternal transmission. The recent reported cases of mtDNA paternal transmission in a human sporadic patient (Schwartz and Vissing, 2002) and bird (great tit) (Kvist *et al.*, 2003) might be rare and it would not alter the bedrock principles of mitochondrial genetics.

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