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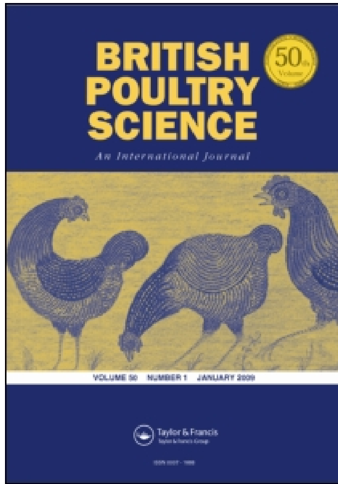

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Large-scale mitochondrial DNA deletions in weak goslings

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Abstract 1. Evidence has accumulated in mammals to support the idea that mitochondrial DNA (mtDNA) deletions and mutations might contribute to ageing and reproductive failure. White Roman geese were monitored to evaluate the effect of large-scale deletions of mtDNA in an avian species.
2. A total of 340 samples from 114 dead embryos, 111 weak goslings, and 115 normal goslings were used in this experiment. The regions of these two large-scale mtDNA deletions, ΔmtDNA6829 and ΔmtDNA6992, were between the COI and ND5 genes. A 3·6% (4 out of 111) positive sample was detected in the weak goslings. In contrast, no large-scale mitochondrial DNA deletions were detected in either the dead embryos (0 out of 114) or the normal goslings (0 out of 115).
3. Large-scale mtDNA deletions may be a factor causing weak goslings.

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INTRODUCTION

Mitochondrial functions in the oocyte affect embryonic development. (Gibson *et al.*, 2005; May-Panloup *et al.*, 2005). Deleted mtDNA were introduced into mouse zygotes and most of the mice carrying large-scale mtDNA deletions were dead (Inoue *et al.*, 2000). The mitochondrial deficient mice reproduced successfully only when the ES cells carried 17% or less deleted mtDNA. No chimera or F1 mice could be obtained when the ES cells had more than 61% deleted mtDNA (Ishikawa *et al.*, 2005). Obviously, zygotes carrying a lower ratio of deleted mtDNA might contain enough functional mitochondria to provide sufficient energy to enable successful reproduction of offspring.

More than 150 mtDNA point mutations and sequence rearrangements have been characterised in humans (Wallace, 1993). The "common deletion", the most frequent large-scale mtDNA deletion found in humans (ΔmtDNA4977) occurs between two 13-bp (base-pair) direct repeats at two nucleotide-break points (Mita *et al.*, 1989; Cortopassi *et al.*, 1992). Based on mammalian studies, we proposed that the large-scale mtDNA deletions may also appear in birds and influence reproductive performance, although there is lack of information indicating the relationship between these two. The purpose of this study was to investigate the proportion of birds with mtDNA deletions and to evaluate the ratio of the mtDNA deletions in geese. In this report we provide evidence for and characterise mtDNA deletions in White Roman geese.

MATERIALS AND METHODS

Sample and DNA purification

Samples of White Roman geese were collected from the Hon-Shin hatching station. A total of 111 web samples of weak goslings from 46 000

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goslings in 4 batches (one day of age), where the goslings died in the next three days. Another 115 normal gosling samples were taken from shell membranes and 114 dead embryonic samples were chosen by candling on day 7 post-hatch.

Total genomic DNA was purified from the tissues with an Easy Tissue and Cell Genomic DNA Purification Kit (GeneMark™, Gmbiolab Co. Ltd, Taiwan), using a solution-based approach to isolate the genomic DNA.

Long polymerase chain reaction (long PCR)

The long PCR strategy was used to identify large-scale mtDNA deletion for the geese. The primer sequences were designed to detect large mtDNA deletion of White Roman geese between nt 5700 and 13913 of mtDNA, as follows:

Forward: DEL-F5700 (5′-TCGGTGCCCCCG ACATAGCATTCCCG)
Reverse: DEL-R13913 (5′-GCACCGTTGGCG TGGAGATTGCGG)

The FailSafe™ PCR system (EPICENTRE® Biotechnologies) with solution A (PCR buffer) was used to amplify the 8212 bp mtDNA fragment. The 50 µL PCR reactions contained total genomic DNA 40 ng, 2 × A solution (FSP995) 25 µL, FailSafe™ PCR Enzyme Mix 1·25 U and 10 µM of each primer using the following amplification profile: 1 cycle at 95°C for 2 min; 1 cycle at 85°C for 5 min (add FailSafe PCR Enzyme Mix for hot start); 35 cycles at 94°C for 20 s; 66°C for 30 s and 70°C for 10 min and then a hold at 4°C. An 8 µL long PCR products and 2 µL of blue/orange loading dye were separated on 1·0% agarose gel and stained with ethidium bromide. The DNA fragments of positive mtDNA deletion were isolated with gel extraction and sequenced with ABI PRISM 3730 DNA sequencer (BI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kits).

Common PCR strategy

Because the FailSafe™ PCR system is time-consuming and costly, a common PCR condition was developed and used to screen the ΔmtDNA6829 and ΔmtDNA6992 deletions in White Roman geese. Common PCR conditions were developed to screen these two mtDNA deletions, with forward primer DEL-F5606 (5′-ATGTAATCGTTACCGCTCACGCCTTTGT) located at nt 5579–5606 bp in COI gene and reverse primer DEL-R13913 (5′-GCACCGTTG GCGTGGAGATTGCGG) at nt 13936–13913 bp in cyt b gene. The 50 µL PCR reactions volume consisted of total genomic DNA 40 ng, 5 mM each dNTP, ExSel high fidelity DNA polymerase 0·5 U and 10 µM of each primers, 5 µL of 10X PCR buffer (JMR-490), using the following amplification profile: 1 cycle at 95°C for 2 min; 1 cycle at 85°C for 5 min (add 1·0 µL ExSel polymerase for hot start); 33 cycles at 95°C for 20 s; 65°C for 30 s and 72°C for 1·5 min; followed 1 cycle at 72°C for 7 min; and then a hold at 4°C. 5 µL common PCR products and 1 µL of blue/orange loading dye were separated on 1·5% agarose gel and stained with ethidium bromide. The positive DNA fragment was isolated and sequenced with the same methods.

RESULTS

The results show that the large-scale mtDNA deletion, ΔmtDNA6829, was detected in two individuals (Lanes 4 and 6; Figure a). The positive large-scale mtDNA deletion was shown in a shorter DNA band. Two shorter DNA fragments (two individuals (Lanes 4 and 6; Figure a) had the same sequence. The nucleotide-breakpoint sites were at nt 5895 (in COI gene) and nt 12725 (in ND5 gene) compared with the complete mtDNA sequence of the White Roman geese (EU932689). The two shorter DNA fragments (ΔmtDNA6829) had a 6829 bp DNA deleted region with a 3 bp (GCC) direct-repeat sequence at the breakpoints.

Another large-scale mtDNA deletion, ΔmtDNA6992, was also detected. The nucleotide-breakpoint sites were at nt 6036 (in COI gene) and at nt 13029 (in ND5 gene) having in total 6992 bp DNA deletions. ΔmtDNA6992 had only one bp direct-repeat sequence (A) at the breakpoints, but an additional 10 bp repeat sequence (ACCTCATCAT) appeared at the ligated position in deleted mtDNA. The breakpoint sequences of ΔmtDNA6829 and ΔmtDNA6992 were as follows:

ΔmtDNA6829, the breakpoint sequence:

5′-CATCCTTGGG(GCC)-GCCCTAACCC (COI—ND5, 5895-12725).

ΔmtDNA6992, the breakpoint sequence:

5′-CCGGTATTACA(A)-***ACCTCATCAT***-A CCTCATCATCGAAAGCTTAAACACA (COI—ND5, 6036-10-13029).

The letters in parenthesis (GCC) and (A) represent imperfect direct repeats flanking the breakpoints of mtDNA. The insert repeat is shown in bold italics in deleted mtDNA. The positive samples, two individuals (Lanes 4 and 6; Figure a), could also be detected and showed a positive DNA band (Figure b) using the common PCR strategy. The nucleotide sequences of the two individuals (Lanes 4 and 6; Figure a) from

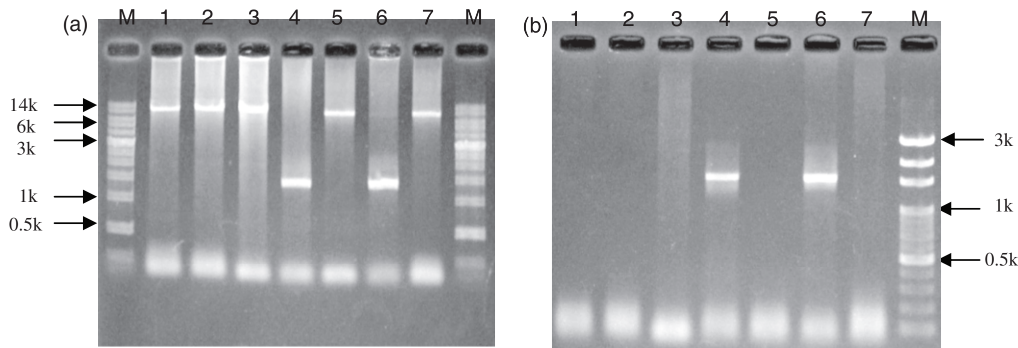

Figure. *PCR products. (a) the long PCR method, using the FailSafve™ PCR system with solution A, was used to detect large-scale mtDNA deletions in White Roman geese. (b) the common PCR method was used to screen large-scale mtDNA deletions. 1-7, No. of sample from weak goslings. M, Marker.*

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the common PCR method were the same as the sequences from the long PCR method.

DISCUSSION

In previous studies, the breakpoints of large-scale mtDNA deletions were concentrated in the ND5, Cytb, COI, and COIII genes in humans (Bua *et al.*, 2006). The breakpoint sites of both the ΔmtDNA6829 and the ΔmtDNA6992 also occurred in ND5 and COI genes. The results imply that the breakpoint sites of mtDNA deletions in birds might be similar to human mtDNA deletions concentrated in some genes. Therefore, DEL-F5700 (in COI gene) and the DEL-R13913 primers (in cyt b gene) were designed and used to detect the large-scale mtDNA deletions for White Roman goose. The breakpoint sites of both the ΔmtDNA6829 and the ΔmtDNA6992 also occurred in ND5 and COI genes.

At the breakpoint of large-scale mtDNA deletions, a direct repeat was an identical repeat of DNA sequence occurring in the flanking breakpoint sites. The size of the direct repeat in mtDNA deletions typically has a 4–16 bp repeat, whereas a imperfect direct repeat only has a 1 or 2 bp repeat (Bua *et al.*, 2006). The common mtDNA deletion ΔmtDNA4977 (a typical deletion) of humans having two 13-bp direct repeats accumulated in somatic cells with age (Sugiyama *et al.*, 1991; Cortopassi *et al.*, 1992). In this study, the direct repeat sequences of the ΔmtDNA6829 and the ΔmtDNA6992 only have three (GCC) and one (A) bp imperfect direct-repeats at the flanking ends of the breakpoint, respectively. It is obvious that these two mtDNA deletion types of White Roman geese are not the same as human ΔmtDNA4977, which accumulated in somatic cells with age and occurred commonly in adult tissues. The frequency of human mtDNA4977 deletion was estimated at 3% and 9% at age 80 and 90, respectively (Sugiyama *et al.*, 1991). At middle age, the frequency of the mtDNA4977 deletion was 0·1% in heart and brain tissues, but not observed in foetuses (Cortopassi and Arnheim, 1990; Cortopassi *et al.*, 1992).

Table. *Ratio of large-scale mtDNA deletions in White Roman geese*

Sample of geese	Number in sample	Positive number of ΔmtDNA	Type of ΔmtDNA (positive number)
Weak gosling	111	4	ΔmtDNA6829(2) ΔmtDNA6992(2)
Normal gosling	115	0	ND
Dead embryos	114	0	ND

ND, non detected.

We were using the common PCR method to screen the other samples. The results show that in a total of 111 weak goslings, 4 large-scale mtDNA deletion samples were found (Table). In contrast, neither of the two large-scale mitochondrial DNA deletions were detected in either dead embryos or normal goslings. Bacterial penetration in clean nest eggs was low in dirty floor eggs on poultry farms (Barbour, *et al.*, 1984). The large variation of hatchability in White Roman geese in Taiwan ranged from 65 to 90%, which was influenced by egg quality and hatching technology. A high ratio of the ΔmtDNA4977 was found in dead human oocytes (50·5%) and embryos (32·5%) (Barritt *et al.*, 1999). In this study we did not find these two types of large-scale mtDNA deletions in 114 dead geese embryo and we concluded that of these many factors, large-scale mtDNA deletions are not a major factor affecting the embryonic development of goslings. In contrast, mtDNA deletions were detected in the weak goslings, implying that large-scale mtDNA deletions were a factor causing early mortality.

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