Complete mitochondrial genomes of five introduced strains of common carp (Cyprinus carpio) in Japan with 29 diagnostic SNPs distinguishable by restriction enzyme analysis

Kohji Mabuchi
Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwa, Chiba, Japan

ABSTRACT
Complete mitochondrial genome sequences were determined for three common carp individuals captured at Lake Kasumigaura, Japan. They represent three of the five introduced strains of common carp in Japan, having the mtDNA D-loop haplotypes c1, d2 and e1. The three obtained mitogenome sequences were compared with two previously published mitogenomes that are identical in the D-loop region to haplotypes b1 and f1, representing the remaining two of the five introduced strains in Japan. Alignment (16,585 base pairs) of these five mitogenomes revealed 29 SNPs that are diagnostic for the five strains, detected through positive digestion by “rare-cutter” restriction enzymes.

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The Japanese native strain of common carp is being seriously affected by the invasion of conspecific domesticated strains from Eurasia. A recent mtDNA survey of complete D-loop region sequences revealed that in Japanese common carp populations, nearly half or more of the haplotypes originated from Eurasia (Mabuchi et al. 2008). These Eurasian haplotypes were classified into five clades, B–F, each of which included only a small length variation of the dinucleotide (AT) repeat region, with no nucleotide substitutions observed. Thus, the introduced Eurasian strains in Japan can be represented by only five main haplotypes, namely, b1, c1, d2, e1 and f1 (Mabuchi et al. 2008). Complete mitochondrial DNA sequences for these D-loop haplotypes will be useful for effective discrimination among the five introduced strains.

BLAST searches at the National Center for Biotechnology Information website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) revealed that two of the five main haplotypes, b1 and f1, were identical to the D-loop regions of the mitogenomes JX188253 from Chinese Oujiang colour carp and KP993139 from German mirror carp, respectively. Complete mitochondrial DNA sequences for the remaining three of the five main D-loop haplotypes (c1, d2 and e1) were thus determined here by a polymerase chain reaction (PCR)-based technique (Mabuchi et al. 2006) using three specimens collected at Lake Kasumigaura, Japan on 29 May 2006 (DDBJ accession nos. AP017364, AP017365 and AP017363, respectively). Phylogenetic positions of these five mitogenomes among the other previously published ones of common carp were analyzed using the neighbour-joining method (Saitou & Nei 1987). The resultant tree (Figure 1) indicated multiple origins of the introduced strains in Japan.

The five (three presently obtained and two previously published) mitogenomes were aligned into a 16,585-base pair (bp) matrix, showing 209 nucleotide substitution sites (and five insertion/deletion sites). Among these 209 sites, 163 were diagnostic for the five Eurasian strains. Among these 163 diagnostic sites, online searches for restriction enzyme recognition sites using TaKaRa Cut-Site Navigator (http://www.takara-bio.co.jp/enzyme/enzyme_search.php) found 29 single-nucleotide polymorphisms (SNPs) that were detected through positive digestion by “rare-cutter” restriction enzymes (Table 1). Here, the term “rare-cutter” means that the enzyme cleaves the whole mitogenome at fewer than 40 sites; in other words, its digestion results in fragments with an average length of more than ca. 400 bp.

These SNPs are reasonably suitable for PCR-restriction fragment length polymorphism (PCR-RFLP) genotyping of the five representative haplotypes. A PCR-based genotyping method distinguishing between the Japanese native and Eurasian introduced haplotypes has already been reported (Mabuchi &
Figure 1. Neighbour-joining tree of previously published 16 and newly determined three mitogenomes of common carp with two Carassius species as outgroups. Phylogenetic positions for mitogenomes of the five introduced strains in Japan (including D-loop haplotypes, b1, c1, d2, e1 and f1, respectively) are indicated by arrows. In the present analysis, pairwise distances among ingroup mitogenomes under the model of Jukes and Cantor (1969) were less than 0.05. Thus, phylogenetic relationships were estimated using uncorrected $p$ distances as the evolutionary distances, according to the recommendation by Nei and Kumar (2000). Calculations were conducted in MEGA6 (Tamura et al. 2013). Bootstrap values (Felsenstein 1985) are based on 1000 replicates for nodes with at least 70% support. Accession numbers with asterisk refer to the new mitogenomes determined in this study. Specimens used for sequencing are shown on the right of the asterisked numbers.
Table 1. Twenty-nine single-nucleotide polymorphisms (SNPs) in the mitochondrial genomes of the five introduced strains of common carp in Japan. Only the SNPs that were diagnostic for the five strains (B–F) and detected through positive digestion by "rare-cutter" restriction enzymes are presented. Here, the term "rare cutter" means that the enzyme cleaves the whole mitogenome of the target strain at fewer than 40 sites (see the "No." column). The SNP sites are underlined in the recognition site sequences.

| Position | Strain | Sequence | No. | Restriction enzyme |
|----------|--------|----------|-----|---------------------|
| 426      | E      | GACCC/C  | 14  | Ban I (Hpy I)       |
| 3278     | B      | G/GGCC   | 24  | Bsp I                |
| 3491     | E      | T/CTAGT  | 7   | Spe I               |
| 4082     | C      | CC/TGG   | 16  | Bcl I (EcoR II)     |
| 4232     | E      | CC/TGG   | 16  | Bcl I (EcoR II)     |
| 5351     | F      | AATT/TTT | 9   | Ssp I               |
| 5841     | F      | C/TCGG   | 25  | Taq I (ThH8B I)     |
| 6111     | C      | T/GCA    | 25  | Tag I (ThH8B I)     |
| 6639     | D      | GCTTTA/TGATG | 46 | Bgl II (Hpa II)   |
| 7593     | B      | T/CAGA   | 4   | Xba I               |
| 7746     | E      | CG/CG    | 16  | Acc II (FnuD II)    |
| 8321     | C      | GC/GTC   | 25  | Hha I               |
| 8481     | B      | C/ATTTG  | 7   | Mun I (Mfe I)       |
| 8576     | C      | G/GGCC   | 31  | BmgT120 I (Cr/13 I, Asu I) |
| 8621     | B      | T/TATTA  | 20  | Psh I (Vsp I)       |
| 9888     | B      | G/GACC   | 11  | VpK11B I (Ava II)   |
| 10199    | D      | T/CTAGC  | 6   | Nhe I               |
| 11725    | F      | CA/TATG  | 4   | Nde I               |
| 11868    | F      | C/CATGG  | 25  | EcoT14 I (Sly I)    |
| 11900    | D      | CCTCCG/TGG | 8  | Van91 I (MIM I)    |
| 12313    | B      | TTT/AAA  | 4   | Dra I (Aha III)     |
| 13456    | D      | A/CTAGT  | 6   | Spe I               |
| 13606    | B      | T/GCCG   | 8   | Eco I (Cfr I)       |
| 13657    | F      | CT/ATTG  | 9   | BpuI102 I (Esp I)   |
| 13765    | F      | TG/GCCA  | 4   | Bst I               |
| 14065    | E      | T/GCCGA  | 7   | Eco I (Cfr I)       |
| 14137    | F      | G/GACC   | 10  | VpK11B I (Ava II)   |
| 14143    | E      | C/CGAGG  | 25  | EcoT14 I (Sly I)    |
| 14247    | D      | GG/GTCTC | 11  | Eco109 I (Dra II)   |
| 14248    | D      | GG/GTCC  | 11  | VpK11B I (Ava II)   |
| 14249    | D      | GG/GTCC  | 31  | BmgT120 I (Cr/13 I, Asu I) |

Position: Position of the SNP in the 16585-bp alignment of the five mitogenomes.

Strain distinguished from the other four strains by the restriction enzyme cleavage being close to the SNP.

Number of cut sites in the whole mitogenome of the target strain.

Nishida (2006). Using these methods together, haplotype frequencies in common carp populations in Japanese waters can be effectively estimated, providing a useful basis for conservation of the native Japanese strain.

Disclosure statement
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References
Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 39:783–791.
Jukes TH, Canter CR. 1969. Mammalian protein metabolism. New York: Academic Press. Evolution of protein molecules. p. 21–132.
Mabuchi K, Miya M, Senou H, Suzuki T, Nishida M. 2006. Complete mitochondrial DNA sequence of the Lake Biwa wild strain of common carp (Cyprinus carpio L.): further evidence for an ancient origin. Aquaculture. 257:68–77.
Mabuchi K, Nishida M. 2006. PCR-based single tube genotyping of mitochondrial DNA of Lake Biwa wild common carp. Suisan ikuusu. 35:19–23.
Mabuchi K, Senou H, Nishida M. 2008. Mitochondrial DNA analysis reveals cryptic large-scale invasion of nonnative genotypes of common carp (Cyprinus carpio) in Japan. Mol Ecol. 17:796–809.
Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. New York: Oxford University Press.
Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4:406–425.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 30:2725–2729.