Sequence Variation in the Mitochondrial DNA Genome of Two Domesticated Strains of Rainbow Trout *Oncorhynchus mykiss*

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Rainbow trout were introduced to Japan in the early part of the twentieth century from North America. It is an intriguing question as to how the mitochondrial DNA sequences of domesticated populations in Japan are related to those of the wild populations in North America. The 2,214 base pairs of the mitochondrial DNA sequences have already been reported for four wild populations and a domesticated strain of the North American rainbow trout.1-3)

We examined the partial sequence of the mitochondrial DNA from two domesticated strains reared in our Institute and compared them with those of the North American populations. We amplified the mitochondrial DNA by means of the polymerase chain reaction (PCR).4) The DNA region amplified was 948 base pairs long, which is a part of the 2,214 base-pair region determined by Beckenbach et al.2) and Thomas and Beckenbach.1) We report here newly found polymorphisms in our domesticated strains, as well as substitutions at the sites previously reported by Beckenbach et al.2)

Two strains of domesticated rainbow trout *Oncorhynchus mykiss* were used. The Fuji strain was introduced to Shiga Prefecture from the U.S.A. in 1909 and subsequently released in Fuji in 1933. This strain has been maintained in the National Research Institute of Aquaculture (our Institute) since 1985. The Nagano strain was first introduced to Nikko, Japan, from the U.S.A. (Colorado State) in 1908 and released in Nagano in 1925, and has been kept in our Institute since 1980.

Mitochondrial DNA samples and two pairs of PCR primers (P01/P02 and P11/P12) were prepared as described previously.5) The PCR-amplified region spanned from the 3' region of the gene for the ATPase subunit 6 (ATPase 6) to the 5' region of the gene for the cytochrome oxdase subunit 3 (CO III).

In order to sequence the amplified products using the universal primer -21M13 (ABI), primers containing the universal sequence (5'-TGTAAGACGACCCAGT-3') in their 5'-region were also synthesized. In total, 8 primers were prepared: four each with and without the universal sequence. The combinations of the 4 sets of primers were as follows: UnivP01/P02, P01/UnivP02, UnivP11/P12 and P11/UnivP12, where UnivP01 means the primer P01 with the universal primer sequence on its 5' region. PCR, sequencing, and the analysis of sequence data were carried out as described previously.2)

Sequences for 4 fish from each strain were determined and were found to be identical, suggesting that a bottleneck effect may occur in the process of reproduction of domesticated strains.

Taking the rainbow trout determined by Thomas and Beckenbach1) as a standard, our domesticated strains of rainbow trout have a 3 base pair insertion from base 440 to 442, and a 9 base pair insertion from base 485 to 493. These correspond to 3 bases between 453 and 454, and 9 bases between 495 to 496 (Fig. 2A in Thomas and Beckenbach1)).

Table 1 summarizes the base substitution polymorphisms found among the two domesticated strains and the previously reported five populations from North America.

For convenience, the table is divided into two parts: the left part lists the polymorphic sites previously reported, and the right part lists the newly found polymorphic sites.

<table>
<thead>
<tr>
<th>Site number</th>
<th>Previously reported*1</th>
<th>Newly found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consensus**</td>
<td>C T A A</td>
<td>G C A</td>
</tr>
<tr>
<td>Fuji strain</td>
<td>— — G</td>
<td>C — G</td>
</tr>
<tr>
<td>Nagano strain</td>
<td>T C — —</td>
<td>C A G</td>
</tr>
<tr>
<td>McCloud River</td>
<td>— — — —</td>
<td>— — —</td>
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<tr>
<td>Qualicum River</td>
<td>— — G</td>
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</tr>
<tr>
<td>Wanopus Creek</td>
<td>— — — —</td>
<td>— — —</td>
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<tr>
<td>Fraser River</td>
<td>T C — —</td>
<td>— — —</td>
</tr>
<tr>
<td>Golden*3</td>
<td>— — — — G</td>
<td>— — —</td>
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<tr>
<td>codon position*4</td>
<td>1 3 3 3 2 1 3</td>
<td>S S S S N N S</td>
</tr>
</tbody>
</table>

Table 1. Base substitutional polymorphisms in rainbow trout mitochondrial DNA

*1 Previously reported in Beckenbach et al.2)
*2 Consensus bases determined by Beckenbach et al.2) The same nucleotides as in the consensus are marked by hyphens.
*3 A domesticated strain studied by Beckenbach et al.2)
*4 Codon position: first (1), second (2) or third (3) codon position for protein coding regions.
*5 Syn/Non-: synonymous or non-synonymous substitution for protein coding sequences.
There are three polymorphic sites newly found in this study, among which two sites (bases 45 and 340) show common bases between our domesticated strains, whereas one site (base 278) shows a substitution only in the Nagano strain.

Between our two domesticated strains, four sites of base substitution polymorphisms were found, among which one is base 278 as mentioned above. For the other three sites (bases 92, 283 and 541), the Fuji strain has identical bases with the wild population from Wanpus Creek, while the Nagano strain has identical bases with the Fraser River population. During the 90 years of domestication, the mitochondrial DNA sequence has not changed so much.

Our results suggest that the mitochondrial DNA polymorphism is useful as a genetic marker to distinguish between domesticated strains of rainbow trout.

The 948 base pairs of DNA sequences reported in this paper have been submitted to the DDBJ/Genebank/EMBL Data bank, with the following accession numbers: D83946 for the Fuji strain; D83947 for the Nagano strain.

References