Evolution of heteromorphic sex chromosomes in the order Aulopiformes

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Abstract

The fish order Aulopiformes contains both synchronously hermaphroditic and gonochoristic species. From the cytogenetic viewpoint, few reports show that gonochoristic Aulopiformes have heteromorphic sex chromosomes. Because fish in this order give us a unique opportunity to elucidate the evolution of sex chromosomes, it is important to examine a phylogenetic relationship in Aulopiformes by both molecular evolutionary and cytogenetic methods. Thus, we conducted molecular phylogenetic and cytogenetic studies of six Aulopiform species. Our results suggested that hermaphroditic species were evolutionarily derived from gonochoristic species. It follows that the hermaphroditic species might have lost the heteromorphic sex chromosomes during evolution. Here, we suggest a possibility that heteromorphic sex chromosomes can disappear from the genome, even if they have appeared once in evolution. Taking into account Ohno’s hypothesis that heteromorphic sex chromosomes might have emerged from autosomes, we propose the hypothesis that heteromorphic sex chromosomes may have undergone repeated events of appearance and disappearance during the course of fish evolution. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

According to Ohno’s hypothesis (Ohno, 1967), heteromorphic sex chromosomes such as XY andZW chromosomes might have emerged from autosomes. This hypothesis is thought to be applicable to vertebrates in general. Although these kinds of heteromorphic sex chromosomes are widely observed in vertebrates, some species lack such chromosomes. There are two mutually exclusive explanations. One is that, in such species, heteromorphic sex chromosomes have not appeared yet in evolution. The alternative explanation is that heteromorphic sex chromosomes have secondarily disappeared. There is little literature in which the disappearance process of heteromorphic sex chromosomes is discussed, because it is usually difficult to investigate the disappearance process, particularly in higher vertebrates.

Thus, it is of particular interest to know which explanation is more reasonable in order to understand the evolutionary process of sex chromosomes. Fish is one of the most interesting groups in which to study such an evolutionary issue, because fish lack a firmly established system of sex chromosomes (Ohno, 1974). Hence, we decided to investigate the evolution of sex chromosomes in fish.

The order Aulopiformes consists of 13 families, in which 42 genera are composed of 219 species (Nelson, 1994). This order has a characteristic feature of synchronous hermaphroditic and gonochoristic species: the former tends to inhabit the deep-sea and the latter inhabits shallow waters. There are a few cytogenetic reports that gonochoristic species have heteromorphic sex chromosomes that are distinguishable from autosomes under the microscope (Chen and Ebeling, 1974; Nishikawa and Sakamoto, 1978). In morphological and ecological studies, Smith (1975) proposed that the gonochoristic group of Aulopiformes was evolutionarily derived from hermaphroditic species. Smith’s proposal appears to be supported by the fact that...
hermaphroditic species are dominant in number, while gonochoristic species are fewer. According to Smith’s proposal, it is assumed that heteromorphic sex chromosomess are derived from the genome of hermaphroditic species. However, Smith’s proposal should be evaluated more carefully, in that there is no valid evidence for a minor group derived from a dominant group. Therefore, it is important to examine a phylogenetic relationship between gonochoristic and hermaphroditic groups of Aulopiformes by both molecular and cytogenetic methods, because it gives us a unique opportunity to elucidate the evolution of sex chromosomes. Thus, we conducted molecular phylogenetic and cytogenetic analyses of six species in the order Aulopiformes.

2. Materials and methods

2.1. Sample collection and gonadal sexing

We collected 50 individual samples in six species of the order Aulopiformes. Two species, Chlorophthalmus albatrossis and Chlorophthalmus sp., were trawled in deep basins of Suruga gulf in Shizuoka prefecture, Japan. Four species, Saurida elongata, Symmodus udas, Symodus hoshononis and Trachinocephalus mysops, were captured off Wakayama prefecture, Japan. All of the samples were sexed by macroscopic examination of the gonads.

2.2. Phylogenetic analysis

We determined the nucleotide sequences of the complete mitochondrial cytochrome b gene for two synchronous hermaphroditic species (C. albatrossis and Chlorophthalmus sp.) and four gonochoristic species (S. elongata, S. udas, S. hoshononis, and T. mysops). Total blood was diluted in TNE-urea buffer (Asahida et al., 1996). After several days to weeks at room temperature, 20–60 μl proteinase K was added and the solution was incubated for 1–4 h at 60°C. This was followed by two phenol-chloroform and two chloroform extractions. After precipitation with two volumes of ethanol, the pellet was rinsed in 70% ethanol, moderately dried and dissolved in TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA].

Cytochrome b gene regions were amplified by polymerase chain reaction (PCR). The primers used were AJG (CAAAACCATGTTGAATTCACAATT), H5 (GAATTTATGCTTGGGAG), eso-f-472 (YTDTC-YGCMGTCCTCACTGT), eso-R-768 (RTTVGCB-GGCGAARAGATT), eso-o-286 (CCCGDGCRATGT-GHTATA), and eso-o-960 (ARCGDGGMYTWAYWTTCCG). The thermal cycle profile was as follows: denaturation for 10 s at 94°C, annealing for 10 s at 48–52°C, and extension for 12 s at 72°C. PCR products were purified by filtration kit (Mo Bio Laboratories) and used for direct cycle sequencing with dye-labeled terminators (Applied Biosystems). The primers used were the same as those for PCR. Labeled fragments were analyzed on a Model 310 DNA sequence (Applied Biosystems).

Sequence alignment was performed by ClustalX (Thompson et al., 1997), and this alignment was improved by visual inspection. The total number of nucleotides for the alignment was 1137, which contains a gap of three nucleotides in the two hermaphroditic A. A phylogenetic tree was reconstructed by the neighbor-joining (NJ) method (Saitou and Nei, 1987). In the tree, we used 10 teleost fishes as outgroups. DDBJ/EMBL/GenBank accession numbers for the representative sequences used in the phylogenetic analysis were as follows: NC000861 Salvelinus alpinus, AF125213 Brachymysstes lenok, U12143 Sahno sdnar, AF125212 Oncorhynchus keta, NC002081 Gadaus morhua, AF009930 Amphipholus affinis, AF009937 Parametopra siebold, AF009941 Tomocichla tuba, AF047346 Pocilopus monacha, and AF031516 Sebastes ambros. For the NJ analysis, Kimura’s two-parameter distance correction (Kimura, 1980) was made by using all positions of a codon and excluding gap sites. The ratio of transition to transversion ranges from 1.0 to 2.0, depending upon the species of Aulopiformes and the outgroups examined. Although we also used only the first and second codon positions, the tree topology obtained was virtually the same. Statistical confidence of the phylogenetic tree was assessed using bootstrapping (1000 iterations).

2.3. Cytogenetic analysis

In order to investigate cytogenetic features of these species of Aulopiformes, we prepared chromosomes in S. udas, S. hoshononis, T. mysops and C. albatrossis. To increase the number of metaphase cells, animals were colchicinized by an intraperitoneal injection of colchicine solution 3 h before preparation. Kidneys were then removed and minced. Kidney cell suspension was treated by hypotonic solution (75 mM KCl) for 30 min at room temperature and fixed with methanol and acetic acid (3:1). The fixed materials were dropped onto precleaned slides and air-dried. Slides were stained by Giemsa solution. In metaphase cells, the nuclear organizer regions (NORs) were observed by the silver staining method (Howell and Black, 1979), and the constitutive heterochromatin was demonstrated by the C-banding method (Sumner, 1972). We observed 20 to 30 cells of metaphase for each species.

3. Results

3.1. Phylogenetic analysis

The topology of the phylogenetic tree suggested that the six Aulopiform species examined formed a monophyletic group, although the bootstrap value at the branching
Fig. 1. Phylogenetic tree of cytochrome b in the order Aulopiformes reconstructed by the neighbor-joining method. A gray box indicates hermaphroditic species. A white box indicates gonochoristic species. The numbers in parentheses indicate the number of diploid chromosomes.

point between S. elongata and the other five species was a bit low (Fig. 1). We also found that the two synchronous hermaphroditic species of the order Aulopiformes were grouped as a single cluster with a high bootstrap value (97.5%). On the other hand, four gonochoristic species were separated into three clusters. One cluster (called 'cluster 1') contained T. myops and the two hermaphroditic species. The second cluster (called 'cluster 2') was composed of the two gonochoristic species, S. ulas and S. budimont. The last cluster (called 'cluster 3') contained the other gonochoristic species, S. elongata.

3.2. Cytogenetic analysis of sex chromosomes

It is generally believed that heteromorphic sex chromosomes of fish are indistinguishable under the
microscope, whereas *S. elongata* was suggested to have one microchromosome as a possible sex chromosome in the female (Nishikawa and Sakamoto, 1978). At first, we inspected cytogenetic features in three gonochoristic species (*S. ulae*, *S. hoshinonis* and *T. myops*; boxed in Fig. 1). Surprisingly, we found that heteromorphic sex chromosomes are present in all three species. Herein, we investigated these heteromorphic sex chromosomes in detail by the C-band and the silver staining methods, and we obtained clearly stained metaphases in several preparations. In fact, the microchromosome in the female of *S. ulae* was stained in the whole region by the C-band staining method (Fig. 2). Likewise, by the silver staining method, the microchromosome in the female of *S. hoshinonis* was clearly detected in the entire region, except the centromeric region (Fig. 3). Because these microchromosomes stained by the C-band or silver staining methods were not detected in the males of *S. ulae* and *S. hoshinonis*, we propose that these microchromosomes are heteromorphic sex chromosomes. We also found that only female individuals had heteromorphic sex chromosomes in *T. myops*, because single acrocentric
and subtelocentric chromosomes in only females were stained by the C-band staining method (Fig. 4). As a result, gonochoristic species were found to have generally heteromorphic sex chromosomes. On the other hand, heteromorphic chromosomes were not found in any of the hermaphroditic species (Fig. 5), implying that the sex chromosomes do not exist in the hermaphroditic species.

3.3. The number of diploid chromosomes

We counted the number of diploid chromosomes in three gonochoristic species and one hermaphroditic species. In both males and females, *S. ulae* and *S. hoshinonis* had 48 chromosomes (Figs. 2 and 3). For *T. myops*, we found a difference in the number of chromosomes between males and females: Males had 26

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Fig. 4. C-band staining metaphase chromosomes of a female in *T. myops*. Arrows indicate the heterochromatin regions. A bar indicates the length of 5 μm.

Fig. 5. Silver staining metaphase chromosomes of *C. albatrossi*. Arrows indicate the NORs regions. A bar indicates the length of 5 μm.
chromosomes (data not shown) whereas females had 27 chromosomes (Fig. 4). For the hermaphroditic species (C. albatrossis), we found 36 chromosomes (Fig. 5).

Because a female of S. elongata has been reported to have 48 chromosomes (Nishikawa and Sakamoto, 1978), all the three species, S. ulce, S. hoshonis and S. elongata, have the same number of chromosomes. Thus, the common ancestor of the two genera of Saurida and Synodus was considered to have 48 chromosomes. Since the numbers of chromosomes for T. myops and C. albatrossis differ from those of the Saurida and Synodus species significantly, it is highly probable that the lineage leading to cluster 1 has been subjected to extensive chromosomal rearrangements, so that the chromosome numbers may have been reduced.

4. Discussion

According to Smith’s proposal (Smith, 1975), gonochoristic species were expected to have been derived from hermaphroditic species. However, our molecular phylogenetic analysis clearly indicated that hermaphroditic species were evolutionarily derived from gonochoristic species. Although it is difficult to estimate the time of emergence of hermaphroditic species in Aulopiformes, the phylogenetic tree obtained (Fig. 1) suggests they emerged immediately after other Aulopiform species had diverged from the common ancestor.

Our cytogenetic data indicates that gonochoristic species had hermaphroditic sex chromosomes. According to a previous paper (Nishikawa and Sakamoto, 1978), S. elongata was reported to have one microchromosome as a possible sex chromosome in a female. Those facts indicated that the microchromosomes were retained in the genus of Saurida and Synodus. Thus, it can be inferred that the common ancestor of the genus of Saurida and Synodus possessed the microchromosomes. In other words, heteromorphic sex chromosomes have been established once in the evolutionary course of the order of Aulopiformes. However, our cytogenetic analysis, as well as molecular phylogenetic analysis, indicated that the hermaphroditic species might have lost the heteromorphic sex chromosomes during evolution. Thus, we suggest a possibility that hermaphroditic sex chromosomes can disappear from the genome even if they have appeared once in evolution.

Here, we focused on the number of chromosomes to examine the disappearance process of sex chromosomes. We pointed out that chromosome numbers of the species in cluster 1 (2n = 26 to 36) differ from clusters 2 and 3 (2n = 48), indicating that large scale rearrangement of chromosomes has occurred in the lineage of cluster 1. Thus, it is quite possible that the disappearance of sex chromosomes was accomplished along with the large scale rearrangement of chromosomes.

Taking into account Ohno’s hypothesis (Ohno, 1967) that heteromorphic sex chromosomes might have emerged from autosomes, we propose the hypothesis that heteromorphic sex chromosomes have undergone repeated events of appearance and disappearance in the evolutionary course of fish.

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