

GENETIC ANALYSIS OF XY FEMALE FROM WILD POPULATIONS OF THE MEDAKA, *ORYZIAS LATIPES*

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The medaka has an XX/XY mechanism of sex determination. Y-specific DM-domain gene, *DMY* is the medaka sex-determining gene. We compared the phenotypic sex of individuals with their genetic sex (*DMY*-positive or -negative) in wild populations. The phenotypic sex of each specimen was identified by the external morphology, and its genetic sex was determined by detecting *DMY* using PCR. The phenotypic sex agreed with their genetic sex in most individuals. We found 24 *DMY*-positive females from 13 localities. Fourteen fishes from nine localities were analyzed genetically, and were judged to be Y-linked mutants. Progeny with the mutant Y chromosome were all female in nine XY females from six localities, while five fishes from three localities produced both males and females with the mutant Y chromosome. We determined nucleotide sequence of the protein-coding region of *DMY* by direct sequencing analysis for all mutants, and the sequence were compared with normal XY males. Twelve fishes from six localities had frame-shift mutation in 3rd Exon. Three fishes from a locality had base substitutions in 6th Exon causing five amino acid substitutions. Other XY males had no mutation in ORF of *DMY*.

MAPPING OF A FACTOR THAT CAUSES MALE SEX-REVERSAL OF XX MEDAKA

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In the medaka, *Oryzias latipes*, a Y-specific gene, *DMY* is a prime candidate for the sex-determining gene. We have found males without *DMY* (XX males) in a wild population of Shirone, Niigata prefecture. Genetic analyses of these males suggested that a recessive factor was responsible for sex-reversal in XX individuals. In order to determine the chromosomal location of the factor, we examined genotypes at 93 EST (expressed sequence tag) markers in 54 males of backcross progeny between the original Shirone XX male and an F1 (Hd-rR XX females \times the Shirone XX male) female. Seventy to eighty-one percent of the backcross males were homozygous for the Shirone allele at nine markers located on linkage group (LG) 8. These ratios were significantly higher than the expected 50% ($p < 0.01$). These results indicate that a major factor that causes sex-reversal of XX individuals is located on LG 8, suggesting a novel gene involved in sex determination.

ORIGIN OF *DMY*, A SEX-DETERMINING GENE OF THE MEDAKA, *ORYZIAS LATIPES*

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DMY, DM-related gene on Y chromosome, is the sex-determining gene of the medaka, *Oryzias latipes*. *O. curvnotus*, a closely related species of *O. latipes*, also has *DMY* on the Y chromosome. However, *DMY* has not been found in *O. luzonensis*, the most related species of *O. curvnotus*. Although the genomic region containing *DMY* seems to have originated through duplication of the region containing *DMRT1* on an autosome, it is unclear when *DMY* diverged from *DMRT1*. In order to investigate the origin of *DMY*, we determined nucleotide sequences of *DMY* and *DMRT1* in *Oryzias*, and constructed phylogenetic trees based on the amino-acid sequences of *DMY* and *DMRT1*. The phylogenetic trees demonstrated that *DMY* diverged from *DMRT1* of a common ancestor of *O. curvnotus* and *O. luzonensis* after separation from *O. latipes*. This result suggests that *DMY* originated in the common ancestor of *O. curvnotus*/*O. luzonensis* by duplication of *DMRT1*, and was horizontally transferred from the common ancestor of *O. curvnotus*/*O. luzonensis* to an ancestor of *O. latipes*. *Oryzias* fishes except *O. latipes* and *O. curvnotus* appear to have another sex-determining gene different from *DMY*.

THE LOCUS ON MEDAKA LINKAGE GROUP 17 CONTROLS XY SEX REVERSAL IN INTERSPECIFIC HYBRIDS BETWEEN *ORYZIAS LATIPES* AND *O. CURVNOTUS*

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Two closely related medaka species, *Oryzias latipes* and *O. curvnotus* have an XX/XY mechanism of the sex determination. Both species have *DMY*, which is required for male development in *O. latipes*. It has been reported that all F₁ XY hybrids from the cross between Hd-rR inbred strain females of *O. latipes* and *O. curvnotus* males develop as females. In this study, we analyzed the phenotypic sex (male or female) of the F₁ XY hybrids between HNI females of *O. latipes* and *O. curvnotus* males. As a result, 23% F₁ XY hybrids developed as males and the remainder developed as females. Then we produced G₂ progeny from the cross between G₁ (Hd-rR \times HNI) females and *O. curvnotus* males. The 20% G₂ XY hybrids developed as males. These results suggest that a factor except *DMY* controls XY sex reversal in interspecific hybrids between *O. latipes* females and *O. curvnotus* males. In the linkage analysis using 31 G₂ XY males, the factor was mapped on LG 17 of the medaka. Two medaka EST markers were detected flanking the factor at 3.2 cM each.

ORYZIAS LUZONENSIS HAS SEX-CHROMOSOMES THAT ARE NOT HOMOLOGOUS TO THE MEDAKA SEX-CHROMOSOMES.

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Although *Oryzias luzonensis* is one of the most closely related species to the medaka, *O. latipes*, the medaka sex-determining gene *DMY* has not been identified in *O. luzonensis*. Sex-reversed female produced all female progeny, suggesting that *O. luzonensis* has an XX/XY sex determining system. We searched for sex-linked DNA polymorphisms in *O. luzonensis* using medaka expressed sequence tag (EST) markers to illustrate the sex-chromosomes and to approach the sex-determining gene. We identified five Y-linked markers and confirmed male heterogamety. The linkage map demonstrated that the sex chromosome of *O. luzonensis* is not homologous to that of *O. latipes* but to an autosome (Linkage group 12). It can be expected that *O. luzonensis* has a sex-determining gene different from *DMY*.

THE PROTOTYPE OF SEX CHROMOSOMES FOUND IN KOREAN POPULATIONS OF *RANA RUGOSA*

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The seventh largest chromosome of the frog *Rana rugosa* in Japan is morphologically evolved as a sex chromosome. It is an XX/XY type in one geographic form and a ZZ/ZW type in another. In contrast, the 7th chromosomes are still homomorphic between the sexes in the other two geographic forms: they are more subtelocentric in the Kanto form and subtelocentric in the West-Japan form. In this study, to identify a prototype of the sex chromosomes, we extended our investigation to the Korean form that is supposed to be close to origin in the phylogeny. The karyotype, a sex-linked gene sequence and mechanisms of sex-determination and gonadal differentiation were all examined. Also, phylogenetic analyses were performed based on the mitochondria gene sequence and using cross experiments between the Korean and Japanese forms. As a result, the more subtelocentric 7th chromosome, shared by the Korean and Japanese Kanto forms, was concluded to be the prototype of the sex chromosomes. Starting at the prototype, a whole process of morphological sex chromosome evolution was reconstructed.

SEARCHING FOR TRANSPOSASES THAT CONTRIBUTE TO TRANSDUCTION OF A *XENOPUS* MITE FAMILY, XMIX.

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Xmix is a family of miniature inverted-repeat transposable elements (MITEs) extensively amplified and transposed in the genomes of *Xenopus* species. In addition, the same transposon system which contributed to the amplification and transposition of Xmix also seemed to contribute to the amplification and transposition of tandem arrays of Xstir, which is one of the most dominant tandem repeat sequence found in the *Xenopus* genomes. In order to identify transposases which contribute to the transposition of Xmix family, ORFs of candidate genes were isolated and analyzed using database analyses and PCR.

COMPARATIVE ANALYSIS OF SYNAPTOTAGMIN PROMOTER BETWEEN TWO ASCIDIAN SPECIES

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Synaptotagmin is expressed abundantly in all types of neurons. In the present study, regulation of syt gene is comparatively analyzed between two distally related species of ascidians, *Halocynthia roretzi* (Hr) and *Ciona intestinalis* (Ci). Hr-syt gene is expressed in all types of neurons. A 5' fragment of Hr-syt promoter mimics expression pattern of endogenous syt gene. Deletion experiments have shown that multiple regions of Hr-syt promoter regulate neuronal gene expression. The Hr-syt