

XY SEX REVERSAL CAUSED IN INTERSPECIFIC HYBRIDIZATION BETWEEN *ORYZIAS LATIPES* AND *O. CURVINOTUS* CORRELATES WITH VARIANT EXPRESSION OF *DMY*

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Two closely related medaka species, *Oryzias latipes* and *O. curvinotus*, have *DMY* that is required for male development in *O. latipes*. In reciprocal matings between *O. curvinotus* and two inbred strains (HNI and Hd-rR) of *O. latipes*, some F₁ XY hybrids developed as females. The differences in XY sex reversal ratio in interspecific hybrids depend on the combinations of Y chromosomes and their genetic backgrounds. In this study, we examined *DMY* expression in F₁ XY hybrids just after hatching using RT-PCR assays. *DMY* expression was evident in normal XY individuals of both species. On (cur/Hd-rR) hybrid background, F₁ XY^{HNI} individuals expressed *DMY* (HNI), while *DMY* (Hd-rR) expression was reduced in F₁ XY^{Hd-rR} individuals. Furthermore, *DMY* (cur) expression was not detected in F₁ (Hd-rR/cur and HNI/cur) backgrounds. As a whole, the *DMY* expressions in F₁ XY hybrids just after hatching depended on Y chromosomes. Variant *DMY* expression patterns appear to correlate with XY sex reversal ratios. These results suggest that XY sex reversal in interspecific hybrids is caused by the differences in the regulatory region of *DMY* expression.

NEW KARYOTYPE OF THE FRESHWATER PLANARIAN *SEIDLIA AURICULATA* COLLECTED FROM SHIRAKAMI MOUNTAINS

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Shirakami mountains is one of the World heritage site. The specimens of freshwater planarian *Seidlia auriculata* were collected from the mountains and karyological analysis was performed. The chromosome number of the specimens that we examined this time was $2n = 12$, $n = 6$. 17 kinds of chromosomal polymorphisms have been found in different populations of *S. auriculata*. In the already known 17 karyotypes, the first chromosome is submetacentric (sm). But in the newly discovered karyotype, the first is only one large metacentric chromosome (m), the second is a pair of acrocentric chromosomes (a), the third is consisted of three acrocentric chromosomes (a), the fourth is a pair of subtelocentric chromosomes (st), and the fifth and the sixth are both a pair of small metacentric chromosomes (m). That is $1m + 2a + 3a + 2st + 2m + 2m$. This new karyotype is remarkably different from 17 karyotypes already reported. Shirakami mountains is also an important region in the view point of karyotypic variations of *S. auriculata*.

MUTATIONS OF THE SEX-DETERMINING GENE *DMY* FROM WILD POPULATIONS OF THE MEDAKA, *ORYZIAS LATIPES*

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The medaka has an XX/XY mechanism of sex determination. It was established that Y-specific DM-domain gene *DMY* has crucial role in male development by the finding two wild-derived XY female mutants. To find new mutants of *DMY* we widely surveyed medaka wild populations and found 26 XY sex-reversed females from 13 localities. 19 XY sex-reversals from 12 localities were analyzed genetically and all were judged to be Y-linked mutants. In the present study, we sequenced the protein-coding region of *DMY* and examined *DMY* expression at 0 days after hatching (dah) using RT-PCR. In results these mutants were classified into three groups: (1) A single nucleotide insertion or deletion in exon 3 of *DMY* with a frame shift and premature termination; Aomori, Aizu-bange, Suzu, Awara, Kurobe and Aki. (2) Reduced or eliminated *DMY* expression at 0 dah; Aizu-wakamatsu, Shirone, Kesennuma and Oura. (3) Large insertion or deletion in exon 6; Saigo. So far, we can interpret the Y-linked factor which induces XY sex-reversals as *DMY* mutations. Because *DMY* mutations may not be harmful and survive in natural habitats, screening of wild populations are useful in collecting *DMY* mutants.

THE SEX DETERMINING GENE OF *ORYZIAS MEKONGENSIS* IS LOCATED ON A CHROMOSOME HOMOLOGOUS TO AN AUTOSOMAL REGION OF *O. LATIPES*

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Oryzias curvinotus, *O. luzonensis*, and *O. mekongensis* are most closely related fishes to the medaka, *O. latipes*, and all of them have an XX/XY mechanism of sex determination. Although *DMY*, a Y-linked sex determining gene, has been identified in *O. latipes* and *O. curvinotus*, it has not been found in *O. luzonensis* and *O. mekongensis*. Molecular phylogenetic analysis suggested that *DMY* occurred by duplication of *DMRT1* after *O. mekongensis* was diverged from a common ancestor of the other species, indicating that the sex-determining gene of *O. mekongensis* can be different from *DMY*. In the present study, we searched for sex-linked DNA markers of the *O. mekongensis*, using medaka expressed sequence tags (ESTs). We found that four Y-linked markers mapped on LG2 of *O. latipes* were linked to the sex completely ($n = 80$). These results indicate that the sex determining gene of *O. mekongensis* is located on a chromosome that is homologous to an autosome (LG2) of *O. latipes*, suggesting that the gene is different from *DMY* and the sex determining gene of *O. luzonensis* mapped on LG12.

GENETIC ANALYSIS OF AN XY FEMALE MEDAKA DERIVED FROM SHIRONE, NIIGATA SUGGESTS AN AUTOSOMAL MODIFIER

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Medaka, *Oryzias latipes*, has an XX-XY sex determination system. However, approximately 1% of wild fish were XY females or XX males. In this study, we performed genetic analysis of an XY female derived from a wild population of Shirone, Niigata prefecture. We crossed the XY female with an XY male of the Hd-rR inbred strain. In F₁ XY individuals that have Y chromosome from Shirone, both males and females were obtained. On the other hand, F₁ XY individuals that have Y chromosome of the Hd-rR strain were all males. These results demonstrated that the XY female is a dominant mutant linked to the Y chromosome. Next, a male or a female F₁ XY individual that have Y chromosome of Shirone were mated with the Hd-rR strain. In the progeny of the F₁ XY female, both XY males and females were obtained. On the other hand, XY individuals from F₁ XY male were all males. These results suggest that an autosomal modifier is involved in the occurrence of the XY female.

EITHER *ORYZIAS LUZONENSIS* Y OR *O. CURVINOTUS* Y INDUCES MALE DEVELOPMENT IN THEIR INTERSPECIFIC HYBRIDS

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Three closely related species, *Oryzias latipes*, *O. curvinotus* and *O. luzonensis* have an XX-XY sex determination mechanism. *O. latipes* and *O. curvinotus* have the *DMY* gene, which was first identified as the male determination gene on the Y chromosome (LG1) of *O. latipes*. However, *O. luzonensis* is devoid of *DMY* and the sex chromosome is proved to be homologous to an autosome (LG12) of *O. latipes*. It is expected that an unknown sex determining factor other than *DMY* is exerted in *O. luzonensis*.

We crossed a sex-reversed XY female of *O. luzonensis* with an XY male of *O. curvinotus* to obtain F₁ hybrid of four genotypes. Offspring were sexed by secondary sexual characters and genotyped using *DMY* of *O. curvinotus* and a Y-linked SNP marker of *O. luzonensis*. All individuals with either Y^{cur} or Y^{luz} or both were male. This indicates that either Y^{cur} or Y^{luz} can induce male development in interspecific hybrids. Our results also imply that there are no epistatic or hypostatic interactions between the two loci (LG1 vs. LG12).

Some individuals which have neither Y^{cur} nor Y^{luz} also developed into male, suggesting that additional factors can affect the sex determination in hybrids.

GENE DUPLICATION AND SPECTRAL DIVERSIFICATION OF VISUAL PIGMENT REPERTOIRE IN MEDAKA

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Genus *Oryzias*, represented with medaka (*O. latipes*), is becoming a powerful model animal of developmental and evolutionary genetics. Comparison of its visual pigment (opsin) repertoire to that of zebrafish, the only fish with its visual opsin repertoire completely characterized, would greatly progress our understanding of the evolution of fish visual system, which is greatly diversified with their diverged visual ecology. As a first step toward this goal, we attempted to isolate all the visual opsin genes from the medaka genome and clarify absorption spectra of the pigments by reconstituting them. We show here that medaka has two loci of red (*LWS-1* and *LWS-2*), green (*RH2-1* and *RH2-2*) and blue (*SWS2A* and *SWS2B*) and single-copy ultraviolet and rod opsin genes in the genome, among which *LWS-1*, *RH2-2* and *SWS2A* are novel. The peak absorption spectra of the reconstituted 11-*cis* retinal photopigments differed greatly between the two green opsins, 453 (*RH2-1*) and 490 nm (*RH2-2*), and also between the two blue opsins, 439 (*SWS2A*) and 408 nm (*SWS2B*). These findings of functional divergence constitute a solid basis for further evolutionary and developmental studies of fish visual system.