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### Endocrine mechanism of the Sex Change in the Serial Sex Changing Gobiid Fish *Trimma okinawae*

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Teleosts may be classified as either gonochorists, *i.e.*, species with distinct separation of the sexes, or hermaphrodites. Hermaphroditic species may be subdivided into simultaneous (individuals that have functional ovotestis) or sequential (individuals that change from one sex to the other) groups. Sequential hermaphrodites may change from male to female (protandry), female to male (protogyny), or back and forth repeatedly (serial sex change). Regardless of the pattern, sequential hermaphroditism not only involves redifferentiation of the gonads, but also changes in the brain, behavior, and body. Therefore, studies of sex change address all aspects of sex differentiation and sex determination simultaneously.

Despite numerous reports describing hermaphroditic gonads, behavior, and mating strategies, information on the endocrine mechanisms of sex change remains scarce. Since the ability to change sex has evolved independently several times in teleosts, a conserved endocrine mechanism is unlikely. Consequently, no ideal model species has been singled out for extensive research. Clearly, protandry and protogyny will differ substantially unless one is simply the reverse of the other. However, a serial hermaphrodite changes in both directions and may represent a basic endocrine mechanism of sex change.

Recently, serial sex change (Male→Female→Male→Female...) was confirmed in the gobiid fish *Trimma okinawae*, which is a small orange goby distributed from Kagoshima south to the Ryukyu Islands in Japan. Its habitat includes cave ceilings, rock slopes, holes, and the underside of table coral. Individuals maintain home ranges in these habitats, where they feed on planktonic copepods. Field observations revealed that a dominant male controls a harem of several females. Removal of this dominant male results in sex change by the largest female in the harem. If placed with a larger male, the once dominant male reverts to a female. As the serial sex change can be manipulated socially, the goby provides an excellent animal model to elucidate the general mechanisms of sex change. To clarify the endocrine mechanism of sex change, we conducted various experiments with the serial sex changing goby *Trimma okinawae*.

First, we observed the gonad structures histologically and tried to induce sex change by manipulating social structure. Female and male phase fish were observed to have both ovaries and testes simultaneously. Pairing females caused sex change in the larger female (protogyny) within 5 days. By contrast, protandrous (male→female) sex change was observed in the smaller individual of a male pair within 10 days.

Next, we focused on cytochrome P450 aromatase (P450arom), a critical enzyme for

ovarian development and function. First, we isolated two types of P450arom cDNA (P450aromA and B) and characterized their expression profiles. Real-time quantitative RT-PCR analyses showed that there were no differences in the P450aromA mRNA levels during sexual transitions, while the P450aromB mRNA levels, although lower than P450aromA, correlated with the female sexual phase.

Finally, we focused on the gonadotropin receptor expression in the gonads to obtain information on the pituitary-gonadal axis. Based on the expression levels and localization of the two receptors, the goby has location-specific mechanisms to regulate transcription in gonadal tissues. These results suggest that gonadotropin receptors have a critical role in the sex change of this goby.

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