Sex determination system of the rosy bitterling, *Rhodeus ocellatus ocellatus*

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Synopsis

The rosy bitterling, *Rhodeus ocellatus ocellatus*, is the only fish species known in which artificial triploids are always male, regardless of the kind of polyploidization technique used. In order to elucidate the genetic sex determination system of the rosy bitterling, two kinds of gynogenesis were carried out: retention of the second polar body (GRSPB) and suppression of the first cleavage (GSFC). The sex ratio of progeny was nearly 7:1 (♂:♀) for both GRSPB and GSFC, while those of control and parental fish were almost 1:1. In backcrosses of female progeny by GRSPB and normal diploid males, male progeny were observed at low frequency (one or two individuals in each experiment), except in one experiment where the appearance rate of males was about 50%. From results of gynogenesis and backcrosses, the following conclusions can be made. The genetic sex determination system of the rosy bitterling is a heterogametic female system (ZW). Survival rate of super-females (WW), produced by gynogenesis, is much lower than that of males (ZW). There is a possibility that crossovers between sex determining genes and a centromere occur in the first meiosis. With respect to the mechanism of unisexuality (male) of artificial triploids of the rosy bitterling, only males (ZZZ and ZZW) are presumed viable, while females (ZWW) are probably inviable.

Introduction

The genetic sex determination system of teleost fishes is variable, thereby differing from those of mammals and birds (Yamazaki 1983). The rosy bitterling, *Rhodeus ocellatus ocellatus*, is a small cyprinid fish which inhabits small ponds and brooks, and is distributed in temperate regions of Eurasia (Nichols 1943, Okada 1960). It was accidentally introduced into Japan from China during the 1940s, mixed in with the grass carp, *Ctenopharyngodon idella*, and the silver carp, *Hypophthalmichthys molitrix* (Nakamura 1955). Because bitterlings are beautiful ornamental fishes, many aquarists and researchers have attempted to make even more beautiful strains using hybridization. Contrary to their expectations, hybrid bitterlings tended to be male, and in many crossbreedings offspring became strictly male (Suzuki & Hibiya 1986, Suzuki & Jeon 1988a). These unisexual bitterlings (male) are probably sterile, not producing motile spermatozoa that can fertilize eggs (Suzuki 1961, Suzuki & Jeon 1988b). In addition to the unisexuality of hybrids, artificial triploids of the rosy bitterling, produced by cold shock technique, were also reported to be all male (Ueno & Arimoto 1982). Although unisexuality has been reported in many wild populations of fish species (Vrijenhoek et al. 1989), in artificially produced triploid fishes it has only been reported in the rosy bitterling. The unisexuality of hybrid or triploid bitterlings may provide valuable information regarding the mechanism
and evolution of sex determination systems in teleost fishes.

Bitterlings are easy to feed and breed, and their reproductive cycle can be controlled (Asahina & Hanyu 1983, Shimizu & Hanyu 1982). For some biting species, maturation may occur within three months. The sex of mature bitterlings is easily identified by secondary sexual characters, such as the nuptial colorations of males and the development of a long ovipositor in females. Thus, bitterlings seem to be good experimental fishes, especially for developmental studies. This study aims to elucidate two points: the sex determination system of the rosy bitterling, including artificial triploids, and the mechanisms of unisexualism and sterility in hybrid bitterlings. We report on a series of experiments related to triploidy and gynogenesis, and attempt to explain the mechanism of unisexualism in artificial triploids of the rosy bitterling.

Materials and methods

Parental fish

Parental fish of the rosy bitterling used in this study were obtained in the suburbs of Ogaki City, located in the northwest region of central Honshu, Japan, on 15 April 1993. Fish were raised in aquaria at 21–24 °C, with a photoperiod of L14:D10 hours. The timing of ovulation in females could be judged with considerable precision by the length of the ovipositor. Eggs and milt were collected in separate Petri dishes (90 mm in diameter × 15 mm in height) containing physiological saline by pressing the abdomens of parental fish, using the methods of Ueda et al. (1990). Insemmination was done by mixing eggs and milt in Petri dishes, with rearing water that was sterilized by boiling. After insemination, eggs were rinsed twice with rearing water, and kept in small dishes in a dark, temperature-controlled incubator at 22 °C. Fertilized eggs and embryos were kept in the incubator for approximately four weeks until free swimming. As they became juveniles, fish were transferred to fish tanks (15 cm in width × 30 cm in depth × 30 cm in height), and fed nauplii of brine shrimp, Artemia salina, and pellets.

Production of artificial triploids

Artificial triploids of the rosy bitterling produced by a cold shock technique have been reported to be all male (Ueno & Arimoto 1982). In order to examine the influence of the actual techniques which lead to the retention of the second polar body on the sex of artificial triploids, artificial triploids of the rosy bitterling were produced from 20 April to 30 June 1993, using three different techniques: cold shock, heat shock, and hydrostatic pressure shock.

The cold shock technique of Ueno & Arimoto (1982) was slightly modified such that eggs were placed in cold water of 0 to 4 °C for 60 minutes, 5 minutes after insemination. In the heat shock technique, eggs were placed at 40 °C for 3 minutes, 5 minutes after insemination. In the hydrostatic pressure shock technique, inseminated eggs were subjected to hydrostatic pressure of 650 kg cm⁻² for 6 minutes (Hyon-Sob et al. 1991). These experiments were conducted five times, each using four batches of eggs including a control. To ascertain the ploidy of shock-treated fish, the DNA volume of erythrocytes was measured by microfluorometry (Komaru et al. 1988).

Extremely high and low water temperatures are known to strongly affect sex differentiation in some species of teleost fishes (Oshiro 1987, Yamamoto 1995). To examine the influence of water temperature on sex differentiation in artificial triploids of the rosy bitterling, controls and artificial triploids were produced by cold shock on 10 May 1993. Each experiment was conducted by using 30 batches of eggs. Progeny were reared at four different temperatures (16, 21, 25 and 32 °C) for 6 months. The constant temperature, regulated by thermometers, was maintained after insemination until the 'sex check', except for the time required for the cold shock treatment.

Gynogenesis and backcross

Gynogenesis is one of the most powerful methods to examine the genetic sex determination system of bisexual species. In this study, two kinds of gynogenesis of the rosy bitterling were performed from 20