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MANIPULATION OF THE MECHANISMS OF SEX DETERMINATION AND SEX DIFFERENTIATION IN FISH BY USING GENETIC AND PHYSIOLOGICAL TECHNIQUES

Francesc Piferrer (1,2), Edward M. Donaldson (2), Silvia Zanuy (1) and Manuel Carrillo (1).

1, Institut d'Aqüicultura de Torre de la Sal (CSIC). 12595 Ribera de Cabanes, Plana Alta, Castelló; 2, West Vancouver Laboratory, Department of Fisheries and Oceans, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6, Canada.

Resum

Manipulació dels mecanismes de determinació i diferenciació sexual en peixos mitjançant tècniques genètiques i fisiològiques

Els teleostis presenten uns avantatges únics entre els vertebrats que faciliten l'estudi dels mecanismes que regulen l'expressió del sexe. La manipulació de l'expressió sexual en els peixos va començar amb fins purament experimentals, seguint els estudis similars que s'estàben duent a terme amb els amfibis. Als darrers anys, però, amb l' expansió dels cultius marins, s'ha demostrat que aquestes manipulacions poden ser de gran interès en el marc de certes estratègies d'explotació. Els aspectes de la biologia de la reproducció dels teleostis sobre els quals s'incideix són els mecanismes de determinació i diferenciació sexual. Les tècniques emprades per controlar aquests processos poden ser genètiques o fisiològiques, i inclouen la inducció a la triploidia i ginogènesi, així com l'administració d'esteroids sexuals durant les primeres fases del desenvolupament. S'ha arribat al moment en què investigacions sobre aspectes fonamentals de la biologia de la reproducció poden beneficiar-se de l'actual grau de desenvolupament de les esmentades tècniques. Es discuteix com ara és possible abordar certs estudis (uns casos prometedors: gonado, gameto i esteroidogènesi) amb uns plantejaments i mitjans fora de l'abast de mà només fa uns anys.

Key words: teleosts, sex manipulation, triploidy, monosex, gynogenesis.

Introduction

Attempts to manipulate sex in fish began more than half a century ago with research only on fundamental aspects. In the past years, however, with the worldwide development of fish cultivation it became increasingly apparent that these manipulations could be of advantage under certain production strategies. This fact has and still is promoting a good amount of research on controlled sex expression in economically important species, mainly Cichlids, Cyprinids and Salmonids. This impressive research effort has further refined these techniques. The point we would like to make here is that a good opportunity exists for scientists in both fundamental and applied fields to endeavour and embark upon collaborative research to try to solve basic problems of fish reproductive biology by taking advantage of this situation. As a matter of fact, today it is possible to approach some aspects of fish reproductive biology with procedures not available some years ago.

In this paper the peculiarities that make the study of sex expression in fish to be more approachable than in other vertebrates are pointed out. Then a brief historic perspective is given on the research that lead to the establishment of the modern techniques, which are also outlined. Finally, the aspects of the reproductive biology that are manipulated are explained and we explore what kind of unsolved problems could be assessed based on recent findings.

Advantages of teleosts

Advantages of teleosts among other vertebrates for the study of the mechanisms that control the expression of sex, including its determination and differentiation are: a) fecundity considerable higher than in any other terrestrial vertebrates, hence allowing rearing full-sib and half-sib families with statistically large numbers of offspring, b) external fertilization, hence allowing full control on gamete management facilitating creation of hybrids, c) metabolism closely linked to ambient temperature, hence allowing easy control of development rate during ontogenesis. Further, teleosts can be subjected to some manipulations very difficult, unsuitable or impossible to perform with other vertebrates. Such manipulations include: a) production of complete and functional sex-reversed individuals by early steroid treatment (HUNTER and DONALDSON, 1983), b) production of self-fertilizable induced hermaphrodites and viable YY males (CHEVASSUS et al., 1988), c) production of viable (even fertile) hybrids, and d) production of polyploid, gynogenetic and androgenetic individuals. All this besides a great amount of diversity, typical of poikilothermic aquatic organisms (WILKINS, 1981), available for classic genetic selection. It is clear then that aquatic vertebrates present means for genetic improvement beyond those available for terrestrial vertebrates.

Background

The determination of sex is established at the moment of fertilization by the combination of chromosomes from maternal and parental origin, whereas the differentiation of sex takes place later during development. The first manipulations of the former process date from the attempts to induce gynogenesis. Gynogenesis is a special type of parthenogenesis where an egg is stimulated to divide by a genetically inactive spermatozoon. Sperm can be inactivated by gamma, X or UV irradiation. Gynogenesis was first achieved in fish by OPPERMANN (1913). Resulting individuals produced in this way were haploid and therefore not viable beyond early stages of development. Paralleling studies carried out with frogs by ROSTAND (1936), ROMASHOV et al. (1960) showed that thermal shocks could restore diploidy in gynogenetic fish and thus increase their viability. Gynogenetic diploids may be fully homozygous or slightly heterozygous, depending wether diploidy restoration has been by blocking the first mitotic division of the embryo or by blocking the extrusion of the second polar body. Since gynogenetic fish carry only maternal chromosomes, gynogenesis will always produce all-female offspring, provided that the species in which it is induced presents female homogamety.

Triploidy was first induced in fish by SWARUP (1959) by applying thermal shocks just after normal fertilization to prevent the extrusion of the second polar body. Triploidy permits mitotic divisions and growth, since it does not affect the duplication of chromosomes, but it blocks the pairing of homologous chromosomes during meiosis, thus conferring genetic sterility.

On the other hand, manipulation of the mechanisms that control sex differentiation in fish began with the administration of steroid hormones during early development (PADOA, 1937), although only intersex fish were produced. Complete sex reversal was first achieved by YAMAMOTO (1953). The same author later established the criteria for effective steroid treatment (YAMAMOTO, 1969) that still prevail, and that have been the working principle in hormonal sex control in modern fish farming. Treatment with exogenous steroids mimics the natural hormones, thus redirecting gonadal development towards the sex that typically produces the hormone administered. Hence estrogens are used for feminization purposes and androgens are used either to masculinize or to sterilize, depending if they are administered at relatively low or high concentrations, respectively. It should be noted, however, that there is a method called indirect feminization that consists in the fertilization of normal ova with sperm produced by masculinized females. This approach requires more than one generation to be completed, but has the advantage that 100% genotypic females are produced without being exposed to steroids at all.

Exhaustive reviews on the field of genetic and hormonal sex control are those of THORGAARD (1983), and HUNTER and DONALDSON (1983), respectively.

Use of sex control techniques in fish culture

With the enormous expansion of fish culture in the past fifteen years, it sooner became apparent that it would be desirable to enhance the expression of the sex with associated morphological, physiological or ethiological characteristics that could be of advantage under certain culture strategies. Globally, the objectives that can be achieved by employing these techniques are: a) to reduce the number of broodstock necessary to obtain a given egg take or, conversely, to increase egg take by rearing mainly females, b) to eliminate the sex that shows less growth, c) to prevent precocious sexual maturation in males and d) to prevent sexual maturation in both species, i.e., by sterilization and thus 1) to permit the commercialization of larger fish, 2) to permit marketing fish during the entire year, beyond the maturation season, 3) to eliminate weight loss associated with gonadal maturation, and 4) to maintain optimal quality of flesh.

Recent developments and future prospects

Genetic and physiological sex control techniques have already found important applications in fish cultivation. It is expected that their use will be tried in a number of new species in the next few years as diversification of cultured fish continues.

The application of these techniques to approach basic problems of fish reproduction should be seriously considered. According to CHOURROUT (1988), tetraploid hybrids will be of great interest for basic research if they prove to be fertile, since the gametes produced will be diploid. Back crossing tetraploid hybrids with their parental species may provide the chance to transfer desirable characteristics from one species to another. So far tetraploidy has only been achieved in rainbow trout (CHOURROUT, 1984). Also, gynogenesis and triploidy may provide rapid inbred clones that can be of great interest in immunological studies because their homogamety will permit the acceptance of allografts (CHOURROUT, 1988). As far as we are aware, clone fish have been produced only in three species: the zebrafish, Brachydario rerio, (STREISINGER et al., 1981); the rainbow trout, Salmo gairdneri, (CHOURROUT, 1984), and the medaka, Oryzias latipes, (MARUSE et al., 1985).

Intersexes are obtained if sex reversal is incomplete. This type of fish thus produced exhibit male and female gonads simultaneously, and can be self-fertilized by collecting ova and sperm (CHEVASSUS et al., 1988). Inbreeding under these circumstances would be twice as rapid as that obtained by brother-sister mating.

With the production of stocks of fish 100% female both genotypically and phenotypically it might be possible, by comparing with 1:1 male:female stocks, the cytological study of the process of sex differentiation on a batch of fish with homogeneous and previously known sex. These comparisons would perhaps help in finding new clues indicating sex differentiation at a cytological level that currently may not be apparent because both sexes are present. Also, the same procedure, i.e., using simultaneously normal 1:1 male:female and monosex stocks could be of advantage in studies dealing with sex differences in early steroidogenic capabilities of developing fish embryos.

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