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FERTILITY PROBLEMS IN THE SECOND GENERATION OF A FOUR-SPECIES TILAPIA CROSS

Gideon Hulata[1]*, Avner Cnaani[1], Tatiana Slossman[1] and Graham A.E. Gall[2]**

[1]Agricultural Research Organization, Institute of Animal Science, Department of Aquaculture, P.O. Box 6, Bet Dagan 50250, Israel

[2]Department of Animal Science, University of California, Davis, CA 95616, USA

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Abstract

Reproductive problems were encountered in attempting to produce the second generation of a four-way cross of tilapia from interspecific F_1 hybrids. The cross (*Oreochromis mossambicus* x *O. aureus*) x (*Sarotherodon galilaeus* x *O. niloticus*) successfully bred for one generation, however, not even a single batch of progeny was obtained in the subsequent generation. It was concluded that the complex genetic structure of this cross caused the fertility problem. Any similar breeding programs that are based on multi-species crosses should take into consideration that reproductive problems may occur.

Introduction

A program aimed at breeding new synthetic populations of tilapia adapted to a temperate climate and saline environment was initiated in 1995 at the Department of Aquaculture's Agricultural Research Organization in Israel. Taking advantage of the ease of producing interspecific hybrids among tilapia (Wohlfarth and Hulata, 1983), systematic interspecific crossings of tilapia species were carried out to produce a synthetic stock (artificial center of origin - ACO) of tilapia.

The use of interspecific composite (or complex) crosses is an old practice in plant breeding and is used mainly as a way to

* Corresponding author. Fax: +972-3-9605667, e-mail: vlaqua@volcani.agri.gov.il

** Current address: Hubbard Center for Genome Studies, University of New Hampshire, Gregg Hall, 35 Colovos Road, Durham, NH 03824, USA

obtain wide genetic and phenotypic variability. A new breeding method, designed to develop new cultivars of carnations (Umiel, 1993), was termed Multiple ReSpeciation (MRS). This method is based on creating an ACO via composite interspecific crosses. The ACO contains a wide genetic diversity and opportunities for genes to recombine and interact with genes from other species, obtaining a gene pool that is impossible in any pure species.

Our ACO involved four tilapiine species: *Oreochromis niloticus* (wild-type and red strains), *O. aureus*, *O. mossambicus*, and *Sarotherodon galilaeus*. Two-way-crosses (2WC; F_1 and F_2), 3-way-crosses (3WC) and 4-way-crosses (4WC) were produced. Several groups were used to construct a composite linkage map for tilapia (Agresti et al., 2000; Moen et al., 2004) using a fluorescence approach to amplified fragment length polymorphism (AFLP) and microsatellite primers that were identified when the first genetic linkage map for Nile tilapia, *O. niloticus*, was created (Kocher et al., 1998).

Materials and Methods

Fish species. Four species were hybridized: *Oreochromis aureus* (Steindachner; *Oa*) of the local Mehadrin strain described by Hulata et al. (1993); *O. niloticus* (Linnaeus; *On*) of the Ghana strain (Mires, 1977; Hulata, 1988); red *O. niloticus* (r*On*) originating in Lake Manzala, Egypt (McAndrew et al., 1988) and obtained from the Institute of Aquaculture, University of Stirling, Scotland; *O. mossambicus* (Peters; *Om*) originating in Natal, South Africa (Hulata, 1988); and *S. galilaeus* (Linnaeus; *Sg*), an endemic species originating in Lake Tiberias (also called Lake Kinneret and Sea of Galilee; Ben-Tuvia, 1959; Goren, 1974).

Creation of artificial centers of origin - ACO. Broodstocks used throughout the experiment were individually tagged with colored, numbered plastic discs that were attached to the fish by nylon thread through the dorsal muscle and knotted. Breeding was carried out at the Agricultural Research Organization of the Department of Aquaculture in Bet Dagan, Israel, unless otherwise stated. Reproduction procedures were similar to those described in Rosenstein and Hulata (1992) except that eggs removed from buccal cavities of females were transferred to hatching jars (Rothbard and Hulata, 1980) to complete incubation. Breeding families consisted of one male and 4-6 females of another species. At least two breeding families were established for each possible cross. F_1 hybrids of *S. galilaeus* females and *Oreochromis* spp. males were produced by artificial fertilization (Don and Avtalion, 1986; Yehezkel and Avtalion, 1988) at Prof. R.R. Avtalion's Laboratory of Fish Immunology and Genetics at Bar Ilan University, Ramat Gan, Israel, and samples were transferred to Bet Dagan for breeding. Crosses of these F_1 hybrids (ACO) were obtained by regular, spontaneous mating. Maternal mouth brooding was observed in all these spawnings.

Induction of spawning. When reproductive problems were encountered, males were injected with salmon GnRH analog ([D-Ala^6, Pro^9-NEt]-mammalian GnRH) at a dose of 15 µg/kg body weight to induce them to perform. In one case, the GnRH was combined with dopamine antagonist metoclopramide (5 mg/kg; Sigma).

Results

Table 1 shows the successful crosses. In the first stage (2-way-crosses), we failed to produce any hybrids from female r*On*, apparently due to some behavioral barrier as suggested by Hulata et al. (1995). Successful combinations were discontinued when enough spawns from different parents were obtained. Of the 4-way-crosses, several attempted combinations did not spawn at all. Fig. 1 shows the crossings that led to production of the ACO. The 3-way cross was produced for genome mapping purposes and was not part of creating the ACO.

The next stage was to propagate the ACO to allow recombination of blocks of chromosomes contributed by the original parental species and shuffle the genetic material coming from each species. In 2000, matings were performed among individuals of each ACO in several breeding families. Ten single-pair progeny of [(*Om* x *Oa*) x (*Sg* x *On*)] from vari-

Table 1. Two-way, three-way and four-way crossings (female x male) of four tilapia species.

Type	*Year of production*	*Genetic combination (female x male)*	*Number of viable spawns*
2-way	1995	*On* x *Oa*	4
	1995	*Oa* x r*On*	2
	1996	*Om* x *Oa*	3
	1996	*Sg* x *Oa*	2
	1996	*Sg* x *On*	1
	1996	*Sg* x r*On*	1
	1996	*Sg* x *Om*	1
	1997	*Om* x r*On*	2
	1997	*On* x *Om*	1
3-way	1997	*Om* x (*Oa* x r*On*)	7
	1996	*Om* x (*On* x *Oa*)	6
	1997	*On* x (*Om* x *Oa*)	4
	1997	*Om* x (*Sg* x *On*)	5
F_2	1997	*Om* x *Oa*	3
	1997	*Sg* x *Om*	1
	1997	*Oa* x r*On*	7
4-way	1998-9	(*Om* x *Oa*) x (*Sg* x *On*)	9
	1998-9	(*Sg* x *Om*) x (*On* x *Oa*)	9
	1998-9	(*Sg* x *On*) x (*Om* x *Oa*)	0/5*
	1998-9	(*Sg* x r*On*) x (*Om* x *Oa*)	1/5*
	1998-9	(*Oa* x r*On*) x (*Sg* x *Om*)	0/4*
	1998-9	(*Om* x *Oa*) x (*Sg* x r*On*)	0/4*
	1998-9	(*Om* x r*On*) x (*Sg* x *Oa*)	3
Generation-0	2000	(*Sg* x *On*) x (*Om* x *Oa*)	10
	2000	(*Sg* x *Om*) x (*On* x *Oa*)	0/13*
Generation-1	2001-2	(*Sg* x *On*) x (*Om* x *Oa*)	0/~25*

Oa - Oreochromis aureus
On - O. niloticus
r*On* - red *O. niloticus*
Om - O. mossambicus
Sg - Sarotherodon galilaeus

* Number of viable/total spawns obtained.

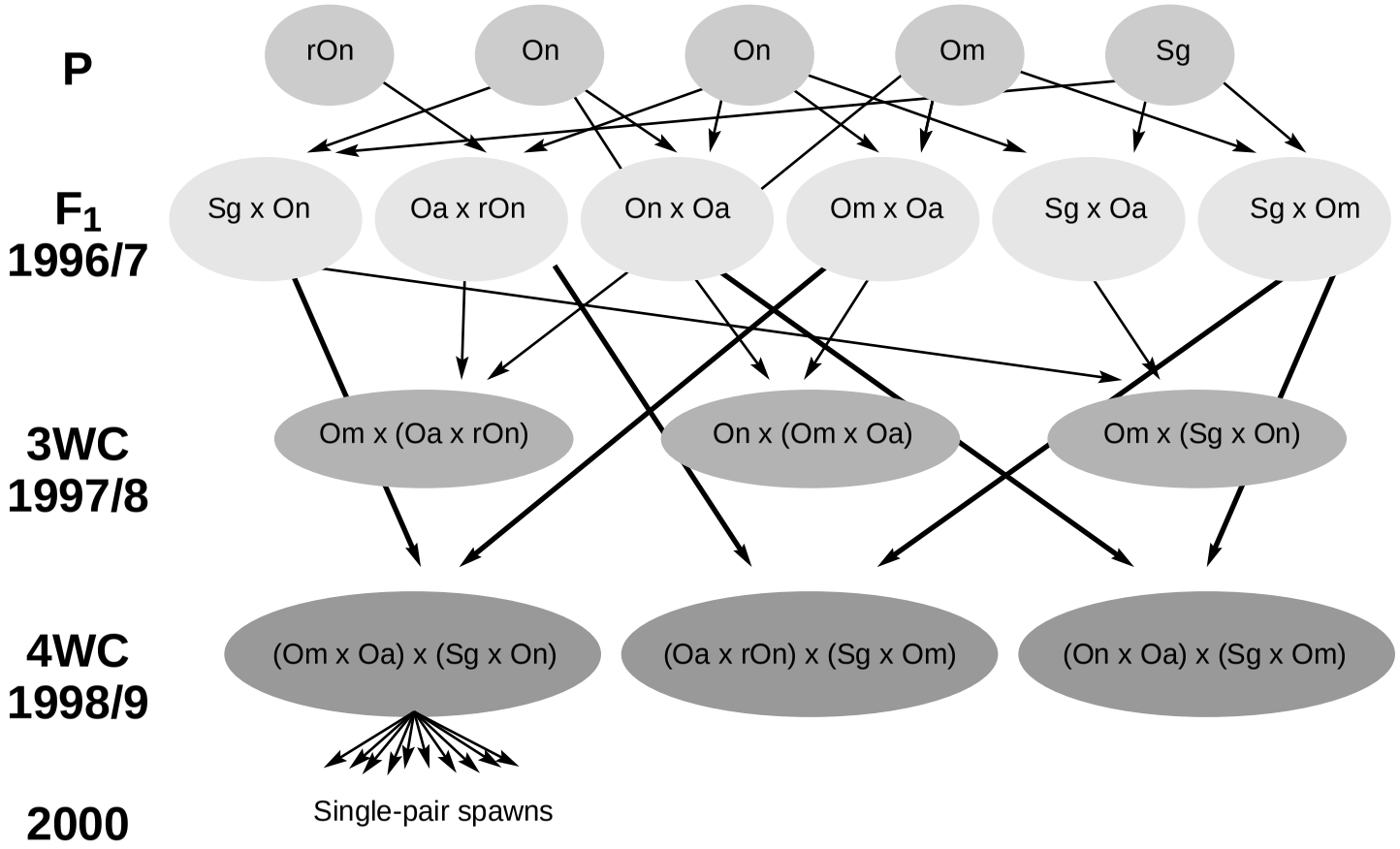

Fig. 1. Scheme of the breeding program performed to produce Generation 0 from 4-way crossed tilapia species. Some crosses made during the project are not shown.

ous families were obtained (Generation-0). These progeny reached maturity in the spring of 2001. There was a surplus of males in all families, and some of the females were small. Sixteen breeding families, each consisting of one male and 4-5 females (all tagged) were established. The females were taken from different ACO families than their male mates, to minimize inbreeding. The expected progeny should have constituted Generation-1 of the selective breeding program. Attempts to propagate a second 4-way cross [(*Sg* x *Om*) x (*On* x *Oa*)] failed (Table 1).

Reproductive problems were encountered in the 2001 spawning season. Not even a single batch of progeny was obtained, so we delayed production of Generation-1 until the spawning season of 2002. The 10 breeding families were over-wintered and re-established in spring 2002. To our disappointment, the fertility problems encountered in 2001, and attributed then to the young age of the females, persisted in the second year. In most of our breeding families, spawning, courting by males, egg laying and collection of eggs into the mouth looked normal, but none of the approximately 25 spawns was fertile. We then suspected that there was some fertility problem with the males. External examination by rubbing the abdomens of the males released very small quantities of semen. Males were injected with GnRHa to stimulate their sexual activity, but most did not run semen even after this treatment. A few males were sacrificed. Dissecting and regular microscopes showed that their testes were under-developed (Fig. 2) and contained a small number of motile sperm. It, thus, was concluded that the complex genetic structure of the base-population (the second generation of the four species cross of *O. aureus, O. niloticus, O. mossambicus* and *Sarotherodon galilaeus*) caused the fertility problem.

Discussion

Fertility problems were first noticed during the production of the 4-way crosses when some combinations could not be produced. Two ACOs were obtained in a reasonable number of repeated spawns and the [(*Om* x *Oa*) x (*Sg* x *On*)] ACO was successfully bred for another generation. Thus, we were astonished when no viable breeding occurred in the following Generation-1.

Fertility problems in interspecific fish hybrids have been demonstrated earlier (see recent review by Bartley et al., 2001). In most cases of hybrid sterility, the F_1 is sterile, often due to triploidy. Park et al. (2003) reported on reduced fertility in hybrid flounder in both

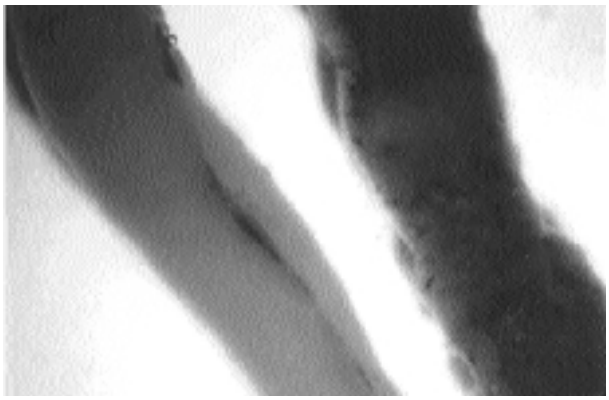

Fig. 2. Gross external morphology of a normal pair of tilapia testes (left) and testes from one of the sacrificed males from an infertile breeding family (right).

males and females that was not associated with triploidy. Goodwin et al. (1994) reported on reduced fecundity in golden shiners (*Notemigonus crysoleucas*) x rudd (*Scardinius erythrophthalmus*) hybrids in which deformities were more pronounced in the testes than in the ovaries. Rarer were the cases where reduced fertility occurred in F_2 (Hooe et al., 1994) or back-crosses of F_1 with parental species (Hulata et al., 1980). In the latter case, ovary abnormalities were mostly observed.

In conclusion, we would like to caution planners of similar breeding programs based on multi-species crosses of the potential reproductive problems that may arise.

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