ISRAELI AQUACULTURE GENETIC IMPROVEMENT PROGRAMS

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ABSTRACT

Genetic improvement programs in Israel have focused mainly on common carp, tilapias, and recently on marine fishes. A breeding program to develop tilapia strains that are cold tolerant and fast growing in fresh and saltwater environments is in progress. Efforts are also being made to produce disease-resistant carp seed. Selective breeding program for sea bream and sea bass has been initiated. Interspecific hybridization experiments were also carried out to improve their culture performance.

Investigations on color pattern inheritance, chromosome-set and sex manipulations of various aquaculture species have been conducted or are in progress. Genetic engineering and transgenesis of a few candidate genes; development of DNA markers and the use of QTL mapping to support the fish breeding programs are underway. Other efforts include development of methods for gamete cryopreservation of common carp, marine fishes and shrimp and initiation of gene bank.

Introduction

The history and status of aquaculture genetics in Israel were reviewed a few years ago (Hulata 1995). The current report reviews trends and progress of the second half of the 1990s, and provides the vision and perspective for the next couple of years. Selective breeding work is currently focused mainly on tilapias (*Oreochromis* sp.) and gilthead seabream (*Sparus aurata*). Edible and ornamental common carp (*Cyprinus carpio*) are used, among other species, in the application of biotechnological methods.

Breeding programs

A program aiming at breeding new, synthetic populations of tilapia specifically adapted to temperate climates and saline environments was initiated in 1995 at the Department of Aquaculture (ARO, Volcani Center). It is based on plant breeders' approach, and takes benefit of the ease of producing interspecific hybrids among tilapias. The use of interspecific composite (or complex) crosses is an established practice in plant breeding for achieving wide genetic and phenotypic variability. This approach is based on the

creation of an artificial center of origin (ACO) via composite interspecific crosses. The ACO contains wide genetic diversity and presents opportunities for genes to recombine and interact with other genes originating from different species, combinations and interactions which are impossible in any of the pure species. Israeli cut flower breeders recently applied an adaptation of this approach, termed Multiple Re-Speciation, to develop new cultivars of carnations.

The ACO (Hulata et al. 1999; Agresti et al. 2000) was produced by inter-crossing four tilapiine species: *Oreochromis niloticus* [wild type (*On*) and red (R*On*) strains], *O. aureus* (*Oa*), *O. mossambicus* (*Om*), and *Sarotherodon galilaeus* (*Sg*). All hybrids were obtained by natural spawning, except for the *S. galilaeus* x *Oreochromis* sp. F_1 hybrids that were produced by artificial fertilization. A series of cold-water challenge tests were performed, involving *O. mossambicus*, *O. aureus* and their F_1 and F_2 hybrids, to study the genetic basis of cold tolerance in tilapias (Cnaani et al. 1999, 2000). All of the different two-way (F_1 hybrids) crosses required to establish the synthetic stock of tilapia (ACO) have been obtained, as well as a set of three four-way-crosses (4WC) derived from five strains in four species and two genera. Full-sibs of the [(*Om* x *Oa*) x (*Sg* x *On*)] 4WC have been successfully bred to enable mixing of the inherited gene blocks; thus, we are ready to start selective breeding for cold tolerance and growth rate in freshwater and for growth rate in saltwater, from this base population. Selection will be based on a combination of individual and family performance; an approach that was used successfully for the GIFT breeding program.

A tilapia breeding program is also conducted at the Nir David Fish Breeding Farm, aiming at creating a fast growing, salinity tolerant, uniformly red tilapia hybrid (Lahav and Ra'anan 1997), and other commercial strains.

A first step in a breeding program to improve growth rate in gilthead seabream (*Sparus auratus*) carried out at the National Center for Mariculture (IOLR, Eilat) was strain evaluation (Knibb et al. 1997a). The research team then moved on to prepare for family selection, encountering difficulties in efficiently producing single-pair offspring groups (full- and half-sibs) and leading to the conclusion that family mating designs are inappropriate for the group spawning *S. aurata* (Gorshkov et al. 1997). Progeny testing, where single males were stocked with groups of females, which more closely simulate the natural group-spawning behavior of the species, yielded genetically related groups more successfully. The small number of families obtained (4 each for full- and half-sibs) did not allow obtaining reliable estimates of heritability for growth, yet large (14-29%) sire components of the offspring weight variance were evident (Knibb et al. 1998a). Mass selection proved more effective and resulted in significant heritability estimates for growth (Knibb et al. 1997b; 1998a,b). A new program has recently been initiated to assess strains and crossbred among strains of cultured sea bass (*Dicentrarchus labrax*) in Israel. This involves comparative assessment of the performance and culture potential of a new Egyptian and existing domesticated strains, in order to identify the most suitable to commence a long-term selective breeding program for sea bass.

Interspecific hybrids between *S. aurata* and red seabream, *Pagrus major* (both belonging to the Sparidae family) have been produced (Knibb et al. 1998a). The hybrids developed only vestigial gonads at the age 2 and 3 years and were sterile. Subsequently, similar vestigial gonads were observed in offspring of the reciprocal crosses. No consistent growth (and survival) superiority until sexual maturity was detected in the reciprocal crosses, compared with parental species. Hybridization between European sea bass (*D. labrax*) females and striped bass (*Morone saxatilis*) males was carried out. Viable hybrid larvae were produced, but surprisingly 28% were triploids, and apparently only triploids survived to age of 6 months. At the age 8 months, surviving (triploid) hybrids showed poor growth compared to diploid *D. labrax*. Interspecific sterile hybrids of marine cultured fish might be of commercial interest when production of fertile fish is restricted for ecological reasons (Gorshkov, pers. comm.).

A severe viral disease has affected Israeli (edible and ornamental) common carp (*Cyprinus carpio*) stocks a couple of years ago. Commercial farms as well as the Dor station, which is holding the breeding nuclei of the parental stocks used for producing the commercially cultured crossbred, have reported massive mortalities. No final census is available yet of surviving broodstocks, and it may well be that years of genetic improvement work had been lost. So far it is not clear whether survivors from affected ponds can inherit tolerance to this disease to their offspring. Farmers are currently trying to produce resistant seed by hybridizing surviving carp broodstocks with Crucian carp (*Carassius carassius*), even at the cost of obtaining a slower growing fish. It is possible that a breeding

program for common carp will have to be restarted to cope with the catastrophe.

Color inheritance

Investigations on color pattern inheritance are being conducted in various aquaculture species, including ornamental (koi) carp (Gomelsky et al. 1995; 1996; 1998a; David et al., work in progress); goldfish (Rothbard et al. 1999a); grass carp, *Ctenopharyngodon idella* (Rothbard and Shelton 1999); tilapia (Shirak et al. 2000) and guppies, *Poecilia reticulata* (Froyman and Hulata, work in progress).

Various mutations which affect body coloration (e.g., 'golden', 'albino' and 'ebony') were isolated in *S. aurata*, some having pleiotropic effects (Knibb et al. 1996; 1998a). While 'ebony' homozygotes are semi-lethal, heterozygotes show strong heterosis for growth.

Chromosome-set and sex manipulations

Methods of chromosome-set manipulations are employed for genetic improvement of various aquaculture species: ornamental (koi) and edible common carp, black carp (*Mylopharyngodon piceus*), tilapias (*O. aureus* and *O. niloticus*), white bass (*M. chrysops*), gilthead seabream (*S. aurata*), European sea bass (*D. labrax*) and white grouper (*Epinephelus aeneus*). Work on edible common carp, carried out at the Dor station (ARO), was directed towards establishing broodstock producing all-female populations (Cherfas et al. 1996). This involved sex reversing XX gynogenetic females to males (Gomelsky et al. 1994), and using these XX males for breeding. Such all-female seed was released to commercial farms and resulted in 10-15% yield improvement over existing commercial stocks. Triploid common carp were produced and their culture potential evaluated (Cherfas et al. 1994). The survival in shock treated progenies (predominantly triploids) was about 70% of that in diploid control. Most of the one-year-old triploid males and females had undeveloped gonads and were sterile. Triploids grew slower than their diploid sibs under all investigated conditions. The results did not reveal the expected positive effect of sterility on somatic growth in triploid common carp, thence the potential of sterile triploid common carp for aquaculture is questionable.

Work on ornamental (koi) carp was aimed at investigating the inheritance of color patterns (Gomelsky et al. 1995; 1996; 1998a). Manipulations are aimed at producing inbred lines, and as a tool in application of DNA-markers for establishment of improved koi broodstock (research in progress). Successful meiotic and mitotic gynogenesis was achieved when the eggs were exposed to, either, heat/cold, or to pressure shocks. An interesting phenomenon detected during the investigation was the presence of males among gynogenetically produced fish. These, when sib-mated with their sisters, produced all-female progeny [Rothbard (Gan Shmuel Fish Breeding Center), pers. comm.]. The objectives of androgenesis were to produce homozygous koi individuals (XX and YY) of both sexes, and to preserve koi genetic traits in a sperm bank. Androgenesis of koi was induced in edible carp eggs, immersed in carp ovarian fluid to avoid stickiness, by UV-irradiation followed by insemination with koi sperm. Low numbers (2-12) of diploid androgenotes, recognizable at the fry stage by light pigmentation, were obtained (Rothbard et al. 1999b).

Studies involving black carp (*M. piceus*) are aimed at producing triploid, presumably sexually sterile fish, as well as mono-sex female or male populations (through application of chromosome-set manipulations, e.g. gynogenesis, androgenesis, triploidy and tetraploidy). Combined technology of gynogenesis and hormonal sex-reversal, enables production of XXX-triploid fish that are completely sterile, unlike the XXY- or YYX-triploids that possess fragments of testes, and are thus able to produce some active sperm (Rothbard et al., 1997). The black carp is a high quality edible fish, which is also highly valued for its ability to control snail populations [e.g., the golden snail (*Pomacea* sp.), zebra mussel (*Dreissena polymorpha*), or snail infestation in the Israeli National Water Carrier system, causing filter and pump clogging](Rothbard and Rubinstein 1999). The methodology enables producing ecologically and economically important exotic species that can potentially serve for biocontrol, without endangering local fish fauna.

Gynogenesis and sex-reversal were successfully induced in *Morone* sp. in an attempt to obtain broodstocks producing monosex populations to avoid limitations on transfers of this exotic species (Gomelsky et al. 1998b; 1999). This project was unfortunately terminated before reaching commercial application.

A meiogynogenetic line of *O. aureus* was established and gynogenetically propagated for 5 generations at the Faculty of Life Sciences, Bar-Ilan University. A successive increase in viability recorded over these 5

generations was interpreted as a gradual elimination of lethal genes. Mitogynogenetic *O. aureus* were produced (Shirak et al. 1998) using third generation meiogynogenetic females from this stock. Three generations of gynogenetic *O. niloticus* were also produced. Males from the gynogenetic *O. aureus* line were used for hybridization with gynogenetic *O. niloticus* females, resulting in consistent production of 100% male hybrids (Avtalion, pers. comm.).

Triploids and meiotic gynogenetic *D. labrax* and *S. aurata* were efficiently produced using temperature shocks (Gorshkova et al. 1995; 1996; 1998). Gorshkova et al. (1996) found nearly 20% males in meiotic gynogenetic *D. labrax* cohorts. Recently, a high incidence (up to 60%) of severe cranial bone deformities were recorded in gynogenetic cohorts at age 10 months (Knibb et al. 1998a). Fertilizing heat-shocked *S. aurata* eggs with untreated heterologous sperm from *Pagrus major* produced triploid hybrids (Gorshkova et al., 1995). However, no growth superiority of triploid hybrids, compared to either parent, was evident for fish up to age 2 and 3 years. *D. labrax* is characterized with sexual dimorphism, with females tending to grow faster than males, so there is considerable interest in culturing only females. Monosex populations were produced by administration of estradiol (yielding all-females) and 17a-methyltestosterone (yielding all-males) to mixed-sex juveniles (Gorshkova et al. 1996).

Karyological analyses of the major maricultured species under cultivation in Israel are being conducted. Recently, series of experiments were undertaken to attempt the cytological technique for examination of early embryogenesis for the purpose of understanding the genetic reasons of high egg and larval mortality in the white grouper (*E. aeneus*). The proportion of cytogenetically abnormal embryos carrying different types of chromosomal aberrations vary significantly in different spawnings of the parental fish. Cytological monitoring of early embryogenesis is carried to support the selective breeding program and the genetic management (stock identification and production of monosex populations) in the white grouper and other cultured marine species (Gorshkova et al. in preparation).

No karyological evidence for a chromosomal mechanism responsible for sexual differentiation was found in European sea bass. However, males usually have a heteromorphic pair of subtelocentric chromosomes, while females usually have a homologous pair of those chromosomes. Temperature manipulation experiments are considerably effective in changing sex ratio in offspring (at the age of 11 months). While data could not identify any particular genetic and/or environmental reason for the observed deviations of sea bass sex ratios, they do illustrate possible plasticity of sex determination in this species (work in progress; Gorshkov and Gorshkova, pers. comm.).

Genetic engineering and transgenesis

Investigations on a few candidate genes have been initiated and/or are in progress. Most noteworthy is the work on the effect of growth hormone (GH), engineered for expression using various promoters, on common carp and gilthead seabream growth (Cavari et al. 1993a,b; Moav et al. 1995; Fine et al. 1996; Hinits and Moav, 1999). Moav (Dept. of Zoology, Tel Aviv university) and coworkers have demonstrated 20% growth improvement in transgenic carp reared in experimental earthen ponds. Current efforts are aimed at constructing new expression vectors to improve efficiency of producing transgenic fish. Further potential economically important genes considered for genetic engineering in marine fish include genes for D^5 and D^6 fatty acid desaturase enzymes (Knibb et al. 1996).

DNA markers and QTL mapping

Collaborative work with US scientists (Hulata et al. 1999; Agresti et al. 2000) using the [*Om* x (*Oa* x R*On*)] cross resulted in mapping 191 AFLP and 26 UNH microsatellite markers to 24 linkage groups. Twenty UNH microsatellite markers were used to search for quantitative trait loci (QTL) associated with cold tolerance and body weight in an F_2 family of *O. aureus* x *O. mossambicus* hybrid. Two markers putatively associated with cold tolerance and three markers putatively associated with body weight were found. Two of these markers are located on the same linkage group, which is apparently a chromosomal region, which affects growth and survival in tilapia (Cnaani et al. 1999, 2000). In a similar study (Agresti et al., in preparation) using cold tolerance-tested [(*Om* x *Oa*) x (*Sg* x *On*)] 4WC, one UNH microsatellite and two AFLP markers from the female gave evidence of association with body weight; these were all from different linkage groups. Seven markers (3 UNH microsatellite and 4 AFLP) from the male also were found to be associated with body weight and six of them were restricted to

two linkage groups. Based on shared microsatellite markers, 12 composite linkage groups have been identified from the combined mapping data of Kocher et al. (1998), Agresti et al. (2000) and the 4WC family. These likely represent 12 of the 22 tilapia chromosomes.

Attempts are also being made at detecting sex linkage and non-Mendelian segregation of microsatellite DNA markers in a meiogynogenetic family of *O. aureus* (Palti et al. 1999, 2000), and at analyzing genetic variation in immunological parameters associated with stress resistance (Palti et al. 2000, and work in progress).

Work is currently under way for development of DNA markers in edible and ornamental (koi) carp. This work is aimed at detecting linkage between DNA markers and color patterns in the ornamental carp (David and Rothbard, pers. comm.).

Studies are under way to apply molecular biology to support the classical breeding program (specifically for determining parenthood in mass-spawning of *S. aurata* and *D. labrax*, and for genetic testing programs) by developing AFLP and microsatellite DNA profiling techniques (Gorshkova, pers. comm.).

Gamete cryopreservation and gene bank

Methods have been developed at the National institute of Oceanography (IOLR, Haifa), for cryopreservation of carp sperm (Lubzens et al. 1993; 1997), leading to the formation of an operating 'sperm bank' for *C. carpio* (mainly ornamental carp) in Israel. Subsequently, methods for cryopreservation of gilthead seabream, mullet (*Mugil cephalus*) and grouper (*E. aeneus*) sperm have also been developed (Lubzens, pers. comm.).

Attempts are currently directed towards developing cryopreservation methods for gilthead seabream (Lubzens and Pekarsky 1998) and marine shrimp (*Penaneus semisulcatus*) embryos. Successful cryopreservation of yolk-laden eggs containing developing fish embryos has yet to be achieved. Obstacles encountered so far are the low permeability of cryoprotectants into the embryos and sensitivity of fertilized eggs to concentrations above 0.15M of DMSO or methanol. Attempts are currently being made at evaluating possible cryopreservation of ovarian oocytes, as an alternative (Lubzens and Pekarsky 1998).

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