METHODS FOR STRAIN VARIATIONS IN SEX RATIO INHERITANCE AND METHODS FOR THE CONTRIBUTION FROM THE MALE AND FEMALE GENOME TO SEX INHERITANCE

Eighth Work Plan, Reproduction Control Research 1A and 1C (RCR1A and 1C)
Progress Report

Ronald P. Phelps, J.T. Arndt, and R.L. Warrington
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

ABSTRACT

Effective and practical control of reproduction is the major constraint in tilapia culture. Uncontrolled reproduction can result in less than 25% of the adults being greater than 250 g after a six-month culture period, with the majority of the population being progeny less than 10 g each. Intraspecific breeding programs have been developed to exploit the sex inheritance mechanism in the tilapia *Oreochromis niloticus*. Females are said to be homogametic (XX) and males heterogametic (XY), but the sex inheritance of the progeny from a single pair often does not conform to the expected 50:50 ratio. This lack of conformity to a simple XX:XY sex inheritance pattern complicates the intraspecific breeding approach of developing YY males that would give all-male progeny. The identification of tilapia populations with minimal variation in progeny sex ratios from individual spawns would be a significant contribution to the development of a YY male breeding program. Three strains of *Oreochromis niloticus*—Egypt, Ghana, and Ivory Coast—were spawned in outdoor hapas at 28 to 32°C. A total of 44, 34, and 52 spawns from the Egypt, Ghana, and Ivory Coast strains, respectively, were successfully reared to a sexable size and the sex ratio of each spawn established. The mean percentage of males, females, or intersex fish did not differ among the three strains evaluated. A given male did not give consistent sex ratios when mated with different females. Multiple spawns from a given female also had variable progeny sex ratios.

INTRODUCTION

Effective and practical control of reproduction is the major constraint in tilapia culture. Uncontrolled reproduction can result in less than 25% of the adults being greater than 250 g after a six-month culture period, with the majority of the population being progeny smaller than 10 g each. Inter- and intraspecific breeding programs can result in populations with highly skewed sex ratios, but often give inconsistent results. Interspecific crosses have not proven to be practical due to difficulties in maintaining the parent species integrity.

Intraspecific breeding programs have been developed to exploit the sex inheritance mechanism in the tilapia *Oreochromis niloticus*. Females are said to be homogametic (XX) and males heterogametic (XY), but the sex inheritance of the progeny from a single pair often does not conform to the expected 50:50 ratio. This lack of conformity to a simple XX:XY sex inheritance pattern complicates the intraspecific breeding approach of developing YY males that would give all-male progeny. The sex ratios of individual spawns have been studied in only a limited number of strains of *O. niloticus*, and the observed variance in sex ratios may be related to strain differences. The identification of tilapia populations with minimal variation in progeny sex ratios from individual spawns would be a significant contribution to the development of a YY male breeding program.

METHODS AND MATERIALS

Three strains of *Oreochromis niloticus* were used during the study: Egypt, Ghana, and Ivory Coast. Brooders used ranged in size from 50 to 250 g. The populations have been maintained at Auburn University since 1982, 1982, and 1974, respectively. Paired matings by strain were accomplished using a 1:3 ratio, male to female inside 2-m^2 net hapas located in outdoor tanks. Hapas were checked on a ten-day cycle for the presence of fry or eggs in the mouths of the females. Females which spawned were tagged using Floy® tags as identifiers and placed with other males at random within the same strain for additional spawns.

The procedure for inspection was to crowd the brooders to one side of the hapa, remove the male to the unoccupied side of the hapa, and extract each female using a small dip net. Upon inspection of each female's mouth, eggs or fry were removed when present. Any extraneous fry were removed from the hapa upon completion of inspection. Eggs collected were incubated in McDonald jars until hatching occurred. Fry collected were also brought indoors for temporary rearing. Fry collected or hatched were then raised to an average size of 2 cm and were then relocated to outdoor hapas or grow-out ponds with a 29°C average daily temperature. Fry were stocked at 250 fry per hapa and 1000 fry per grow-out pond and raised to an average length of 7 cm, of which samples of 110 fish were preserved in formalin.

Additionally, randomly chosen groups of 250+ siblings averaging 8.7 mm in length were split into aquaria and raised under two temperature conditions. One half of the fry were raised at an elevated average daily temperature of 35.3°C for 45 days and the other half at ambient temperature with an average daily temperature of 28.3°C for 45 days. Fish were grown out in outdoor tanks at an average temperature of 29.8°C. When they reached a length of approximately 7 cm they were preserved in 10% formalin.

Determination of sex was accomplished using the gonadal squash method (Guerrero and Shelton, 1974). Each gonad was stained using Harris' hematoxylin with a 10X dilution and then

Table 1. Mean and range of sex ratios of progeny from pair spawns of Egypt, Ghana, and Ivory Coast strains of *Oreochromis niloticus.*

Strain	Number of spawns	% Males	% Females	% Intersex	Range in % males
Egypt	44	52.9	46.3	0.8	26-72
Ghana	34	54.7	44.9	0.4	16-77
Ivory Coast	53	50.6	49.0	0.4	7-100

crushed by placing an additional slide on top and pressing the two together. Visual inspection was then made at 100X magnification along the length of each gonad, which was determined to be teste, ovary, or intersex with the % ovarian tissue recorded.

Results and Discussion

A total of 44, 34, and 52 spawns from the Egypt, Ghana, and Ivory Coast strains, respectively, were successfully reared to a sexable size and the sex ratio of each spawn established. The mean percentage of males, females, or intersex fish did not differ among the three strains evaluated (Table 1). There was considerable variation in the sex ratios of individual spawns, with the Ivory Coast strain the most variable, followed by Ghana and Egypt (CVs of 33.2, 21.2, and 19.4%, respectively). The frequency distribution of percent males per spawn for each strain is given in Figure 1.

Multiple spawns by a given male or female mated to a new fish were often highly variable. Seventeen males spawned with three or more females, with one individual producing nine spawns. Of these males, only 3 of 17 produced progeny where the percentage of males was between 40 and 60% in all sets. Seventy-six percent of the males gave one or more spawns with > 60% males, and 18% gave one or more spawns with < 40% males. A given male did not give consistent sex ratios when mated with different females. Only 3 of 17 males with three or more spawns gave progeny where the CV in percent males among spawns was 10% or less. The one male that sired nine sets of progeny gave one that was 16.3% males, four between 40 and 60%, and one that was 100% male.

Multiple spawns from a given female also had variable progeny sex ratios. Seven females gave three or more sets of progeny. Only two females consistently gave progeny where

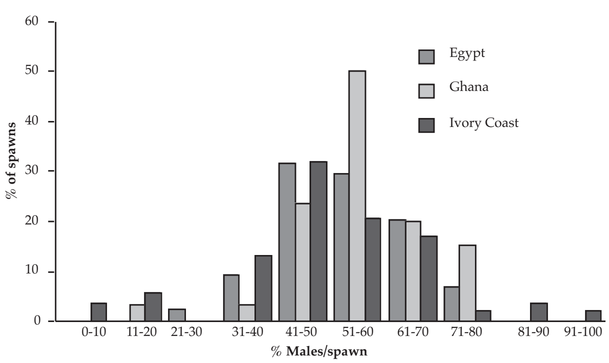

Figure 1. Variation in percentage of males in the progeny of pair spawns of Egypt, Ghana, and Ivory Coast strains of *Oreochromis niloticus*.

the frequency of males was between 40 and 60%. Three of seven gave one or more spawns with < 40% males, and three gave spawns with > 60% males. The CV in percent males among sets of progeny was 10% or less for only one female.

No one sex could be said to determine sex inheritance based on the observations to date. Where two or more spawns were obtained from a given pair, there were differences of 20% or more in the percentage of males among spawns from three of eight such pairs.

Nine spawns were successfully reared for 45 days at two temperatures (two Ghana, four Egypt, and three Ivory Coast). There was no difference in the percent males obtained when reared at 28.3°C and 35.3°C (Table 2). No one strain was more sensitive to the effect of temperature on sex ratios. Individual spawns did respond differently to temperature. In four spawns, siblings reared at the higher temperature had an increase in percent males of more than 10%, while the percent males from siblings from four spawns differed 10% or less at the two temperatures. The percent males in one set of siblings at 35.3°C was 20% less than the siblings at 28.3°C.

The variation in sex ratios among individual spawns was not unexpected from the Ivory Coast strain. Variation from 1:1 ratios of individual spawns has also been reported by Calhoun and Shelton (1983) from Ivory Coast strain Nile tilapia. The Ivory Coast strain of *O. niloticus* used in this study is highly inbred. The founder stock introduced in 1974 consisted of 100 to 200 juveniles from five to ten pairs of brooders at the Centro de Pesquisas Ictiologicas, Pentecoste, Ceara, Brazil, whose stock was founded from 50 to 100 fish introduced from the Station de Recherches Piscicoles, Bouake, Ivory Coast, in 1972 (Tave and Smitherman, 1980). Additional bottlenecks have occurred since that time. Abdelhamid (1988) compared the Auburn Ivory Coast strain of *O. niloticus* to six others and found 13.2% of the 38 loci to be polymorphic while the other strains were > 26% polymorphic. He found a mean heterozygosity of 0.018 for the Ivory Coast strain; the others ranged from 0.037 to 0.069. Tave and Smitherman (1980) established a predicted heritability of 0.04 ± 0.14 for the Auburn Ivory Coast strain. Teichert-Coddington and Smitherman (1988) found the realized heritability to be -0.10 ± 0.02 for the strain. These low levels of heritability reflect the significant level of inbreeding that has occurred in this line. Such an inbred line should also have a very inbred sex inheritance mechanism with little variation in observed ratios, but this was not the case. The high variability in sex ratios also occurred in the Egypt and Ghana lines.

The high degree of variation in individual spawns within a population and among repeat spawns of the same individual does not support a simple XX:XY sex-determining mechanism in any of the strains examined. Such lack of conformity has been encountered in other tilapia studies and explained as autosomal influence (Avtalion and Hammerman, 1978),

Table 2. Mean sex ratio of *Oreochromis niloticus* siblings of the Egypt, Ghana, and Ivory Coast strains reared 45 days at 28.3 and 35.3°C during the period of gonadal differentiation.

Strain	Temperature (°C)	% Males	% Females	% Intersex
EGYPT (N=4)				
	28.3	51.7	46.3	2.0
	35.3	57.8	41.4	0.8
GHANA (N=2)				
	28.3	53.5	45.5	1.0
	35.3	56.5	43.5	0
IVORY COAST (N=3)				
	28.3	55.6	43.9	0.5
	35.3	64.1	35.9	0

unequal potencies of X and Y (Shelton et al., 1983), multi-factorial (Lester et al., 1989), crossing over (Wohlfarth and Wedekind, 1991), and monofactorial with autosomal or environmental sex-modifying factors (Mair et al., 1995). In any case, the variation in sex ratios from individual matings makes the development of YY breeding programs more complicated. The same events which produce skewed sex ratios in untreated populations of *O. niloticus* may also produce females in the expected all-male offspring of YY broodstock. Mair et al. (1997) found that YY males would on occasion sire sets of progeny that were not all male.

The variation in response to temperature further illustrates the degree of individual variation that may occur. Mair et al. (1990) and Baroiller et al. (1995) demonstrated how sex ratios of Nile tilapia could be altered by temperature and how the degree of response was dependent on the individual spawn. The variability in sex ratios in response to temperature by individual pairs and the variability of repeated spawns of a given male at ambient conditions in this study emphasize the need for selection of individual fish of known spawning histories for use in a YY breeding program.

Additional pairings will be made in the 1998 season of males and females with known progeny sex ratio history. Progeny from selected pairs will be nursed at two temperatures to determine the influence of temperature on such progeny.

ANTICIPATED BENEFITS

The variation in sex ratios among sets of progeny was not a function of strain, but appears instead to be a characteristic of the species. For a YY breeding program to be successful, individual fish will have to be tested with a number of mates and under a range of environmental circumstances in an attempt to develop a line of fish which conform to a simple XX:XY sex inheritance pattern. Work to date as part of this project has helped to provide insight as to the variation among individuals and the possibility of selecting for individuals that breed true to a given sex ratio. Continued selection based on the individuals identified to date may be able to provide the true breeding lines of fish needed to make a YY breeding program practical.

LITERATURE CITED

Abdelhamid, A.A. 1988. Genetic homogeneity of seven populations of *Tilapia nilotica* in Africa, Central America, and Southeast Asia. M.S. thesis, Auburn University, Alabama, USA.

Avtalion, R.R. and I.S. Hammerman, 1978. Sex determination in *Sarotherodon* (*Tilapia*). I. Introduction to the theory of autosomal influences. Bamidgeh, 30(4):110-115.

Baroiller, J.F., D. Chourrout, A. Fostier, and B. Jalabert, 1995. Temperature and sex chromosomes govern sex ratios of the mouthbrooding cichlid fish *Oreochromis niloticus*. J. Exper. Zool., 273:216-223.

Calhoun, W.E. and W.L. Shelton, 1983. Sex ratios of progeny from mass spawnings of sex-reversed broodstock of *Tilapia nilotica*. Aquaculture, 33:365-371.

Guerrero, R.D., and W.L. Shelton, 1974. An acetocarmine squash method of sexing juvenile fishes. Prog. Fish-Cult., 36(1):56.

Lester, L.J., K.S. Lawson, T.A. Abella, and M.S. Palada, 1989. Estimated heritability of sex ratio and sexual dimorphism in tilapia. Aquacult. Fish. Manage., 20:369-380.

Mair, G.C., J.A. Beardmore, and D.O.F. Skibinski, 1990. Experimental evidence for environmental sex determination in *Oreochromis* species. In: R. Hirano and I. Hanyu (Editors). The Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp. 555-558.

Mair, G.C., A. Scott, D.J. Penman, J.A. Beardmore, and D.O.F. Skibinski, 1991. Sex determination in the genus *Oreochromis*: I. Sex reversal, gynogenesis, and triploidy in *O. niloticus* L. Theor. Appl. Genet., 82:144.152.

Mair, G.C., J.B. Capili, D.O.F. Skibinski, J.A. Beardmore, L.P. Pascual, J.S. Abucay, J.C. Danting, and R.A. Reyes, 1997. Sex ratios and growth performance of crossbred genetically male tilapia. In: K. Fitzsimmons (Editor), Proceedings from the Fourth International Symposium on Tilapia in Aquaculture, 9-12 November 1997, Orlando, Florida. Northeastern Regional Agricultural Engineering Service, Cooperative Extension, Ithaca, New York, pp. 262-271.

Shelton, W.L., F.H. Meriwether, K.J. Semmens, and W.E. Calhoun, 1983. Progeny sex ratio from intraspecific pair spawnings of *Tilapia aurea* and *Tilapia nilotica*. In: L. Fishelson and Z. Yaron (Editors), Proceedings from the International Symposium on Tilapia in Aquaculture, 8-13 May 1983 Nazareth, Israel. Tel Aviv University Press, Tel Aviv, pp. 270-280.

Tave, D. and R.O. Smitherman, 1980. Predicted response to selection for early growth in *Tilapia nilotica*. Trans. Am. Fish. Soc. 109:439-445.

Teichert-Coddington, D.R. and R. Oneal Smitherman, 1988. Lack of response by *Tilapia nilotica* to mass selection for rapid early growth. Trans. Am. Fish. Soc., 117:297-300.

Wohlfarth, G.W. and H. Wedekind, 1991. The heredity of sex determination in tilapia. Aquaculture, 92:143-156.

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