# Monosex Tilapia Production through Androgenesis: Selection of Individuals for Sex Inheritance Characteristics for Use in Monosex Production

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## Abstract

Intraspecific breeding programs have been developed to exploit the sex inheritance mechanism in the tilapia *Oreochromis niloticus* to produce male populations. These programs are built on the premise that the mechanism of sex inheritance must conform closely to a monofactorial sex determination with a heterogametic male. Sex inheritance in tilapia, however, appears to be more complicated. The sex ratio of an individual spawn often does not conform to the expected 1:1 ratio. A better understanding of sex inheritance in tilapia and the identification of tilapia populations with a minimum variation in progeny sex ratios from individual spawns is needed for a successful intraspecific breeding program to produce male tilapia.

Nine families of *O. niloticus*, each from individual pair spawns, were selected based on the sex ratio in the family. Two families were highly skewed to male (100% male), three were near 50% male, and four were skewed to female. Fish within the same family were mated, and the sex ratios of the progeny were determined. In the two families that were all males, the males were mated to females from a family with a sex ratio near 1:1. Ten sets of progeny per family were sexed, with the exception of one family, from which only eight sets were available.

Sex ratios did not appear to be passed on from one generation to another in the fish used in our study. A realized heritability for sex ratio of –0.09 was calculated. No family with skewed sex ratios produced progeny from sibling matings with similarly skewed sex ratios. Family VIII, which had a 1:1 male:female ratio, had a range of 43 to 68% male in its sets of progeny. Family V, which was 22% male, gave 10 sets of progeny, of which five sets were > 70% male. Families II and VII, which were 100% male, when crossed with females from Family III gave sets of progeny ranging from 23 to 79% male. When the percentage of males in the female parent family (III, 40% male) was considered in matings with Families II and VII, 40 and 50% of the spawns, respectively, differed in sex ratios from the female parent family.

## Introduction

Tilapia culture is one of the fastest-growing forms of finfish aquaculture in the world. It has broadened its base from being a subsistence-oriented technology to being a component of world commerce as a high-quality fillet product exported to Europe and the US. The commercial market requires a large fish suitable for providing fillets or being sold whole. 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, with few to no marketable fish.

Monoculture of males prevents reproduction while allowing the culture of the faster-growing sex. Male populations of tilapia can be created by hormone sex reversal, but there are concerns about consumer perceptions of eating hormone-treated fish. Inter- and intraspecific breeding programs can result in populations with highly skewed sex ratios, but these programs 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). The presence of the Y chromosome establishes the sex as male. Selective breeding programs have been developed to produce YY males, where the progeny of such males mated with a normal female (XX) would be all male. For this approach the mechanism of sex inheritance must conform closely to a monofactorial sex determination with a heterogametic male. Sex inheritance in tilapia, however, appears to be more complicated. The sex ratio of an individual spawn often does not conform to the expected 1:1 ratio. This lack of conformity to a simple XX:XY sex inheritance mechanism complicates any intraspecific breeding program used to produce all-male progeny. The identification of tilapia populations with a minimum variation in progeny sex ratios from individual spawns would be a significant contribution to the development of an intraspecific breeding program. The following study addressed the question of the heritability of sex in *O. niloticus*.

## Methods and Materials

### Broodstock

Nine families of *O. niloticus*, each from individual pair spawns, were selected based on the sex ratio in the family. Two families were highly skewed to male, three were near 50% male, and four were skewed to female. The total number of fish for each family, the strain, and the male:female ratio are provided in Table 1.

Fish were kept in $1 \times 1 \times 2$ m hapas of 1.5-mm mesh in 20-$m^2$ concrete tanks. Four hapas were placed in each tank and

suspended by a frame of treated 2 × 2 lumber. Water was kept at a 70-cm depth by an adjustable PVC pipe outlet. The water source was the station's reservoir of rainwater. Tank water was not fertilized. Daily maintenance of the tank water consisted of adjusting water flow in or out and removal of any algae build-up in the water or on the hapas. Dissolved oxygen and temperatures recorded from May to September 2000 averaged 9.8 mg $l^{-1}$ and 28.7°C, respectively, and ranged from 1.1 to 15 mg $l^{-1}$ and 19.9 to 33.7°C.

Fish of each family were sexed and separated equally into the four hapas at stocking, the exception being Family IX, for which only two hapas were needed. Once sorted, five males were placed in each female hapa. All fish were fed once a day with floating catfish feed to apparent satiation. Feed offered was adjusted daily as needed. In the case of all-male families, II and VII, ten females from Family III were used. Females from Family III were not transferred until after Family III had met its goal of 15 successful spawns.

### Seed Collection

Adult females' mouths were checked for eggs every eight to ten days starting on 20 May 2000. Females with eggs, or fry, were placed individually in 19-l buckets and transferred to 75-l aquaria at the hatchery of the Fisheries Research Station, Auburn University, Alabama. Females were allowed to incubate the eggs and were then transferred to 19-l buckets for return to their respective families' tank. Females were not necessarily returned to the same hapa, but even numbers per hapa were maintained. In families that showed little spawning activity over two spawning cycles, the males were replaced by five different males from the same family. The last date females were examined for the spawns was 31 July 2000.

The hatchery was equipped with ninety 40-l aquaria on a recirculating system. Aeration was provided to each aquarium by a low-pressure blower through a single air stone. Average water temperature in the hatchery was 28°C. No feed was offered to females or the sampled spawns during the incubation and swim-up period.

### Fry and Juvenile Management

Aquaria were checked for swim-up fry daily. Spawns were removed from aquaria upon swim-up. Each spawn was identified with a tag in a sealed vial. Those spawns that were estimated to contain over 100 fish were transferred via a 19-l bucket to either a 20-$m^2$ tank or a small suspended hapa. The 20-$m^2$ tank was used for grow-out. When one was not available, fry were kept in a 1.5-mm-mesh hapa, with a volume of 0.5 $m^3$, until a grow-out tank was ready. Fifteen small hapas were suspended per 20-$m^2$ tank. Fry were fed three times daily to apparent satiation, starting with a #0 floating trout feed. As fry grew larger, feed changed to #2, and finally #4 floating trout feed was fed twice a day. Each spawn was grown until fish were approximately 4 cm long. Upon the completion of the grow-out period, each spawn was harvested.

### Harvest

Each tank was fully drained with a screen placed in the drain. Fish were then removed from the catch basin with a fine mesh net and placed in a 19-l bucket. Spawns were then transferred to the hatchery for processing. Each spawn was anesthetized in a solution of MS-222, counted, and 110 randomly selected fish preserved in a 10% formalin solution in a labeled jar. If fewer than 110 fish survived, then all were preserved. Preserved spawns were held a minimum of ten days before being sexed.

### Sexing

Each spawn was rinsed in water for a minimum of 24 hours before dissecting. Sexing consisted of removing the gonads from each fish, placing each pair on a glass slide, staining the gonads with Fast Green, squashing the gonads with a second slide, and then determining sex by microscopic examination.

## RESULTS

### Families and Spawns

A total of 88 spawns were collected and sexed from the nine families of adults. Each family produced ten spawns for sexing except family VI, which produced eight spawns for the season. No family gave progeny sex ratios that closely conformed to that of the family. Family sex ratios and sex ratios of progeny

Table 1. Nine families of known sex ratios* selected for determining the heritability of sex and the number of siblings of each sex available for matings on 17 May 2000.

| Family | Strain | Number of Males | Number of Females | Parent M:F Ratio* |
|---|---|---|---|---|
| I | Ivory Coast | 59 | 61 | 45:55 |
| II | Ivory Coast | 111 | 0 | 100:0 |
| III | Ivory Coast | 34 | 42 | 40:60 |
| IV | Ivory Coast | 41 | 38 | 25:75 |
| V | Egypt | 57 | 38 | 22:78 |
| VI | Ghana | 13 | 72 | 19:81 |
| VII | Egypt | 94 | 0 | 100:0 |
| VIII | Ghana | 41 | 38 | 50:50 |
| IX | Egypt | 6 | 35 | 18:82 |

* Note that the parent male:female ratio is that of the family after sexual differentiation and not the number on hand at stocking.

Table 2. The percentage of males found and the percentage of males in each set of progeny for the nine parent families, where the percentage of males in the parent families is known.

| Family | Males (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Parent* | *Per Spawn* | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| I | 45 | 23 | 24 | 26 | 48 | 50 | 51 | 52 | 54 | 58 | 61 |
| II | 100 | 23 | 40 | 42 | 44 | 48 | 48 | 51 | 52 | 53 | 56 |
| III | 40 | 25 | 40 | 43 | 44 | 47 | 54 | 56 | 56 | 59 | 59 |
| IV | 25 | 25 | 39 | 40 | 41 | 46 | 56 | 58 | 59 | 63 | 74 |
| V | 22 | 43 | 48 | 48 | 63 | 64 | 70 | 71 | 71 | 79 | 86 |
| VI | 19 | 19 | 38 | 53 | 56 | 57 | 62 | 63 | 68 | -- | -- |
| VII | 100 | 27 | 33 | 35 | 43 | 49 | 51 | 54 | 59 | 65 | 79 |
| VIII | 50 | 43 | 43 | 48 | 52 | 53 | 55 | 56 | 56 | 57 | 68 |
| IX | 18 | 31 | 34 | 41 | 43 | 53 | 59 | 66 | 69 | 76 | 80 |

Table 3. Percent of spawns that had a sex ratio different from the parent family sex ratio and from the mean sex ratio of its sibling pair spawns as determined by Chi square.

| Family Number | Spawns Differing from Parent Sex Ratio (%) | Spawns Differing from Mean Sex Ratio of Sibling Family (%) |
|---|---|---|
| I | 40 | 40 |
| II | 100 | 10 |
| III | 60 | 10 |
| IV | 90 | 20 |
| V | 100 | 40 |
| VI | 88 | 25 |
| VII | 100 | 50 |
| VIII | 10 | 10 |
| IX | 100 | 40 |

from matings within each family are given in Table 2, and the percentages of spawns that had a mean percentage of males different than that of the parent family are given in Table 3. When the original families were considered as to the skewness of their sex ratio and categorized as skewed to male, female, or neutral, the progeny varied in sex ratio but had category means of 47.6 ± 12.8, 55.6 ± 15.9, and 48.7 ± 11.4% male, respectively. No one family had a narrow distribution of sex ratios. The progeny from sibling matings within each family had a range of sex ratios from a low of 19% in Family VI to as high as 86% in Family V. The percentages of spawns in which the mean percentage of males was different than that of the sibling spawns within the same family are given in Table 3.

Values for inheritance were calculated using a linear regression between parents and offspring as described by Kirpichnikov (1981). Values for the regression were for all spawns percentage male (Y) compared to the parent percentage male (X), where $h^2$ is equal to the slope (b). Calculations revealed that $h^2$ was –0.09 with an $R^2$ value of 0.04.

### Environmental Effects on Sex Ratios

High temperature did skew the sex ratio of spawns from Families VI and VIII but not Family V. The means are shown in Figure 1. Temperature data for each spawning and rearing cycle for fish cultured outdoors, along with the mean percent male for that period, are given in Table 4. There was no trend in percentage of males over this period related to ambient temperature.

## Discussion

The heritability of sex ratio is a fundamental question in tilapia breeding programs. Variability in sex ratios among individual spawns has been noted by a number of authors (Shelton et al., 1983; Mair et al., 1991; Al Hafedh, 1994; Tuan et al., 1999). Shelton et al. (1983) proposed that it might be possible to select for specific sex ratios. This would require that sex ratio be a heritable trait. Wohlfarth and Wedekind (1991) discuss the heritability of sex determination in tilapia using the data of Wedekind (1987), who found that selected males from spawns that were skewed to male gave progeny which were also skewed to male and that control males from families with normal ratios gave progeny with normal ratios. Al Hafedh (1994), using a strain of *O. niloticus* originally collected from Lake Manzala, Egypt, selected brooders from families with known sex ratios and mated various combinations of fish. Two pair spawns from males of a family skewed to males (75%), when crossed to females from that family, gave 68 and 75% male progeny. In three spawns from pairs in which the males from the male-skewed family were crossed with females from a family highly skewed to female (99.4%), the progeny were 27, 27, and 69% male. However, using males and females from more normal ratio families (males from 44 to 46% male families × females from 42 to 46% male families), seven spawns were obtained, of which five had sex ratios different than that of the parent family.

Sex ratios did not appear to be passed on from one generation to another in the fish used in our study. No family with skewed sex ratios produced progeny from sibling matings with similarly skewed sex ratios. Family VIII, which had a 1:1 male:female ratio, had a range of 43 to 68% male in its sets of progeny. Family V, which was 22% male, gave ten sets of progeny, of which five sets were > 70% male. Families II and VII, which were 100% male, when crossed with females from Family III gave sets of progeny ranging from 23 to 79% male. When the percentage of males in the female parent family (III, 40% male) was considered in matings with Family II and VII, 40% and 50% of the spawns, respectively, differed in sex ratios from the female parent family.

The low value of $h^2$ calculated from a linear regression between parents and offspring suggests little to no heritability of the trait sex ratio. This is contrary to studies concerning other populations where greater levels of heritability for sex ratio have been calculated. Lester et al. (1989), studying *O. niloticus* strains that may have been mixed with *O. mossambicus*, give a heritability value of 0.26 for half-sib families. Al Hafedh (1994), using a sire-dam model to determine sex ratio heritability, found 0.43 ± 0.05 in the first generation and 0.56 ± 0.10 in the second. The strains of *O. niloticus* used in our study have been inbred for a number of generations, experiencing several bottlenecks along the way. This may have been a factor

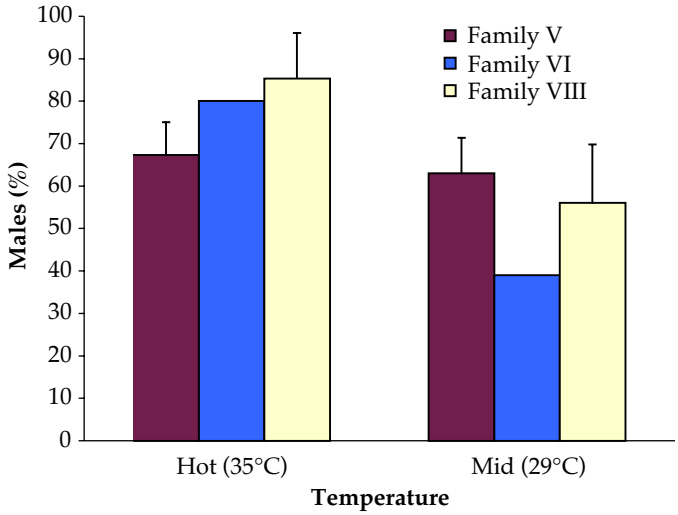


Figure 1. Percent males of fry from Families V, VI, and VIII held at a mid-range temperature (29°C) or at a hot temperature (35°C) during the period of gonadal differentiation.

Table 4. Temperature and percent male data for spawning and rearing periods.

| Cycle | Spawning Period | Average Temperature (°C) | Rearing Period | Average Temperature (°C) | Mean Percent Male |
|---|---|---|---|---|---|
| A | 5/1–6/3 | 26.90 ± 1.97 | 6/3–7/3 | 28.88 ± 2.34 | 51.17 ± 16.41 |
| B | 5/7–6/7 | 27.08 ± 1.55 | 6/7–7/7 | 29.23 ± 2.22 | 52.80 ± 10.48 |
| C | 5/14–6/14 | 27.93 ± 1.61 | 6/14–7/14 | 29.43 ± 1.08 | 55.71 ± 16.78 |
| D | 5/19–6/19 | 28.37 ± 2.38 | 6/19–7/19 | 29.59 ± 1.00 | 46.91 ± 10.14 |
| E | 5/26–6/26 | 28.72 ± 2.38 | 6/26–7/26 | 29.52 ± 0.87 | 44.00 ± 11.62 |
| F | 6/6–7/6 | 29.10 ± 2.32 | 7/6–8/6 | 29.48 ± 0.97 | 60.25 ± 17.02 |
| G | 6/10–7/10 | 29.53 ± 2.12 | 7/10–8/10 | 29.54 ± 1.00 | 50.33 ± 8.06 |
| H | 6/18–7/18 | 29.57 ± 0.98 | 7/18–8/18 | 29.42 ± 1.08 | 53.40 ± 22.70 |
| I | 6/25–7/25 | 29.52 ± 0.87 | 7/25–8/25 | 29.22 ± 1.13 | 52.25 ± 16.25 |
| J | 6/30–7/30 | 29.61 ± 0.92 | 7/30–8/30 | 29.07 ± 1.13 | 42.00 ± 20.07 |
| K | 7/3–7/31 | 29.45 ± 0.86 | 8/3–9/3 | 29.07 ± 1.19 | 38.00 ± 0.00 |

contributing to the low heritability. Low heritability for growth has been found in both the Ivory Coast and Ghana lines. Teichert-Coddington and Smitherman (1988) found a low realized heritability for fast growth in the Ivory Coast strain and attributed that to a small founder stock and subsequent generations of inbreeding. Hulata et al. (1986) found no response to selection for rapid growth using the Ghana strain of *O. niloticus*.

Water temperature during the time of gonadal differentiation did appear to affect sex ratios in two of the families tested. High water temperatures have been shown to skew sex ratios to male in other studies with *O. niloticus* and *O. aureus*. Baroiller et al. (1995) skewed the sex ratio of *O. niloticus* to more males by holding fry at 36°C during gonadal differentiation. Desprez and Mélard (1998) found similar results with *O. aureus*, where the sex ratio was altered to 97.8% male by holding non-hormone-treated fry at 34°C. Mair et al. (1990) also found that some matings of *O. niloticus* were more sensitive to the effects of temperature on sex ratio than others. This variation in response among individual fish to temperature is another factor to be considered in sex determination.

When all the matings from all families are considered as a whole, the percentage of males is normally distributed around a mean of 51.4 ± 14.2% (Figure 2).

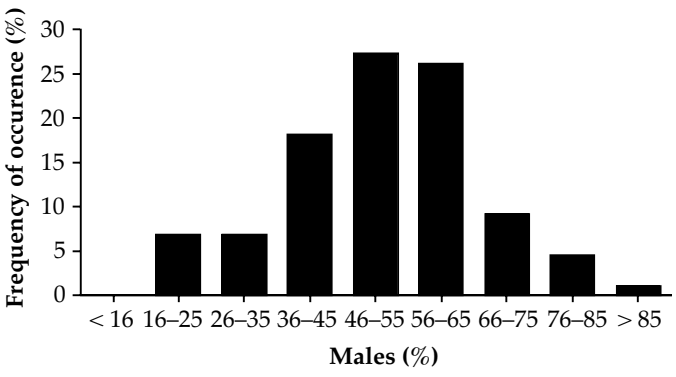


Figure 2. The frequency of sex ratios observed from the combined sets of progeny, where the parent sex ratios were skewed to male or female or not skewed.

This distribution pattern is similar to that seen in studies in which the sex ratios of individual spawns of normal fish are given (Shelton et al., 1983; Mair et al., 1991; Al Hafedh, 1994; Tuan et al., 1999; Warrington, 1999). This pattern suggests a polygenic mechanism of sex determination susceptible to environmental influences, where in any one mating a sex ratio that does not closely conform to 1:1 might be possible. None of the nine parent families when mated to siblings gave progeny sex ratios that closely matched that of the parent family. The diversity of sex ratios in such closed populations reflects a complex mechanism of sex determination making it difficult to produce a true-breeding line of fish for use in a YY breeding program.

## CONCLUSIONS

The sex ratio produced by one set of parents was not a trait inherited and expressed by the progeny. The lack of consistency from one generation to another and among sibling matings to give a consistent sex ratio in their progeny provided evidence that a YY breeding program to produce male progeny will not be effective for the strains of *O. niloticus* evaluated.

## ANTICIPATED BENEFITS

The above study illustrates that sex determination in tilapia is a complicated mechanism of genetic expression not following a monofactoral mode of control. This degree of complication suggests that even a highly selective program for developing brooders for a YY breeding program will not be effective. This study illustrates the need for developing other methods of controlling tilapia reproduction.

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