METHODS OF IDENTIFYING MALES WITH YY GENOTYPE IN *Oreochromis niloticus* L.

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Abstract

Two studies were conducted, during the development of the YY male technology for production of genetically male tilapia, to test hypotheses related to maximizing the probability of identifying males with YY genotype during progeny testing. The first study investigated the hypothesis of differential feminization of XY and YY genotypes following only partial sex reversal of progeny from XY x YY crosses. The second study compared the morphometric and meristic characters in sibling XY and YY male genotypes using discriminant function analysis. In the first study non-reversed males and sex reversed females from three treated families of XY x YY crosses in the Egypt-Swansea strain were progeny tested. Among the three families tested only one family seems to support the hypothesis ($P<0.05$), the rest have genotypes in accordance with the expected (1:1) in both sex-reversed females and non-reversed males. In the second study, out of 42 males characterized, the selected predictor variables correctly classified 95% of XY-males and 95.5% of YY-males.

Introduction

A great deal of research effort has been invested in the development of monosex male populations of tilapia to exploit their benefits for aquaculture. One of the developments from this research is the YY male technology for the production of genetically male tilapia (GMT) derived from novel YY "supermales". The development of this technology is well described by Mair *et al.* (1997). However, the time to generate and identify YY-males using standard methods of breeding and progeny testing is quite long requiring up to 52 generations. One step in the development of this technology involves the testing of progeny from crosses of XX x XY genotypes to identify the YY male genotypes. The absence of easily identifiable sexual dimorphisms in tilapia sex chromosomes (Harvey *et al.*, 2003) and the lack of obvious phenotypic characters of YY-males creates a limitation in the development of this technology. The usual method of progeny testing to identify YY-males, in which males are crossed to females of known genotype and progeny are on-grown for three months prior to sexing, is both time consuming and labour intensive. As a result relatively few YY-males can be identified at a time causing a genetic bottleneck in the breeding programme. The development of a technique which can be effectively

applied to discriminate or increase our ability to identify YY males from a pool of XY and YY-males would be highly beneficial in the development of this technology.

This study tests two hypotheses that were formulated to address this constraint in the breeding programme. The first hypothesis relates to the potential differential feminization of XY and YY genotypes. This hypothesis was developed following the poor results from feminization treatments during the initial attempts to sex reverse progeny from XY x YY crosses by exogenous application of estrogens through the diet. It was hypothesized that genetic males with a YY genotype are more difficult to feminize and thus would occur in a higher frequency in non-reversed males than in feminised females or untreated fish. The second hypothesis relates to the possible existence of genotype correlation with one or more morphological and/or meristic characters. The identification of a character or combination of characters that can discriminate the genotypes would be highly desirable in improving the efficiency of this stage of the breeding programme.

Materials and methods

Source of broodstock

Families of three XY x YY crosses from an Egypt-Swansea strain were used in this study. This strain which originated from Lake Manzala in Egypt was introduced to the Philippines from a laboratory population held in the U.K. and was the main strain used in the initial development of the YY male technology. Males from this cross are expected to have either a YY or XY genotype in a ratio of 1:1. During the process of attempting to produce larger numbers of YY males, several families from an XY female crossed to three different YY males were produced. To generate YY females, one half of the progenies from these families were feminized by oral application of Diethylstilboestrol (DES) at a dosage of 1000 mg.kg^{-}○ (Mair and Santiago, 1994; Vera Cruz *et al.* 1996) of feed in cages in an outdoor concrete tank . The remaining untreated fish were left as a control. Both treated and untreated families were then on-grown to broodstock size (mean weight of 30 grams) under similar conditions in cages in a pond. A sub-sample of 1/3 of each group was sexed using a gonad squash technique described by Guerrero and Shelton (1974) when they attained a mean weight of at least two grams.

The hypothesis of differential feminization

Hormone treatment of three families resulted in only partial feminization (Table 1). A number of non-reversed males and sex reversed females from the DES treated families were progeny tested, with the exception of Family 3, where all the females were lost during the grow-out. Prior to progeny testing the fish were tagged to provide individual identification. Each male to be tested was stocked with three females in a 1 m; fine mesh hapa. Females for testing were stocked with single male. Collection of fry or eggs followed after every 7 days until most of these males and females had spawned. Progeny were sexed by gonad squash when they attained an average weight of at least two grams.

Table 1. Number, sex ratio and percentage of females from DES treated progeny (and their untreated controls) indicating the extent of partial feminisation.

Family	No of samples	Sex Ratio M:F	% Female
Family 1	40	16:24	60.0
Control 1	32	32:0	0.0
Family 2	72	64:8	11.1
Control 2	60	60:0	0.0
Family 3	30	12:18	60.0
Control 3	30	30:0	0.0

The hypothesis of morphological and meristic differentiation

Males from a single XY x YY cross were reared together for one year in a cage in pond. A total of 63 of these sibling males were characterized for 21 morphological and meristic characters as shown in Table 3. Following characterization, males were tagged for identification. Progeny testing was done following procedures described for the first study.

Data analysis

In the identification of genotypes, the progeny sex ratios were tested against an expected ratio of 1:1 using the chi-squared test. In the assigning of genotypes, the following criteria were followed:

Level of significance of $\chi 5$ test against 1:1 ratio	Genotype assigned
$P<0.05$	XY
$0.05<P<0.01$	??
$P<0.001$	YY

In the correlation of morphometric and meristic characters with genotype, discriminant function analysis was applied to the data from the 21 characters, to determine the weighted linear composite of the predictor variables that most accurately separated the two genotypes.

Results

The hypothesis of differential feminization

The results of progeny testing of males and females from three partially feminized XY x YY families are summarized in Table 2. In Family 1, all nine females tested were found to have XY genotypes which is significantly different from the expected 1:1 ($P<0.01$) and among the eight non-reversed males tested, seven YY-males were identified again significantly different from 1:1 ($P<0.05$). The genotype ratios from the other two families tested did not differ from the expected ($P<0.05$).

Hypothesis of morphological and meristic differentiation

A total of 20 XY-males and 22 YY males were identified from among the characterized males. The proportion is within the expected (1:1). A discriminant function determined from 21 predictor variables correctly classified 95% of XY-males and 95.5% of YY-males.

Table 2. Summary of the results of progeny testing of non-feminised males and feminised females from three partially feminized families from XY x YY crosses.

	Family 1		Family 2		Family 3[a]
	female	male	female	male	male
Total number of fish tested	9	8	12	13	48
Total number of fingerlings sexed	431	396	572	824	2957
Total number of YY genotypes identified	0	7	4	5	28
Total number of XY genotypes identified	9	1	8	8	20
Expected number of YY genotypes	4.5	4	6	6.5	24
Chi-squared value of observed vs. expected	9.00 **	4.50 *	1.33 ^{ns}	0.69 ^{ns}	1.33 ^{ns}
Mean of % male from YY genotype	N/A	96.0	98.0	88.6	94.8

* = P< 0.05 ** = P< 0.01 ns = not significant a = females were not tested

Table 3. Variable means (standard deviation) of XY and YY genotypes, standard canonical discriminant function coefficient (SCDFC) and pooled within group correlation between discriminating variables and canonical discriminant function(r5).

Variable	Description	XY Mean (+/- sd)	YY Mean (+/- sd)	SCDFC	r5
AFR	Number of anal fin rays	10.50(0.50)	10.50(0.51)	0.147	0.000
DFR	Number of dorsal fin rays	12.80(0.41)	12.82(0.39)	0.454	0.158
DFS	Number of dorsal fin spines	15.85(0.59)	15.68(0.65)	0.568	0.095
LLSCL	Lower lateral line scales count	13.95(1.19)	13.41(1.10)	1.124	0.166
LLSCU	Upper lateral line scales count	21.35(0.81)	21.09(0.87)	0.223	0.108
CAUDBROK	Caudal fin bar (broken)	0.35(0.49)	0.45(0.51)	0.453	0.073
CAUDOK	Caudal fin bar (not broken)	0.50(0.51)	0.27(0.46)	0.639	0.165
GENPAPL	Genital papilla length	0.35(0.49)	0.36(0.49)	0.021	0.009
GENPAPW	Genital papilla width	0.45(0.51)	0.27(0.46)	1.031	0.129
BODDEP	Body depth (mm)	35.96(2.36)	34.64(2.60)	4.533	0.186
ED	Eye diameter (mm)	10.67(0.72)	10.28(0.45)	0.742	0.230
HEADL	Head length (mm)	37.42(1.78)	36.00(1.86)	2.697	0.272
IOW	Inter-orbital width (mm)	11.44(0.74)	11.13(0.81)	1.033	0.139
LAS	Length of anal spine (mm)	17.55(1.13)	16.90(1.15)	1.084	0.200
LCP	Length of caudal peduncle (mm)	11.96(0.89)	11.47(0.90)	0.101	0.192
LDS	Length of dorsal spine (mm)	17.04(0.91)	16.30(1.33)	0.289	0.224
LS	Length of snout (mm)	10.00(0.74)	9.63(0.75)	0.658	0.173
SL	Standard length (mm)	99.23(5.17)	96.06(6.53)	0.918	0.187
TL	Total length (mm)	125.77(6.97)	121.67(7.53)	3.574	0.198
WCP	Width of caudal peduncle(mm)	15.01(0.83)	14.43(1.00)	1.073	0.220
WEIGHT	Wet weight (g)	32.44(4.86)	28.70(5.82)	4.977	0.243

The standardized discriminant function coefficients reveal the composite is composed primarily from variance in size related morphometric variables such as WEIGHT, BODDEP, TL, with some contributions from GENPAPW, HEADL, IOW, LAS, LLSCL, SL and WCP. The canonical correlation (0.8245) showed that the discriminating function is highly correlated to the genotypes. A computed Wilk's Lambda (0.3202) significantly ($P<0.05$) supports the strength of the derived discriminant function.

Discussion and conclusions

In aiming to increase the chances of identifying a YY male from a pool of XY and YY males, progeny testing of non reversed males from previously feminized families might be effective considering the results from the Family 1 which supported our hypothesis of differential feminization. However, the results from the other two families did not support the hypothesis. The variable results of the tested families might be due to the inconsistency of the results using this sex reversal method, as shown in Table 1 and it is only under certain conditions that differential sex reversal takes place. Also the failure to test the genotypes of the females in Family 3 masked our capacity to further evaluate the hypothesis. In practical terms, if feminisation is being applied to the progeny of XY x XY or XY x YY crosses, it may be worthwhile concentrating progeny testing on non-reversed males. However, it clearly cannot be assumed, in all cases, that these non-feminised males will have higher than expected proportions of YY males among them. Following on from this work, feminisation methods were reoptimised resulting in

higher rates of feminisation (Mair and Santiago, 1994). This subsequently enabled production, and identification, by progeny testing, of YY females (Vera Cruz *et al.,* 1996 and Karayücel *et al.,* 2003), which then enabled mass production of YY males in YY x YY crosses (Mair *et al.*, 1997).

The discriminant function based on a number of morphometric and meristic characters was efficient in discriminating the two genotypes although not with 100% efficiency. This study should be repeated using other families of the same type of cross with varying size and age to verify the extent of its effectiveness and applicability. Calculation of the discriminant function is itself time consuming and progeny testing would still be required to be sure that selected fish were YY-male. However a high percentage of discrimination would make progeny testing much more effective.

Due to the remaining doubts of the universal applicability of either of these methods, neither are used routinely for identifying YY genotypes. The need for such technique is now reduced due to the availability of YY females but could nevertheless be applied to improve the efficiency of identifying YY genotypes in new strains.

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