Bulletin of the Japanese Society of Scientific Fisheries 51(8), 1219–1224 (1985)

Introgressive Hybridization in Cultured Tilapia Stocks in the Philippines*1

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(Accepted December 20, 1984)

Tilapia populations, assumed by culturists to be Nile tilapia *Oreochromis niloticus*, were sampled from five farms and one research center around Laguna de Bay, Philippines and their identity were investigated by starch gel electrophoresis of polymorphic and monomorphic isozymes and by isoelectric focusing of sarcoplasmic protein markers. A reference stock of *O. mossambicus* from natural waters was similarly examined.

Data from morphological characters were inconclusive. However, from 20 biochemical loci investigated, five isozyme and two sarcoplasmic protein loci were found to be reliable species specific markers and from this evidence the introgressive hybridization by *O. mossambicus* was found in all the assumed Nile tilapia populations. The percentage of pure *O. niloticus* individuals with no *O. mossambicus* biochemical markers ranged from 0–40 between populations. Individuals heterozygous for all the biochemical marker loci (assumed to be F_1 hybrids) were rare. The mean percentage frequency of *O. mossambicus* marker alleles in the six populations was 13.6 (range 8.2 to 17.9). Their genic variabilities, indicated by proportion of polymorphic loci ($P=0.30$ to 0.40) and mean expected heterozygosity ($H^e=0.047$ to 0.104), were almost all higher than published values for Egyptian *O. niloticus* ($P=0.18$, $H^e=0.061$). For the Philippine *O. mossambicus* examined and for wild Egyptian fish, the respective values of P and H^e were 0.10 and 0.021 compared to 0.05 and 0.002. For the six Philippine *O. niloticus* populations, the ratios of observed to expected heterozygosities (H^o: H^e) were all >1.000 (range 1.021 to 1.103).

The importance of conservation of tilapia genetic resources, the need to ascertain the identity of farmed and experimental populations, the provision of single species and hybrid seed for culturists and future approaches to genetic improvement are discussed relative to these results,

The identity of Philippine cultured tilapia has been little studied. The principal species farmed is the Nile tilapia *Oreochromis niloticus* but other species, notably *O. mossambicus*, are well established in fish ponds and natural waters. The current status of the industry is described by Smith *et al.*[1] A decline in the culture performance of some farmed stocks, reported by farmers but not well documented, has stimulated interest in studying the genetics of cultured tilapias in the Philippines and initiating stock improvement programs. It is widely suspected that interbreeding of wild *O. mossambicus* with cultured *O. niloticus* stocks may be a factor in their decline in performance. Hybrids between these two species can be readily identified by biochemical markers.[2–5]

MACARANAS *et al.**5 found *O. mossambicus* alleles at sarcoplasmic protein and glucose phosphate isomerase (GPI) loci in assumed Philippine *O. niloticus* and suggested a more detailed study using additional markers and sampling additional populations. This study was therefore undertaken to investigate assumed *O. niloticus* populations sampled from five farms and one research center/government hatchery in the Philippines. It formed part of a continuing program of research cooperation between the Fish Ecology Laboratory of Kochi University, the Marine Science Center of the University of the Philippines (UPMSC) and the International Center for Living Aquatic Resources Management (ICLARM).

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Table 1. Standard lengths and meristic counts for tilapia, assumed to be *O. niloticus*, sampled from five farms and one research center/hatchery (SEAFDEC, Laguna Lake, Philippines and *O. mossambicus* from Concepcion market, Malabon, Manila

Farm populations assumed to be *O. niloticus*	Year population established at sample location	No. of fish sampled	Standard length range (cm)	Dorsal spine				Mean
				XV	XVI	XVII	XVIII	
Farm No. 1	1978	20	17.7–21.6	0	7	13	0	16.7
2	1981	16	14.0–20.5	0	5	11	0	16.7
3	1981	20	10.6–12.9	1	8	11	0	16.5
4	1980	20	14.4–20.9	0	6	14	0	16.7
5	1982	20	8.4–11.2	0	4	15	1	16.9
SEAFDEC population (assumed to be *O. niloticus*)	1979	20	12.6–18.4	1	10	9	0	16.4
O. mossambicus	N/A	20	9.7–12.5	3	16	1	0	15.9

Dorsal rays				Mean	Anal rays						Mean	Dark colour bars of caudal fin									Mean
11	12	13	14		8	9	10	11	12	13		<5	6	7	8	9	10	11	12	13<	
0	9	10	1	12.6	0	7	13	0	0	0	9.7	0	0	0	1	2	3	5	5	4	11.4
1	7	7	1	12.5	1	9	6	0	0	0	9.3	0	0	0	4	6	1	1	2	2	9.8
0	8	12	0	12.6	0	12	8	0	0	0	9.4	2	1	8	5	4	0	0	0	0	7.4
1	7	10	2	12.7	0	11	9	0	0	0	9.5	0	4	4	6	2	3	1	0	0	7.9
1	7	9	3	12.7	1	14	5	0	0	0	9.2	8	10	2	0	0	0	0	0	0	5.6
1	2	16	1	12.9	0	6	14	0	0	0	9.7	1	2	4	8	0	1	2	1	1	8.3
3	15	2	0	12.0	0	0	3	14	2	1	11.1	—	—	—	—	—	—	—	—	—	—

Materials and Methods

Six populations of cultured tilapias, assumed to be Nile tilapia *Oreochromis niloticus*, from five farms around Laguna Lake, Philippines and one research center/government hatchery—the Freshwater Fisheries Station, Binangonan, Rizal, Philippines of the Aquaculture Department of the Southeast Asian Fisheries Development Research Center (SEAFDEC)—were sampled in October–November 1983. The farm populations examined all originated from stocks maintained and distributed by the Philippine Bureau of Fisheries and Aquatic Resources, SEAFDEC and other commercial farmers, and were established in the locations sampled between 1978 and 1982 (Table 1). All the farms sampled are involved in seed supply to other farmers. A population of *O. mossambicus* from Philippine natural waters was sampled at Concepcion market, Malabon, Metro Manila for comparison.

The numbers of dorsal spines, dorsal and anal fin-rays and dark colour bars on the caudal fin were counted for 16–20 fish from each population. Tissue samples (skeletal muscle, liver, heart and eye) were taken from 16–20 fish from each population and subjected to horizontal starch gel electrophoresis and polyacrylamide gel isoelectric focusing, following the procedures given in previous papers.[6,7] For reliability and quality of separation and stability, the following enzymes and proteins were chosen: aspartate aminotransferase (AAT, E.C. 2.6.1.1.), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), esterase (EST, E.C. 3.1.1.1.), glucosephosphate isomerase (GPI, E.C. 5.3.1.9.), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42.), lactate dehydrogenase (LDH, E.C. 1.1.1.27.), malate dehydrogenase (MDH, E.C. 1.1.1.37.), malic enzyme (Me, E.C. 1.1.1.40.), phosphoglucomutase (PGM, E.C. 2.7.5.1.), sorbitol dehydrogenase (SDH, EC. 1.1.1.14), superoxide dismutase (SOD, E.C. 1.15.1.1.), and sarcoplasmic proteins (SP). The loci identified and tissue selected were as described by BASIAO and TANIGUCHI[5] and MACARANAS *et al.**

* see page 1219.

Table 2. Allele frequencies for isozyme and sarcoplasmic protein loci in tissue samples from five farm populations and one research center/hatchery population (SEAFDEC) of tilapia around Laguna Lake, Philippines, assumed to be *Oreochromis niloticus*

Locus*1	Allele	Farm					SEAFDEC	Total
		1 n=20	2 n=16	3 n=20	4 n=20	5 n=20	n=20	n=116
1. Isozymes								
Aat-1	100	0.975	0.969	0.875	0.950	1.000	0.950	0.918
	46	0.025	0.031	0.125	0.050	0.000	0.050	0.082
Aat-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Adh	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Est-e**2	100	0.975	0.969	0.950	0.850	0.875	0.950	0.917
	105	0.025	0.031	0.050	0.150	0.125	0.050	0.083
Gpi-1	100	0.750	0.750	0.850	0.875	0.925	0.775	0.823
	120	0.250	0.250	0.150	0.125	0.075	0.225	0.177
Gpi-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-1	100	1.000	1.000	1.000	.1000	1.000	1.000	1.000
Ldh-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ldh-3	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Mdh-1**3	100	0.100	0.125	0.175	0.275	0.100	0.250	0.172
	80	0.900	0.875	0.825	0.725	0.900	0.750	0.828
Mhd-2	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-3	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sdh	100	0.875	0.906	0.725	0.925	0.875	0.875	0.853
	133	0.125	0.094	0.275	0.075	0.125	0.125	0.147
Sod	100	0.950	0.875	0.850	0.850	0.975	0.900	0.901
	60	0.050	0.125	0.150	0.150	0.025	0.100	0.099
2. Sarcoplasmic proteins								
Sp-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sp-2	100	0.950	0.938	0.775	1.000	0.825	0.850	0.888
	80	0.050	0.062	0.225	0.000	0.175	0.150	0.112
Sp-3	100	0.875	0.906	0.875	0.925	0.900	0.850	0.888
	80	0.125	0.094	0.125	0.075	0.100	0.150	0.112

*1 For detail of tissue and loci see text and Basiao and Taniguchi[5].

*2 *Est-e*: specific in eye tissue.

*3 *Mdh*-1,2 and 3 here correspond to *m-Mdh*, *s-Mdh*-1 and *s-Mdh*-2 of Basiao and Taniguchi[5].

Results

Table 1 gives the meristic characters for samples of assumed. *O. niloticus* from the five tilapia farms and SEAFDEC and the *O. mossambicus* from natural waters. Meristic data were not useful for discriminating the two "species", though the mean values in these characters were significantly different with each other.

Among the 20 loci investigated, as biochemical markers, eight were polymorphic in the assumed *O. niloticus* stocks sampled: the isozymes *Aat-1*, *Est-e*, *Gpi-1*, *Mdh-1*, *Sod*, *Sdh* and the sarcoplasmic proteins *Sp-2* and *Sp-3* (Table 2). Table 3 compares these with information on species-specific markers published previously. It appears that *Aat-1*, *Gpi-1*, *Sdh*, *Sod*, *Sp-2* and *Sp-3* are the most reliable markers for separating *O. niloticus* and *O. mossambicus*. They can be used for estimating degree of introgression of wild *O. mossambicus* into cultured *O. niloticus* stocks, by calculating allele frequencies (Table 4). The frequencies of *O. mossambicus* alleles in the assumed *O. niloticus* populations sampled here were relatively stable between loci compared, except for *Aat-1* which was not completely divergent between the species.

Genic variability in the six assumed *O. niloticus* populations, as indicated by the proportion of polymorphic loci (0.30–0.40) was high in comparison with published values for supposedly pure species[4] and also fluctuated between populations (Table 5). These high values and the fact that the ratios of observed: expected heterozygosity (H^o: H^e) were greater than 1.0 also indicate the presence of hybrids in the Philippine populations. The frequency distribution of the numbers of heterozygous loci per individual shows that *O. mossambicus* alleles are well introgressed into the *O. niloticus* gene pools. However, only two F_1 hybrid individuals, heterozygous in the almost of all polymorphic loci examined, were observed in Farm 3 and 4 and individuals lacking any *O. mossambicus* alleles were also rare (Table 6).

Table 3. Allele frequencies of species-specific isozyme and sarcoplasmic protein marker loci for *Oreochromis niloticus* and *O. mossambicus*

Locus	Allele	*O. niloticus* 1*1	*O. niloticus* 2*2	*O. mossambicus* 1*1	*O. mossambicus* 3*3
Isozymes					
Aat-1	*100*	1.000	0.944	0.000	0.250
	46	0.000	0.056	1.000	0.750
Gpi-1	*100*	1.000	1.000	0.000	0.000
	120	0.000	0.000	1.000	1.000
Mdh-1	*100*	—	0.582	—	0.975
	80	—	0.418	—	0.025
Sdh	*100*	—	0.982	—	0.000
	133	—	0.018	—	1.000
Sod	*100*	1.000	1.000	0.000	0.000
	60	0.000	0.000	1.000	1.000
Sarcoplasmic proteins					
Sp-2	*100*	—	1.000	—	0.000
	80	—	0.000	—	1.000
Sp-3	*100*	—	1.000	—	0.000
	80	—	0.000	—	1.000

*1 Auburn University, Alabama populations[5].
*2 Osaka population[5].
*3 Present study.

Table 4. Mean frequencies of alleles derived from *O. mossambicus* in the samples taken from six populations of five farms and one research center (SEAFDEC) of tilapias, assumed to be *O. niloticus* in the Philippines

Locus	Frequencies of allele derived from: *mossambicus*	*niloticus*
Isozymes		
Aat-1	0.082	0.918
Gpi-1	0.179	0.821
Mdh-1	0.171	0.829
Sdh	0.136	0.864
Sod	0.100	0.900
Sarcoplasmic proteins		
Sp-2	0.110	0.890
Sp-3	0.120	0.880
Grand mean frequency	0.136	0.864

Table 5. Genic variability in wild and cultured populations of *Oreochromis niloticus* and *O. mossambicus* as indicated by their proportion of polymorphic loci and average heterozygosity

Population	Number of fish sampled	Number of loci investigated	Proportion of polymorphic loci	Average observed heterozygosity	Expected heterozygosity	H^o/H^e
Philippine assumed *O. niloticus* (present study)						
Farm 1	20	20	0.40	0.075	0.068	1.101
Farm 2	16	20	0.40	0.081	0.071	1.018
Farm 3	20	20	0.40	0.108	0.104	1.034
Farm 4	20	20	0.40	0.095	0.092	1.038
Farm 5	20	20	0.30	0.048	0.047	1.011
SEAFDEC	20	20	0.40	0.090	0.088	1.023
Japanese *O. niloticus*[5]	55	35	0.34	0.091	0.088	1.034
Egyptian *O. niloticus*[4]	50	22	0.18	0.058	0.061	0.818
Philippine *O. mossambicus* (present study)	20	20	0.10	0.018	0.021	0.826
Egyptian *O. mossambicus*[4]	40	22	0.05	0.002	0.002	1.000

Table 6. Frequency distribution (%) of heterozygous loci per individual in Philippine tilapia populations*1

Number heterozygous loci per individual	Assumed *O. niloticus* populations						*O. mossambicus*
	Farms						
	1 n=20	2 n=16	3 n=20	4 n=20	5 n=20	SEAFDEC n=20	Malabon, Manila n=20
0	15.0	0.0	10.0	20.0	40.0	10.0	65.0
1	45.0	56.3	20.0	30.0	35.0	35.0	35.0
2	20.0	25.0	40.0	10.0	15.0	35.0	0.0
3	15.0	18.7	20.0	30.0	10.0	10.0	0.0
4	5.0	0.0	5.0	5.0	0.0	5.0	0.0
5	0.0	0.0	0.0	0.0	0.0	5.0	0.0
6	0.0	0.0	0.0	5.0	0.0	0.0	0.0
7	0.0	0.0	5.0	0.0	0.0	0.0	0.0
H*2	0.075	0.081	0.108	0.095	0.048	0.090	0.018

*1 Five farm populations and one research center (SEAFDEC) population of assumed *O. niloticus* from around Laguna Lake and *O. mossambicus* from Malabon, Manila.

*2 Average observed heterozygosity.

Discussion

Our results support and enlarge on the conclusions of MACARANAS *et al.*[*1] that Philippine farmed and experimental populations, assumed to be *O. niloticus*, have experienced widespread introgression of *O. mossambicus* genes. The implications of this for culture performance require further investigation. F_1 hybrids of these species were produced for a time by Taiwanese culturists because of their fast growth.[8-9] However, their use has been largely discontinued in favor of higher performance *O. niloticus* ♀ × *O. aureus* ♂ hybrids and various hormonally sex-reversed single species and hybrids.[10]

The introgression of *O. mossambicus* genes into Philippines *O. niloticus* populations is unlikely to be beneficial. Experience in the Philippine tilapia culture industry (albeit largely unsupported by quantitative data) suggests that the more such introgression that has occurred, as judged by the *O. mossambicus*-like appearance of the fish, the lower their culture performance.[*2] Indeed it would be difficult to conceive how uncontrolled mixing and interbreeding of wild and cultured fish could produce populations with stable high performance traits like the above-mentioned *O. niloticus* × *O. aureus* hybrids, in which the beneficial cross to produce nearly all-male progeny is unidirectional and reciprocal crosses and backcrosses must be eliminated by rigorous segregation of broodstocks.

The maintenance of pure species is very important for fish breeding and stock improvement work and the use of introgressed hybrid populations mixed with wild fish (which are inherently unstable) can not be condoned for experimental farming studies. For the future, new founder stocks of commercial tilapia strains should be established and monitored regularly for distribution to fishseed suppliers wishing to upgrade their broodstocks. The requirements for this are discussed by MIRES[11] and WOHLFARTH and HULATA[12] using the Israeli system of hybrid fry production as an example.

The present paper also shows that Philippine populations of assumed *O. niloticus* have higher level of genic variability than those published for wild Egyptian fish, a result of creation of new genotypes through genetic recombination, a characteristic of introgressed populations. The lack of F_1 hybrids, observed from the meristic and electrophoretic data, is further indication that introgression is well established in the *O. niloticus* gene pools. Some farmers have attempted to clean their *O. niloticus* stocks by removing and destroying fish which resemble *O. mossambicus*. They do not realize that this can not be a successful method. However, this selection against *O. mossambicus* characters has involved unconscious selection against the other characters, which were therefore not as discriminating as the electrophoretic data obtained in our study.

Further investigations of phenotypic variability

*1 See page 1219,

*2 Personal communication from M. C. BROUSSARD.

in other morphological characters and performance traits, such as growth rate and salinity tolerance, are needed to understand fully the status and culture potential of these Philippine stocks. It is possible that some of them may be useful for selective breeding programs in working farms if systematic selection can be applied to a wide range of variation in culture performance traits. However, the substitution for introgressed stocks by well-defined strains and hybrids from reliable breeding centers is preferable for selective breeding and hybridization programs and is vital for experimental studies.

Acknowledgement

The authors wish to thank Dr. E. D. GOMEZ (UPMSC), Dr. R. A. NEAL (ICLARM), Dr. A. OCHIAI (Kochi University) for their encouragement and support during these studies. The services of Misses Nelda A. DANETARAS, Josefa R. PANTE (UPMSC) and Josephine B. CAPILI (ICLARM), research assistants to the project, are also gratefully acknowledged. The UPMSC-ICLARM program on fish genetics, of which this study formed part, is supported by a grant from the International Development Research Center of Canada. The visit of the senior author to the Philippines for this work was supported by the Japan Society for the Promortion of Science (JSPS). The authors also wish to thank Mrs. Z. U. BASIAO, SEAFDEC and the farmers who provided fish samples for their help and cooperation.

References

1) I. R. SMITH, E. B. TORRES, and E. O. TAN: ICLARM Conference Proceedings No. 10, pp. 1–45 (1983).
2) L. KORNFIELD, U. RITTE, C. RICHLER, and J. WAHRMAN: *Evolution*, **33**, 1–4 (1979).
3) R. E. BRUMMET: M. Sc. Thesis of Auburn University, Auburn, Alabama, 1982, pp. 1–40.
4) B. J. MACANDREW and K. C. MAJUMDAR: *Aquaculture*, **30**, 249–261 (1983).
5) Z. U. BASIAO and N. TANIGUCHI: *Aquaculture*, **38**, 335–345 (1984).
6) N. TANIGUCHI and K. NUMACHI: *Bull. Japan. Soc. Sci. Fish.*, **44**, 1351–1355 (1978).
7) N. TANIGUCHI, K. SUMANTADINATA, A. SUZUKI, and J. YAMADA: *Bull. Japan. Soc. Sci. Fish.*, **48**, 139–141 (1982).
8) H. KUO: Jt. Comm. Rural Recontr. (Chinese-American) Taiwan Fish. Ser. No. 8, 116–117 (1969).
9) T. P. CHEN: Aquaculture practices in Taiwan, Fishing News Books Ltd., Farnham, Surrey, England, 1976, pp. 1–161.
10) R. S. V. PULLIN: in "International Symposium on Tilapia in Aquaculture" (compiled by L. FISHELSON and Z. YARON), Proceedings Tel Aviv University, Tel Aviv, 1983, pp. 64–76.
11) D. MIRES: In "The biology and culture of tilapias" (ed. by R. S. V. PULLIN and R. H. LOWE MCCONELL), ICLARM Conference Proceedings No. 7, Manila, Philippines, 1982, pp. 317–329.
12) G. W. WOHLFARTH and G. HULATA: ICLARM Studies and Review No. 6, 1–26 (1983).