

**Karyotype Analysis of the Hybrid, Thai Red Tilapia  
(*Oreochromis niloticus* Linn. X *Oreochromis mossambicus* Linn.)**

Jiradej Manosroi, <sup>1</sup>Keravit Petchjul, <sup>2</sup>Umnat Mevatee and Aranya Manosroi  
Division of Pharmaceutical Sciences, Faculty of Pharmacy/Pharmaceutical Cosmetic Raw  
Materials and Natural Products Research and Development Center (PCRNC),  
Institute for Science and Technology Research and Development (IST),  
Chiang Mai University, Chiang Mai, Thailand,  
<sup>1</sup>Department of Fisheries, Faculty of Agriculture,  
Rajamangala University of Technology, Kalasin Campus, Kalasin, Thailand  
<sup>2</sup>Department of Anatomy, Faculty of Medicine,  
Chiang Mai University, Chiang Mai, Thailand

---

**Abstract:** Thai Red Tilapia is the hybrid fish between *Oreochromis niloticus* Linn. and *O. mossambicus* Linn. The chromosomal constitution of this hybrid were investigated using high quality metaphase preparations obtained from their bone marrow. The diploid chromosome of Tilapias consisted of 22 chromosome pairs ( $2n = 44$ ). However, the morphology of their chromosome types were different. The karyotype of *O. niloticus* consisted of 18 subtelocentric (st), 26 acrocentric (a) whereas 6 submetacentric (sm), 10 st, 28 a and 2 sm 6 st, 36 a were observed in *O. mossambicus* and Thai Red Tilapia respectively. The arm number of *O. niloticus*, *O. mossambicus* and Thai Red Tilapia were 62, 60 and 52 respectively. The heterochromatic of the short arms of all Tilapias were also observed. The karyotypic differences between *O. niloticus*, *O. mossambicus* and the hybrid, Thai Red Tilapia will be useful for strain classification and the improvement of commercial Tilapias production.

---

**Key words:** Thai red tilapia, *Oreochromis niloticus*, *O. mossambicus*, karyotype

---

**Introduction**

Tilapias are native fresh water fish of Africa (Trewawas, 1983). They are one of the commercial important perch-like fishes in the family Cichlidae. Tilapias are one of the largest taxonomic groups of fishes, with more than 1500 species in Africa were found (Nelson, 1994). Tilapias are one of the most important species in aquaculture with the world-wide production exceeds 1 million metric tons per year (FAO, 1997). They have been introduced either accidentally or deliberately to many countries around the world in the last five decades (Pillay, 1990). This is due to the fact that Tilapias are easily growing fish species since they eat a variety of foods, resist to diseases and grow well in poor quality water with low dissolved oxygen (Mair and Roberts, 1988). Some Tilapias can either survive in fresh, brackish or sea water. Several culturing methods of Tilapias have been developed in Asia. Thai Red Tilapia is the hybrid between

*Oreochromis niloticus* Linn. and *O. mossambicus* Linn. (Jarimopas, 1988). It has been originally found from the Ubonratchathani Freshwater Fisheries Station, in North-Eastern Thailand in 1968. This hybrid Tilapia can grow well in various favorable environments. It has red color which is distinctly different from the greyish black color of its parents (Jarimopas, 1988). In Thailand, Thai Red Tilapia is one of the most popular Tilapias because of its economic values. The organoleptic test compared between the Thai Red Tilapia and Tilapia indicated about 80% of the testers preferred the Thai Red Tilapia to the Tilapia for its nice-looking colour, softness and fatty test of the flesh. Thus, commercial culture of Tilapias has focused on Thai Red Tilapia and *O. niloticus*. The most important breeding goal of Tilapias is to improve growth rate and feed conversion efficiency, which are the prime factors for the possible commercialization of this fish. The karyotypic of this fish has never been investigated. Infact, this information is valuable for strain classification and cross-breeding between different species for the improvement of its commercial production (Purdom, 1993). For fish karyotypic analysis, samples can be prepared from several organs such as kidney, testis, gill filament, fin and fish embryos (Denton, 1973). So far, the direct methods using the squash technique for chromosomal preparation has been widely used. Nevertheless, only a small number of mitotic cells which are frequently broken is obtained (Hartley and Horne, 1985; Al-Sabti, 1985). In this study, the fresh flush cut bone marrow technique which has never been applied in fish chromosome study is used to obtain high quality metaphase spread for karyotypic identification of the hybrid, Thai Red Tilapia.

#### Materials and Methods

Tilapias used were 100-150 g in the body weight and the length of 8-12 cm. *O. niloticus* and the hybrid, Thai Red Tilapia were reared and obtained from the Department of Fisheries Technology, Faculty of Agricultural Production, Maejo University, Chiang Mai. *O. mossambicus* was obtained from the National Aquaculture Genetic Research Institute, Department of Fisheries, Pathumthanee, Thailand.

Specimens for chromosomal studies were obtained from bone marrow of 8 fish. Cell division was arrested at metaphase using colchicine solution (Sigma Co. USA) at the concentration of 1 µg/100 g of body weight. Flush cut bone marrows obtained from the central vertebrae were resuspended in RPMI 1640 (Gibco BRL, Life Technologies). Cells were incubated with cold 0.075 M KCl solution for 30 mins prior to fixing in a fresh solution of acetic acid/methanol (1:3) for 10 mins, 20 mins and 30 mins respectively as described by Fukasawa *et al.* (1997) with some modifications. Cell suspension was dropped on the slides, air dried and stained with 5% Giemsa solution for 15-20 mins.

Slides were examined under a light microscope (Zeiss Axioskop, Germany) equipped with a 35 mm camera (Zeiss M35W 476012, Germany). Metaphase chromosomes were examined at 1000x magnification, photographed and counted. Representative metaphases were printed on high contrast paper and the karyotypes were arranged according to chromosomal classification of Levan *et al.* (1964).

## Results

Tilapia 150 metaphases were analysed. The modal number of chromosome of all Tilapias was 44 ( $2n$ ) with the frequency of about 90% from the 150 analysed metaphases (Fig. 1). In some metaphase spreads, the numbers of chromosome were 42 and 43, which were likely due to the loss during chromosome preparation and handling. The karyotypes of *Oreochromis niloticus*, consisted of 18 subtelocentric (st), 26 acrocentric (a) and 62 arm number (NF) whereas 6 submetacentric (sm), 10 st, 28 a, 60 NF and 2 sm, 6 st, 36 a, 52 NF were observed in *O. mossambicus* and Thai Red Tilapia respectively (Table 1). Examples of the photomicrographs of the metaphase spreads and the karyogram were shown in Fig. 2 and 3.

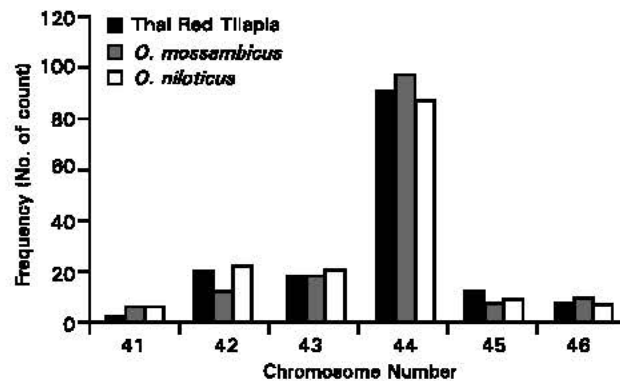


Fig. 1: Frequency distribution of chromosome counts of 150 metaphase spreads obtained from bone marrow of Tilapias.

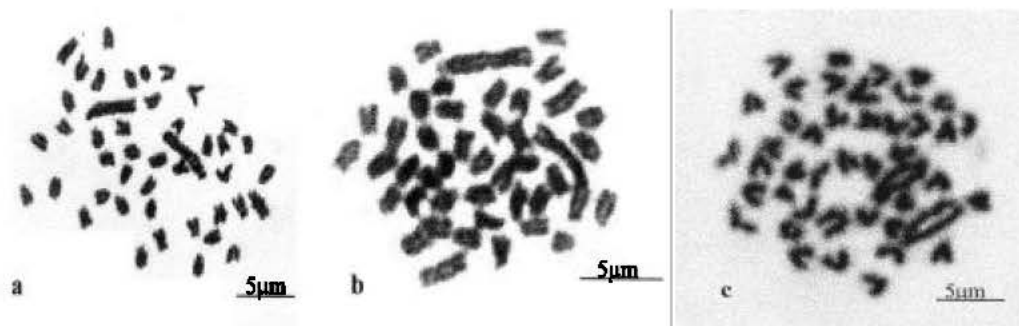


Fig. 2: Photomicrographs of the metaphase spreads of Tilapias  
 (a) *O. niloticus* (b) *O. mossambicus*  
 (c) Thai Red Tilapia (*O. niloticus* × *O. mossambicus*)

## Discussion

As known, chromosomes of the Tilapias are small and generally less than 5 microns. Some of them are much smaller than those of mammalian chromosomes (Carrasco et al., 1999). Hence,

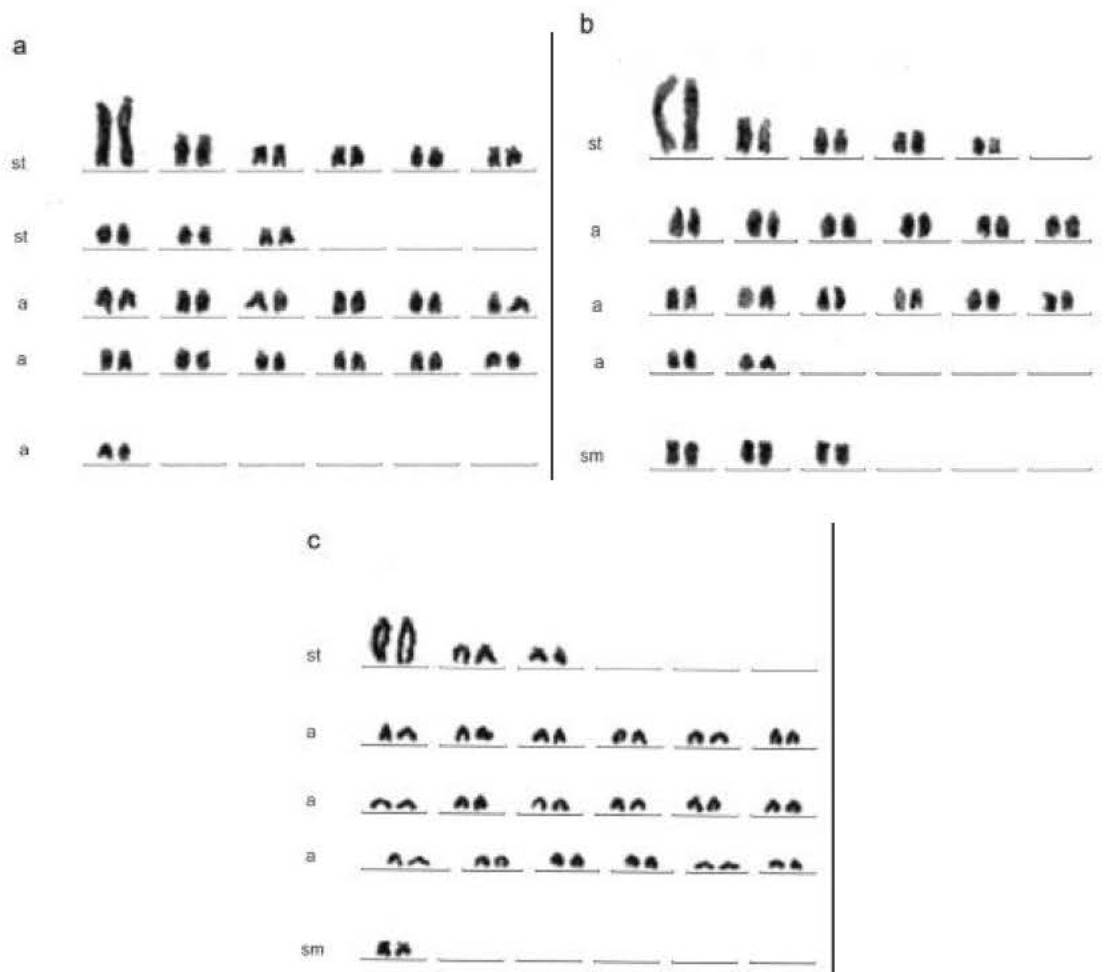


Fig. 3: Karyogram of Tilapias  
 (a) *Oreochromis niloticus*  
 (b) *O. mossambicus*  
 (c) Thai Red Tilapia (*O. niloticus* x *O. Mossambicus*)

Table 1: The Morphological comparison of chromosome type of Tilapias

| Type of Tilapias                                                | No. of Chromosome Types |    |    |    |
|-----------------------------------------------------------------|-------------------------|----|----|----|
|                                                                 | sm                      | st | a  | NF |
| <i>O. niloticus</i>                                             | -                       | 18 | 26 | 62 |
| <i>O. mossambicus</i>                                           | 6                       | 10 | 28 | 60 |
| Thai Red Tilapia<br><i>O. niloticus</i> x <i>O. mossambicus</i> | 2                       | 6  | 36 | 52 |

sm = submetacentric      st = subtelocentric      a = acrocentric      NF = arm number

methods which yield an adequate numbers of well spread metaphase chromosomes for the investigation of fish chromosomes are needed. In this study, dividing bone marrow cells were used. About 1 to 3% of these cells were in metaphase and the chromosomes can be distinguished. They were well spread without overlapping and suitable for karyotypic arrangement.

Jarimopas (1988) concluded that Thai Red Tilapia is the hybrid of *Oreochromis niloticus* Linn. and *O. mossambicus* Linn. Thus, the gene frequency of *O. niloticus* and *O. mossambicus* were 78% and 22% respectively as detected by Sterling University and The University of Philippines using the electrophoresis method. In this study, the analysis of chromosomes of the Tilapias by fresh flush cut bone marrow technique was relatively convenient since well spread metaphases can be obtained easily. The chromosome numbers of all Tilapias were found to be 44 (diploid, 2n) which was the same as that of *Oreochromis niloticus* previously described (Oliveira and Wright, 1998). However, one major karyotypic difference was observed. The karyotype of hybrid, Thai Red Tilapia could be differentiated karyotypically from the parental species *O. niloticus* and *O. mossambicus* on the basis of the relative lengths of the three largest chromosome pairs (Fig. 3). Karyological analysis of hybrids has been shown to be an accurate method to define the genetic structure of other hybrid fishes (Chevassus, 1983). The diploid karyotypes of the reciprocal hybrids are composed of a haploid set from each parental species. Karyotypes of Thai Red Tilapia exhibited the large paired acrocentric chromosome which inherited from its parent. This hybrids also possed two more additional acrocentric chromosomes which were different from *O. mossambicus*. Infact, the relative length difference between the largest chromosome pairs of *O. niloticus*, *O. mossambicus* and the hybrid, Thai Red Tilapia was obscured by the karyotypic diversity among populations. These data suggest that the hybrid was a true biparental diploid with no spontaneous gyro-or androgenesis.

Although the hybrid, Thai Red Tilapia showed a high growth rate in favorable environments under less optimal condition, the hybrid fish tends to have stress and had a relatively low survival rate with only average fecundity (Macaranas *et al.*, 1997). The difference in karyotype of Thai Red Tilapia from the *O. niloticus* and *O. mossambicus* might be due to the genotype interaction. The results obtained from this study will be useful for the determination of karyotypic diversity and the breeding improvement of Tilapias as well.

#### Acknowledgements

This work was supported by grants from the Graduate School, Chiang Mai University, Chiang Mai, Thailand and Rajamangala Institute of Technology, Kalasin Campus, Kalasin, Thailand. The authors would like to thank Dr. Sabine Mai for her suggestions in this study.

#### References

- Al-Sabti, K., 1985. Chromosomal studies by blood leukocyte culture technique on three salmonids from Yugoslavian waters. J. Fish Biol., 26 : 5-12.

- Carrasco, L.A.P., D.J. Panman, A.S. Villalobos and B. Niall, 1999. The effects of oral administration with 17  $\alpha$  - methyltestosterone on chromosomal synapsis in *Oreochromis niloticus* (Pisces, Cichlidae). *Mutation Res.*, 430: 87-98.
- Chevassus, B., 1983. Hybridization in fish. *Aquaculture*, 33: 245-262.
- Denton, T.E., 1973. Fish chromosome methodology. Charles C. Thomas Publisher, 29-30.
- FAO., 1997. Review of the state of world aquaculture. FAO Fisheries Circular. No. 886, Rev.1. FAO, Rome, pp: 163.
- Fukasawa, K., F. Wiener, G.F. Vande Woude and S. Mai, 1997. Genomic instability and apoptosis are frequent in p53 deficient young mice. *Oncogene* 15 : 1295-1302.
- Hartley, S.E. and M.T. Horne, 1985. Cytogenetic techniques in fish genetics. *J. Fish Biol.*, 26: 575-582.
- Jarimopas, P., 1988. Thai Red Tilapia. *J. Thai Fish. Gazet.*, 41: 41-43.(In Thai).
- Levan, A., K. Fredkaand and A.A. Sandberg, 1964. Nomenclature for centromeric position on chromosome. *Hereditas*, 52: 201-220.
- Macaranas, M., B. Mather, N. Lal, V. Tavenisa, L. Maciu and F. Capra, 1997. Genotype and environment:A comparative evaluation of four Tilapia stocks in Fiji. *Aquaculture*, 150: 11-24.
- Mair, J.F. and R.J. Roberts, 1988. Recent advances in aquaculture, pp: 407.
- Nelson, J.S., 1994. Fishes of the world, 3<sup>rd</sup> ed. New York. John Wiley and Sons.
- Oliveira, C. and J.M. Wright, 1998. Molecular cytogenetic analysis of heterochromatin in the chromosomes of Tilapia, *Oreochromis niloticus* (Teleostei : Cichlidae). *Chromosome Res.*, 6: 205-211.
- Pillay, T.V.R., 1990. Aquaculture principles and practices. Aquaculture development and coordination programme. Food and agriculture organization of the united nations. Italy, pp: 376.
- Purdom, C.E., 1993. Genetic and fish breeding. Ministry of Agriculture, Fisheries and Food, pp: 133.
- Trewavas, E., 1983. Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia. London : British Museum (Natural History).