UNIVERSITI PUTRA MALAYSIA

COLOUR INHERITANCE IN TIGER BARB (PUNTIUS TETRAZONA)

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COLOUR INHERITANCE IN TIGER BARB (*Puntius tetrazona*)

By

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COLOUR INHERITANCE IN TIGER BARB (*Puntius tetrazona*)

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A study to identify the colour inheritance of Tiger barb (*Puntius tetrazona*) through Mendelian inheritance was conducted in the Biotechnology Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for the duration of 10 months. Three varieties of Tiger barb that is the normal, yellow and green were subjected to three experiments consisting of breeding within and between the varieties and egg and larval observation. The result of the first breeding experiment produced 11 to 153 fries after 5 weeks with 15% to 85% survival. The normal and yellow variety produced phenotypes which were the same as their parents which indicated that these varieties were true breeding. The green variety showed a segregation producing normal and green variety with a N:G ratio of 15:1, 1:1, 3:1, 1:3 and 9:7. Breeding of the normal variety produced 5 different normal variations with mating number 5 producing 84% fries with type 2 pattern. The study has succeeded in producing a new type of normal variety.

Crossbreeding experiments between the 3 varieties of Tiger barb produced 15 to 182 fries after 5 weeks with survival ranging from 60.0% to 86.3%. The breeding resulted 100% normal phenotype in N×Y and G×Y crosses which showed that the normal variety was the dominant phenotype. Crosses between N×G however produced 2 and 3 phenotypes that is the normal and green with 2 N:G ratios of 9:7 and 3:1 and normal, green and yellow with N:G:Y ratio of 7:6:3. The results proved the occurrence of epistasis during the breeding between these 2 varieties and further indicated that colour was controlled by 2 autosomal genes with a simple Mendelian inheritance for the normal and yellow mating and epistatic interaction in the green mating.

Egg and larval observations of Tiger barb were carried out. The size of the eggs ranged from 1.07 mm to 1.22 mm and hatched within 27 to 30 h. The newly hatched larvae ranged from 2.89 mm to 3.12 mm. The mouth was functional 4 days after hatching. The egg yolk was totally absorbed 8 days after hatching. Pigmentation of the eyes began on the second day. The larvae reached juvenile stage 21 days after hatching.

In the normal larvae, the banding patterns of the bands began to develop as spots of melanophore located along the body where the tail was the first to accumulate. The movement of melanophore was slowly distributed and the 4 bands on the body was seen developing 15 days after hatching. In the green larvae, the melanophore was observed dispersed all over the body while there was no melanophore development in the yellow larvae. The colour of the fins was present 40 days after hatching. The presence of melanin eye indicated that the yellow variety was not an albino. The result of the experiment indicated that the larval stage could be used as biological markers to the determine the varieties.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEWARISAN WARNA DALAM IKAN TIGER BARB (*Puntius tetrazona*)

Oleh

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Satu kajian menentukan pewarisan warna badan pada ikan Tiger barb (*Puntius tetrazona*) melalui pewarisan Mendel telah dijalankan selama 10 bulan di Makmal Bioteknologi, Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia dengan menggunakan 3 jenis variasi iaitu normal, hijau dan kuning. Tiga eksperimen telah dijalankan iaitu pembiakan di dalam setiap variasi, kacukan di antara variasi dan penelitian perkembangan telur dan larva ikan Tiger barb

Di dalam eksperimen pertama, jumlah fri yang terhasil selepas 5 minggu ialah di antara 11 hingga 153 fri dengan 15% hingga 85% kemandirian. Pembiakan variasi normal dan kuning menghasilkan fenotip yang sama dengan induk. Ini menunjukkan bahawa kedua-dua variasi ini adalah pembiak tulin. Pemerhatian yang lebih lanjut mendapati variasi kuning bukan albino kerana terdapat kehadiran melanin pada mata. Variasi hijau pula menghasilkan 2 jenis fenotip iaitu normal dan hijau dengan nisbah N:G 15:1, 1:1, 3:1, 1:3 dan 9:7. Kacukan variasi normal menghasilkan 5 jenis corak. dengan kacukkan ke-5 menghasilkan 84% fri mempunyai corak jenis kedua. kajian ini telah berjaya menghasilkan variasi baru.

Eksperimen kacukan di antara variasi ikan Tiger barb menghasilkan di antara 15 hingga 182 fri dengan 60.0% hingga 86.3% kemandirian. Kacukan N×Y dan G×Y menghasilkan 100% variasi normal menunjukkan bahawa variasi normal ialah variasi dominan. Kacukan N×G pula menghasilkan 2 dan 3 jenis fenotip iaitu normal dan hijau dengan nisbah N:G 9:7 dan 3:1 serta variasi normal, hijau dan kuning dengan nisbah N:G:Y 7:6:3. Ini membuktikan kewujudan epistasis dalam kacukan N:G yang seterusnya menunjukkan bahawa warna badan pada ikan Tiger barb dikawal oleh 2 gene dengan mengikut pewarisan Mendel pada variasi normal dan kuning manakala mengikut tindakbalas epistasis pada hijau.

Perkembangan telur serta larva ikan Tiger barb mendapati saiz telur ialah di antara 1.07 mm hingga 1.22 mm dan mengambil masa di antara 27 hingga 30 jam untuk menetas. Larva yang baru menetas berukuran di antara 2.89 mm hingga 3.12 mm. Mulut mula berfungsi 4 hari selepas menetas. Yolka telur diserap sepenuhnya 8 hari selepas menetas. Pigmen pada mata mula terbentuk 2 hari selepas menetas. Larva mencapai peringkat juvenil 21 hari selepas menetas.

Dalam larva normal, corak jalur mula terbentuk sebagai satu garisan bintik melanofor di sepanjang badan di mana bahagian ekor ialah tempat pengumpulan yang pertama. Melanofor kemudian bergerak membentuk 4 jalur hitam pada ikan Tiger barb yang jelas kelihatan pada fri yang berumur 15 hari. Melanofor pada larva hijau tersebar keseluruh badan manakala tiada melanofor kelihatan semasa perkembangan larva kuning. Warna pada sirip ikan mula kelihatan 40 hari selepas menetas. Hasil penelitian mendapati peringkat larva boleh digunakan sebagai penanda biologi bagi menentukan jenis variasi yang terhasil daripada pembiakan.

CHAPTER I

INTRODUCTION

Ornamental fish include a considerable number of species and variety of colours and colour patterns within each species. The more exotic or unique an aquarium fish is, the higher is its commercial value. The exotic colours and striking varied forms of fishes mainly account for the worldwide interest in ornamental fish. Now through current advance in biotechnology and genetics, a lot of varieties can be obtained.

The ornamental fish industry plays an important role in the fisheries sector in Malaysia by their increasing profit from RM 14 million in 1991 (Malaysia, 1991) to RM 61 million in 1997 (Thalathiah, 1998). There are many local species that are potentially marketable as ornamental fish such as Harlequin fish (*Rasbora heteromorpha*), Arowana (*Scleropages formosus*) and Tiger barb (*Puntius tetrazona*).

Ornamental fish are kept in captivity for their aesthetic appeal. Breeding such fish for sale is of very great importance world-wide as a trade and as a hobby. The breeding of fancy fish is conducted professionally on large fish farms and also on a very small scale by individuals who rate it as a hobby. It probably involves far more practitioners than other forms of fish farming and generates highly significant financial turnover in rich and poor nations alike (Purdom, 1993).

The ornamental fish industry has been in Malaysia for a very long time. Although there are many local farmers who have been doing selective breeding to get new varieties, no local breakthrough has been reported. The question whether the fish is sustainable, cannot be determined. Having paucity of information, there is an urgent need to improve or maintain the produced variety.

In order to sustain the interests of fish hobbyists, it is crucial to improve the quality of the fish in terms of colour and patterns. This can be done by screening and carefully picking out spontaneous mutants that may arise in the stocks and subsequently by fixing the genes and building up a new line over several generations. Another way is by hybridization between the varieties to achieve new combinations of colour patterns (Phang and Fernando, 1989).

The success of production of these superior offspring is due to proper breeding programmes. Breeding methods are diverse and tailored to the needs of individual species but one thing they all seem to have in common is that years of effort have generated distinct genetic varieties in most of the species bred. There are various breeding programmes that are useful in improving a population of fish genetically. Selective breeding and crossbreeding are the two traditional methods commonly used for these purposes (Tave, 1995).

Selective breeding is actually referred to as the genetic manipulation of a cultured species for the purpose of improving certain traits of interest to man (Newkirk, 1980). Fish offer advantages over other livestock for selective breeding procedures because of their high reproductive potential and low reproductive barriers (Ihssen, 1976). However, it is not widely practised on aquatic species (Refstie, 1990) and much of the huge range of variation seen in the colour of pet fish and also in some farm fish remains of unknown genetic derivation (Purdom, 1993).

There are many ways to study colour inheritance from selective breeding and to investigate genetic variability among the varieties since the progeny produced from the breeding programme generally follows a simple Mendelian inheritance. The understanding of this inheritance can be useful in developing a suitable breeding plan to maintain a desired variety (Koren *et al.,* 1994). Studies on colour inheritance have been done intensively by several researchers in Japanese carp (Gomelsky *et al.,* 1996; Cherfas *et al.,* 1992; Wohlfarth and Rothbard, 1991), goldfish (Rothbarth *et al.,* 1997), guppy (Phang and Fernando, 1989) and tilapia (Koren *et al.,* 1994; Wohlfarth *et al.,* 1990). The colouration of certain mutants derived from the present variety has been used as genetic markers in some of the breeding investigations (Gomelsky *et al.,* 1996; Wohlfarth and Rothbarth, 1991).

The application of genetic study can be a very useful tool in order to get accurate information about the newly produced varieties. Understanding the gene control of the colour phenotypes of different varieties is essential in improving the existing varieties (Phang and Fernando, 1989).

Although more sophisticated molecular techniques have been implemented in the surge of producing new products, we cannot exempt the importance of egg and larval development in any improved variety of fish. Studies of egg and larval development have attracted considerable interest from aquaculturists to biotechnologists. Many of the genetic improvements in fish through genetic engineering have been done through the manipulation of the genes from the egg.

Two basic fields of practical importance involve the processes of parthenogenesis and induced polyploidy both derived conceptually from the work of embryologists during the early years of this century (Purdom, 1983). Both methods are becoming popular and the use of these methods in ornamental fish are just waiting to be exploited.

Many local species that can be categorized as ornamental fish have been fully exploited for this industry. Most of them were obtained from the wild. One of the most popular kept aquarium fish is the Tiger barb (*Puntius tetrazona*) which is highly coloured, hardy and very adaptable to aquarium life. Small in nature with a maximum size of 6 cm, they are found mostly in Sumatra and Borneo (Axelrod, 1988). They are inexpensive and their striking colour has triggered fish hobbyist to own them. Up to date there are three varieties of Tiger barb that is the normal variety, the green variety and the yellow variety.

In Malaysia, several colour morphs are cultured mainly for export to the international market. Several mutants derived from Tiger barb culture were reported by Dinesh *et al.* (1992) which showed the possibility of producing new varieties from the present varieties. Although there are possibilities of producing various colourations and patterns from the existing varieties, it is difficult to sustain them without information of their genetic background.

A selective breeding programme on Tiger barb was reported by Gibbs (1992) to have produced a semi-translucent coral red variety. However no such variety was found in Malaysia. So far there have been few studies on the genetics of domesticated varieties of the Tiger barb. By understanding the colour inheritance and formulating a breeding programme, new varieties can be produced in mass production and thus increase the market value of this fish. In this context, it is necessary to investigate the heredity of the body colour in Tiger barb by proper selective breeding within the present varieties.

A research programme entitled "Development of DNA Markers in Tiger Barb (*Puntius tetrazona*)" has been started at Universiti Putra Malaysia. It was a topdown project supported by the Ministry of Science, Technology and Environment. The first phase in the genetic manipulation aspect was to determine the colour inheritance of Tiger barb.

As this research in ornamental fish is at the infancy stage in Malaysia, the logical starting point of research was to do a selective breeding programme within and among the existing varieties. In addition, the study of egg and early larval development was also initiated for further research on genetic manipulation.

Three experiments were conducted in this study. The first experiment was to run single pair matings within each variety to understand the breeding of Tiger barb. The second experiment done was mating between each variety to identify the phenotypic expression from each mating. In the third experiment, the successful matings were then studied for their egg and larval development. In addition, the larvae of each variety were observed for their pattern formation and colour development.

The study of inheritance could provide a better understanding on the body colouration of the Tiger barb. The study can be useful in encouraging the utilisation of selective breeding combined with genetic information to boost the production of other ornamental fish species in Malaysia.

Based on the above perspectives, the research has the following objectives :

1. To identify the phenotypic expression of the existing varieties.
2. To determine the colour inheritance of new strains or varieties of Tiger barb.
3. To identify the egg and larval development on the present varieties of Tiger barb.
4. To determine the pattern formation and colour development on the present varieties of Tiger barb.

CHAPTER II

LITERATURE REVIEW

Morphology

Taxonomy

Tiger barb is one of the most popular kept aquarium fish. They have always been popular. This is because they are highly coloured, generally peaceful, breed readily and are very adaptable to aquarium life (McInerny and Gerard, 1966). They are hardy and their flashy colours make them one of the most beautiful of the barbs (Axelrod, 1967). Tiger barb is a tropical freshwater fish which originated from Sumatra and Borneo (Axelrod, 1967; Gibbs, 1992; Ramshorst and Nieuwenhuizen, 1991; Short, 1992; Mills, 1991) but can also be found in Thailand (Ambak and Mohsin, 1990; Dreyer and Keppler, 1996).

The Scientific name of Tiger barb is *Puntius tetrazona*. They are members of the genus Puntius and comes under the family of Cyprinidae. Some reference and aquarium books have identified them either as *Barbus tetrazona* or *Capoeta tetrazona* (Axelrod, 1967). To some, the name barbus has become generally accepted but the name puntius has been widely used in some reference books and some scientific reports (Ramshorst and Nieuwenhuizen, 1991).

External Features

The commonly found Tiger barb is *Puntius tetrazona* (Plate 1). This species is also known as *Barbus sumatrana* on the basis of Bleeker's redescription from Sumatra but this name is a synonym of *Puntius tetrazona* (Axelrod and Schultz, 1990). Tetrazona refers to four distinct zones or four black bars on its body (Short, 1992). The first is from the top of the skull through the eye and covers the greater part of the cheek below. The second is more or less cone-shaped, just forward of the dorsal fin down the side of the body to just before the pelvic fins. The third stripe starts immediately behind the dorsal fin and continues into the first anal fin rays whereas the fourth starts at the beginning of the caudal peduncle crosses the base of the caudal fin.

McInerny and Gerard (1966) found that these bands reflect a deep bottle green colour when fish is exposed to some light. This might be due to the colour of the scales in which if the scale lies within the bands, it will have a beautiful shiny golden green edges (Ramshorst and Nieuwenhuizen, 1991).

Tiger barb has no barbel (Dreyer and Keppler, 1996; Gibbs, 1992). The body is deep and laterally compressed. The length of the Tiger barb ranged from 5 cm to a maximum of 7 cm (Axelrod, 1967; Ramshorst and Nieuwenhuizen, 1991; Sandford, 1995). The structure of the fins and scales is as follows, dorsal iv,8; anal iv,5; pectoral I,13 or 14; pelvic I,8; scales 23 (Axelrod and Schultz, 1990).

Body Colour

The body colour ranges from orange brown pales to a silvery hue below (Short, 1992) and some with red brown fading to silver (Mills, 1991) and golden brown (Gibbs, 1992), golden pink (Axelrod, 1967) and bronze or tan (Mills, 1981).

The pectoral and ventral fins are bright red. The dorsal fin is black at the base and is red beyond the black bar. The anal fin is bordered with red and intense red streaks appear in the upper and lower lobes of the tail. The other fins are transparent but tinged with red.

Plate 1: The Morphology of Tiger Barb (*Puntius tetrazona*)

Sex

The sexes can easily be distinguished. The males are slim and brightly coloured with more red in the fins and usually have a bright red or cherry red nose.

The females are plumper, more rounded and are less brightly coloured with heavier bodies. The pigmentation of the dorsal and anal fin rays is less vivid compared to the male. Generally the male is slightly smaller than the female with a size of about 5 cm while the female is approximately 5.6 cm (McInerny and Gerard, 1966).

Habitat

In the wild, they live in tropical streams where rivers that run through dense tropical rain-forest regions are generally soft and acidic (Mills, 1991). Generally Tiger barbs are kept in water with a temperature between 20-29 °C (Sandford, 1995; Axelrod, 1967), slightly acidic with pH between 6.5-6.8 and a soft to medium hard water with hardness up to 12 °DH (106.8 ppm). Although they live in soft acidic water, the fish is able to tolerate various ranges of pH (from 6.5 to 7.5) and water hardness.

Behaviour

The fish has a lively disposition and always keeps its fins erect and well spread. When resting, the body is often inclined nose downwards (McInerny and Gerard, 1966). They are frisky and fast swimmers and are inclined to playfully nip the fins of slower species (Axelrod, 1967). They should be kept in schools to dissuade them from bothering other fish (Axelrod, 1967; Gibbs, 1992; Sandford, 1995).

Tiger barb are omnivorous fish. They have excellent appetites and will eat both live and prepared food. They can take flake, frozen, live and green food