On the inheritance of the red colour in the Mozambique Tilapia *Oreochromis mossambicus*

J.T. FERREIRA

Department of Zoology, Rand Afrikaans University, P.O. Box 524, Johannesburg, 2000, South Africa

ABSTRACT

Several options for the inheritance of the red colour in the Mozambique tilapia, *Oreochromis mossambicus* are discussed. Breeding with only red mutants resulted in 100 % red progeny, while crossing typically coloured individuals and red mutants provided silvery coloured offspring. Interbreeding this offspring resulted in a mixture of red, red typical and typical coloured individuals. It is obvious that the red colour is not due to albinism nor is it the result of a single recessive gene. It is more likely the result of multiple gene interaction, involving several structural genes and at least one regulatory gene. The fact that this mutant breeds pure red makes it, for conservational purposes, a useful substitute for the red tilapia hybrid.

Verskillende opsies vir die oorerwing van die rooi kleur by die bloukurper, *Oreochromis mossambicus*, word bespreek. Uit die broei eksperimente het dit geblyk dat rooikleurige ouers 'n 100 % rooi nageslag lewer, terwyl die kruising daarvan met normale kleur individue, 'n silwerkleurige nageslag tot gevolg gehad het. Onderteling van laasgenoemde nageslag het 'n mengsel van slegs rooi, rooi-normale kleur en normale kleur individue opgelewer. Dit blyk duidelik dat die rooi kleur nie oorge-erf word as synde die resultaat van 'n enkele resessiewe geen nie. Dit is ook nie 'n geval van albinisme nie. Dit is meer waarskynlik die resultaat van poligeniese oorerwing waarby verskeie strukturele gene en ten minste een regulatoriese geen betrokke is. Die feit dat hierdie mutant slegs 'n rooi nageslag oplewer maak dit vanuit 'n natuurbewaringsoogpunt, 'n handige plaasvervanger vir die rooi tilapia hibried.

INTRODUCTION

This study deals with permanent colour differences which are the result of genetic polymorphism (polyphasy or polychromatism) or permanent colour differences which are the result of rare mutations. Exploitation of this genetic polymorphism and rare mutants in general, has led to the production of many different strains of a particular species. This is very much the situation in the aquarium trade and species such as *Carassius auratus*, the common goldfish, are now available in more than 120 different strains (Papworth 1984) — admittedly not all of which are colour varieties.

Genetic polymorphism, or more specifically, polychromatism, is also a relatively common phenomenon in the Cichlidae. Studies done by Fryer and Iles (1972) and Ribbink *et al.* (1983) on natural populations, indicate the important role of genetic polymorphism when considering the taxonomy and identification of species from several genera of the Cichlidae.

Different colour morphs are also present in the genera *Tilapia* and *Oreochromis*, i.e. typically coloured individuals — that is the ones that display the colour that characterises the species, as well as a red-orange strain.

The interest in this red coloured *Oreochromis* is due to the several advantages it holds for aquaculture and was therefore selected for by fish culturists. The red mutant with its bright red colour and absence of black colour on the peritonium is highly preferred by consumers in several countries because it resembles the very popular seabream, *Chrysophrys major* (Safriel & Bruton 1984). It is even reputed to have a better taste than the normally coloured fish (Trewavas 1983). It is this red mutant that was used to produce the now well known red tilapia — a hybrid which was obtained from crossing a red mutant of *O. mossambicus* and *O. niloticus* (Liao & Chen 1983). None of the red tilapia strains cultured in Taiwan and the Philippines, however, produce an all-red progeny. The Philippine strains is claimed to produce 70 % red progeny and the Taiwanese strain less consistent results (Safriel & Bruton 1984). Introduction of this hybrid into South African waters may pose a serious thread to conservation.

The uncontrolled distribution of several *Oreochromis* species together with the similarities among these species as well as their capacity to interbreed, has led to widespread contamination of some of the wild and cultured stocks (Lowe-McConnell 1982). Thus, instead of introducing an alien species, it seems sensible to use an indigenous species which will rule out any problems regarding the contamination of breeding stocks and will also enhance the chances of conserving the genetic purity of the indigenous species. It was therefore decided to investigate the inheritance of the red colour in *O. mossambicus*, a species indigenous to southern Africa to see whether a suitable strain can be established.

MATERIALS AND METHODS

Experimental Stocks

Five breeding families of 4 females and 1 male of the red mutant of *O. mossambicus* were selected. The fish weighed between 32 and 40 g and were kept in 400 ℓ aquaria. The aquaria were supplied with internal biological gravel filters. Every second week the aquaria were cleaned and 30 % of the water replaced. The water was continuously aerated and heated by means of thermostatically controlled, immersion heaters. Water temperature was maintained at 28° ± 1°C. The

fish were fed commercial fish pellets as a maintencance diet at 1−2 % of body mass per day. The aquaria were checked daily for nesting behaviour, spawning and mouthbrooding. The fertilized eggs were taken from the females, and hatched in breeding funnels connected to a recirculating system in which the water temperature was also maintained at 28° ± 1°C. After hatching the fry were transferred to 200 ℓ aquaria which were maintained in the same manner. At this stage the fish were fed with ground commercial fish pellets. When the fingerlings reached a mean mass of 10−25 g they were put into plastic pools, 1,0 m deep and 3,5 m in diameter. The pools were supplied with a continuous flow of air and the water quality was maintained through a recirculating biological filter.

After four months the fish were collected from the plastic pools and breeding families (five consisting of five red mutant females and two typically coloured males each and five families consisting of five typically coloured females and two red mutant males each) were established in 400 ℓ aquaria. The typically coloured O. mossambicus were recruited from stocks that were collected in Roodeplaat Dam near Pretoria, Transvaal. The aquaria were maintained in the same manner as for the previous families. The offspring obtained were also transferred to plastic pools and after four months another set of five breeding families were put together from this red mutant and typically coloured crosses. As before, each breeding family consisted of five females and two males. The offspring of this cross were treated in the same manner as in the previous experiments.

Before transfer of the offspring to the pools, all fish were inspected individually, counted and their colour noted.

RESULTS AND DISCUSSION

From the results (Table 1) it is obvious that the red mutant of O. mossambicus breeds true red. This immediately distinguishes this strain from the red tilapia hybrid which does not produce 100 % all red progency. Thus anything other than all red offspring will indicate contamination of the breeding stock.

It can further be seen from the results that the inheritance of the red colour does not seem to follow a simple Mendelian pattern. Before considering the possible underlying genetic mechanism, it might be helpful to refer very briefly to the manner in which colours are produced in fish. The skin contains cells called chromatophores. These may be divided into melanophores, xanthophores and erythrophres. Normally the melanin produced by the melanophores dominates whence in the absence of melanin, the red and yellow colours produced by the erythrophore and xanthophores will be detected. Thus, the colours seen in an individual fish depend to a large extend on the numbers and the degree of expansion of the different types of chromatophores. Colours are also produced by reflecting cells called iridiocytes which contain guanin

(Fryer & Iles 1972). The colour differences in O. mossambicus seem to originate in the chromatophores (McAndrew pers. comm.)

Let us now then consider the different possibilities of inheritance of this red colour. Dzwillo (1962) remarked that colour variants are normally the result of single recessive alleles. Such a situation is found in the so-called blue carp where the blue colour is inherited as a simple recessive trait (Probst 1949; Moav & Wohlfarth 1968). From this single gene situation it is obvious that only the homozygous recessive genotype would produce the trait. The fact that in breeding programmes with red tilapia, normal colour progeny were also obtained, rules out this possibility, although experiments with pure O. mossambicus mutants produced all red progeny. However, according to the results (Table 1) crossing the typical colour with the red mutant gave an F1 progeny more silvery in colour (less melanin). Interbreeding this F1 progeny produced quite unexpected results (Table 1). Out of a total of 1 172 fish, 839 displayed silvery to normal colour, 241 the red colour and then 92 displayed the red colour with 7−9 dark vertical bands. This gives a ratio of 9,1 : 2,6 : 1 which does not fit the expected Mendelian ratio of 3 : 1 for a recessive allele at a single locus. It is therefore obvious that the red mutant in O. mossambicus is not inherited as a simple recessive trait.

The second possibility considered was that of incomplete dominance as is the case in the four o'clock plant where in the heterozygous state an intermediate phenotype is produced (Dustman 1971). The intermediate silvery colour obtained in the F1 progeny after crossing a normal O. mossambicus with a red mutant suggested that this option warranted consideration. However, the results obtained interbreeding the F1 population indicate that there is more to it than just incomplete dominance.

The inheritance of colour proves to be complicated and seems to be controlled by several genes − that is a multiple gene interaction. We may find a situation of collaboration where two or more genes interact to produce single character phenotypes that neither gene could produce alone, e.g. the combs in chickens (Crow 1983; Dustman 1971). Another option is allelic gene interaction through epistasis. This refers to genic interactions whereby one gene may or may not be expressed because of the presence of another gene, e.g. in mice; the gene coding for melanin (A-melanin; a-albino) and colour (B-agouti; b-intense uniform colour). "A" must be present otherwise neither B nor b will be expressed (Dustman 1971). Judging from the results (Table 2) it seems that epistasis may indeed be involved in the distribution and intensity of expression of the red colour as such. The recessive homozygous state (dd) of the epistatic gene would have no effect and the normal colour would be produced. The heterozygous state of the epistatic gene (Dd) would only lead to a partial inhibition of melanin production and melanophore development and distribution producing the silvery

colour or even red/black combinations, while the dominant homozygous state (DD) of the epistatic gene would have a very high inhibitory effect on melanin formation and melanophore development and therefore result in an all red specimen.

Against this background the following explanation is proposed: The presence and density of chromatophores in the skin is coded for by at least two pairs of alleles which are incompletely dominant. Epistasies is involved in the occurrence of albinism and in the distribution and intensity of expression of chromatophores, but not in the expression of the red color as such.

Several genes, which are incompletely dominant, code for the density of chromatophores. Fish without or with few chromatophores in the skin are red. Alleles that code for the absence of chromatophores are very rare in natural populations because of selection against silvery or red individuals (apparently through predation). Let us assume that alleles A and B code for the presence of chromatophores and alleles a and b code for the absence of chromatophores (more than two loci could be involved). Alleles a and b were probably absent in the experimental group of normal coloured fish (outcome of F1). The red individuals used in the trials probably all had the genotype aabb (because they bred true). If this was so, all the F1 individuals (silvery) were AaBb. The theoretical outcome of the F1 × F1 will depend on whether the two loci involved are situated on the same or on different chromosomes (occurrence or not of crossing over) and the distance between the loci (frequence of crossing over). In all the trials, except trial no. 3, approximately 20% of the offspring were red. This is more than the expected frequency of aabb, and if the reasoning is correct, some of the individuals identified as "red", must have had the genotype Aabb of aaBb. Further crosses with the F2 "red" individuals will show whether this is the case. The fact that the Taiwanese red tilapia does not breed true for red colour (which must be determined by the same genes: developed from "red" *O. mossambicus*), substantiates the hypothesis that red individuals are not homozygous. This is furthermore supported by the fact that red coloured individuals do vary in their amount of redness.

To conclude – the red colour in *O. mossambicus* seems to be coded through multiple gene interaction, involving several structural genes and at least one regulatory gene. Parents producing the all-red progeny, much sort after in aquaculture can, however, be achieved through careful selection programmes. Thus working with the indigenous *O. mossambicus* rules out the possibility of genetic contamination. Furthermore, red mutants that are by accident released into natural waterbodies would find it difficult to become established as a separate strain due to this intricate multiple gene interaction. Fishelson (pers. comm.) has further

TABLE 1: The number and colour of the offspring obtained in the different breeding experiments.

Pure red mutant breeding families					
Breeding families	1	2	3	4	5
No. of offspring Colour of offspring	152 True red	143 True red	157 True red	193 True red	211 True red

Cross between red mutant and typically coloured males					
Breeding families	1	2	3	4	5
No. of offspring Colour of offspring	193 Silvery	308 Silvery	267 Silvery	217 Silvery	245 Silvery

Cross between red mutant males and typically coloured females					
Breeding families	1	2	3	4	5
No. of offspring Colour of offspring	231 Silvery	219 Silvery	287 Silvery	185 Silvery	226 Silvery

TABLE 2: The offspring obtained by interbreeding of the red mutant and the typically coloured cross

Breeding families Total No. of offspring	1 167			2 341			3 172			4 239			5 253		
No. according to colour Colour of offspring* Ratio of different types	122 B/S 8,71	31 R : 2,21	14 B/R : 1,00	252 B/S 13,27	70 R : 3,69	19 B/R : 1,00	108 B/S 4,91	42 R : 1,91	22 B/R : 1,00	168 B/S 7,29	47 R : 1,97	24 B/R : 1,00	189 B/S 14,53	51 R : 3,92	13 B/R : 1,00

* B/S = Black (normal) to Silvery
R = Red
B/R = Red with 7–9 black vertical bars

found that whenever red mutants are kept with typically coloured fish, they are extremely susceptible to cannibalism and also predation by birds.

REFERENCES

BAREL, C.D.N., VAN OIJEN, M.J.P, WITTE, F. & WITTEMAAS, E.L.M. 1977. An introduction to the morphology of the haplochromine Cichlidae from Lake Victoria. *Neth. J. Zool.* 27: 33-389

CROW, J.F. 1983. Genetics notes. 8th ed. Minneapolis: Burgess Publishing Co. 306p.

DUSTMAN, J.H. 1971. General Biology. New York: McGraw-Hill. 119p.

DZWILLO, B. 1962. Über künstliche Erzeugung funktioneller Männchen zweiblichen Genotyps bei *Lebistes reticularis. Biol. Zentralbl.* 82(2): 575-584.

FRYER, G. & ILES, T.D. 1972. The cichlid fishes of the great lakes of Africa. Edinburgh: Oliver & Boyd. 641p.

LIAO, I.-C. & CHEN, T.-P. 1983. The status and prospect of tilapia culture in Taiwan. In: L. FISHELSON & Z. YARON (eds.). Proceedings of the International Conference on tilapias in aquaculture. Tel Aviv: Tel Aviv University: 588-598.

LOWE-McCONNELL, R.H. 1982. Tilapias in fish communities. In: R.S.V. PULLIN and R.H. LOWE-McCONNELL (eds.). The biology and culture of tilapias. *ICLARM Conference Proceedings* 7. Manila, Philippines: International Centre for Living Aquatic Resources Management: 83-113.

MOAV, R. & WOHLFAHRTH, G.W. 1968. Genetic improvement of yield in carp. *FAO Fish. Rep.* 4(44): 12-19.

PAPWORTH, D. 1984. A fishkeepers guide to garden ponds. London: Salmander Books Ltd. 117p.

PROBST, E. 1949. Der bläling-Karpen. *Allg. Fisch-Z.* 74(13): 232-238.

RIBBINK, A.J., MARSH, B.A., MARSH, A.C., RIBBINK, A.C. & SHARP, B.J. 1983. A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. *S. Afr. J. Zool.* 18(3): 149-310.

SAFRIEL, O. & BRUTON, M.N. 1984. Aquaculture in South Africa: A co-operative research programme. *CSIR Spec. Rep.* 59: 79p.

TREWAVAS, E. 1983. Tilapiine fishes of the genera *Sarotherodon, Oreochromis* and *Danakila.* London: British Museum for Natural History. 583p.