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RESEARCH NOTE

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Introduction

Of the numerous subtropical and tropical freshwater species of fish, labyrinth fish are among the most varied in body markings and colouration patterns. Four taxonomic families (Belontiidae, Anabantidae, Helostomatidae, and Osphronemidae) of the suborder Anabantoidei comprise the 'traditional' labyrinth fishes, a group of about 80 African and Southeast Asian species (Linke 1991). These Anabantoids are popular with aquarists due to their interesting reproductive behaviours, with males of most species brooding eggs in their mouths or in floating bubble nests (Vevers 1980; Linke 1991; Axelrod and Vorderwinkler 1995; Mills 2000). They have also been the focus of genetic, environmental, and morphological studies (Sommer 1982; Gosline 1985; Klinkhardt *et al*. 1995; Wakiyama *et al*. 1997; Frankel 1992, 2001, 2005).

The honey gourami, *Trichogaster chuna* Hamilton (Osphronemidae), is a popular labyrinth fish due to its peaceful nature, and the bright and attractive appearance of sexually mature males. Interestingly, the 'honey gourami' frequently offered for sale to the hobbyist is not *T. chuna* but, instead, a colour variety of *Colisa labiosa* Day (Osphronemidae). Further, while immature fishes of these species can easily be confused, they exhibit distinct genetic differences, e.g., $2n = 46$ and 48 for *T. chuna* and *C. labiosa*, respectively (Klinkhardt *et al*. 1995). Indigenous to northeast India, the habitat of *T. chuna* extends to the floodplain and delta of the Ganges, Jamuna in Bangladesh and to the Hugli river, west of Krishnagar and Kolkata (Linke 1991). Characteristically, sexually matured males exhibit a luminous red-orange hue, with the hypaxial region of the body and the spiny ray segment of the anal fin coloured dark blue to black. The spiny ray segment of the dorsal fin, to the upper part of the soft rays, is coloured in lemon yellow. Females of this species exhibit a grey-yellow hue with a single dark-brown stripe. A striking colour variant of the male honey gourami is a yellow phenotypic form that, as the name indicates, has a golden yellow body colouring with an orange-red hue on the caudal fin and the soft ray regions of both the dorsal and anal fins. Indeed, this is particularly an interesting colour variant of males in this species, since these yellow individuals lack the strong colour pattern intrinsic to the sexual dimorphism of the species.

As a result of ongoing work on the inheritance of colouration and banding patterns of teleostean fishes (Frankel 1992, 1997, 1998, 2001, 2002, 2004, 2005), studies on the mode of inheritance of the 'yellow' colour variant in *T. chuna* were undertaken. The segregation patterns observed in the male progeny from fifteen different crosses are consistent with a hypothesis that the inheritance of these colour phenotypes is controlled by the action of a single, autosomal locus acting in a sex-limited fashion, with dominance required for the expression of the characteristic red-orange '*chuna*' colouration.

Materials and methods

Healthy adult males of *T. chuna*, possessing either the characteristic of red-orange hue or the variant yellow colouration, and adult grey-yellow females, were obtained from a local wholesale distributor in Maryland, USA, and maintained in 76 l capacity holding tanks at 25°C. Sexually mature pairs exhibiting these phenotypes were selected at random from stock specimens and placed in separate 76 l capacity breeding tanks. All the fry for this study were obtained from natural mating as previously utilized (Frankel 1992, 2005). Optimal water conditions were provided for all sexually mature pairs (i.e., water hardness 5° or less, pH slightly acidic at 6.5, and temperature of 25°C) (Linke 1991). After each spawning, the female was removed from the breeding tank

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and the male was allowed to attend to the brood. After swim-up of the fry, individuals from each mating were placed in their own 76 l capacity rearing tank and allowed to develop until sexual maturity and their phenotypes visually discernable. Males exhibiting the red-orange (R) or lemon yellow (Y) phenotype, along with selected F1 progeny (F), were subsequently used in a series of controlled crosses, and the phenotypic data from matings exhibiting segregation ratios subjected to chi-square analysis.

Results and discussion

Probable genotypes, observed phenotypic numbers, expected ratios, chi-square values, and probability of fit for *T. chuna* analysed for the mode of inheritance of the yellow male variant of this species are given in table 1. All

parental, F_1 and F_2 male fishes displayed either the red-orange or yellow phenotypes, while females always exhibited the grey-yellow hue. Red-orange parental males R-1, R-2, R-3, and R-4 were scored as homozygous dominant, since all crosses involving these individuals resulted in red-orange male progeny (crosses 1–14). Parental red-orange males R-5, R-6, R-7, and R-8 were scored as heterozygous for a dominant allele, as crosses of these individuals with presumptive heterozygous females (crosses 15–19) confirmed from backcrosses (crosses 23–26), consistently resulted in a satisfactory fit to the expected male proportions of three red-orange: one yellow. The heterozygous nature of the R-5, R-6, R-7, and R-8 males was further confirmed from backcrosses with presumptive homozygous recessive females (F-III, F-IV, and F-V) which resulted in the expected colour proportions of one red-orange:

Table 1. Probable genotypes (PG), observed phenotypic numbers, expected male ratios, chi-square values (χ^2) and probability of fit (*P*) for crosses employing red-orange and yellow male *T. chuna*.

Cross no.	♂ (PG)	♀ (PG)	Red ♂ (PG)	Yellow ♂ (PG)	Grey ♀ (PG)	Expected ♂ ratio	χ^2	P
1	R-1 (*AA*)	G-I (*AA*)	26	0	14	1 : 0	-	-
2	R-2 (*AA*)	G-I (*AA*)	20	0	22	1 : 0	-	-
3	R-3 (*AA*)	G-II (*AA*)	31	0	36	1 : 0	-	-
4	R-2 (*AA*)	G-II (*AA*)	32	0	22	1 : 0	-	-
5	R-4 (*AA*)	G-II (*AA*)	37	0	34	1 : 0	-	-
6	R-4 (*AA*)	G-I (*AA*)	23	0	20	1 : 0	-	-
7	R-1 (*AA*)	G-III (*Aa*)	26	0	28	1 : 0	-	-
9	R-2 (*AA*)	G-III (*Aa*)	30	0	35	1 : 0	-	-
10	R-3 (*AA*)	G-IV (*Aa*)	33	0	40	1 : 0	-	-
11	R-4 (*AA*)	G-V (*Aa*)	14	0	10	1 : 0	-	-
12	R-1 (*AA*)	F-III (*aa*)	28	0	15	1 : 0	-	-
13	R-3 (*AA*)	F-V (*aa*)	22	0	25	1 : 0	-	-
14	R-4 (*AA*)	F-VI (*aa*)	36	0	29	1 : 0	-	-
15	R-5 (*Aa*)	G-III (*Aa*)	35	10 (F-3-*aa*)	42 (F-III-*aa*)	3 : 1	0.185	0.6669
16	R-6 (*Aa*)	G-III (*Aa*)	44	12 (F-4-*aa*)	37 (F-IV-*aa*)	3 : 1	0.381	0.5371
17	R-6 (*Aa*)	G-IV (*Aa*)	25	9 (F-5-*aa*)	24 (F-V-*aa*)	3 : 1	0.039	0.8430
18	R-7 (*Aa*)	G-III (*Aa*)	40	10 (F-6-*aa*)	45 (F-VI-*aa*)	3 : 1	0.667	0.4142
19	R-8 (*Aa*)	G-V (*Aa*)	44	14 (F-7-*aa*)	60	3 : 1	0.023	0.8795
20	R-5 (*Aa*)	F-III (*aa*)	23	21	44	1 : 1	0.091	0.7630
21	R-6 (*Aa*)	F-IV (*aa*)	34	29	50	1 : 1	0.397	0.5287
22	R-7 (*Aa*)	F-VI (*aa*)	26	33	51	1 : 1	0.831	0.3261
23	F-3 (*aa*)	G-III (*Aa*)	14	23	30	1 : 1	2.189	0.1390
24	F-4 (*aa*)	G-III (*Aa*)	22	19	25	1 : 1	0.220	0.6394
25	F-5 (*aa*)	G-IV (*Aa*)	34	39	61	1 : 1	0.342	0.5584
26	F-7 (*aa*)	G-IV (*Aa*)	12	10	25	1 : 1	1.200	0.2733
27	Y-1 (*aa*)	G-IV (*Aa*)	14	12	20	1 : 1	0.154	0.6945
28	Y-2 (*aa*)	G-III (*Aa*)	20	27	40	1 : 1	1.043	0.3072
29	Y-4 (*aa*)	G-III (*Aa*)	13	20	30	1 : 1	1.485	0.2230
30	Y-2 (*aa*)	G-II (*AA*)	30	0	37	1 : 0	-	-
31	Y-3 (*aa*)	G-I (*AA*)	14	0	19	1 : 0	-	-
32	Y-1 (*aa*)	F-IV (*aa*)	0	24	30	0 : 1	-	-
33	Y-2 (*aa*)	F-V (*aa*)	0	33	27	0 : 1	-	-
34	Y-3 (*aa*)	F-VI (*aa*)	0	40	33	0 : 1	-	-
35	Y-4 (*aa*)	F-III (*aa*)	0	21	14	0 : 1	-	-

*R, red-orange parental males; Y, yellow parental males; G, grey-yellow parental females; F, F₁ offspring. +The probability for all χ^2 tests is > 0.05; thus, observed results for all matings fit the expected ratio according to Mendelian inheritance.

one yellow of their male offspring (crosses 20–22). Males exhibiting the yellow colour variant (Y-1, Y-2, Y-3, and Y-4) were scored as homozygous recessives, as crosses between these individuals with presumptive heterozygous females resulted in a one red-orange: one yellow ratio of male progeny and in only red-orange males when mated with presumptive homozygous dominant females (crosses 27–29 and 30–31, respectively). Further, when mated with F1 females (F-III, F-IV, F-V, and F-VI) presumed to be homozygous for the 'yellow' allele, the resulting male progeny exhibited only the yellow phenotype (crosses 32–35); commensurate with the recessive nature of the sex-limited yellow colouration in male *T. chuna* (i.e. A_- is required for the red-orange phenotype in males of the species).

Since neither the number of loci affecting colouration patterns nor their chromosome positioning has been determined for *T. chuna*, the genetic basis for the inheritance of these patterns were inferred from breeding data. While epigenetic factors or sex-linkage limited to males cannot be ruled out as possible modes of inheritance (Basolo 2006), the ratios observed for progenies of *T. chuna* are clearly consistent with those expected for the alleles segregating on autosomal chromosomes. The results presented here support the hypothesis that the red-orange and yellow phenotypes in male *T. chuna* are due to the segregation of alleles at a single autosomal locus exhibiting sex-limited inheritance, with dominance required for the expression of the characteristic '*chuna*' red-orange colouration. Further, observations suggest complete expressivity of the *chuna* phenotype, since there are no perceived differences in either colouration patterns or intensities in presumptive homozygous or heterozygous male fishes.

In sexually mature males, the red-orange body hue with a lemon yellow dorsal and dark blue to black hypaxial region provides a striking sexual colour dimorphism with respect to females of this species, while yellow males and female fishes exhibit little of this dimorphic contrast. Yet, it is interesting to note that, based on preliminary laboratory observations of mate selection in *T. chuna*, there appears to be neither a positive nor negative assortative mating mate preference with regard to this trait.

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