

RESEARCH NOTE

Digenic control of colouration in the two-spot gourami *Trichogaster trichopterus trichopterus*

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Introduction

Labyrinth fishes of the families Anabantidae, Belontiidae, Helostomatidae, and Osphronemidae comprise the traditional anabantoids, a group of about 80 relatively small African and southeast Asian species (Linke 1991). Many of the anabantoids are popular with aquarium hobbyists because of 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 several environmental, morphological, and genetic investigations (Sommer 1982; Gosline 1985; Wakiyama *et al.* 1997; Frankel 1992, 2001). The two-spot gourami, *Trichogaster trichopterus trichopterus* Pallas (Osphronemidae), is one of the more popular labyrinth fishes due to its peaceful nature and attractive appearance of gold spotting on its dorsal and anal fins. Indigenous to southeast Asia, the distribution of *T. t. trichopterus* extends over a wide range, covering Thailand, Cambodia, Vietnam, Malaysia, Sumatra, and many of the surrounding islands (Linke 1991).

The subspecies *T. t. trichopterus* characteristically exhibits a bluish-grey hue and is further distinguished by the presence of two dark spots, one in the middle of the trunk and the other on the caudal peduncle. However, this spotting pattern is not a consistent feature of all *T. trichopterus*, as some subspecies clearly lack these markings. One striking variant of the two-spot gourami is a golden phenotypic form that, as the name indicates, has a very intense golden body colouring. Indeed, this is a particularly interesting colour variant of this subspecies, since these golden individuals lack the protective colouration afforded by the more bluish-grey phenotype. As a

part of ongoing work on the inheritance of colouration and banding patterns of teleostean fishes (Frankel 1992, 1997, 1998, 2001, 2002, 2004), studies on the mode of inheritance of the colour variants in the two-spot gourami were undertaken, and are reported here. Segregation patterns observed in the progeny from twelve different matings support the hypothesis that the inheritance of the blue-grey and gold phenotypes is controlled by the action of two independently assorting autosomal loci acting in a complementary fashion, with dominance at both loci required for the expression of the characteristic “trichopterus” blue-grey colouration.

Materials and methods

Healthy adults of *T. t. trichopterus*, possessing either the characteristic bluish hue (B) or the gold (G) colouration, were obtained from a local wholesale distributor in Virginia, U.S.A. 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 fry for this study were obtained from natural matings, as previously described (Frankel 1992). Optimal water conditions were provided for all sexually mature pairs as recommended for *Trichogaster* (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 and the male allowed to attend to the brood. After swim-up of the fry (72–120 h post-hatching), individuals from each mating were placed in their own 76 L capacity rearing tank and allowed to develop until their phenotype could be visually discerned. Parentals of both phenotypes (B and G) and selected F₁ progeny (F) were subsequently used in a series of controlled crosses, and the phenotypic data from all progeny recorded and subjected to chi-square analysis.

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Results and discussion

Probable genotypes, observed phenotypic numbers, expected ratios, chi-square values, and probability of fit for *T. t. trichopterus* analysed for the mode of inheritance of the gold variant of this subspecies are given in table 1. All parental, F₁ and F₂ fishes displayed either the blue-grey or golden phenotypes. Blue-grey parental females B-I, B-II, B-III, and B-IV and males B-1, B-2, and B-3

were scored as homozygous dominants, since all crosses involving these individuals resulted in blue-grey progeny (crosses 1-5, 11-16). Parental fishes exhibiting the gold colour variant (G-I, G-II, and G-III females and G-1, G-2, G-3, and G-4 males) were scored as homozygous recessives, as all crosses between these individuals consistently bred true (crosses 6-10). Furthermore, reciprocal crosses between homozygous parentals exhibiting the two distinct colouration phenotypes resulted exclusively

Table 1. Probable genotypes (PG), observed phenotypic numbers, expected ratios, degrees of freedom (*df*), chi-square values (*c*²) and probability of fit (*P*) for crosses among blue-grey and gold *Trichogaster trichopterus trichopterus*.

Cross no.	Parents*		Phenotypic numbers		Expected ratio	<i>df.</i>	<i>c</i> ²	<i>P</i> ⁺
	♀ (PG)	♂ (PG)	Blue-grey	Gold				
1	B-I (AABB) × B-1 (AABB)		26	0	1 : 0	—	—	—
2	B-II (AABB) × B-2 (AABB)		20	0	1 : 0	—	—	—
3	B-III (AABB) × B-1 (AABB)		22	0	1 : 0	—	—	—
4	B-I (AABB) × B-3 (AABB)		18	0	1 : 0	—	—	—
5	B-IV (AABB) × B-2 (AABB)		24	0	1 : 0	—	—	—
	Pooled		110	0	1 : 0	—	—	—
6	G-I (aabb) × G-1 (aabb)		0	18	0 : 1	—	—	—
7	G-I (aabb) × G-2 (aabb)		0	12	0 : 1	—	—	—
8	G-II (aabb) × G-2 (aabb)		0	16	0 : 1	—	—	—
9	G-III (aabb) × G-3 (aabb)		0	20	0 : 1	—	—	—
10	G-III (aabb) × G-4 (aabb)		0	19	0 : 1	—	—	—
	Pooled		0	85	0 : 1	—	—	—
11	B-I (AABB) × G-1 (aabb)		24(F-I & F-1)	0	1 : 0	—	—	—
12	B-II (AABB) × G-2 (aabb)		20(F-II & F-2)	0	1 : 0	—	—	—
13	G-I (aabb) × B-1 (AABB)		21(F-III & F-3)	0	1 : 0	—	—	—
14	G-II (aabb) × B-2 (AABB)		18(F-IV & F-4)	0	1 : 0	—	—	—
15	B-III (AABB) × G-3 (aabb)		15(F-V & F-5)	0	1 : 0	—	—	—
16	B-IV (AABB) × G-4 (aabb)		19(F-VI & F-6)	0	1 : 0	—	—	—
	Pooled		117	0	1 : 0	—	—	—
17	G-I (aabb) × F-2 (AaBb)		5	14	1 : 3	1	0.0175	0.8947
18	G-III (aabb) × F-3 (AaBb)		7	16	1 : 3	1	0.3623	0.5472
19	F-I (AaBb) × G-1 (aabb)		3	15	1 : 3	1	0.6666	0.4142
20	F-II (AaBb) × G-2 (aabb)		8	17	1 : 3	1	0.6533	0.4189
21	F-V (AaBb) × G-3 (aabb)		1	9	1 : 3	1	1.1999	0.2733
22	G-II (aabb) × F-1 (AaBb)		5	18	1 : 3	1	0.1304	0.7180
23	G-III (aabb) × F-6 (AaBb)		4	15	1 : 3	1	0.1578	0.6911
	Pooled		33	104	1 : 3	1	0.0608	0.8052
	Total					7	3.1878	0.8671
	Heterogeneity					6	3.1270	0.7927
24	F-I (AaBb) × F-1 (AaBb)		10	9	9 : 7	1	0.1010	0.7506
25	F-II (AaBb) × F-5 (AaBb)		13	9	9 : 7	1	0.0721	0.7883
26	F-III (AaBb) × F-2 (AaBb)		14	10	9 : 7	1	0.0423	0.8370
27	F-IV (AaBb) × F-4 (AaBb)		15	9	9 : 7	1	0.3809	0.5371
28	F-V (AaBb) × F-6 (AaBb)		12	12	9 : 7	1	0.3809	0.5371
	Pooled		64	49	9 : 7	1	0.0068	0.9342
	Total					5	0.9772	0.9643
	Heterogeneity					4	0.9704	0.9142

*(B) = blue-grey parentals; (G) = gold parentals; (F) = F₁ blue-grey offspring.

⁺The probability for all *c*² tests is > 0.05; thus, observed results for all matings fit the expected ratio according to Mendelian inheritance.

in blue-grey fry (crosses 11–16). When crossed to presumptive heterozygous fry (F–I, F–II, F–V females and F–1, F–2, F–3, F–6 males), gold parental females (G–I, G–II, G–III) and males (G–1, G–2, G–3) produced blue-grey and gold progenies in a ratio of 1 : 3 (crosses 17–23). Furthermore, commensurate with a modified 9 : 3 : 3 : 1 ratio resulting from dominant complementary gene action (i.e. $A_B_$ is required for the blue-grey phenotype), offspring from $F_1 \times F_1$ blue-grey heterozygotes conformed to the expected F_2 proportions of 9 blue-grey: 7 gold (crosses 24–28).

The results presented here clearly illustrate that segregation of the blue-grey and gold phenotypes in *T. t. trichopterus* fits an independently assorting autosomal dihybrid pattern of inheritance, controlled in a complementary fashion, with dominance at both loci required for the expression of the characteristic “trichopterus” blue-grey colouration. Further, the data suggest complete dominance of this phenotype, since there is no difference in the colouration between homozygous and heterozygous fishes. A similar pattern of inheritance has been reported for a blue colouration pattern in the *T. t. sumatranus* subspecies (Frankel 1992). In *T. t. trichopterus*, the bluish-grey body with prominent flank and caudal peduncle spotting most likely serves as a protective pattern, as compared to the distinctive colouration of its golden variant. Indeed, golden male and female *T. t. trichopterus* exhibit little, if any, of the protective and disruptive colouration patterns which characterize the species. Lastly, based on our laboratory observations of mate selection in *T. t. trichopterus*,

there appears to be no mate preference in either a positive or negative assortative manner with regard to this trait.

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