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SHORT COMMUNICATION

Inheritance of Humeral Spotting in the Croaking Gourami (Osphronemidae: *Trichopsis vittatus*)

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Abstract

The croaking gourami (*Trichopsis vittatus*) exhibits two phenotypes associated with humeral spotting. Fish possess a prominent, dark humeral patch or spot located behind the operculum or lack this spotting pattern. Segregation patterns observed from the progenies of eleven different crosses support the hypothesis that the inheritance of humeral spotting in *T. vittatus* is controlled by the action of a single autosomal locus, with complete dominance of the allele controlling the spotted phenotype.

Key Words: *Trichopsis vittatus,* croaking gourami, humeral spotting, Osphronemidae.

Introduction

Labyrinth fishes of the teleost families Osphronemidae, Helostomatidae, Belontiidae, and Anabantidae comprise the traditional anabantoids, a group of approximately 80 relatively small African and southeast Asian species (Linke, 1991). The anabantoids are quite popular with aquarium hobbyists for their interesting reproductive behaviors, with males of most species brooding eggs in their mouths or in a floating bubblenest (Linke, 1991; Axelrod & Vorderwinkler, 1995). The croaking gourami, *Trichopsis vittatus* (Cuvier, 1831), is named for its ability to produce an audible chirping noise, accomplished via the utilization of specialized adaptations of the pectoral fins (Henglmuller & Ladich, 1999). These chirping sounds are produced by both sexes during breeding and well-mated individuals can continue producing sounds for a few hours. Males alone may also produce such sounds during the establishment of dominance hierarchies (Ladich, 1998; Ladich et. al., 1992). The croaking gourami is an Asian anabantoid with a widespread distribution throughout Sumatra, the Sunda Islands, Malaysia, Thailand, and Vietnam (Linke, 1991; Axelrod & Vorderwinkler, 1995). This species occupies bodies of water ranging in size from pond-like accumulations of water to small rivers. In addition to its unique sound-producing ability, both female and male fishes in natu-

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Table 1. Probable genotypes (PG), observed phenotypic numbers, expected ratios, degrees of freedom (df), chi-square values (X^2) and probability of fit (P) for crosses amongst humeral spotted and unspotted *Trichopsis vittatus*.

Cross No.	Parents* ♀ (PG) x ♂ (PG)	Phenotypic Numbers Humeral Spotting	No Spotting	Exp. ratio	*df*	X^2	P^+
1	HI (*AA*) x H1 (*AA*)	24(FI&F1)	0	1:0			
2	HII (*AA*) x H2 (*AA*)	17(FII&F2)	0	1:0			
3	HIII (*AA*) x H3 (*AA*)	12	0	1:0			
4	HIV (*AA*) x H4 (*AA*)	26	0	1:0			
5	HV (*AA*) x H5 (*AA*)	20	0	1:0			
		Pooled 99	0	1:0			
6	NI (*aa*) x N1 (*aa*)	0	22	0:1			
7	NII (*aa*) x N2 (*aa*)	0	21(FIII&F3)	0:1			
8	NIII (*aa*) x N3 (*aa*)	0	19(FIV&F4)	0:1			
9	NIV (*aa*) x N3 (*aa*)	0	14(FV&F5)	0:1			
		Pooled 0	76	0:1			
10	HI (*AA*) x F1 (*AA*)	25	0	1:0			
11	FI (*AA*) x H1 (*AA*)	27	0	1:0			
12	HIV (*AA*) x F2 (*AA*)	16	0	1:0			
13	FII (*AA*) x H4 (*AA*)	18	0	1:0			
		Pooled 86	0	1:0			
14	NII (*aa*) x F3 (*aa*)	0	12	0:1			
15	NIV (*aa*) x F5 (*aa*)	0	10	0:1			
16	NIII (*aa*) x F4 (*aa*)	0	15	0:1			
17	FIII (*aa*) x N2 (*aa*)	0	11	0:1			
18	FV (*aa*) x N3 (*aa*)	0	22	0:1			
		Pooled 0	70	0:1			
19	HII (*AA*) x N1 (*aa*)	21(FVI&F6)	0	1:0			
20	HIII (*AA*) x N2 (*aa*)	32(FVII&F7)	0	1:0			
21	HV (*AA*) x N3 (*aa*)	10(FVIII&F8)	0	1:0			
22	NI (*aa*) x H2 (*AA*)	12(FIX&F9)	0	1:0			
23	NIII (*aa*) x H5 (*AA*)	15(FX&F10)	0	1:0			
		Pooled 90	0				
24	FVI (*Aa*) x F9 (*Aa*)	15	6	3:1	1	0.1429	0.7054
25	FVII (*Aa*) x F8 (*Aa*)	19	7	3:1	1	0.0513	0.8208
26	FVIII (*Aa*) x F7 (*Aa*)	12	5	3:1	1	0.1765	0.6743
27	FIX (*Aa*) x F6 (*Aa*)	16	5	3:1	1	0.0159	0.8996
28	FX (*Aa*) x F10 (*Aa*)	15	4	3:1	1	0.1579	0.691
29	FVI (*Aa*) x F10 (*Aa*)	22	7	3:1	1	0.0115	0.9146
30	FVII (*Aa*) x F6 (*Aa*)	21	8	3:1	1	0.1034	0.7477
		Total			7	0.6593	0.9986
		Pooled 120	42	3:1	1	0.0741	0.7854
		Heterogeneity			6	0.5852	0.9966
31	FVI (*Aa*) x F4 (*aa*)	18	15	1:1	1	0.2727	0.6015
32	FVII (*Aa*) x F5 (*aa*)	14	15	1:1	1	0.0345	0.8526
33	FIV (*aa*) x F7 (*Aa*)	12	10	1:1	1	0.1818	0.6698
34	FV (*aa*) x F8 (*Aa*)	9	11	1:1	1	0.2	0.6547
		Total			4	0.689	0.9526
		Pooled 53	51	1:1	1	0.0385	0.8444
		Heterogeneity			3	0.6505	0.8847

* (H) = humeral spotted parental fishes; (N) = unspotted parental fishes; (F) = F_1 offspring. + The probability for all X^2 tests is > .05; thus, results fit expected ratios according to Mendelian inheritance.

ral populations of *T. vittatus* typically exhibit a black patch or spot located caudally to the operculum. While this humeral spot is a feature of most individuals, there is an alternate phenotype lacking this humeral spot. The mode of inheritance of this spot is of particular interest, as it likely serves as an eyespot to confuse predators. As a continuation of our interest in the inheritance of both banding and spotting patterns in freshwater teleosts (Frankel, 1985, 1991, 1998, 2001, 2002, 2004, 2009, 2011), the present study was undertaken to ascertain the mode of inheritance of humeral spotting in the croaking gourami.

Materials and Methods

Healthy adult specimens of *T. vittatus* were obtained from a wholesale distributor in Maryland, USA, and maintained in separate 76 liter holding tanks equipped with aerators at 25° C. Male and female fishes exhibiting either the characteristic humeral spotting phenotype or an aberrant, unspotted phenotype were selected at random from stock specimens, placed in separate 76 liter tanks, and allowed to develop at 25°C until sexually mature. Optimal water conditions were provided for all fish (i.e. low water hardness of 5° dGH, pH 7.5, and temperature 25°C). Sexually mature fishes were transferred to 36 liter tanks with temperature raised to 30°C (Linke, 1991).

All progeny for this study were obtained utilizing artificial fertilization techniques. Artificial fertilizations were performed at a constant temperature of 30°C. Eggs were collected and inseminated in fish Ringer's solution prepared with 6.50 g NaCl, 0.250 g KCl, 0.20 g $NaHCO_3$, and 0.30 g $CaCl_2$ in one liter of distilled water. Gametes were obtained by cycling individual females through at least one natural breeding period in the presence of three males. Each gravid female was transferred to a 250 ml fingerbowl containing Ringer's solution. A small piece of nylon mesh netting was used to secure the female, bellyside down, against the side of the fingerbowl. Gentle and even pressure was then applied to the abdominal flank, resulting in the release of eggs. Sperm was obtained by gently compressing the abdomen of a male fish against the side of a 500 ml fingerbowl containing tank water. The milt, clearly visible at the urogenital aperture as a thin white stream, was collected using a narrow-mouthed pipette and expelled over the eggs. The total volume of sperm suspension, collected as "dry" as possible, was approximately 0.5 ml. The time interval from egg collection to sperm addition was between 20 and 40 seconds.

Parental fishes, exhibiting either the spotted (H) or unspotted (N) phenotype, along with F_1 progeny (F), were used in a series of 34 crosses (Table 1). Embryos from all crosses were transferred to 250 ml fingerbowls containing tank water and incubated at 30°C. Dead or developmentally arrested individuals were removed daily until fry were free-swimming. Progeny groups then were placed in separate 36 liter rearing tanks, fed initially on Pure Aquatic Brand Premium Fry Food, and allowed to develop until their phenotype could be visually determined. Since humeral spotting is more defined in young adults, determination of spotting was only scored for those individuals. Phenotypic data of all progeny were recorded and subjected to chi-square analysis. Pooled and heterogeneity chi-square tests were also performed, treating the progenies from reciprocal F_1 x F_1 crosses as single large progenies in an analysis of overall goodness of fit.

Results and Discussion

Table 1 presents data for the proposed genotypes of parental fishes, observed phenotypic numbers, expected ratios, and probability of fit for *T. vittatus* analyzed for the mode of inheritance of humeral spotting. Parental fishes and progeny from all crosses clearly displayed either the humeral spotted or unspotted phenotype. Females and males exhibiting a humeral spot HI, HII, HIII, HIV, HV and H1, H2, H3, H4, H5, respectively, were scored as homozygous dominants, as crosses involving these individuals always resulted in spotted progeny (crosses 1-5, 10-13, 19-23). Parental fishes lacking a humeral spot (females NI, NII, NIII, NIV and males N1, N2, N3) were scored as homozygous recessives, as crosses amongst these individuals consistently bred true (crosses 6-9). Further, when FIII and FV females, and F3, F4, and F5 males were mated with their parent, the resulting offspring consistently lacked a humeral spot (crosses 14-18). In addition, reciprocal crosses between spotted and unspotted parental fishes always resulted in spotted progeny (FVI-FX and F6-F10) (crosses 19-23).

Crosses amongst F_1 fishes resulting from parental spotted (H) and unspotted (N) matings always resulted in a satisfactory fit to a 3:1 phenotypic ratio of F_2 progeny (crosses 24-30), commensurate with the action of a single autosomal gene locus exhibiting complete dominance for humeral spotting; the A_ genotype results in expression of humeral spotting. This mode of Mendelian inheritance was further supported by crosses between presumptive F_1 homozygous recessives (FIV, FV, F4, F5) and F_1 heterozygotes (FVI, FVII, F7, F8) (crosses 31-34). Both spotted and unspotted fry resulted from these crosses and, based on chi-square analyses, conformed to the expected 1:1 phenotypic ratio.

Results of this study support the hypothesis that humeral spotting in the croaking gourami is controlled by a single locus, with dominance at this locus required for the expression of the spotted phenotype. Segregation patterns for the spotted and unspotted phenotypes of *T. vittatus* clearly fit an autosomal pattern of inheritance, as chi-square tests do not deviate significantly from expectations. The data also suggest complete dominance for humeral spotting, since there is no discernable difference in the appearance of spotting between presumptive heterozygous (Aa) and homozygous (AA) fishes. Further, results of heterogeneity tests also support the acceptance of the null hypothesis for these data. This prominent, dark humeral spot in the croaking gourami most certainly serves as an eyespot and, therefore, would give a selective advantage to those individuals possessing this feature by providing them with an interspecific marking to minimize predation. A monogenic mode of inheritance has also been reported for the expression of a caudal peduncle marking in the labyrinth fish *Pseudosphromenus cupanus* (Frankel, 2001). Here, the presence of a caudally located band is also controlled by a dominant allele determining this eyespot phenotype. It is interesting to note, however, that markings serving to provide fishes with disruptive patterns are not always the preferred or prominent phenotypic alternative. Indeed, studies investigating a melanic (mottled-black) body spotting pattern in the mosquitofish (*Gambusia holbrooki*) (Bisazza & Pilastro, 2000; Horth, 2006), have shown that this phenotype is expressed in very low frequency or is completely absent from populations of this poeciliid.

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