SHORT COMMUNICATION

Inheritance of Shoulder Spotting in the Red-base Tetra (Characidae: *Hemigrammus stictus*)

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Abstract

The Red-base tetra (*Hemigrammus stictus*) exhibits two pheno-types associated with shoulder spotting. Fish either possess a prominent black shoulder spot located directly behind the operculum or lack this spotting pattern. Segregation patterns observed from the progenies of eleven different crosses suggest that the inheritance of shoulder spotting is controlled by the action of two autosomal loci acting in a complementary recessive fashion, with dominance at either locus resulting in the expression of the spotted phenotype.

Key Words: *Hemigrammus stictus*, Red-base tetra, shoulder spotting, Characidae.

Introduction

Fishes in the teleost genus *Hemigrammus* (Characidae) exhibit a broad spectrum of body colorations and marking patterns (Axelrod and Vorderwinkler, 1995; Riehl et al., 1997; Frankel, 2000, 2002). The Red-base tetra (*H. stictus*), so named due to the prominent red coloration found on both the caudal peduncle and caudal fin, is a South American characin that is easily main-tained and bred in captivity. While not a commonly imported species, *H. stictus* routinely appears in catches of the more popu-lar South American characins collected from Venezuela, Guy-ana, and Brazil and, as such, is usually available to aquarists. In addition to this strikingly red caudal region, both female and male fishes in natural populations of *H. stictus* characteristically exhibit a prominent black spot or patch located caudally to the operculum. While this shoulder spot is a feature of virtually all individuals of *H. stictus*, a rare alternate phenotype is occasion-ally found which lacks this shoulder spot. The inheritance of this spotting pattern is of particular interest, since it most probably serves a prominent role in protecting individuals from preda-tion by acting as an eye-spot. As a result of our interest in the

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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 spotted and unspotted *Hemigrammus stictus*.

Cross No.	Parents* ♀ (PG)	♂ (PG)	Phenotypic numbers Spotting	No Spotting	Exp. Ratio	df	X^2	P^+
1	SI (*AABB*)	x S1 (*AABB*)	40(FI&F1)	0	1:0			
2	SII (*AABB*)	x S2 (*AABB*)	51	0	1:0			
3	SIII (*AABB*)	x S3 (*AABB*)	50	0	1:0			
4	SIV (*AABB*)	x S4 (*AABB*)	38	0	1:0			
5	SV (*AABB*)	x S5 (*AABB*)	42(FII&F2)	0	1:0			
6	SI (*AABB*)	x S4 (*AABB*)	46	0	1:0			
	Pooled		267	0	1:0			
7	NI (*aabb*)	x N1 (*aabb*)	0	35(FIII&F3)	0:1			
8	NII (*aabb*)	x N2 (*aabb*)	0	46(FIV&F4)	0:1			
9	NIII (*aabb*)	x N3 (*aabb*)	0	47 (FV&F5)	0:1			
	Pooled		0	128	0:1			
10	SI (*AABB*)	x F1 (*AABB*)	32	0	1:0			
11	FI (*AABB*)	x S1 (*AABB*)	30	0	1:0			
12	SV (*AABB*)	x F2 (*AABB*)	41	0	1:0			
13	FII (*AABB*)	x S5 (*AABB*)	44	0	1:0			
	Pooled		147	0	1:0			
14	NI (*aabb*)	x F3 (*aabb*)	0	43	0:1			
15	NII (*aabb*)	x F4 (*aabb*)	0	29	0:1			
16	NIII (*aabb*)	x F5 (*aabb*)	0	47	0:1			
17	FIV (*aabb*)	x N2 (*aabb*)	0	33	0:1			
18	FV (*aabb*)	x N3 (*aabb*)	0	35	0:1			
	Pooled		0	187	0:1			
19	SI (*AABB*)	x N1 (*aabb*)	37(FVI&F6)	0	1:0			
20	SII (*AABB*)	x N2 (*aabb*)	32(FVII&F7)	0	1:0			
21	SIII (*AABB*)	x N3 (*aabb*)	44(FVIII&F8)	0	1:0			
22	N1 (*aabb*)	x S4 (*AABB*)	31(FIX&F9)	0	1:0			
23	NII (*aabb*)	x S5 (*AABB*)	35(FX&F10)	0	1:0			
	Pooled		179	0				
24	FVI (*AaBb*)	x F6 (*AaBb*)	38	2	15:1	1	.107	.7440
25	FVII (*AaBb*)	x F7 (*AaBb*)	37	5	15:1	1	2.292	.1300
26	FVIII (*AaBb*)	x F8 (*AaBb*)	48	3	15:1	1	.012	.9136
27	FIX (*AaBb*)	x F9 (*AaBb*)	37	1	15:1	1	.849	.3568
28	FX (*AaBb*)	x FX (*AaBb*)	33	4	15:1	1	1.314	.2518
29	FVI (*AaBb*)	x F8 (*AaBb*)	28	1	15:1	1	.389	.5331
30	FVII (*AaBb*)	x F9 (*AaBb*)	60	6	15:1	1	.909	.3404
	Total					7	5.872	.5547
	Pooled		281	22	15:1	1	.528	.4673
	Heterogeneity					6	5.344	.5005
31	FIX (*AaBb*)	x F3 (*aabb*)	28	8	3:1	1	.148	.7003
32	FX (*AaBb*)	x F4 (*aabb*)	35	10	3:1	1	.185	.6670
33	FIV (*aabb*)	x F8 (*AaBb*)	30	9	3:1	1	.077	.7815
34	FV (*aabb*)	x F9 (*AaBb*)	15	7	3:1	1	.545	.4602
	Total					4	.955	.9165
	Pooled		108	34	3:1	1	.085	.7713
	Heterogeneity					3	.870	.8326

* (S) = spotted parental fishes; (N) = unspotted parental fishes; (F) = F1 offspring. + The probability for all X2 tests is > .05; thus, all observed results fit the expected ratio according to Mendelian inheritance.

inheritance of disruptive banding and spotting patterns in several genera of freshwater teleosts (Frankel, 1985, 1991, 1998, 2001, 2002, 2004, 2005, 2009), the present study was undertaken to ascertain the mode of inheritance of shoulder spotting in *H. stictus*.

Materials and Methods

Healthy juvenile specimens of *H. stictus* were obtained from a wholesale distributor in Maryland, USA, and maintained in separate 76 liter holding tanks at 26°C. Male and female fishes exhibiting either the characteristic shoulder spotting phenotype or the unspotted phenotype, were selected at random from stock specimens, placed in separate 76 liter tanks, and allowed to develop at 26°C until sexually mature. Optimal water conditions for Hemigrammus were provided for all fish (i.e. low water hardness of 5° dGH, pH 6.5, and temperature 26°C) (Riehl et al., 1997). All progeny for this study were obtained from artificial fertilizations as previously described (Frankel, 1985). Parental fishes, exhibiting either the spotted (S) or unspotted (N) phenotype, along with F1 progeny (F), were used in a series of 34 crosses (Table 1). Embryos from all crosses were incubated at 26°C in 250 ml fingerbowls containing tank water. Dead or developmentally arrested embryos were removed daily. Fry hatched 24-36 hours post-fertilization and were free-swimming 72-96 hours post-hatching. Progeny groups were placed in separate 36 liter rearing tanks, fed initially on rotifers and allowed to develop until their phenotype could be visually determined. Since spotting is more defined in mature individuals, determination of spotting was only scored for mature 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 F1xF1 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 *H. stictus* analyzed for the mode of inheritance of shoulder spotting. Parental fishes and progeny from all crosses clearly displayed either the spotted or unspotted phenotype. Spotted females SI, SII, SIII, SIV, SV and males S1, S2, S3, S4, S5 were scored as homozygous dominants, as crosses involving these individuals always resulted in spotted progeny (crosses 1-6, 10-13, 19-23). Parental fishes lacking shoulder spots (NI, NII, NIII, females and N1, N2, N3 males) were scored as homozygous recessives, as crosses amongst these individuals consistently bred true (crosses 7-9). Further, when FIV and FV females, and F3, F4, and males were crossed with their parents, the resulting offspring consistently lacked a shoulder spot (crosses 14-18). In addition, reciprocal crosses between spotted and unspotted parental fishes always resulted in spotted progeny (crosses 19-23).

Crosses amongst the F1 fishes resulting from parental matings

always resulted in a satisfactory fit to a 15:1 phenotypic ratio of F2 progeny (crosses 24-30), commensurate with a modified 9:3:3:1 ratio resulting from recessive complementary gene action; the aabb genotype resulting in fishes absent shoulder spotting. This mode of inheritance was further substantiated by matings between presumptive F1 homozygous recessives and F1 heterozygotes (crosses 31-34). Both spotted and unspotted fry resulted from these matings and, based on chi-square analyses, conformed to the expected 3:1 phenotypic ratio.

Results of this study support the hypothesis that shoulder spotting in *H. stictus* is controlled by two loci acting in a complementary fashion, with dominance at either locus required for the expression of the spotted phenotype. Segregation patterns for the spotted and unspotted phenotypes of *H. stictus* clearly fit an autosomal pattern of inheritance, as chi-square tests do not deviate significantly from expectations. Results of heterogeneity tests also support the acceptance of the null hypothesis for this data. Further, observations of the extent of spotting in parental, F1, and F2 fishes also suggest that these loci do not act in an additive fashion, since there is no perceptible difference in the appearance of spotting in presumptive AABB spotted parentals (S) with either presumptive AaBb spotted F1 progeny (FVI-FX and F6-F10) or spotted F2 fishes, some of which would be heterozygous at one of the two loci involved (i.e. Aabb or aaBb individuals).

A digenic mode of inheritance has also been reported for shoulder spotting in the tetra Hyphessobrycon bentosi Characidae (Frankel, 2009), although in this species shoulder spotting results from a pair of autosomal loci exhibiting dominant complementary gene action. As in *H. bentosi*, the prominent dark shoulder spot in the Red-base tetra most certainly serves as an "eye-spot" and, therefore, gives a selective advantage to those individuals possessing this feature by providing them with an interspecific marking to minimize predation. Indeed, the mode of inheritance suggested here for shoulder spotting in *H. stictus* would clearly provide for individuals in populations of this species to exhibit the spotted phenotype (i.e. A_bb, aaB_, and A_B_ all result in the expression of shoulder spotting). Interestingly, spotting is not always the preferred or prominent phenotypic alternative in fishes. For instance, studies on the mosquitofish (*Gambusia holbrooki*) (Bisazza and Pilastro, 2000; Horth, 2006), have shown that the melanic (mottled-black) body spotting pattern is inherited as a Y-linked trait with autosomal modifiers and is either expressed in very low frequency or is completely absent from certain populations of this poeciliid.

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