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Caudal Spotting in the Beacon Fish (*Hemigrammus ocellifer* Characidae)

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needed to determinate the organization of telomeres in this insect order.

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Caudal Spotting in the Beacon Fish (*Hemigrammus ocellifer* Characidae)

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The beacon fish (*Hemigrammus ocellifer*) exhibits two phenotypes associated with spotting at the base of the caudal fin, with fish either possessing (*H. o. ocellifer*) or lacking (*H. o. falsus*) a prominent red spot in this region. Segregation patterns observed from the progenies of 15 different crosses support a hypothesis that caudal spotting in this species is controlled by a single gene with two alleles, for which the caudal spotting allele is completely dominant.

Fishes comprising the Characidae and several other closely related teleost families make up the traditional characins, a group of about 200 African and 1,000 South, Central, and southern North American species (Mills and Vevers 1982). Fishes in the genus *Hemigrammus* (Characidae) are particularly popular with aquarium hobbyists since they not only exhibit a wide variety of attractive coloration and marking patterns, but also are easily maintained and bred. The latter makes them ideally suited to environmental, behavioral, and genetic investigations (Brown et al. 1999, 2000; Buehrnheim and Fernandes 2001; Frankel 2000; Mikheev and Pakul'skaya 1989; Mikheev et al. 1992; Zhujkov and P'yanov 1993).

Within the species complex *Hemigrammus ocellifer* (beacon fish), two subspecies, *H. o. ocellifer* and *H. o. falsus*, are commonly recognized (Mills and Vevers 1982). Both subspecies are indigenous to northern South America. Phenotypically beacon fish are brown to greenish-yellow in color with a silvery iridescence. At the level of the dorsal fin, a dark transverse bar surrounded by striking golden-yellow spots characterizes the species and gives it its popular name. While both subspecies share these basic coloration and marking patterns, a brilliant red spot at the base of its caudal fin further distinguishes *H. o.*

ocellifer. The inheritance of this spotting pattern is of particular interest, since it probably serves as an eyespot, mimicking the red color of the iris of the eye. As a result of recent investigations on the inheritance of coloration patterns in *Hemigrammus* (Frankel 2000) and on caudal peduncle banding in paradisefish (Frankel 2001), the present study was undertaken to ascertain the mode of inheritance of caudal spotting in the beacon fish.

Materials and Methods

Juvenile specimens of *H. o. ocellifer* and *H. o. falsus* were obtained from a local wholesale distributor in Virginia and maintained in separate 76 L holding tanks at 24°C. Male and female fish exhibiting the phenotypes were selected at random from stock specimens, placed in separate 76L tanks, and allowed to develop at 24°C until sexually mature. Optimal water conditions were provided for all fish (i.e., water hardness ≤ 5, pH 6.5, temperature 24°C) (Axelrod and Vorderwinkler 1995; Mills and Vevers 1982). All progeny for this study were obtained from artificial fertilizations as employed for the cypripid fishes *Brachydanio rerio* and *B. albolineatus* (Frankel and Hart 1977; Hart and Messina 1972). Parentals exhibiting either the spotted (S) or unspotted (U) phenotype, along with F₁ progeny, were used in a series of 29 crosses (Table 1). Embryos from all crosses were incubated at 24°C in 250 ml fingerbowls containing tank water. Dead or developmentally arrested embryos were removed daily. Fry hatched 20–24 h after fertilization and were free swimming 48–72 h after hatching. Progeny groups were placed in separate 36 L rearing tanks, were fed initially on rotifers, and were allowed to develop until their phenotype could be visually determined. Phenotypic data of all progeny were recorded and subjected to chi-square analysis. Pooled and heterogeneity chi-square tests were also performed, treating the U × F₁ and F₁ × F₁ progenies as single large progenies in an analysis of overall goodness-of-fit.

Results and Discussion

Table 1 presents data for the proposed genotypes of parentals, and observed phenotypic numbers, expected ratios, and probability of fit for *H. ocellifer* analyzed for the mode of inheritance of caudal spotting for corresponding progeny groups. Parental fish and progeny from all crosses

Table 1. Proposed genotypes (PG) and observed phenotypic numbers, expected ratios, degrees of freedom (df), chi-square values (χ^2), and probability of fit (P) for crosses among spotted and unspotted *Hemigrammus ocellifer*

Cross no.	Parents ^a		Un-spotted	Phenotypic numbers			
	♀ (PG)	♂ (PG)		Expected ratio	df	χ^2	P^b
1	S-I (AA)	× S-I (AA)	16	0	1:0	—	—
2	S-II (AA)	× S-I (AA)	25	0	1:0	—	—
3	S-II (AA)	× S-2 (AA)	20	0	1:0	—	—
4	S-III (AA)	× S-2 (AA)	22	0	1:0	—	—
5	S-III (AA)	× S-3 (AA)	18	0	1:0	—	—
Pooled			101	0	1:0	—	—
6	U-I (aa)	× U-1 (aa)	0	17	0:1	—	—
7	U-II (aa)	× U-2 (aa)	0	24	0:1	—	—
8	U-III (aa)	× U-3 (aa)	0	26	0:1	—	—
9	U-IV (aa)	× U-4 (aa)	0	17	0:1	—	—
10	U-I (aa)	× U-4 (aa)	0	14	0:1	—	—
Pooled			0	98	0:1	—	—
11	S-I (AA)	× U-1 (aa)	14 (F-I and F-1)	0	1:0	—	—
12	S-II (AA)	× U-2 (aa)	20 (F-II and F-2)	0	1:0	—	—
13	U-I (aa)	× S-2 (AA)	24 (F-III and F-3)	0	1:0	—	—
14	U-III (aa)	× S-3 (AA)	19 (F-IV and F-4)	0	1:0	—	—
15	S-III (AA)	× U-4 (aa)	18 (F-V and F-5)	0	1:0	—	—
Pooled			95	0	1:0	—	—
16	U-I (aa)	× F-2 (Aa)	8	12	1:1	1	0.800 .3711
17	U-II (aa)	× F-3 (Aa)	10	12	1:1	1	0.182 .6698
18	F-I (Aa)	× U-2 (aa)	11	10	1:1	1	0.048 .8273
19	F-III (Aa)	× U-4 (aa)	14	11	1:1	1	0.360 .5485
20	F-IV (Aa)	× U-1 (aa)	13	10	1:1	1	0.391 .5316
21	F-V (Aa)	× U-3 (aa)	11	15	1:1	1	0.615 .4328
22	U-III (aa)	× F-4 (Aa)	10	9	1:1	1	0.053 .8185
23	U-IV (aa)	× F-1 (Aa)	12	15	1:1	1	0.333 .5637
Total					8	2.782 .9472	
Pooled			89	94	1:1	1	0.137 .7117
Heterogeneity					7	2.645 .9157	
24	F-I (Aa)	× F-2 (Aa)	13	5	3:1	1	0.074 .7855
25	F-II (Aa)	× F-1 (Aa)	19	7	3:1	1	0.051 .8208
26	F-III (Aa)	× F-4 (Aa)	24	7	3:1	1	0.097 .7557
27	F-III (Aa)	× F-5 (Aa)	15	8	3:1	1	1.174 .2786
28	F-IV (Aa)	× F-3 (Aa)	22	9	3:1	1	0.269 .6041
29	F-V (Aa)	× F-4 (Aa)	12	1	3:1	1	2.077 .1495
30	F-V (Aa)	× F-5 (Aa)	20	6	3:1	1	0.051 .8208
Total					7	3.793 .8032	
Pooled			125	43	3:1	1	0.032 .8586
Heterogeneity					6	3.761 .7089	

^a S designates *H. o. ocellifer* parentals; U designates *H. o. falsus* parentals; F designates F_1 offspring.

^b The probability for all chi-square tests is greater than .05, thus observed results for all matings fit the expected ratio according to Mendelian monohybrid inheritance.

displayed either the spotted or unspotted phenotype. *H. o. ocellifer* females S-I, S-II, and S-III, and males S-1, S-2, and S-3 were scored as homozygous for a dominant allele, as crosses involving these individuals always resulted in spotted progeny (crosses 1–5, 11–15). *H. o. falsus* (U-I, U-II, U-III, U-IV females and U-1, U-2, U-3, U-4 males) were scored as homozygous recessives, as crosses between these individuals consistently bred true (crosses 6–10). Reciprocal crosses between *H. o. ocellifer* and *H. o. falsus* parentals always resulted in spotted fry (crosses 11–15). Further, when F_1 fishes (F-I, F-III, F-IV, F-V, F-1, F-2, F-3, and F-4) were mated with *H. o. falsus* parentals, spotted and unspotted progeny resulted in a satisfactory fit to a 1:1 ratio (crosses 16–23), indicative of mating between heterozygous and homozygous recessive individuals. Complete dominance of caudal spotting was further substantiated by $F_1 \times$

F_1 crosses that resulted in the expected 3:1 proportions (crosses 24–30).

Results of this study illustrate that segregation for the spotted and unspotted phenotypes of *H. ocellifer* clearly fit an autosomal monogenic pattern of inheritance, as chi-square tests for individual and pooled progenies do not deviate significantly from expectations. Results of heterogeneity tests also support the acceptance of the null hypothesis for this data. Further, the data also suggest complete dominance of the “*ocellifer*” phenotype, since there is no perceptible difference in the appearance of spotting between *H. o. ocellifer* parentals and the spotted F_1 heterozygous fish. This prominent red spot at the base of the caudal fin most likely serves as an eyespot, as it appears to mimic the coloration of the iris of the eye in this species and probably provides some selective advantage by giving the

fish a false “head” to confuse predators. A similar pattern of inheritance has been reported for caudal peduncle banding in the spike-tailed paradisefish (*Pseudosphromenus cupanus* Belontiidae), which is also controlled by a completely dominant allele that specifies the banded phenotype (Frankel 2001). Since the two subspecies are traditionally distinguished on the basis of their coloration patterns, it would appear that a revaluation of the *ocellifer-falsus* complex is warranted within the genus *Hemigrammus*.

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