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Polygenic Inheritance of Background Body Colour in Hybrids of *Poecilia latipinna* and *Poecilia sphenops*

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ABSTRACT

Inheritance of background body colour in molly, *Poecilia* is not well documented despite being an economically important aquarium fish. This study was attempted to understand the inheritance of background body colour between crosses of *Poecilia latipinna* (non-black) and *Poecilia sphenops* (black) through controlled breeding. A total of 13 breeding crosses which consist of two pure bred crosses, six parental hybrid crosses, two backcrosses, two test crosses and one full-sib cross were successfully initiated. In molly, *Poecilia,* non-black body colour is completely dominant over black and is not sex-linked. Multiple genes interaction which acted nonadditively was also found to be influencing this phenotypic trait. However, such interaction effect was restricted in crosses generated between genetically related fish (backcross and F_2 full-sib) and was absent in crosses between distantly related fish (testcross). Perhaps, such occurrence was a natural mechanism to maintain the vibrancy of non-black colouration.

Key words: Polygenic inheritance, background body colour, *P. latipinna*, *P. sphenops*

INTRODUCTION

Mollies are poeciliids, a livebearer group of fishes that bear young through internal fertilization. Sperm from male is transferred into the female via gonopodium, a modified structure of anal fin in the male (Basolo, 2006). This fish originated from the southeastern United States of America (USA) particularly along the coastal drainage of Mexico (Hankinson and Ptacek, 2008) and brought into Malaysia 15 years ago (Phang, Aquatic International, pers. comm. 10 September 2009). There are three main species of mollies that could be found in the local market namely *Poecilia latipinna, P. velifera* and *P. sphenops*. *Poecilia latipinna* and *P. velifera* are commonly known as sailfin molly, named after a special feature of their enlarged dorsal fin resembling a fan (Ptacek and Breden, 1998). Such appearance is absent in *P. sphenops*. In terms of body colouration, the *P. latipinna* is more vibrant and could be found in variable colour while *P. sphenops* is largely black in colour.

An attempt was initiated to develop a mapping population for linkage map construction by hybridizing between a non-black *P. latipinna* and a black *P. sphenops*. Concurrently, studies on mode of inheritance in background body colour on F_1 hybrid, backcross, test cross and F_2 full-sib cross were also undertaken. The inheritance of background body colour through selective and controlled breeding on molly fish had not been documented to date. Hence, this attempt was made to understand the inheritance of background body colour in this live-bearer fish by controlled breeding between *P. latipinna* (sailfin molly) and *P. sphenops* (black lyretail molly). The genetic inheritance through classical Mendelian dominant and recessive, additive and epistatic fashion was postulated. This study assumed that effect of environment on this phenotypic trait was of minor and non influential.

MATERIALS AND METHODS

Parental stocks (n = 40) used in this project were purchased from Aquatic International, Subang Jaya, Malaysia and maintained in Aquaculture Research Centre, Universiti Putra Malaysia, Puchong in the middle of October 2009. These parental fishes were separated according to gender and housed in glass aquaria (43×23×22 cm) for 3 months prior to breeding trials. The water temperature was kept in a range of 26 to 28°C by an electric-operated aquarium heater throughout the experiment. Newborn were removed from the breeding glass aquaria immediately and were fed with live *Artemia* sp for the first two weeks and later fed with pellet feed. The breeding fish were alternately fed with frozen blood worm, freshly cooked peas and imported pellet feed.

A total of 13 breeding trials were carried out as listed in Table 1. The first-generation of hybrids (F_1) were created between matured male sailfin molly, *P. latipinna* and female black lyretail molly, *P. sphenops*. A reciprocal cross was also carried out. For simplicity in description, colours other than black were designated as non-black. Backcrossed hybrids were generated by either backcrossing F_1 hybrids with female or male recurrent parents. Test crosses were carried out by crossing F_1 male hybrid with genetically unrelated *P. sphenops*. Full-sibling cross between a sexually matured F_2 male (non-black) with a F_2 female (black) between F_2 hybrids of F_2-1 were also attempted. The phenotypic data of background body colour segregation from each mating were subjected to chi-square (χ^2) analysis at significance level of p = 0.05.

RESULTS AND DISCUSSION

Two control crosses (Pure1 and Pure2) were generated by crossing between a male and female *P. latipinna* (non-black) and *P. sphenops* (black), respectively. The F_1 progenies derived from control crosses Pure1 were non-black whereas those derived from Pure 2 were black. The F_1 progenies from both control crosses were advanced for full-sib mating to produce F_2. The F_2 progenies of both Pure1 and Pure 2 crosses likewise were non-black and black, respectively without any segregation and variation in background body colour.

Males of *P. latipinna* used in the breeding trials in general were white in body colour except for those used to produce F_1 hybrids of F1-E and F1-F, in which the body colour was orange and milky with orange spots, respectively (Table 1). All six hybrid breeding trials (F1-A to F1-F) successfully produced offsprings with an exception of a cross to produce F_1 hybrids (F1-C), in which both parents died three weeks after pairing. Collectively, all F_1 hybrids produced from the four separate crosses (F1-A, F1-B, F1-E and F1-F) consistently showed non-black in background body colouration. Hybrids from reciprocal cross (F_1-D), likewise were also non-black in background body colouration. These observations confirmed the complete dominant character of non-black over black and are not sex-linked. Our results on molly fish were found to be similar to those observed by Majumdar *et al.* (1997) in tilapia species which showed lighter colouration (pink) was complete dominance over black and was not sex-linked.

Table 1: Possible crosses, crossing patterns and number of fish used in each cross between *P. latipinna* and *P. sphenops*

		No. of brood fish		
Crosses	Crossing patterns	Female	Male	Hybrid symbol
Pure Breed	Female *P. latipinna*×male *P. latipinna*	1	1	Pure 1
	Female *P. sphenops*×male *P. sphenops*	1	1	Pure 2
F_1 hybrid	Female *P. sphenops*×male *P. latipinna*	1	1	F1-A
	Female *P. sphenops*×male *P. latipinna*	1	1	F1-B
	Female *P. sphenops*×male *P. latipinna*	1	1	F1-C
	Female *P. latipinna*×male *P. sphenops*	1	1	F1-D
	Female *P. sphenops*×male *P. latipinna*	1	1M	F1-E
	Female *P. sphenops*×male *P. latipinna*	1F	1	F1-F
Backcross	Female (F1-E) x male *P. latipinna*	6	1M	BC_1-A
	Female *P. sphenops*×male (F1-F)	1F	1	BC_1-B
F_2 hybrid	Female *P. sphenops*×male (F1-E)	1	1	F_2-1
	Female *P. sphenops*×male (F1-E)	1	1	F_2-2
Full-sib	Female F_2 (F_2-1)×male F_2 (F_2-1)	1	1	F_3

F: Filial generation; BC: Backcross; 1F: Same female; 1M: Same male

Table 2: Body colour segregation in the backcross, test cross and full sib crossing of *Poecilia* spp.

		Colour of progeny										
		Non-black			Black							
Cross code	Total No. of progeny	Dark orange	Orange	Light orange	Light grey	Grey	Black	Dark black	Expected ratio	χ^{2*}	df	p-value
Backcross												
BC_1-A	6	1	2	3	-	-	-	-	1 : 0	-	-	-
BC_1-B	50	4	3	19	3	5	7	9	1 : 1	0.080*	1	0.777
Total	56	5	5	22	3	5	7	9	-	-	-	-
Testcross												
F_2-1	23	-	-	12	-	-	11	-	1 : 1	0.043	1	0.835
F_2-2	26	-	-	19	-	-	7	-	1 : 1	5.538	1	0.019
Total	49	-	-	31	-	-	18	-	1 : 1	3.449	1	0.063
Full-sib	37			15			22		1 : 1	1.324	1	0.250

-: Not applicable, df: Degree of freedom, *χ^2 test is done by considering colour as two category (non-black and black) and significance is checked at p = 0.05, Nuo. in bold indicates distortion from Mendelian expectation of 1:1, Cross code refer to Table 1

In order to further confirm black as a recessive character, two test-crosses (F_2-1 and F_2-2) and a backcross (BC_1-B) were attempted. Collectively, results from backcross of BC_1-B and testcross of F_2-1 showed segregation of non-black and black were in the expected 1:1 ratio and was confirmed by the χ^2 analysis (Table 2). The performed χ^2 analysis showed deviation in testcross of F_2-2. Pooled data from both testcrosses were merely in the expected 1:1 ratio. Perhaps if more progenies produced and evaluated in testcross of F_2-2, the segregation of background body colour might be in the expected 1:1 ratio. The presence of two colour variants observed in this study further proved the homozygosity of black colour as a recessive character.

Upon careful observation, interestingly, the background body colour seems to exhibit various degrees of colour shades in both the backcross progenies (BC_1-A and BC_1-B) (Fig. 1, 2) but such occurrence were absent in both testcross progenies (Fig. 3). The precise characterization of colour

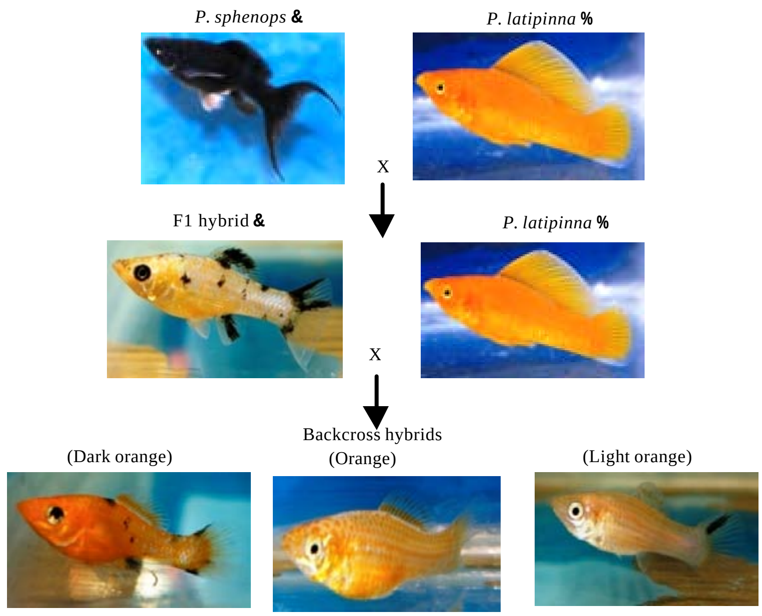

Fig. 1: Controlled crosses between *P. latipinna* and *P. sphenops* of BC_1-A. Parentheses indicate description of colour

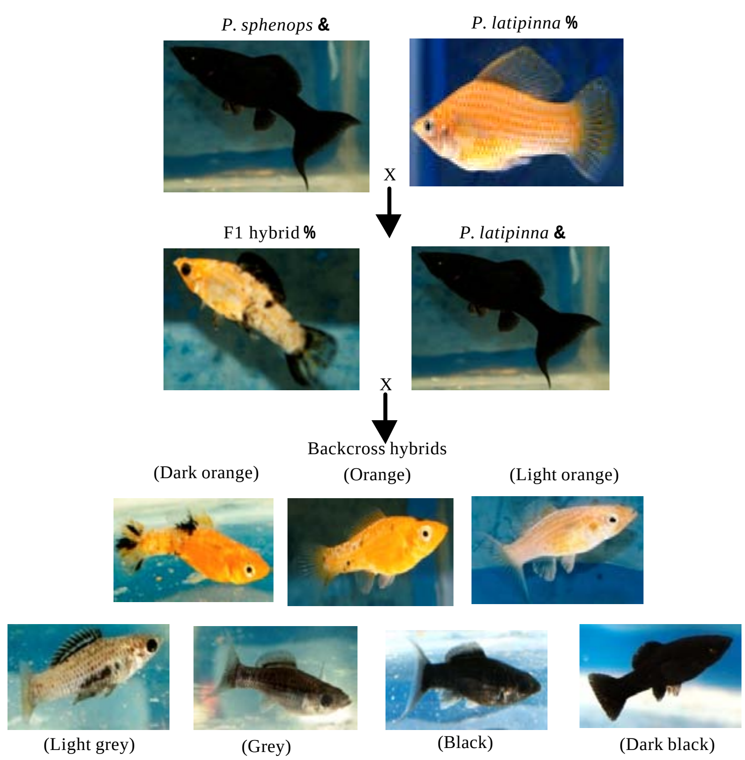

Fig. 2: Controlled crosses between *P. latipinna* and *P. sphenops* of BC_1-B. Parentheses indicate description of colour

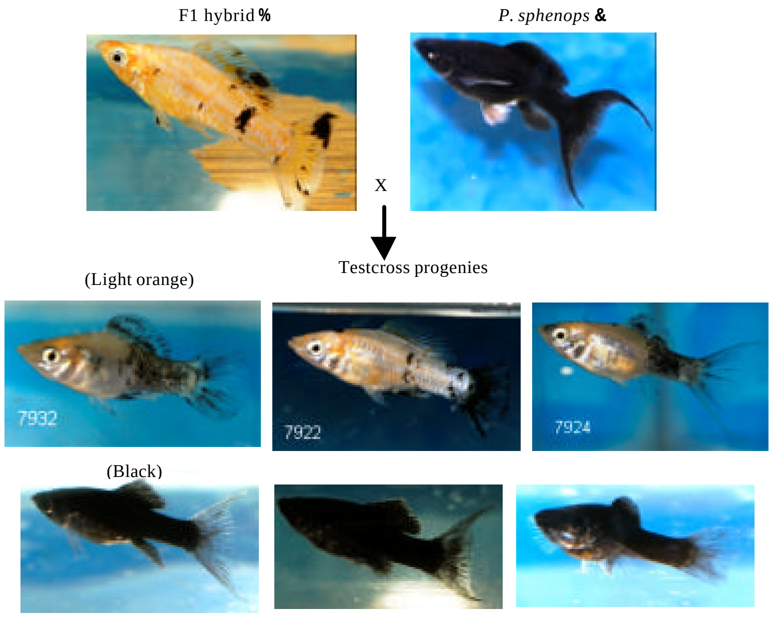

Fig. 3: Controlled crosses between F_1 male hybrid and genetically unrelated *P. sphenops* of F_2-1. Parentheses indicate description of colour

in this study seems to be impossible as colour variants were diverse without any two identical colour clones but this result strongly illustrates an example of multiple genes acting in additive or nonadditive manner. A dilution in black colouration as seen in some of the progenies of BC_1-B cross was the most prominent observation as both black and grey colours could be easily differentiated. Hence, it could be postulated that backcrossing in molly contributed nonadditively to black colouration, causing dilution of black to grey. Further to this, similar observation was found in the F_2 full sib crossing, in which two of the non-black progenies showed reduced body and eye colouration. Our study showed that sexual crossing between genetically related fishes either through backcrossing or full sibling crosses seemed to exhibit dilution in the body colour of molly, *Poecilia*. The exact reason for such occurrence was still unclear. The possible explanation would be s ets of interacting genes in a network that control the colouration might be different in the testcross and backcross (Darvasi, 2006). Perhaps, such occurrence is crucial as a natural mechanism to maintain the vibrancy of non-black colouration.

This study provides an insight into the mode of colour inheritance in one of the most economically important traits in aquarium fish of which research on it had not been undertaken for the last few decades.

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