Interaction between the Autosomal Recessive *bar* Gene and the Y-Linked Snakeskin Body (*Ssb*) Pattern Gene in the Guppy, *Poecilia reticulata*

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Interaction between the Autosomal Recessive *bar* Gene and the Y-Linked Snakeskin Body (*Ssb*) Pattern Gene in the Guppy, *Poecilia reticulata*

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ABSTRACT—Many color varieties of the guppy, *Poecilia reticulata*, are commercially cultured in Singapore for the aquarium industry. In the group of guppy varieties called Snakeskin, males characteristically have snakeskin-like reticulations on the body and caudal fin. The snakeskin pattern on the body of male Snakeskin guppies is due to a Y-linked gene (*Ssb*). Female guppies, being homogametic (XX), do not carry the *Ssb* gene. About 90% of Yellow Snakeskin males have the typical snakeskin pattern on their bodies and tails. The remaining males are different in that the snakeskin body pattern has been modified into four or five vertical bars on the caudal-peduncle region. F_1 and F_2 results of single-pair reciprocal matings of the Yellow Snakeskin variety show that a single gene is responsible for the vertical bar pattern. This gene, *bar*, is autosomal recessive. In the homozygous condition (*barbar*), it interacts with the Y-linked *Ssb* gene to give vertical barring patterns on the caudal-peduncle of Yellow Snakeskin males. This pattern is not expressed when the dominant allele, bar^+, is present.

INTRODUCTION

The guppy is unique among other teleosts in that almost all the genes responsible for pigmentation and color patterns, with the exception of background body coloration genes, are sex-linked and sex-limited. It is the first organism in which Y-linked inheritance of color genes was demonstrated (Schmidt, 1920; Winge, 1922a, b, 1927). The guppy has 23 pairs of chromosomes, of which 22 are autosomal and one pair the sex chromosomes. Males are heterogametic (XY) while females are homogametic (XX) (Winge, 1922a, b; Winge and Ditlevsen, 1947). Expression of color patterns in domesticated varieties is due to dominant sex-linked genes (Dzwillo, 1959; Nayudu, 1979; Fernando and Phang, 1989; Phang *et al*., 1989a, b, 1990; Phang and Fernando, 1991; Khoo *et al*., 1999a, b). The color patterns of these varieties were initially selected from a large gene pool in wild-type populations. Kirpichnikov (1981), in his review, documented 17 Y-linked genes that are passed from father to son through the Y-chromosome, 15 that are X- and Y-linked (found in both males and females but expressed only in males because they are sex-limited and hormone-mediated), and one that is autosomal dominant. In contrast, genes responsible for background body coloration such as blond (*b*), gold (*g*), albino (*a*) and blue (*bl*) are autosomally inherited and recessive to their wild-type alleles (Kirpichnikov, 1981).

Color patterns on the body and caudal fin of domesticated guppies take the form of single bright colors, snakeskin-like reticulations and variegated mosaic patterns of two or more colors (Fernando and Phang, 1985, 1989; Phang *et al*., 1989a, b, 1990; Phang and Fernando, 1991; Khoo *et al*., 1999a, b). Recent surveys of guppy farms in Singapore show

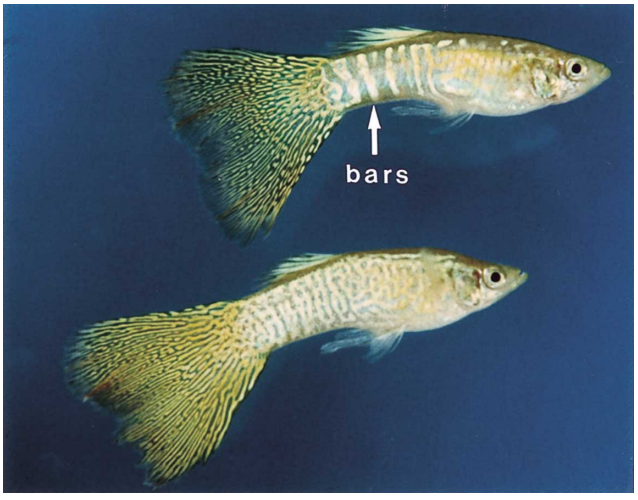

Fig. 1. An adult Yellow Snakeskin (YSSbar) male guppy showing the vertical bar pattern (arrow) on its caudal-peduncle region (top) and a normal Yellow Snakeskin (YSS) male without the bar pattern (bottom).

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the popularity of snakeskin-like and variegated patterns among guppy varieties that are cultured for export (Khoo *et al.*, 1999a). The iridescent snakeskin pattern on the body and tail is the result of two closely linked genes, Snakeskin-body (*Ssb*) and Snakeskin-tail (*Sst*), that are expressed only in males (Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991). *Ssb* and *Sst* are thus absent in the homogametic (XX) females as these genes are Y-linked. Observations of Yellow Snakeskin males obtained from stocks in Singapore guppy farms show that about 10% of them differ from normal snakeskin males in having at least four to five prominent iridescent vertical bars on the caudal peduncle region instead of the characteristic r-eticulated snakeskin-like network pattern over the entire body (Fig. 1). Using the Yellow Snakeskin guppy variety as a genetic model, we undertook this study to investigate the genetic basis of the bar pattern.

MATERIALS AND METHODS

Source of the fish

Three- to four-week old fry of the Yellow Snakeskin guppy variety were obtained from Swee Hing & Brothers Aquarium Co. in Singapore. YSS juveniles were separated according to sex and cultured as in Khoo *et al.* (1999a, b).

Description of the fish

The common name, Yellow Snakeskin (YSS), is given by commercial guppy breeders to males (♂♂) and females (♀♀) of this guppy variety. The color phenotypes of adult Yellow Snakeskin males are Yellow Snakeskin without bar pattern (YSS ♂♂) and Yellow Snakeskin with bar (YSSbar ♂♂) as shown in Fig. 1. YSS and YSSbar males have pale yellowish background body coloration and a yellow caudal fin that is overlaid with delicate snakeskin-like reticulations. The two phenotypes differ in that YSS males have the characteristic reticulated snakeskin pattern over the whole body while the snakeskin pattern of YSSbar males is modified to four or five prominent vertical bars on the caudal-peduncle region (Fig. 1). All Yellow Snakeskin females (YSS ♀♀) show only pale yellow background coloration on the body and tail.

Reciprocal matings

Single-pair reciprocal matings of males (YSS ♂♂ and YSSbar ♂♂) and females (YSS ♀♀) of the Yellow Snakeskin variety were set up to determine the inheritance of the bar pattern. The following notations were used: YSS ♂♂ × YSS ♀♀ (Mating 1) and YSSbar ♂♂ × YSS ♀♀ (Mating 2) (Table 1). Single-pair full-sib F_1 males and females were mated to obtain the F_2 generation. Each mating pair (two-month old mature virgin fish) was kept in a 3.5-liter breeding tank. Broods were usually produced 4-6 weeks after mating. F_1 and F_2 offspring were segregated according to phenotypes and sex after about eight weeks of age. Maintenance and grow-out of newly born fry and juveniles were as described by Khoo *et al.* (1999a, b).

Statistical analyses

Observed phenotypic distributions were tested for goodness-of-fit with predicted proportions using the chi-square (χ^2) test (Sokal and Rohlf, 1981; Strickberger, 1990). Since observed and expected numbers in the phenotypic classes and sample sizes were small ($n < 200$), Yates' (1934) correction for continuity was included in the calculation of χ^2 to improve the approximation to the χ^2 distribution, as shown by the χ^2_{adj} values.

RESULTS AND DISCUSSION

Segregation of *bar* in F_1 and F_2 offspring of YSS ×YSS

Single-pair matings of Yellow Snakeskin (YSS) males and females gave two different groups of F_2 progenies (Table 1, Fig. 2). The first group (Mating 1a) of eight single-pair matings produced F_1 and F_2 offspring where all the males had the reticulated snakeskin-like body pattern on a pale yellowish body and yellow caudal fin that is characteristic of the Yellow Snakeskin phenotype (Figs. 1, 2). All the females were also

Table 1. Segregation data of the F_1 and F_2 generations of single-pair reciprocal matings of: normal Yellow Snakeskin males (YSS ♂♂) and Yellow Snakeskin females (YSS ♀♀) (Mating 1a), YSS ♂♂ and YSS ♀♀ (with *bar* pattern gene that was unexpressed due to the absence of the Y-linked *Ssb* gene) (Mating 1b), Yellow Snakeskin males with bar pattern (YSSbar ♂♂) and YSS ♀♀ (Mating 2a), and YSSbar ♂♂ and YSS ♀♀ (with unexpressed *bar* gene) (Mating 2b). Observed segregation numbers for F_2 male offspring with and without the bar pattern were subjected to chi-square (χ^2) goodness-of-fit analyses. Female progenies were excluded from χ^2 tests (*) because those that carried the *bar* gene could not be distinguished from those with the *bar*$^+$ allele.

Mating (Mating no.)	Generation	No. of matings (No. of broods)	Observed no. for each phenotypic class (Expected no.) ♂♂ YSS	♂♂ YSSbar	♀♀ YSS	Expected ratio	Chi-square Goodness-of-fit Test (*df*=1) χ^2	χ^2_{adj}
YSS♂♂×YSS♀♀	F_1	8 (10)	65 (61.50)	0	58 (61.50)	1:1	0.398	0.293
(Mating 1a)	F_2	8 (10)	88 (90.50)	0	93 (90.50)	1:1	0.138	0.088
YSS♂♂×YSS♀♀	F_1	3 (5)	17 (17.50)	0	18 (17.50)	1:1	0.029	0.000
(Mating 1b)	F_2	3 (5)	20 (19.50)	6 (6.50)		3:1*	0.051	0.000
			20 + 6 = 26 (29.00)		32 (29.00)	1:1	0.621	0.431
YSSbar♂♂×	F_1	7 (7)	85 (81.50)	0	78 (81.50)	1:1	0.301	0.221
YSS♀♀	F_2	6 (6)	24 (23.25)	7 (7.75)		3:1*	0.097	0.011
(Mating 2a)			24 + 7 = 31 (30.50)		30 (30.50)	1:1	0.016	0.000
YSSbar♂♂×	F_1	6 (6)	0	16 (14.50)	13 (14.50)	1:1	0.310	0.138
YSS♀♀	F_2	5 (5)	0	9 (10.50)	12 (10.50)	1:1	0.429	0.190
(Mating 2b)								

* : expected ratio (3:1) of normal YSS ♂♂ to YSSbar ♂♂. All other hypothetical ratios (1:1) were for male to female progenies.

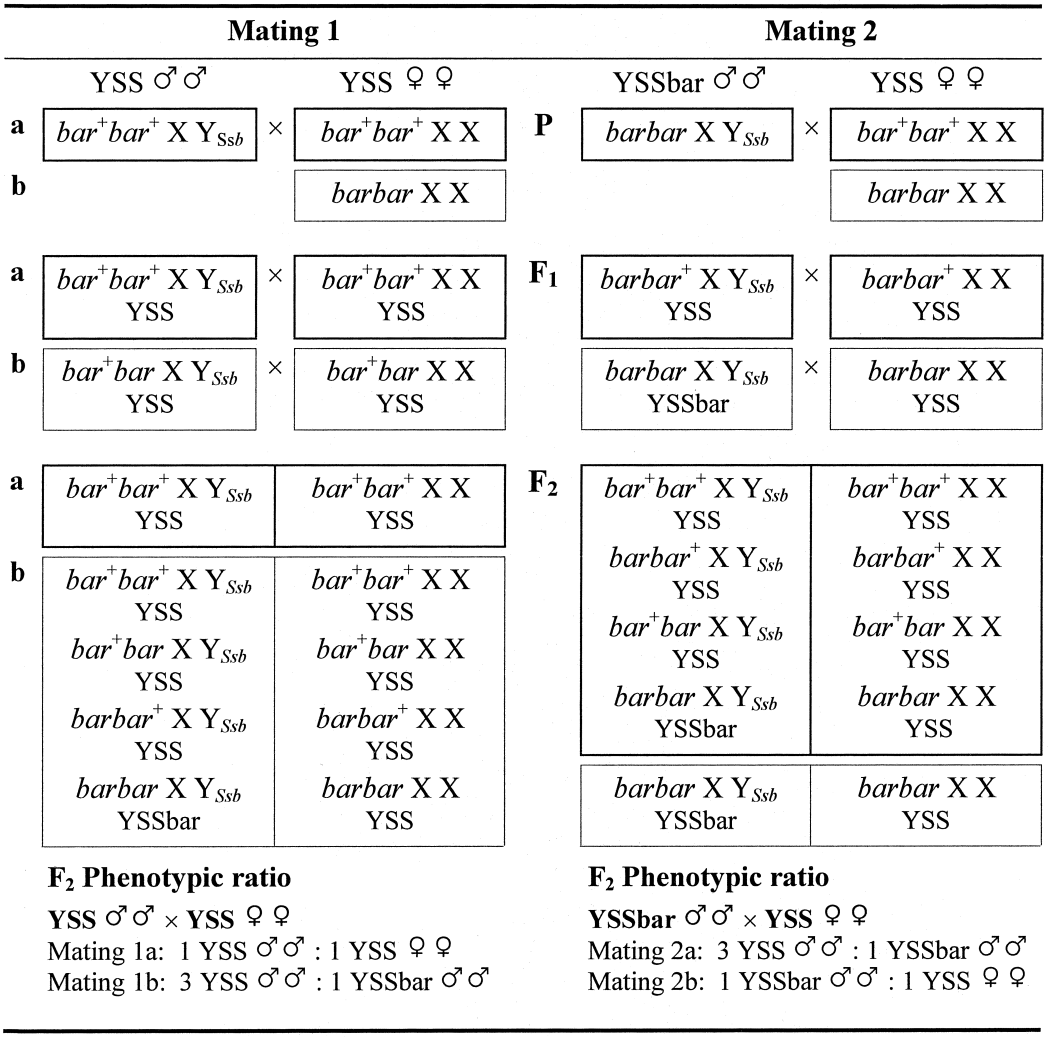

Fig. 2. Segregation of the bar (*bar*) and Snakeskin body pattern (*Ssb*) genes in the $\mathbf{F_1}$ and $\mathbf{F_2}$ progenies of Mating 1a: normal Yellow Snakeskin male (YSS ♂♂) ×Yellow Snakeskin female (YSS ♀♀), Mating 1b: YSS ♂♂ × YSS ♀♀ (with bar gene that was unexpressed due to the absence of the Y-linked *Ssb* gene), Mating 2a: Yellow Snakeskin bar male (YSSbar ♂♂) × YSS ♀♀ and Mating 2b: YSSbar ♂♂ × YSS ♀♀ (with unexpressed *bar* gene). Matings 1b and 2b, and their F_1 and F_2 offspring are shown within thin borders to distinguish them from those of the normal YSS ♂♂ × YSS ♀♀ (non-bar) and YSSbar ♂♂ × YSS ♀♀ (non-bar) matings.

YSS but did not express the snakeskin pattern on the body and tail since these patterns are determined only by the Y-linked Snakeskin-body (*Ssb*) and Snakeskin-tail (*Sst*) genes (Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991). From χ^2 tests, the number of male to female F_1 and F_2 offspring was consistent with the expected ratio of 1:1 (Table 1).

The second group, Mating 1b, of three mating pairs, produced F_1 progenies where the males also exhibited the typical color patterns of the YSS phenotype (Table 1, Fig. 2). Females were devoid of snakeskin-like patterns due to the lack of the *Ssb* and *Sst* genes (Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991). In the F_2 generation, 20 of the males were YSS while the rest had four to five iridescent vertical bars on the caudal-peduncle region (Fig. 1, Table 1). These males were designated as the Yellow Snakeskin bar phenotype (YSSbar) (Table 1, Fig. 2). Chi-square tests showed that the number of F_2 males fit the hypothetical ratio of 3 YSS : 1 YSSbar (Table 1). F_1 and F_2 results gave evidence that the bar pattern in YSSbar males was inherited from the YSS female parents (Table 1, Fig. 2). The 3:1 ratio also indicates that Mating 1b was a simple Mendelian monohybrid cross in which a single autosomal recessive gene was responsible for the bar pattern. As such, parental YSS females in Mating 1b were possibly homozygous for this gene (Fig. 2).

From the results of Matings 1a and 1b (Table 1, Fig. 2), we propose the designation of *bar* for this autosomal recessive gene that, when in homozygous condition (*barbar*), interacts with the *Ssb* gene to produce iridescent vertical barring patterns on the caudal-peduncle of male snakeskin guppies. In Mating 1a, YSS male parents are inferred to have the $bar^+bar^+XY_{Ssb}$ genotype (Fig. 2). Similarly, the putative genotype of Yellow Snakeskin females in this case is likely to be bar^+bar^+XX. Our observations, however, do not indicate that YSS male and female parents of each mating pair were het-

erozygous for *bar*, i.e., *bar*$^{+}$*bar* (Table 1). Thus, parental females of Mating 1b were homozygous for this gene (genotype: *barbar*XX) (Fig. 2). Only male guppies are able to express the bar pattern because they possess the Y-linked *Ssb* gene. The dominant allele of this locus, *bar*$^{+}$, does not modify the snakeskin body pattern of YSS males.

Segregation of *bar* in F_1 and F_2 offspring of YSSbar × YSS

As described for Mating 1, F_1 and F_2 progenies of Mating 2 could also be separated into Matings 2a and 2b according to their phenotypes (Table 1, Fig. 2). Seven single-pair matings between YSSbar males and YSS females of Mating 2a gave a total of 85 F_1 males and 78 females that were all YSS in the expected 1:1 male to female ratio (Table 1, Fig. 2). In the F_2 generation, however, there were 24 YSS males and seven YSSbar males. These observed numbers of F_2 males concurred with the 3 YSS : 1 YSSbar male ratio expected in the F_2 generation of a monohybrid cross (Table 1, Fig. 2). This shows that the parental YSSbar males in Mating 2a most likely had a genotype of *barbar*XY$_{Ssb}$ while the females were *bar*$^{+}$*bar*$^{+}$XX. For Mating 2b, the F_1 and F_2 offspring comprised only YSSbar males and YSS females that conformed to the male to female ratio of 1:1 (Table 1, Fig. 2). These results prove that YSSbar males and YSS females of the parental generation of Mating 2b were homozygous for the autosomal recessive *bar* gene (Fig. 2).

Inheritance of *bar* and its interaction with the *Ssb* gene

To date, the number of sex-linked color pattern genes described for the guppy far outnumber the autosomal ones (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Dzwillo, 1959; Nayudu, 1979; Kirpichnikov, 1981; Fernando and Phang, 1989; Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991; Khoo *et al.*, 1999a, b). Only four genes, blond (*b*), gold (*g*), albino (*a*) and blue (*bl*), that are responsible for background body coloration have been shown to be autosomally inherited in the guppy (Kirpichnikov, 1981). These genes are recessive to their wild-type alleles. Our observations of all parental and full-sib matings (Mating 1: YSS♂♂×YSS♀♀ and Mating 2: YSSbar♂♂×YSS♀♀) show evidence of Mendelian inheritance of the vertical bar pattern in guppies with reticulated snakeskin-like patterns (Table 1).

This study demonstrates, for the first time, that a single autosomal body pattern gene, *bar*, is responsible for the recessive vertical bar trait in male guppies of Snakeskin varieties. Segregation data for Matings 1 and 2 shows that the occurrence of *bar* in the homozygous condition modifies the Y-linked Snakeskin-body (*Ssb*) gene of Yellow Snakeskin males (Table 1, Fig. 2), resulting in a pattern of four to five vertical bars on the caudal-peduncle region (Fig. 1). Females that are homozygous for the *bar* gene do not express this barring pattern due to absence of *Ssb* (Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991). The *Ssb* gene is not modified by the dominant allele of the bar pattern locus, *bar*$^{+}$.

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