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Authors: Khoo, Gideon, Lim, Tit Meng, Chan, Woon-Khiong, and Phang, Violet P. E.

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Sex-Linkage of the Black Caudal-Peduncle and Red Tail Genes in the Tuxedo Strain of the Guppy, *Poecilia reticulata*

Gideon Khoo[1], Tit Meng Lim[1], Woon-Khiong Chan[1,2] and Violet P.E. Phang[1]*

[1]*Department of Biological Sciences, Faculty of Science, National University of Singapore, Kent Ridge, Singapore 117600, Republic of Singapore and*
[2]*Institute of Molecular Agrobiology, 1 Research Link, National University of Singapore, Kent Ridge, Singapore 117604, Republic of Singapore*

ABSTRACT—Two color patterns of the Tuxedo guppy strain commercially cultured in Singapore were subjected to genetic analyses. Gene control of the black caudal-peduncle and red tail color patterns was elucidated by reciprocal crosses between the Tuxedo (TUX) strain and wild-type (WT) stock. F_1 progenies were produced by single-pair crossing between TUX and WT, while the F_2 generation was obtained from full-sib mating between F_1 males and females. F_1 and F_2 data were segregated according to phenotypes and sex, and tested by chi-square analyses. Both color patterns show single gene inheritance, and are dominantly expressed in both sexes, sex-linked and determined by different loci on the X- and Y-chromosomes. Alleles for the black caudal-peduncle (*Bcp*) and red tail (*Rdt*) loci, are dominant over that of the wild-type, Bcp^+ and Rdt^+, which do not display these color patterns. The typical genotypes for TUX guppies are proposed to be $X_{Bcp,Rdt}Y_{Bcp,Rdt}$ for males and $X_{Bcp,Rdt}X_{Bcp,Rdt}$ for females. Heterozygous TUX males have the $X_{Bcp,Rdt}Y_{Bcp^+,Rdt}$, $X_{Bcp,Rdt}Y_{Bcp^+,Rdt^+}$ and $X_{Bcp,Rdt}Y_{Bcp,Rdt^+}$ genotypes while the females are $X_{Bcp,Rdt}X_{Bcp^+,Rdt}$. The segregation and inheritance of the *Bcp* and *Rdt* genes are illustrated by genetic models. Map distances estimated from F_1 and F_2 recombinants are approximately 3.4, 5.1 and 2.4 map units for the sex-determining region (SdR)–*Rdt*, SdR–*Bcp* and *Rdt*–*Bcp*, respectively. The gene map order is hypothesized to be SdR–*Rdt*–*Bcp*.

INTRODUCTION

The guppy, *Poecilia reticulata* Peters, is a fresh- and brackish-water ovoviviparous poecilid fish native to Trinidad, Barbados, Venezuela, Guyana and North-Eastern Brazil (Haskins and Haskins, 1951; Yamamoto, 1975). This fish is known for its striking sexual dimorphism. Wild-type males are smaller than females and their anal fin is modified into a copulatory organ called the gonopodium. The complex polymorphic spots and patches of color on the body and fins of wild guppies are expressed only by sexually mature males. The female guppy is devoid of color patterns, being a uniform olive-brown with hyaline fins (Haskins and Haskins, 1951). The wild-type guppy was introduced into Singapore and other parts of South-East Asia in the late 1930s for mosquito control (Herre, 1940).

The guppy became popular among aquarists and hobbyists who developed many exotic strains by intensive selection of spontaneous mutant genes that affect the coloration as well as the shape and size of the body and fins (Dzwillo, 1959; Kirpichnikov, 1981; Fernando and Phang, 1985). In Singapore, commercial culture of fancy guppy strains began in the early 1950s. About 30–40 different strains are reared in monoculture farms (Fernando and Phang, 1985). The guppy plays an important role in the ornamental fish industry of Singapore.

The guppy is unique among other teleosts in that almost all the genes encoding for color patterns are sex-linked and sex-limited. There are 23 pairs of chromosomes in the guppy, 22 of which are autosomal and one the sex chromosomes. Male guppies are heterogametic (XY) while the females are homogametic (XX) (Winge, 1922a, b; Winge and Ditlevsen, 1947). It is the first species known to have Y-linked inheritance of color genes (Schmidt, 1920). Kirpichnikov (1981) documented 17 Y-linked genes that are only passed from father to son (one-sided masculine inheritance), 15 that are X- and Y-linked (found in both males and females but expressed only in males as they are sex-limited and hormone-mediated), and one that is autosomal dominant. Several of these genes, e.g., *Maculatus* (*Ma*), *Armatus* (*Ar*) and *Pauper* (*Pa*) influence sex determination in the guppy (Schmidt, 1920; Winge, 1922a,b, 1927, 1934; Winge and Ditlevsen, 1947). These are usually located close to or within a sex-determining region (desig-

* Corresponding author: Tel. (65)-874-2694;
FAX. (65)-779-2486.
E-mail: dbsphang@nus.edu.sg

nated as SdR) on the Y-chromosome, and are presumed to be tightly linked with a gene for maleness (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Kirpichnikov, 1981). The SdR may also represent, by itself, a dominant factor for male-determination and possibly has a recessive female-determining region at a similar position on the X-chromosome. Genes for background body coloration such as blond (*bb*), gold (*gg*), albino (*aa*) and blue (*blbl*) are, however, autosomally inherited and recessive to their wild-type alleles (Haskins and Druzba, 1938; Goodrich *et al.*, 1944, 1947; Kirpichnikov, 1981).

Color patterns on the body and fins of domesticated guppies take the form of single colors, snakeskin-like reticulations and variegated mosaic patterns of two or more colors (Nayudu, 1975, 1979; Kirpichnikov, 1981; Fernando and Phang, 1989, 1990; Phang *et al.*, 1989a,b, 1990; Phang and Fernando, 1991; Khoo *et al.*, 1999). The ease with which new strains can be developed from spontaneous mutation makes the guppy a suitable species for investigating the genetic control of color polymorphism (Dzwillo, 1959; Yamamoto, 1975; Nayudu, 1979; Kirpichnikov, 1981; Fernando and Phang, 1985). Expression of phenotypic color patterns in cultured strains has been found to be determined by dominant X- and Y-linked genes (Dzwillo, 1959; Nayudu, 1975, 1979; Kirpichnikov, 1981; Fernando and Phang, 1989, 1990; Phang *et al.*, 1989a, b, 1990; Phang and Fernando, 1991). These genes may consequently be used as genetic (phenotypic) markers to map the sex chromosomes of the guppy (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Nayudu, 1975, 1979; Kirpichnikov, 1981; Purdom, 1993).

Two color patterns of the Tuxedo guppy strain, namely, black caudal-peduncle and red tail, were genetically investigated in this study. The loci for the black caudal-peduncle and red tail genes on the sex chromosomes are proposed to be *Bcp* and *Rdt*, respectively. Genetic map distances of these genes from the sex-determining region were determined from recombination rates. Our study forms part of an on-going effort to link color pattern genes to the X- and Y-chromosomes and autosomes of the guppy using phenotypic markers (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Dzwillo, 1959; Nayudu, 1975, 1979; Kirpichnikov, 1981; Fernando and Phang, 1989, 1990; Phang *et al.*, 1989a,b, 1990; Phang and Fernando, 1991; Purdom, 1993; Khoo *et al.*, 1999) and recently, molecular markers by Random Amplified Polymorphic DNA (RAPD) fingerprinting (Foo *et al.*, 1995).

MATERIALS AND METHODS

Source of the fish

Three- to four-week old fry of the Tuxedo (TUX) guppy strain were obtained from Chin Lam Brothers Tropical Fish Farm in Singapore. The common name, Tuxedo, for this strain was given by guppy breeders. Wild-type (WT) feral guppies were collected from an isolated hill-stream near the Bukit Timah nature reserve in Singapore. Juvenile TUX were cultured in 180-liter fibreglass tanks (30 fish/tank) in the aquarium area of the Department of Biological Sciences, National University of Singapore, at temperatures of 25–28°C. WT fry were separated from the collected samples and raised in 30-liter clear plastic tanks (20 fish/tank). Under laboratory conditions, sexual maturation of WT fry usually occurs at 4–6 weeks of age. Juvenile WT were checked daily for developing males which are detected by gonopodial formation of the anal fin. Males, when spotted, were immediately removed and reared separately from females as virgin females were essential for the reciprocal crosses.

Description of the fish

Adult males and females of the TUX strain have a total length of 3–4 cm and 5–6 cm, respectively. Adult TUX males have black (melanic) or dark grey pigmentation on the caudal-peduncle region which masks normal wild-type male body coloration, and a caudal fin that ranges from blood-red to orange-red in color (Fig. 1A). Some TUX males may have a metallic blue or green sheen overlying the black caudal-peduncle. TUX females show drab wild-type olive-brown body coloration and grey caudal-peduncle with red tinges of varying intensity on an opaque greyish-white tail (Fig. 1B). Wild-type guppies are smaller than the domesticated TUX strain. Adult WT males are 2–2.5 cm in length and females are about 3–4 cm. As described earlier, WT males have highly polymorphic color patterns on the body and fins (Fig. 1C), while WT females are devoid of color patterns (Fig. 1D).

Reciprocal crosses

Inheritance of the black caudal-peduncle and red tail color patterns was elucidated by single-pair reciprocal crosses between the TUX strain and WT stock, using six-week old mature virgin fish. Each pair was kept in a 3.5-liter breeding tank. Broods were produced 4-6 weeks after mating. Single-pair full-sib F_1 males and F_1 females were mated to obtain the F_2 generation. The following notations were used: TUX♂♂×WT♀♀(Table 1A) and WT♂♂×TUX♀♀(Table 2A) for parental crosses, and F_1♂♂×F_1♀♀(Tables 1B, 2B) for full-sib F_1 crosses. Newly born fry were separated and raised to maturity in 3.5-liter clear plastic tanks (five fish/tank). All F_1 and F_2 progenies were segregated and scored according to phenotypes and sex. Progenies displaying color patterns such as Tuxedo, red tail and black caudal-peduncle were designated as the TUX, RT and BCP phenotypes, respectively, and those without such color patterns, WT phenotype. Tuxedo males of parental crosses were typed using Roman numerals (I, II, III, IV and V) according to their putative alleles following segregation of their F_1 and F_2 progenies. This was to facilitate description of the crosses.

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. The χ^2 test for homogeneity was used to determine whether there were significant differences among the phenotypic frequencies, and if the observations were sufficiently uniform and the population homogeneous after the data was pooled. The correction for continuity was not incorporated into the test for homogeneity because calculated χ^2 values had to be summed and χ^2_{adj} values were not additive (Sokal and Rohlf, 1981; Strickberger, 1990). Following Winge (1922b, 1923, 1927, 1934), Nayudu (1979), Phang *et al.* (1989a, b, 1990), Phang and Fernando (1991), and Khoo *et al.* (1999), individuals with exceptional coloration due to crossing-over of the black caudal-peduncle (*Bcp*) and red tail (*Rdt*) genes between the X- and Y-chromosomes were not considered in chi-square analyses.

Recombination frequencies and map distances between *Bcp* and *Rdt*, and the sex-determining region (SdR) were estimated according to Strickberger (1990), Phang *et al.* (1990), Phang and Fernando (1991), Purdom (1993) and Khoo *et al.* (1999). Winge's (1922b, 1927, 1934) "zig-zag line diagram" method was applied to test all possible linkage combinations between *Bcp*, *Rdt* and SdR, and map these loci

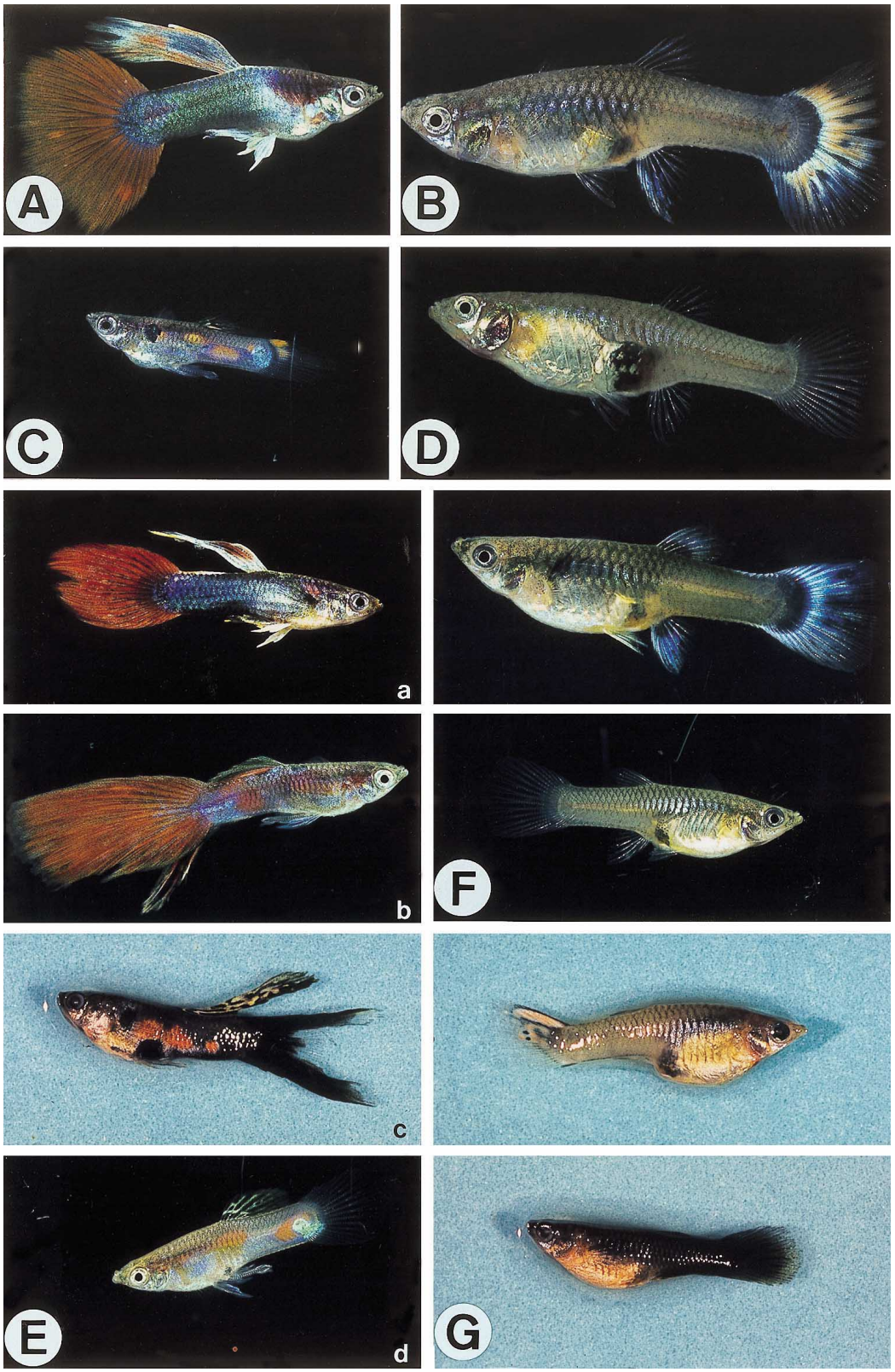

Fig. 1. (**A**) Adult male guppy of the Tuxedo (TUX) strain showing black caudal-peduncle and red tail color patterns. (**B**) Adult female guppy of the Tuxedo strain with grey caudal-peduncle and faint red tinges on an opaque greyish-white tail. (**C**) Adult male feral guppy with typical wild-type (WT) male body coloration. (**D**) Adult female wild-type guppy with a drab olive-brown body that is devoid of any bright color patterns. (**E**) Male progenies (F_1 and F_2) of the (**a**) TUX, (**b**) red tail, (**c**) black caudal-peduncle and (**d**) WT phenotypes. (**F**) Female progenies (F_1 and F_2) with typical TUX (top) and WT (bottom) colorations. (**G**) Recombinant females (F_1 and F_2) displaying red tail (top) and black caudal-peduncle (bottom) color patterns.

in sequential order. Due to the infrequency of single crossing-over in the guppy compared to the fruitfly, *Drosophila* (Winge, 1927, 1934; Purdom, 1993), double crossing-over was excluded from calculations of map distances. All crossovers in this study were thus regarded as single crossovers.

RESULTS

Segregation and recombination in TUX ♂♂×WT ♀♀ F_1 and F_2 offspring

Three mating pairs TUX♂♂×WT♀♀ (PT5, PT9 and PT10) produced 40 male and 34 female F_1 offspring in 8 broods

Table 1. Mating results of crosses between Tuxedo (TUX) males and wild-type (WT) females showing observed and expected numbers for each phenotypic class, expected segregation ratios, chi-square goodness-of-fit to the expected ratios and their corresponding adjusted values (χ^2_{adj}) after application of Yates' correction for continuity, χ^2 test for homogeneity, probable genotypes and recombinants for (**A**) the F_1 generation of single-pair parental crosses, and (**B**) the F_2 generation of single-pair crosses between full-sib F_1 males and F_1 females. Recombinants (§) due to crossing-over of the *Rdt* and *Bcp* genes were not considered in chi-square analyses. (Phenotypes: TUX♂♂=Tuxedo males with black caudal-peduncle and red tail; TUX♀♀=Tuxedo females with grey caudal-peduncle and faint red tinges on an opaque greyish-white tail; RT = red tail without black caudal-peduncle; BCP=black caudal-peduncle without red tail; WT = wild-type coloration without red tail and black caudal-peduncle. Genes: *Bcp*=black caudal-peduncle gene; *Bcp*$^+$=absence of black caudal-peduncle gene; *Rdt*=red tail gene; *Rdt*$^+$=absence of red tail gene).

A. TUX♂♂×WT♀♀ (Parental Cross)

TUX ♂♂ type	Mating pair design-nation	No. of F_1 broods	Observed numbers for each F_1 phenotypic class (expected numbers) ♂♂ TUX	♂♂ RT	♂♂ BCP	♂♂ WT	♀♀ TUX	♀♀ BCP	Expect-ed F_1 Ratio of ♂:♀	Chi-square Goodness-of-fit Test (*df*=1) χ^2	χ^2_{adj}	Total χ^2	Pooled χ^2	χ^2 for Homo-geneity	Putative parental genotypes ♂♂ TUX	♀♀ WT
I	PT5	4	26 (23)				20 (23)		1:1	0.782	0.544					
	PT9	2	7 (6.5)				6 (6.5)		1:1	0.077	0.000	0.926	0.486	0.440	$X_{Bcp,Rdt}$	$X_{Bcp}^{+},_{Rdt}^{+}$
	PT10	2	7 (7.5)				8 (7.5)		1:1	0.067	0.000	(*df*=3)	(*df*=1)	(*df*=2)	$Y_{Bcp,Rdt}$	$X_{Bcp}^{+},_{Rdt}^{+}$
	Pooled:	8	40 (37)				34 (37)		1:1	0.486	0.338					
III	PT2	5		24 (26.5)			29 (26.5)		1:1	0.472	0.302					
	PT3	3		14 (13)			12 (13)		1:1	0.154	0.038					
	PT4	3		30 (35.5)			41 (35.5)		1:1	1.704	1.408	2.512	0.796	1.716	$X_{Bcp,Rdt}$	$X_{Bcp}^{+},_{Rdt}^{+}$
	PT7	3		16 (17)			18 (17)		1:1	0.118	0.030	(*df*=5)	(*df*=1)	(*df*=4)	$Y_{Bcp}^{+},_{Rdt}$	$X_{Bcp}^{+},_{Rdt}^{+}$
	PT8	4	4§	32 (31)			30 (31)		1:1	0.064	0.016					
	Pooled:	18	4§	116 (123)			130 (123)		1:1	0.796	0.686					
IV	PT1	4	2§		2§	33 (34)	35 (34)		1:1	0.058	0.014	—	—	—	$X_{Bcp,Rdt}$	$X_{Bcp}^{+},_{Rdt}^{+}$
															$Y_{Bcp}^{+},_{Rdt}^{+}$	$X_{Bcp}^{+},_{Rdt}^{+}$
V	PT6	3			25 (27)		29 (27)	1§	1:1	0.296	0.166	—	—	—	$X_{Bcp,Rdt}$	$X_{Bcp}^{+},_{Rdt}^{+}$
															$Y_{Bcp,Rdt}^{+}$	$X_{Bcp}^{+},_{Rdt}^{+}$

df : degrees of freedom
§ : recombinant data (not used for chi-square analyses)

B. F_1 ♂♂×F_1♀♀ (Full-sib F_1 Cross)

TUX ♂♂ type	Mating pair design-ation	No. of F_2 broods (No. of F_1 pairs)	Observed numbers for each F_2 phenotypic class (expected numbers) ♂♂ TUX	♂♂ RT	♂♂ BCP	♂♂ WT	♀♀ TUX	♀♀ WT	♀♀ RT	♀♀ BCP	Expected F_2 ratio (*df*)	Chi-square Goodness-of-fit Test χ^2	χ^2_{adj}	Total χ^2	Pooled χ^2	χ^2 for Homo-geneity	Putative F_1 genotypes [phenotypes] ♂♂	♀♀
I	PT5	23 (9)	158 (159.5)	2§		3§	84 (79.75)	77 (79.75)	1§	1§	2:1:1 (2)	0.335	0.245	1.699	0.702	0.997	$X_{Bcp}^{+},_{Rdt}^{+}$	$X_{Bcp,Rdt}$
	PT9	3 (2)	26 (27.5)				14 (13.75)	15 (13.75)	1§	1§	2:1:1 (2)	0.201	0.082	(*df*=6)	(*df*=2)	(*df*=4)	$Y_{Bcp,Rdt}$	$X_{Bcp}^{+},_{Rdt}^{+}$
	PT10	2 (2)	21 (24.5)	1§			15 (12.25)	13 (12.25)			2:1:1 (2)	1.163	0.785				[TUX]	[TUX]
	Pooled:	28 (13)	205 (211.5)	3§		3§	113 (105.75)	105 (105.75)	2§	2§	2:1:1 (2)	0.702	0.602					
III	PT2	5 (4)	18 (19.5)	19 (19.5)			21 (19.5)	20 (19.5)		1§	1:1:1:1 (3)	0.256	0.102					
	PT3	8 (3)	49 (52.5)	50 (52.5)			56 (52.5)	55 (52.5)		1§	1:1:1:1 (3)	0.704	0.494	3.466	0.978	2.488	$X_{Bcp}^{+},_{Rdt}^{+}$	$X_{Bcp,Rdt}$
	PT4	0 (1)	0	0			0	0			1:1:1:1 (3)	—	—	(*df*=9)	(*df*=3)	(*df*=6)	$Y_{Bcp}^{+},_{Rdt}$	$X_{Bcp}^{+},_{Rdt}^{+}$
	PT7	7 (2)	41 (43.5)	52 (43.5)			43 (43.5)	38 (43.5)	4§	1§	1:1:1:1 (3)	2.506	2.138				[RT]	[TUX]
	PT8	0 (1)	0	0			0	0			1:1:1:1 (3)	—	—					
	Pooled:	20 (11)	108 (115.5)	121 (115.5)			120 (115.5)	113 (115.5)	4§	3§	1:1:1:1 (3)	0.978	0.814					
IV	PT1	13 (4)	61 (55.25)			54 (55.25)	58 (55.25)	48 (55.25)			1:1:1:1 (3)	1.714	1.426	—	—	—	$X_{Bcp}^{+},_{Rdt}^{+}$	$X_{Bcp,Rdt}$
																	$Y_{Bcp}^{+},_{Rdt}^{+}$	$X_{Bcp}^{+},_{Rdt}^{+}$
																	[WT]	[TUX]
V	PT6	9 (2)	64 (60.75)		65 (60.75)	7§	54 (60.75)	60 (60.75)		1§	1:1:1:1 (3)	1.230	0.999	—	—	—	$X_{Bcp}^{+},_{Rdt}^{+}$	$X_{Bcp,Rdt}$
																	$Y_{Bcp,Rdt}^{+}$	$X_{Bcp}^{+},_{Rdt}^{+}$
																	[BCP]	[TUX]

df : degrees of freedom
§ : recombinant data (not used for chi-square analyses)

(Table 1A). F_1 males exhibited the black caudal-peduncle and red tail color patterns of their TUX male parents (Fig. 1E), while F_1 females had a grey caudal-peduncle and an opaque greyish-white tail (Fig. 1F). F_1 males and females could have inherited the black caudal-peduncle and red tail color genes only from their TUX male parents (designated as type I). Table 1A also shows three other crosses in which the TUX male parents were heterozygous for black caudal-peduncle and red tail. To facilitate description of these crosses and their offspring, these TUX males were labelled as types III, IV and V. Type II TUX males were not observed in this study although they were found among crosses between the Tuxedo and Green Variegated guppy strains that we carried out in a later study (Khoo *et al*., submitted). For type III, five mating pairs gave 18 broods of 116 red tail (RT) males and 130 TUX females (Fig. 1E, F, Table 1A). Four F_1 broods of 33 WT males and 35 TUX females were produced by the cross between a type IV TUX male and a WT female (mating pair PT1), while 25 BCP males and 29 TUX females were obtained from mating pair PT6 (type V TUX male) (Fig. 1E, F, Table 1A). For all four types (I, III, IV and V) of TUX male parents, the F_1 male to female ratio was consistent with the expected ratio of 1:1 (Table 1A).

The F_2 generation of type I comprised 205 TUX males, 113 TUX females and 105 WT females with the observed numbers conforming to the expected phenotypic ratio of 2:1:1 (Fig. 1E, F, Table 1B). Four F_2 phenotypes that consisted of 108 TUX and 121 RT males, and 120 TUX and 113 WT females were obtained from 11 single-pair full-sib F_1 crosses of type III (Fig. 1E, F, Table 1B). These phenotypes fitted the hypothetical ratio of 1:1:1:1. Mating pair PT1 (type IV) also gave four F_2 phenotypes that were made up of 13 broods consisting of 61 TUX and 54 WT males, and 58 TUX and 48 WT females that agreed with the 1:1:1:1 ratio (Fig. 1E, F, Table 1B). For mating pair PT6 (type V), two full-sib F_1 crosses produced nine broods of 64 TUX males, 65 BCP males, 54 TUX females and 60 WT females that corresponded to the expected 1:1:1:1 ratio (Fig. 1E, F, Table 1B). Homogeneity χ^2 tests carried out for TUX males of types I and III showed that the F_1 and F_2 observations were uniform and did not form heterogeneous populations after being pooled (Table 1A, B).

F_1 and F_2 data for TUX♂♂×WT♀♀ showed that the dominantly expressed black caudal-peduncle and red tail color patterns are due to single genes that are found at two different loci, *Bcp* and *Rdt*, respectively, on the sex chromosomes. Homozygous TUX male parents (type I) were elucidated to have the $X_{Bcp,Rdt}Y_{Bcp,Rdt}$ genotype (Table 1, Fig. 2). When TUX males were heterozygous at the *Bcp* and *Rdt* loci, these individuals possessed the $X_{Bcp,Rdt}Y_{Bcp}^{+}$,*Rdt* (type III), $X_{Bcp,Rdt}Y_{Bcp}^{+}{}_{,Rdt}^{+}$ (type IV) and $X_{Bcp,Rdt}Y_{Bcp,Rdt}^{+}$ (type V) genotypes (Table 1, Fig. 2). The segregation of the *Bcp* and *Rdt* alleles in the TUX♂♂×WT♀♀ cross is presented schematically by a genetic model in Fig. 2.

Crossing-over between the sex-determining region (SdR), and the black caudal-peduncle (*Bcp*) and red tail (*Rdt*) loci at the parental and F_1 levels for TUX♂♂×WT♀♀ produced exceptionally colored F_1 and F_2 recombinants (Table 1). These individuals which expressed TUX, RT, BCP and WT phenotypic colorations in males, and RT and BCP in females, deviated from the predicted phenotypes for types I, III, IV and V. Type I produced three RT and three WT recombinant F_2 males (Fig. 1E, Table 1B). The crossover frequency calculated from the percentage of crossover males out of the total number of F_2 males (3/211×100%) was 1.422% for SdR–*Rdt* and *Rdt*–*Bcp*, respectively. Occurrence of RT recombinant F_2 males indicated that the Y-chromosome of the guppy may possibly have a map order of SdR–*Rdt*–*Bcp* as these recombinants could not be produced using Winge's (1922b, 1927, 1934) "zig-zag line diagram" method if the gene order had been either SdR–*Bcp*–*Rdt* or *Bcp*–SdR–*Rdt* (Figs. 2, 3). A map distance of 1.802 map units was estimated for *Rdt*–*Bcp* from two RT and two BCP recombinant F_2 females of 222 F_2 females for type I (Fig. 1G, Table 1B). Recombinant F_1 TUX males and F_2 RT females of type III were not used to estimate map distances because crossovers could have taken place at SdR–*Rdt* and *Rdt*–*Bcp*, making it impossible to determine the actual region of crossover. Of 240 F_2 females of this cross, three BCP female recombinants gave a crossover frequency of 1.250% between *Rdt* and *Bcp* (Fig. 1G, Table 1B).

For mating pair PT1 (type IV), 5.405 map units were estimated to separate the *Rdt* locus from SdR and *Rdt* from *Bcp*, respectively (Table 1A). Longer SdR–*Rdt* and *Rdt*–*Bcp* map distances for type IV compared to types I and III was due to frequent crossing-over which yielded two TUX and two BCP recombinant F_1 males out of only 37 F_1 males (Fig. 1E, Table 1A). The presence of F_1 BCP male recombinants in this cross also indicated a gene sequence of SdR–*Rdt*–*Bcp* (Figs. 2, 3). An F_1 BCP recombinant female of 30 F_1 females for mating pair PT6 (type V) gave a crossover rate of 3.333% between SdR and *Rdt* (Fig. 1G, Table 1A). Seven F_2 recombinant males that had WT coloration of 136 F_2 males of PT6 further supported an order of SdR–*Rdt*–*Bcp*, giving an approximate distance of 5.147 map units between SdR and *Bcp* (Fig. 1E, Table 1B). The mean genetic map distance between the SdR and *Rdt*, calculated from F_1 and F_2 recombinant data (Table 1), was 3.387±1.992 map units while the Bcp locus appeared to be about 5.147 map units from the SdR (Fig. 3).

Segregation and recombination in WT ×TUX F_1 and F_2 offspring

Three mating pairs (PB2, PB5 and PB6) of the reciprocal cross, WT♂♂×TUX♀♀, gave nine F_1 broods of 69 males and 82 females, all of which had black caudal-peduncle and red tail color patterns typical of the Tuxedo (TUX) phenotype (Fig. 1E, F). The observed numbers of F_1 male to female offspring agreed with the expected ratio of 1:1 (Table 2A). With the exception of four males with BCP phenotype, the F_2 progeny of this cross segregated into 93 TUX males, 93 WT males and 183 TUX females according to the expected phenotypic ratio of 1:1:2 (Fig. 1E, F, Table 2B). Results for mating pair PB4 were not included with those of the above three mating pairs because it produced a very high number of F_2 recombi-

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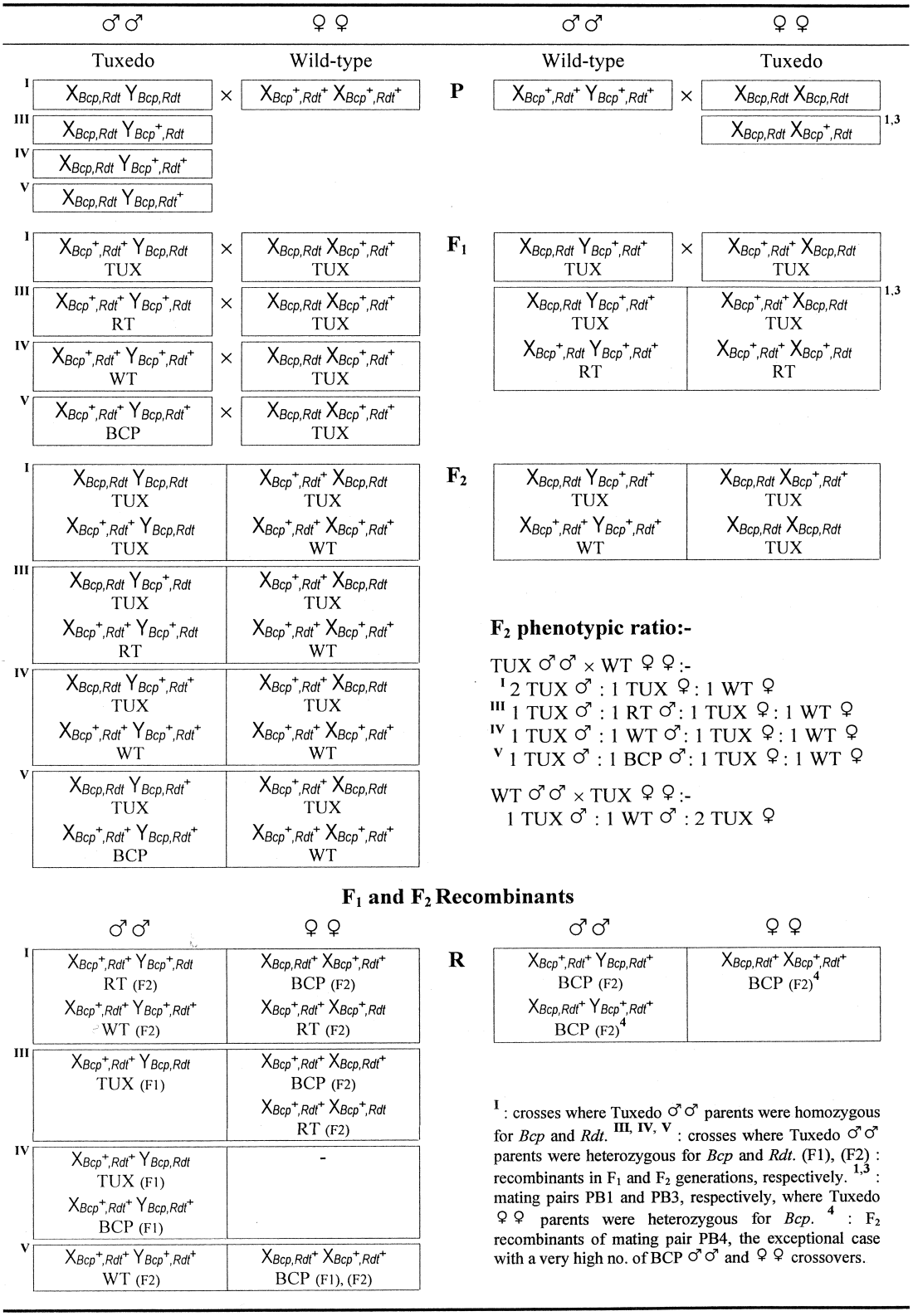

Fig. 2. Schematic diagram of the proposed genetic models showing segregation of the *Bcp* and *Rdt* color genes, and genotypes of the parents (**P**), F_1 and F_2 progenies, and recombinants (**R**) that occur in reciprocal crosses between the Tuxedo (TUX) and wild-type (WT) guppies.

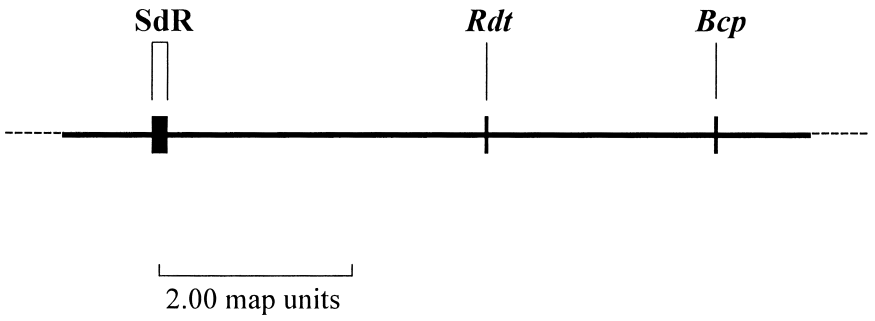

Fig. 3. Genetic map of the Y-chromosome of the guppy, *Poecilia reticulata*, showing the positions of the black caudal-peduncle (*Bcp*) and red tail (*Rdt*) loci relative to the sex-determining region (SdR). Map distances of *Bcp* and *Rdt* from the SdR are based on recombination frequencies estimated from the TUX ♂♂×WT ♀♀ and WT ♂♂×TUX ♀♀ crosses in Tables 1 and 2. *Bcp* and *Rdt* are also inferred to be located at similar positions on the X-chromosome of the guppy. The size of the SdR is not according to scale as the number of male-determining genes within that region is unknown.

Table 2. Mating results of crosses between wild-type (WT) males and Tuxedo (TUX) females showing observed and expected numbers for each phenotypic class, expected segregation ratios, chi-square goodness-of-fit to the expected ratios and their corresponding adjusted values (χ^2_{adj}) after application of Yates' correction for continuity, χ^2 test for homogeneity, probable genotypes and recombinants for (**A**) the F_1 generation of single-pair parental crosses, and (**B**) the F_2 generation of single-pair crosses between full-sib F_1 males and F_1 females. Recombinants ($^{\#}$) due to crossing-over of the *Rdt* and *Bcp* genes were not considered in chi-square analyses. (Phenotypes: TUX♂♂= Tuxedo males with black caudal-peduncle and red tail; TUX♀♀= Tuxedo females with grey caudal-peduncle and faint red tinges on an opaque greyish-white tail; RT = red tail without black caudal-peduncle; BCP = black caudal-peduncle without red tail; WT = wild-type coloration without red tail and black caudal-peduncle. Genes: *Bcp* = black caudal-peduncle gene; Bcp^+ = absence of black caudal-peduncle gene; *Rdt* = red tail gene; Rdt^+ = absence of red tail gene).

A. WT♂♂×TUX♀♀(Parental Cross)

Mating pair designation	No. of F_1 broods	Observed numbers for each F_1 phenotypic class (expected numbers) ♂♂ TUX	♂♂ RT	♀♀ TUX	♀♀ RT	Expected F_1 ratio (*df*)	Chi-square Goodness-of-fit Test χ^2	χ^2_{adj}	Total χ^2	Pooled χ^2	χ^2 for Homo-geneity	Putative parental genotypes ♂♂ WT	♀♀ TUX
PB2	4	21 (23.5)		26 (23.5)		1:1 (1)	0.532	0.340	1.592 (*df*=3)	1.120 (*df*=1)	0.472 (*df*=2)	$X_{Bcp}{}^{+},{}_{Rdt}{}^{+}$	$X_{Bcp,Rdt}$
PB5	2	20 (23.5)		27 (23.5)		1:1 (1)	1.042	0.766				$Y_{Bcp}{}^{+},{}_{Rdt}{}^{+}$	$X_{Bcp,Rdt}$
PB6	3	28 (28.5)		29 (28.5)		1:1 (1)	0.018	0.000					
Pooled:	9	69 (75.5)		82 (75.5)		1:1 (1)	1.120	0.954					
PB4[e]	5	26 (27.5)		29 (27.5)		1:1 (1)	0.164	0.072	—	—	—	$X_{Bcp}{}^{+},{}_{Rdt}{}^{+}$ $Y_{Bcp}{}^{+},{}_{Rdt}{}^{+}$	$X_{Bcp,Rdt}$ $X_{Bcp,Rdt}$
PB1	3	14 (10.5)	11 (10.5)	9 (10.5)	8 (10.5)	1:1:1:1 (3)	2.000	1.333	2.526 (df=6)	1.100 (df=3)	1.426 (df=3)	$X_{Bcp}{}^{+},{}_{Rdt}{}^{+}$	$X_{Bcp,Rdt}$
PB3	3	10 (9.5)	8 (9.5)	9 (9.5)	11 (9.5)	1:1:1:1 (3)	0.526	0.210				$Y_{Bcp}{}^{+},{}_{Rdt}{}^{+}$	$X_{Bcp}{}^{+},{}_{Rdt}$
Pooled:	6	24 (20)	19 (20)	18 (20)	19 (20)	1:1:1:1 (3)	1.100	0.752					

df : degrees of freedom
[e] : exceptional case with a very high number of F_2 recombinants

B. F_1♂♂× F_1♀♀(Full-sib F_1 Cross)

Mating pair designation	No. of F_2 broods (No. of F_1 pairs)	Observed numbers for each F_2 phenotypic class (expected numbers) ♂♂ TUX	♂♂ WT	♂♂ BCP	♀♀ TUX	♀♀ BCP	Expected F_2 ratio	Chi-square Goodness-of-fit Test (*df*=2) χ^2	χ^2_{adj}	Total χ^2	Pooled χ^2	χ^2 for Homo-geneity	Putative F_1 genotypes [phenotypes] ♂♂	♀♀
PB2	8 (4)	37 (38.25)	39 (38.25)	4[#]	77 (76.5)		1:1:2	0.059	0.017	1.303 (*df*=6)	0.024 (*df*=2)	1.279 (*df*=4)	$X_{Bcp,Rdt}$	$X_{Bcp}{}^{+},{}_{Rdt}{}^{+}$
PB5	9 (3)	38 (33.5)	32 (33.5)		64 (67.0)		1:1:2	0.805	0.601				$Y_{Bcp}{}^{+},{}_{Rdt}{}^{+}$ [TUX]	$X_{Bcp,Rdt}$ [TUX]
PB6	5 (4)	18 (20.5)	22 (20.5)		42 (41.0)		1:1:2	0.439	0.250					
Pooled:	22 (11)	93 (92.25)	93 (92.25)	4[#]	183 (184.5)		1:1:2	0.024	0.007					
PB4[e]	13 (3)	63 (54.75)	51 (54.75)	11[#]	105 (109.5)	16[#]	1:1:2	1.685	1.436	—	—	—	$X_{Bcp,Rdt}$ $Y_{Bcp}{}^{+},{}_{Rdt}{}^{+}$ [TUX]	$X_{Bcp}{}^{+},{}_{Rdt}{}^{+}$ $X_{Bcp,Rdt}$ [TUX]

df : degrees of freedom
[e] : exceptional case with a very high no. of F_2 recombinants
[#] : recombinant data (not used for chi-square analyses)

nants (11 BCP males and 16 BCP females) (Table 2B). PB4, however, satisfied the expected F_1 male to female ratio of 1:1 with 26 TUX males and 29 TUX females (Table 2A). In addition, three full-sib single-pair F_1 crosses of PB4 generated 63 TUX males, 51 WT males and 105 TUX females that were consistent with the F_2 phenotypic ratio of 1:1:2 (Table 2B). Tuxedo females of mating pairs PB1 and PB3 were inferred to be heterozygous at the *Bcp* locus since they gave a total of 24 TUX males, 19 RT males, 18 TUX females and 19 RT females that conformed to the expected F_1 ratio of 1:1:1:1 (Table 2A). After pooling of the F_1 and F_2 data for WT♂♂× TUX♀♀, homogeneity χ^2 tests showed that each pooled population was homogeneous and uniform (Table 2A, B).

F_1 and F_2 results for WT♂♂×TUX♀♀confirmed the observations of the reciprocal cross (TUX♂♂×WT♀♀) that two single sex-linked genes, black caudal-peduncle (*Bcp*) and red tail (*Rdt*), are responsible for the dominant expression of the Tuxedo color pattern. TUX female parents (including PB4) used in this study were found to be homozygous for *Bcp* and *Rdt*, and had the $X_{Bcp,Rdt}X_{Bcp,Rdt}$ genotype (Table 2, Fig. 2). Conversely, the putative genotype for heterozygous TUX females of mating pairs PB1 and PB3 was $X_{Bcp,Rdt}X_{Bcp^+,Rdt}$(Table 2, Fig. 2). The segregation of *Bcp* and *Rdt* is illustrated by a genetic model in Fig. 2.

Four F_2 males of mating pair PB2 had a black caudal-peduncle and black tail instead of a red tail (Fig. 1E, Table 2B). In this instance, *Bcp* could have crossed over from the X- to the Y-chromosome in the F_1 male parent, and also from Y to X in the F_1 female parent to produce male BCP recombinants (Fig. 2). Since there were four BCP males of a total 190 F_2 male individuals, the crossover frequency between *Bcp* and *Rdt* was estimated to be 2.105% (2.105 map units) (Table 2B). As mentioned earlier, mating pair PB4 produced a very high number of F_2 BCP male (11) and female (16) recombinants out of 125 F_2 males and 121 F_2 females (Table 2B, Fig. 2). From these crossover data, the map distance between *Bcp* and *Rdt* was averaged to be 11.012±3.128 map units. Simultaneous occurrence of BCP males and females suggested a gene order of SdR–*Bcp*–*Rdt*. These results contradicted those of the reciprocal cross (TUX♂♂×WT♀♀) which pointed to a map order of SdR–*Rdt*–*Bcp*. Moreover, *Bcp* and *Rdt* seemed to be too far apart (11.012± 3.128 map units) for PB4 compared to the 2.397±1.714 map units estimated from mating pairs PB2, PB5 and PB6, and TUX♂♂×WT♀♀ (Tables 1, 2, Fig. 3).

DISCUSSION

Inheritance of the black caudal-peduncle and red tail color patterns

Observations for all parental (TUX♂♂×WT♀♀and WT ♂♂×TUX♀♀, Tables 1A, 2A) and full-sib (F_1♂♂×F_1♀♀, Tables 1B, 2B) crosses indicate that the black caudal-peduncle (*Bcp*) and red tail (*Rdt*) color pattern genes are responsible for the Tuxedo phenotype of the guppy. This study demonstrates that these two color patterns are simple sex-linked traits controlled by single genes: the *Bcp* allele is dominant for black caudal-peduncle over *Bcp*$^+$, and *Rdt* is dominant for red tail over *Rdt*$^+$. Wild-type or feral guppies do not display these color patterns as they have the recessive *Bcp*$^+$ and *Rdt*$^+$ genes (Fig. 1C, D). Using Tuxedo males that were heterozygous for *Bcp* and *Rdt*, we have also shown that the expression of *Bcp* and *Rdt* is dominant in both males and females (Tables 1, 2, Figs. 1, 2). This study confirms our preliminary findings that the black caudal-peduncle and red tail colour genes have a dominant mode of inheritance (Fernando and Phang, 1989, 1990, Phang *et al.*, 1990). The typical genotypes for the Tuxedo guppy are thus inferred to be $X_{Bcp,Rdt}Y_{Bcp,Rdt}$ for males (type I) and $X_{Bcp,Rdt}X_{Bcp,Rdt}$ for females. Tuxedo males that are heterozygous have the $X_{Bcp,Rdt}Y_{Bcp^+,Rdt}$ (type III), $X_{Bcp,Rdt}Y_{Bcp^+,Rdt^+}$ (type IV) and $X_{Bcp,Rdt}Y_{Bcp,Rdt^+}$ (type V) genotypes while the females are $X_{Bcp,Rdt}X_{Bcp^+,Rdt}$. The presence of these heterozygous individuals suggests that the black caudal-peduncle and red tail color patterns have not yet become “fixed” traits among cultured stocks of the Tuxedo strain.

Phenotypic map of *Bcp*, *Rdt* and the SdR

Our results prove that the *Bcp* and *Rdt* alleles are able to cross over from the X- to the Y-chromosome and vice versa since male and female recombinants of the TUX, RT, BCP and WT phenotypes were obtained from both F_1 and F_2 offspring of TUX♂♂×WT♀♀(types I, III, IV and V) and WT♂♂×TUX♀♀(Tables 1, 2, Figs. 1, 2). The phenomenon of alleles migrating between the X- and Y-chromosomes as a result of crossing-over was first documented in the guppy by Winge (1922a, b, 1923). Subsequent analyses by Winge (1927, 1934), Winge and Ditlevsen (1938), Dzwillo (1959), Nayudu (1975, 1979) and Kirpichnikov (1981) showed that the X- and Y-chromosomes of the guppy are cytologically indistinguishable and possibly share undifferentiated homologous regions along the lengths of their chromatids. Therefore, crossing-over occurs between the sex chromosomes, and recombination rates of up to 10% have been recorded between the *Vitellinus* (*Vi*) and *Elongatus* (*El*), and *Doppelschwert* (*Ds*) and *Pigmentierte caudalis* (*Cp*) genes (Winge, 1927, 1934; Winge and Ditlevsen, 1947; Dzwillo, 1959; Kirpichnikov, 1981; Purdom 1993). In our study, the occurrence of recombination shows that *Bcp* and *Rdt* are located within homologous regions on the X- and Y-chromosomes, and are approximately 5.147 and 3.387±1.992 map units, respectively, away from the sex-determining region (SdR) (Tables 1, 2, Fig. 3).

From the F_1 and F_2 recombinant data (Tables 1, 2), genetic map distances of 3.387±1.992, 5.147 and 2.397±1.714 map units were obtained for SdR–*Rdt*, SdR–*Bcp* and *Rdt*–*Bcp*, respectively (Fig. 3). As expected, the map distance between SdR and *Bcp* (5.147 map units) is close to the sum of distances (3.387+2.397=5.784 map units) for SdR–*Rdt* and *Rdt*–*Bcp* (Fig. 3). Estimates for crossing-over between two loci that are far apart are, however, never exactly the sum of the estimates for smaller regions amidst them (Purdom, 1993). This is because a crossover between two loci usually inhibits

a second crossover from occurring in an adjacent region (Strickberger, 1990). Crossing-over at SdR–*Bcp*, SdR–*Rdt* and *Rdt–Bcp* will thus influence each other, thereby affecting their frequency of occurrence. Despite this, our results show that *Rdt* is closer to SdR than *Bcp* because lower crossover frequencies (shorter map distances) were obtained between *Rdt* and SdR than from *Bcp* to SdR for all crosses (Tables 1, 2). Recombinant data for Tuxedo male parents of types I, IV and V also provides evidence that *Rdt* lies between the SdR and *Bcp*, hence indicating a gene map order of SdR–*Rdt–Bcp* (Fig. 3). The exception is mating pair PB4 which suggests a sequence of SdR–*Bcp–Rdt*. The latter may possibly result from translocation of a segment of the sex chromosomes that contain at least one of these color genes onto another, or pericentric inversion of a region that has two of these genes (Purdom, 1993).

In conclusion, the black caudal-peduncle (*Bcp*) and red tail (*Rdt*) genes of the domesticated Tuxedo guppy strain (1) occur as single genes at two different loci, and are (2) dominantly expressed, (3) X- and Y-linked, and (4) fully capable of crossing-over from the Y- to the X-chromosome and vice versa since they are situated in an undifferentiated homologous region on these chromosomes. Genetic map distances for the sex-determining region (SdR)–*Rdt*, SdR–*Bcp* and *Rdt–Bcp* are estimated to be 3.4, 5.1 and 2.4 map units, respectively. We therefore propose a phenotypic map of SdR–*Rdt–Bcp* (Fig. 3) for the Y-chromosome of the guppy. The *Bcp* and *Rdt* loci are also inferred to be at similar positions on the X-chromosome that was postulated by Winge (1927, 1934) to have a corresponding feminine segment of the SdR (Fig. 3). Our findings for the black caudal-peduncle and red tail genes, and the sex-determining region should prove valuable for the mapping of color pattern genes onto the sex chromosomes of the guppy as initiated by Winge (1927, 1934), and carried on by Winge and Ditlevsen (1947), Dzwillo (1959), Nayudu (1975, 1979), Kirpichnikov (1981), Fernando and Phang (1989, 1990), Phang *et al.* (1989a, b, 1990), Phang and Fernando (1991), Purdom (1993) and Khoo *et al.* (1999).

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REFERENCES

Dzwillo M (1959) Genetische Untersuchungen an domestizierten Stämmen von *Lebistes reticulatus* Peters. Mitt Hamburg Zool Mus Inst 57: 143–186

Fernando AA, Phang VPE (1985) Culture of the guppy, *Poecilia reticulata*, in Singapore. Aquaculture 51: 49–63

Fernando AA, Phang VPE (1989) X-linked inheritance of red and blue tail colorations of domesticated varieties of guppy, *Poecilia reticulata*, and its implications to the farmer. Singapore J Pri Ind 17: 10–18

Fernando AA, Phang VPE (1990) Inheritance of the Tuxedo and blond Tuxedo color pattern phenotypes of the guppy, *Poecilia reticulata*. In "Proceedings of the Second Asian Fisheries Forum" Ed by R Hirano and I Hanyu, Asian Fisheries Soc, Manila, Philippines, pp 487–490

Foo CL, Dinesh KR, Lim TM, Chan WK, Phang VPE (1995) Inheritance of RAPD markers in the guppy fish, *Poecilia reticulata*. Zool Sci 12: 535–541

Goodrich HB, Josephson ND, Trinkaus JP, Slate JM (1944) The cellular expression and genetics of two new genes in *Lebistes reticulatus*. Genetics 29: 584–592

Goodrich HB, Hine RL, Lesner HM (1947) Interaction of genes in *Lebistes reticulatus*. Genetics 32: 535–540

Haskins CP, Druzba JP (1938) Note on anomalous inheritance of sex-linked color factors in the Guppyi. Amer Nat 72: 571-574

Haskins CP, Haskins EF (1951) The inheritance of certain color patterns in wild populations of *Lebistes reticulatus* in Trinidad. Evolution 5: 216–225

Herre AWCT (1940) Additions to the fish fauna of Malaya and notes on rare or little known Malayan and Bornean fishes. Bull Raffles Mus 16: 27–61

Khoo G, Lim TM, Chan WK, Phang VPE (1999) Genetic basis of the variegated tail pattern in the guppy, *Poecilia reticulata*. Zool Sci (in press)

Kirpichnikov VS (1981) The genetics of aquarium fish species. In "Genetic Bases of Fish Selection" Translated by GG Gause. Springer-Verlag, Berlin-Heidelberg, Germany, pp 77-103

Nayudu P (1975) Contributions to the genetics of *Poecilia reticulata*. PhD Thesis, Monash University, Australia

Nayudu P (1979) Genetic studies of melanic color patterns, and atypical sex determination in the guppy, *Poecilia reticulata*. Copeia 1979: 225–231

Phang VPE, Ng LN, Fernando AA (1989a) Inheritance of the snakeskin color pattern in the guppy, *Poecilia reticulata*. J Hered 80: 393–399

Phang VPE, Ng LN, Fernando AA (1989b) Genetics of the color of the yellow snakeskin variety of the guppy, *Poecilia reticulata*. Singapore J Pri Ind 17: 19–28

Phang VPE, Fernando AA, EWK Chia (1990) Inheritance of the color patterns of the blue snakeskin and red snakeskin varieties of the guppy, *Poecilia reticulata*. Zool Sci 7: 419–425

Phang VPE, Fernando AA (1991) Linkage analysis of the X-linked green tail and blue tail color genes in the guppy, *Poecilia reticulata*. Zool Sci 8: 975–981

Purdom CE (1993) Genetics and Fish Breeding. Chapman & Hall, London, UK, 277 p

Schmidt J (1920) Racial investigations. IV. The genetic behavior of a secondary sexual character. CR Trav Lab Carlsberg 14: 1–8

Sokal RR, Rohlf FJ (1981) Biometry. The Principles and Practice of Statistics in Biological Research. 2nd ed, WH Freeman and Co, New York, USA, 859 p

Strickberger MW (1990) Genetics. 3rd ed, Macmillan Publishing Co, New York, USA, 842 p

Winge Ö (1922a) A peculiar mode of inheritance and its cytological explanation. J Genet 12: 137–144

Winge Ö (1922b) One-sided masculine and sex-linked inheritance in *Lebistes reticulatus*. J Genet 12: 145–162

Winge Ö (1923) Crossing-over between the X- and the Y-chromosome in *Lebistes*. J Genet 13: 201–219

Winge Ö (1927) The location of eighteen genes in *Lebistes reticulatus*. J Genet 18: 1–43

Winge Ö (1934) The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by *Lebistes*. CR Trav Lab Carlsberg, Ser Physiol, 21: 1–49

Winge Ö, Ditlevsen E (1938) A lethal gene in the Y-chromosome of *Lebistes*. CR Trav Lab Carlsberg, Ser Physiol, 22: 203–210

Winge Ö, Ditlevsen E (1947) Color inheritance and sex determination

in *Lebistes*. Heredity 1: 65–83
Yamamoto T-O (1975) The medaka, *Oryzias latipes*, and the guppy, *Lebistes reticularis*. In "Handbook of Genetics Vol 4" Ed by RC King, Plenum Press, New York, USA, pp 133–149
Yates F (1934) Contingency tables involving small numbers and the χ^2 test. J R Stat Soc (suppl) 1: 217–235

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